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이온화 방사선과 화학물질의 복합작용 해석기술 개발

Mechanism of Interaction between Ionizing Radiation and Chemicals

KAERI

한국원자력연구원

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제 출 문

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	세부(단위)과제명	이온화 방사선과 화학물질의 복합작용해석기술 개발			
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요약(연구결과를 중심으로 개조식 500자이내)	보고서 면수	

○ 이온화 방사선과 화학물질 등과의 복합작용에 의한 생물학적 영향의 평가와 관련 응용기술을 선진화하기 위하여 폴란드핵물리연구소와 공동연구개발을 수행함

○ 면역조직화학 검정법, 홀몬 검정법, 체세포돌연변이 검정법 등 다양한 분석기법을 확립하는 동시에 방사선과 타 요인의 복합생체영향을 정량화함

- 방사선과 수은화합물의 고등동물에 대한 영향평가를 위하여 IHC 등의 조직판별 기술을 적용하는 한편 식물에 대한 영향평가를 위해 TSH assay 기법을 효과적으로 적용함으로써 복합영향 평가의 기술적 수단을 확보함

○ 단세포 실험계인 효모세포 실험을 통하여 다요인 복합작용의 공통적 특성을 검출하고 상승작용에 관한 이론모델을 수립하는 한편 이를 라돈과 흡연의 복합영향평가에 활용함

- 방사선유발 세포손상 회복에 대한 화학물질의 억제효과 해석을 위한 이론체계 정립

- 방사선 등의 복합 상승작용에 관한 이론모델을 수립하고 평가 및 예측기술로 개선

○ 연구결과를 방사선 관련 학술지에 공동게재함은 물론 국내외 학술대회에 발표하여 연구결과의 학술적 인증을 획득함

○ 기관간 공동연구의 경험은 과학기술세계화를 위한 다자간협동연구 등에 적용될 수 있으며 특히, 한국·폴란드 간 과학기술 분야의 호혜적 동반자관계 발전에 기여할 것임

색 인 어 (각 5개 이상)	한 글	이온화 방사선, 화학물질, 생체손상, 손상회복, 복합상승작용, 생체검정법, 데옥시리보핵산, 면역조직화학분석
	영 어	Ionizing radiation, chemicals, biological damage, damage repair, synergistic interaction, bioassay, DNA, immunohistochemistry

빈 면

요 약 문

Ⅰ. 제 목

이온화 방사선과 화학물질의 복합작용해석기술 개발

Ⅱ. 연구개발의 목적 및 필요성

본 공동연구는 한국원자력연구원과 폴란드핵물리연구소가 각각 보유하고 있는 강점기술을 상호전수하고 공동으로 발전시키는 한편 상호보완적 실험연구를 수행하여 그 결과를 공동으로 활용하는데 목적이 있다. 폴란드는 방사선 연구 분야의 오랜 경험을 보유한 선도국의 하나로서 방사선생물학, 방사선물리학 및 방사선화학 등 기초과학 분야가 발전되어 있기 때문에 한국과 폴란드의 방사선 연구기관인 한국원자력연구원과 폴란드핵물리연구소간의 공동협력은 우리나라 방사선 연구개발 기술을 획기적으로 발전시킬 수 있는 계기가 될 수 있다. 모든 생물체는 생활환경에 존재하는 인공 및 자연 방사선과 여러 가지 물리적, 화학적, 사회적 요인의 영향을 받으면서 살고 있다. 여러 요인이 복합적으로 작용할 때 생물체에 유발되는 영향은 복잡하고 다양하기 때문에 평가나 사전 예측이 매우 어렵다. 다중 요인의 복합영향을 연구하기 위해서는 자극에 민감하게 반응하는 실험생물계를 확립하고 이들에 대한 각종 생물종말점을 분석함으로써 복합영향에 의한 생체손상 및 회복기작을 밝히는 것이 선결되어야 한다. 이를 위한 효과적인 생체검정기술의 확립이 필요할 뿐 아니라 이온화 방사선과 다른 물리화학적 요인이 생물체에 동시에 작용하였을 때 나타나는 영향을 입체적으로 해석할 수 있는 기술이 개발되어야 한다.

Ⅲ. 연구개발의 내용 및 범위

한국원자력연구원과 폴란드핵물리연구소가 공동으로 수행한 연구개발에는 다음과 같은 주요 내용과 범위가 포함되었다.

1. 방사선과 화학물질 동시처리에 따른 방사선유발 손상 회복의 억제
2. 방사선과 타 요인 복합작용에 관한 이론모델 수립
3. 상승작용 이론모델을 적용한 라돈과 흡연의 복합작용 해석
4. 상승작용 이론모델을 적용한 화학물질 복합작용 해석
5. 카페인과 아스코브산의 방사선손상 방어에 관한 면역조직화학적 평가
6. 이온화 방사선 및 저농도 염화수은 처리에 따른 영향
7. 이온화 방사선과 염화수은에 의한 고등식물 체세포 돌연변이

Ⅳ. 연구개발결과

1. 방사선과 화학물질 동시처리에 따른 방사선유발 손상 회복의 억제

세포에 있어서 손상복구기작 자체가 피해를 입거나 비가역 손상이 발생하는 경우 손상복구가 억제되어 세포회복의 비율 및 정도가 감소된다. 이 두 가지 가능성을 시험하기 위해서 CHO 세포에 X-선 단일조사, X-선 및 화학물질(pyruvate, novobiocin, lactate, nalidixic acid, 3-aminobenzamide) 동시처리를 하고 이 때 유발된 치사손상의 회복과정을 실험하였다. 유효선량 (effective radiation dose)의 감소는 곧 세포회복으로 이어진다는 개념을 적용하여 정량적인 분석을 시행한 결과 세포회복의 억제는 순전히 비가역 손상의 유발에 기인하는 것일 뿐 손상복구기작 자체는 영향을 받지 않는 다는 것이 밝혀졌다.

2. 방사선과 타 요인 복합작용에 관한 이론모델 수립

물리·하학적 요인이 복합적으로 작용하였을 때 일어나는 상승작용을 이론적으로 해석할 수 있는 수리모델을 수립하였다. 이 모델의 기본이론은 이온화 방사선에 의한 영향과 화학물질 따위의 타 요인에 의한 영향은 각각 치사손상과 준치사 손상을 유발하게 되며 이 때 각 요인에 의해 유발된 준치사 손상 (sublethal damage) 간의 상호작용에 의해 추가적인 치사손상이 발생하고 이것이 상승작용으로 나타난다는 것이다. 배수성 효모세포에 감마선 또는 자외선을 고온과 함께 처리한 다음 실험분석을 실시하였다. 실험결과 방사선 조사시 온도가 높을수록 액체유지회복 (LHR)이 감소하였다. 수리모델을 이용하여 예측한 값들은 실제 실험자료와 잘 일치함으로써 개발한 모델이 타당함을 확인하였다.

3. 상승작용 이론모델을 적용한 라돈과 흡연의 복합작용 해석

라돈과 단반감기의 붕괴산물들은 일반인들에 대한 주된 방사선 피폭 요인이 되고 있기 때문에 흡연과 라돈 흡입에 의해 나타나는 복합작용은 큰 문제이다. 전항의 연구에서 수립된 이론을 확장하여 라돈과 흡연의 복합작용에 따른 발암효과를 평가·예측할 수 있는 모델을 제시하였다. 이 모델 또한 각각의 요인에 의해 유발된 준손상들끼리 상호작용을 일으켜 추가적인 치사손상을 유발하게 되고 결과적으로 상승작용으로 이어짐을 강조하였다. 다른 연구자들에 의해 발표된 자료를 모델 계산과 비교한 결과 모델은 매우 합당하다는 것이 확인되었다. 또한 모델을 이용하여 최대상승작용에 이르게 하는 조건을 예측하는 것이 가능하다. 최대상승작용을 유발하는 요인별 강도가 최적값을 벗어날 경우 상승작용의 폭은 감소하였다.

4. 상승작용 이론모델을 적용한 화학물질 간 복합작용 해석 및 검증

이온화 방사선과 타 요인의 복합작용에 따른 상승작용 해석모델을 방사선 이외의 순수 화학물질의 복합작용에 적용하였다. Fluoride와 xylitol의 동시처리가 치아 구균의 산 (acid) 생산에 미치는 영향과 비교함으로써 개발된 모델의 응용성과 확장성을 입증하였다.

5. 카페인과 아스코브산의 방사선손상 방어에 관한 면역조직화학적 평가

방사선 작업종사자나 암치료 환자에 있어서 이온화 방사선으로부터 정산조직과 세포를 보호하는 것은 매우 중요한 문제이다. 산소가 방사선의 생체손상을 증대시킨다는 점과 연계하여 볼 때 항산화제는 방사선방어제의 기능을 가지고 있다고 볼 수 있다. 본 실험에서는 사람들이 아주 흔히 접할 수 있는 화학물질인 아스코브산과 카페인의 방사선손상 보호효과를 평가하였다. 방사선 조사된 생쥐의 체중변화, 장기 무게 변화를 분석하였고 방사면역법을 이용하여 혈청내 테스토스테론의 농도를 분석하였다. 카페인과 아스코브산은 공히 방사선 조사후의 체중감소를 현저히 경감시키는 효과를 나타내었으며 생식조직인 수정세관의 손상을 감소시키는 효과를 나타내었을 뿐 아니라 정자생성에 대한 방사선방어효과도 나타내었다.

6. 이온화 방사선 및 저농도 염화수은 처리에 따른 영향

생태계는 수은의 화학적 형상 및 산업화에 따른 방출로 지속적이면서 다양하게 오염된다. 수은이 유독한 것이 알려져 있기는 하나 생체가 환경에서 노출 가능한 저농도의 수은 영향에 대해서는 정확한 접근이 이루어지지 않고 있다. 본 실험은 이온화 방사선과 염화수은(II)의 영향을 비교해보고자 수행하였다. 미성숙의 웅성 F344 흰쥐에 음수에 염화수은을 녹여 실험기간 동안 음용시켰으며, 방사선 조사군의 경우는 6.5 Gy의 감마선을 전신조사하여 실험기간 동안 관찰하였다. 2주 후에 실험동물의 체중 및 기관 무게를 조사하고 분석을 위한 시료를 채취하였다. 혈액적 표지 인자들의 농도를 조사하였고, 각 외인성 인자에 의한 세포자연사 양상을 비교하고자 정소와 신장 조직에 대한 역전사중합반응을 실시하였다. 본 실험의 결과 염화수은(II)은 주대상기관이 신장이며 손상 기전은 이온화 방사선의 것과는 다른 양상을 나타나는 것으로 확인하였다.

7. 이온화 방사선과 염화수은에 의한 고등식물 체세포 돌연변이

자주달개비 4430 콜론은 분홍꽃이 피는 *T. subacaulis*와 남색꽃이 피는 *T. hirsutiflora*를 인위적으로 교잡시켜 만든 종간잡종이다. 이 종간 잡종 클론은 이온화 방사선 돌연변이유발물질에 매우 민감하기 때문에 방사선생물학적 연구재료로 각광을 받고 있다. 절화 실험

군을 0~2 Gy의 감마선 조사, 감마선 조사 전후 염화수은 양액 배양, 염화수은함유 양액 배양의 tkar 조로 나누어 실험을 실시하였다. 방사선 조사후 5일 째부터 분홍돌연변이가 크게 증가하기 시작하여 8~10일에 최고조에 달하였다. 수술털체세포 분홍돌연변이는 감마선 선량과 염화수은 농도에 대하여 뚜렷한 선량- 또는 용량-반응을 나타내었다. 본 연구를 통하여 자주달개비는 체세포돌연변이 검정법은 이온화 방사선과 화학물질이 복합적으로 식물체에 미치는 영향을 연구하는데 효과적인 도구로 활용될 수 있음이 확인하였다.

Ⅴ. 연구개발결과의 활용계획

본 연구를 통하여 확립된 생체검정법은 방사선생물학, 핵의학, 환경학, 위해성 평가, molecular epidemiology, human monmitoring 등 폭 넓은 분야의 분석·평가 기술로 적용가능하다. 공동연구기관 간 병립적 실험 및 연계실험을 수행함으로써 각 기관이 보유한 연구 장점을 최대로 활용할 수 있었을 뿐 아니라 한국원자력연구원 보유기술의 수준을 검증할 수 있었다. 연구를 거쳐 획득한 결과들은 저명학술지에 논문으로 게재하거나 국내외 학술대회를 통하여 결과를 공표함으로써 관련 연구자들이 손쉽게 활용할 수 있도록 하였다.

이온화 방사선과 기타 요인의 복합작용을 분석하여 범규칙성을 인출하고 이를 농해 이론모델을 개발하였으며 방사선이 아닌 화학적 요인들 간의 복합작용을 해석하기 위한 적용검증을 하였다. 공동결과를 국제학술지 논문으로 게재함으로써 우리 과학기술의 국제적 인지도를 높이는 한편 개발된 복합상승작용 이론모델에 많은 관심을 보이고 있는 유럽의 연구기관 (Spain Dawnesh Research Institute, Norwegian Radiation Protection Authority 등)과의 공동협력을 위한 발받침으로 활용할 예정이며 지속적인 보완과 개선 과정을 거쳐 다요인 생체영향 평가를 위한 입체평가법으로 활용할 것이다. 또한 본 공동연구의 경험은 한국과 폴란드간 과학기술 분야의 동반자 관계 증진을 위한 자료로 일익을 담당하게 될 것이다.

S U M M A R Y

Ⅰ. Title of Project

Mechanism of Interaction between Ionizing Radiation and Chemicals

Ⅱ. Objectives and Necessity of Research

The objectives of the project were collaboration between KAERI and INP through the parallel and joint experiments in each institute. Since Poland is one of the leading countries with a long experience in radiation research, coperation with INP can provide a great chance for KAERI to upgrade its R&D technologies in radiation research.

Organisms including human are never exposed to one environmental factor. They live in the mixture of natural- and man-made radiations and various physico-chemical factors. The biological effects due to the combined action of ionizing radiation with the other factor are hard to estimate and predict in advanced. It is possible to develop a technology for assessing the combined effects using experimental systems sensitive to radiation and chemicals, as well. For that purpose, it is necessary to study bioassay techniques in relation to a specific biological end-point and to develop a theoretical model for interpretation of synergistic interactions.

III. Work Scopes of Research

The cooperative research consisted of the following work scopes.

1. Irreversible damage production and inhibition of recovery from potentially lethal damage by chemicals
2. Development of a theoretical model for cell recovery after thermoradiation action
3. Description of synergistic interaction of radon and tobacco smoking
4. Description of synergistic interaction of chemical agents
5. Differential functions of caffeine and ascorbate in γ-irradiated male mice
6. Effects of mercury chloride (II) and ionizing radiation in prepubertal male rats
7. Somatic mutations of *Tradescantia* 4430 treated with ionizing radiation and mercury

IV. Results of Research

1. Irreversible damage production and inhibition of recovery from potentially lethal damage by chemicals

The inhibition of cell recovery might be proceeded via either the damage of the mechanism of the recovery itself or via the formation of irreversible damage which could not be repaired at all. Both these processes may take place at the same time. Any of these possibilities would result in a decrease in both the rate and extent of cell recovery. To distinguish them, a quantitative approach describing the process of recovery as a decrease in the effective radiation dose was applied to experimental data on the recovery from potentially lethal damage in CHO cells exposed to X-rays alone or combined with various chemicals (pyruvate, novobiocin, lactate, nalidixic acid, 3-aminobenzamide). For these particular cases, it is concluded that the recovery process itself is not damaged and the inhibition of the recovery is entirely due to the enhanced yield of the irreversibly damaged cells.

2. Development of a theoretical model for cell recovery after thermoradiation action

A theoretical model for the synergistic interaction of physical and chemical environmental agents was suggested for quantitative prediction of irreversibly damaged cells after combined exposures. The model takes into account the synergistic interaction of agents and based on the supposition that additional effective damages responsible for the synergy are irreversible and originated from an interaction of ineffective sublesions. The experimental results regarding the irreversible component of radiation damage of diploid yeast cells simultaneous exposed to heat with ionizing radiation (^{60}Co) or UV light (254 nm) are presented. It was shown that the cell ability ofthe liquid holding recovery decreased with an increase in the temperature, at which the exposure was occurred. A good correspondence between experimental results and model prediction was demonstrated.

3. Description of synergistic interaction of radon and tobacco smoking

Radon and its short-lived decay products are considered as the important sources of public exposure to natural radioactivity. The synergistic interaction between tobacco smoking and radon is known to be an actual problem. This study has provided a mathematical description and prognosis of the carcinogenic effects after

combined action of radon with smoking. A simple model was adjusted for the optimization and prognosis of the synergistic interaction of radon with smoking. The model postulates that the occurrence of synergism is to be expected as a result of additional carcinogenic damage arising from the interaction of sublesions induced by the two agents under consideration. The predictions of the model were verified by comparison with experimental data published by other researchers. The model appears to be appropriate and the predictions valid. The suggested mathematical model predicts the greatest level of synergistic effect and condition under which this level is reached. The synergistic effect appeared to decline with any deviation from the optimal value of the ratio of carcinogenic effective damages produced by each agent alone.

4. Description of synergistic interaction of chemical agents

The potential ability of various physical or chemical agents to enhance their effect when they are applied simultaneously with each other is well-known. The purpose of this study was to adjust a simple mathematical model to describe, optimize and predict a synergistic interaction between fluoride and xylitol on acid production by mutans streptococci. The model suggests that the synergism is caused by the additional effective damage arising from an interaction of sublesions induced by each agent. These sublesions are considered to be ineffective when each agent is used individually. The predictions of the model were verified by comparison with experimental data published by other researchers.It was shown that the model describes the experimental data, predicts the greatest value of the synergistic effect and the condition under which it can be achieved.

5. Differential functions of caffeine and ascorbate in γ-irradiated male mice

Radioprotection is of practical importance for the normal tissues of tumor patients subject to radiotherapy, people with planned or accidental exposure to radiation, and the public and radiation workers. Since oxygen enhances radiation-induced biological damage, antioxidants should be related with the function as a radioprotectors. Ascorbic acid or caffeine is an essential component and antioxidant in the diet of humans and a small range of other mammals. The present study investigates functional radioprotection of caffeine and ascorbic acid against gamma radiation in irradiated C57BL/6N mice.Eight-week-old male C57BL/6N mice were irradiated with

6.5 Gy. A caffeine treated group was administered with 80 mg/ kg body weight by intraperitoneal injection, a single treatment 1 hr before irradiation. Ascorbic acid was administered 330 mg/liter in drinking water through all the experimental period. According to time schedules, animals were sacrificed by cervical dislocation. And the samples were collected 2 weeks after whole-body gamma irradiation. The caffeine treated group showed lower decrement of body and organ weights than the other experimental groups. The qualitative analysis of circulating testosterone in serum was performed by means of radioimmunoassay (RIA). The normal level of circulating testosterone was maintained by the treatment of caffeine and ascorbic acid. The change of weight of body and organ and the appearance of seminiferous tubules were improved by an effect of caffeine of ascorbic acid against irradiation. Taken together, caffeine and ascorbic acid protects impairment of spermatogenesis against gamma radiation and may act as a radioprotector.

6. Effects of mercury chloride (II) and ionizing radiation in prepubertal male rats

Mercury, one of the most diffused and hazardous organ-specific environmental contaminants, exists in a wide variety of physical and chemical states. Although the reports indicate that mercury induces a deleterious damage, little has been reported from the investigations of mercury effects at surrounding levels in living things. The purpose of this study is to evaluate the biological effects of mercury chloride and ionizing radiation. Prepubertal male F344 rats were administered mercury chloride in drinking water throughout the experimental period. Two weeks after whole body irradiation, organs were collected for measuring the induced injury. The blood levels of red blood cells, white blood cells, hemoglobin, and hematocrit were checked in the experimental groups and the cortisol was analyzed in the serum. Relative expressions of mRNA related to mitochondrion-mediated apoptosis were investigated using semiquantitative reverse transcription polymerase chain reaction on gonad and urinary organs of the experimental groups. In conclusion, the target organ of mercury chloride seems to be urinary organs and the pattern of damage induced by mercury differs from that of the irradiated group.

7. Somatic mutations of *Tradescantia* 4430 treated with ionizing radiation and mercury

Tradescantia 4430 clone is an interspecific hybrid made by artificially crossing *T. hirsutiflora* with *T. subacaulis*. It has hereditary heteromorphy in its flower color, blue

dominant and pink recessive. The hybrid clone is exttremely sensitive to ionizing radiation and chemical mutagens. Groups of inflorescence cuttings were irradiated with 0.3, 0.5, 1.0, 1.5 and 2.0 Gy. After irradiation, the cuttings were maintained at 2 4℃ under a controlled light:dark (14:10) cycle. Five days after irradiation, mutations started to increase rapidly and reached a maximum rate during 8~10 days after irradiation. Mutation frequencies increased with radiation dose and with mercury concentration. In conclusion irradiation as well as mercury caused a dose- or concentration-response relationship in the somatic mutation frequencies of *Tradescantia* 4430. *Tradescantia* 4430 proved to be a good material for studying the combined effect of radiation and mercury.

V. Plan for Use of the R & D Results

Through the cooperative project, KAERI and INP have established wide variety of research techniques applicable to radiation bioscience, risk assessment, human monitoring, molecular epidemiology and environmental science. The joint experiment, in special, made it possible to utilize the merits of both institutes and to upgrade and verify KAERI's current technology level. Bioassays such as TSH assay, immunohistrochemical assay and hormone assay developed through joint research would be further elaborated and be continuously used for the collaboration between two institutes.

Using the common regularities from the combined actions, a theoretical model was formulated and applied to experimental data for validation. The results of the cooperative research were published in high standard scientific journals which could make the fundamental role for developing joint collaboration with Spain and Norway whose scientists showed their interest in the model approach. The model will be further elaborated for multidimensional assessment of the combined effects. Experiences of the joint collaboration can exert a favorable influence on improving relationship between Korea and Poland.

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제1장 연구개발과제의 개요

빈 면

제 1 장 연구개발과제의 개요

제 1 절 공동연구 추진 배경

제1차 한·폴 과학기술 공동위원회가 1995년 5월 폴란드 바르샤바에서 개최되어 총 8개 제안과제에 대하여 공동연구를 추진하기로 합의함에 따라 한국과 폴란드간의 과학기술 분야 공동연구가 시작되었다.

한국원자력연구원 (KAERI)과 폴란드원자물리연구소 (The Henryk Niewodniczanski Institute of Muclear Physics; INP) 두 기관은 방사선생물학, 방사선보건환경 분야, 생물물리학, 핵의학 등 다양한 분야에 걸쳐 공동관심사가 있음이 여러 경로를 통해 확인되었다. 제1차 한·폴 과학기술 공동위원회 개최에 따른 후속조치로서 1995년 12월 당시 한국원자력연구소와 폴란드핵물리연구소 (KAERI-INP) 간의 공동연구에 관한 약정 (MOU)을 체결하였다. 이를 근거로 최초의 한·폴 과학기술분야의 공동연구 과제인 '지표생물을 이용한 방사선량 감시' 과제가 착수되어 3년간 수행되었다.

양국간 공동연구가 호혜적이라는 점에 인식을 같이한 KAERI 및 INP 두 기관은 1998년 '방사선과 화학물질의 상호작용에 의한 생체영향 연구'를 공동으로 추진키로 하고 기관간 약정서의 보조약정 (Annex II)에 이 과제를 추가하고 2001년 7월 27일 바르샤바에서 개최된 제3차 한·폴 과학기술공동위원회에서 양기관에서 상정한 의제를 합의함에 따라서 보조약정에 포함된 공동연구 과제가 수행되었다. 또한, 2005년 5월부터는 KAERI-INP 병립적 연구수행을 기본 체제로 하고 주요 연구 사안에 대해서는 공동연계실험을 실시하는 방식으로 "이온화 방사선과 화학물질의 복합작용해석기술 개발" 과제를 수행하게 되었다.

한국원자력연구원은 본 공동연구를 통해 폴란드핵물리연구소의 연구시설과 숙련된 인력을 공동 활용할 수 있었으며 이를 통해 장기간 축적된 방사선생물학 관련기술과 생물검정기법을 공동 활용할 수 있는 계기로 삼을 수 있었다. 연구정보 교환, 연구원 상호교환방문 및 공동연계실험 수행을 거쳐 새로운 기술을 조기·도입하여 정착시킬 수 있었으며 이러한 과정을 통해 습득한 기술을 한국적 실정에 맞도록 개선함으로써 폭넓은 분야에 응용가능한 기술을 확보할 수 있었다.

제 2 절 연구개발의 필요성

최초의 방사성 물질인 라듐을 발견한 폴란드 태생 과학자 퀴리 부인의 명성에 걸맞게 폴란드는 기초과학에 오랜 경험을 보유한 방사선 연구개발 분야 선도국의 하나이다. 특히 방사선생물학, 방사선물리학 및 방사선화학 등 기초과학 분야가 발전되어 있기 때문에 한국과 폴란드의 방사선 연구기관인 한국원자력연구소와 폴란드 핵물리연구소간의 공동협력은 우리나라 방사선 연구개발 기술을 획기적으로 발전시킬 수 있는 계기가 될 수 있다.

인간과 생물체는 생활환경 중에 혼재해 있는 인공 및 자연 방사선과 여러 가지 물리·화학적 요인의 영향을 받으면서 살고 있다. 다양한 산업 분야에서 이온화 방사선이 가지고 있는 특성을 긍정적 이용하고 관련된 다른 기술들과의 융합을 위한 노력이 증대되고 있으며 특히 생물·화학적 이용효율을 증가시켜 특정 목적을 달성하기 위해서 방사선과 화학물질을 병용하는 사례가 증가되는 현실이다. 최근 방사선기술 (RT)를 BT, ET, NT 등의 주요 기술과 접합시킨 RFT (radiation fusion technology)를 정착시키고 이를 국가 주요기술의 하나로 발전시키기 위한 노력이 진행되고 있다.

이온화 방사선과 화학적, 물리적 요인이 복합적으로 작용할 경우 생물체 또는 생체물질에 유발되는 영향을 평가하거나 사전 예측하는 것은 매우 어렵다. 생물체에 미치는 이온화 방사선과 물리·화학적 요인의 복합영향을 판별하고 특성을 파악하는 것은 방사선의 효과를 긍정적으로 이용하기 위한 기술개발에 필수적인 요소로 인정된다. 특히 방사선의 산업적, 의학적 이용이 현저하게 증가하고 있는 추세를 감안할 때 이온화 방사선 및 화학물질의 상호작용에 의해 생물체에 나타나는 영향을 판별할 수 있는 기술개발과 함께 이를 개선한 응용기술의 확립은 과학기술의 국제경쟁력을 높이는 데 크게 기여할 것이다.

이온화 방사선에 민감하게 반응하는 실험생물계를 확립·이용하는 것이 중요하다. 고등동물의 림프구와 생식세포, 체세포 돌연변이 분석법이 확립된 Tradescantia 식물체 그리고 각종 실험에 적합토록 개발된 특성이 다양한 효모균주가 이온화 방사선과 화학요인의 복합작용을 규명하는데 효과적으로 이용되고 있다.

한국원자력연구원과 폴란드핵물리연구소는 1995년부터 2008년까지 방사선생물학, 핵의학 및 방사선환경 분야 등에 공동관심사를 가지고 있기 각기 보유하고 있는 강점 기술을 상호 전수 및 공유하는 등 상호 보완적 협력을 수행하여 왔다. 양국간 공동연구를 통해 호혜적인 협력관계를 구축해 온 것은 물론 폴란드 강점기술 분야의 하나인 방사선 연구기술을 도입, 상호전수 또는 공동 개발함으로써 우리나라 방사선 분야의 발전에 크게 기여할 수 있는 기틀을 마련할 수 있다고 판단된다.

제 3 절 공동연구의 목적 및 범위

KAERI-INP 공동연구는 양 기관이 보유하고 있는 강점기술을 상호전수 및 공유하고 발전시키는 한편 기관간 병립적이며 상호보완적 실험연구를 수행하여 그 결과를 공동으로 활용하는데 그 목적을 두었다.

이온화 방사선과 화학물질의 상호작용을 연구함에 있어 ^{60}Co 감마선 및 X-선을 방사선원으로 사용하였으며 화학물질의 경우 환경유해물질로 잘 알려져 있는 수은화합물을 대상으로 하였다. 수은화합물은 치의학적으로 이용되고 있을 뿐 아니라 사진감광, 물질표면살균 등 농업 및 산업적으로 이용되는 물질로서 이에 불가피하게 노출되는 경우가 발생할 수 있는 유해ㅐ화학물질로서 이를 연구대상물질로 삼았다. 이온화 방사선과 기타 요인 복합작용의 특성 및 공통점을 분석하고 상승작용에 관한 이론모델을 수립하기 위해서 효모세포계를 이용한 실험결과를 이용하였다. 본 연구의 범위는 다음과 같은 실험연구를 포함하고 있으며 이와 관련된 여러 생체검정법들이 이용되었다.

- ㅇ 체세포돌연변이 검정법 (TSH assay) ; 종간잡종 자주달개비 수술털에 나타나는 체세포 분홍돌연변이를 생물종말점으로 삼아 방사선과 수은의 복합영향을 분석하였다.
- ㅇ 면역조직화학 검정법 (IHC assay) ; 방사선 조사 및 염화수은 수용액을 음용시킨 고등동물의 조직에 나타나는 손상을 면역조직화학적 방법을 이용하여 평가하였다.
- ㅇ 모델 검정법 (model assay) ; 배수성 또는 반수성 효모 균주를 이용한 실험을 수행하였다. 복합작용의 규칙성을 검출하고 이론모델을 수립하고 다른 연구자들에 의해 수행된 실험결과를 이용한 검증을 실시하는 한편, 라돈과 흡연에 의한 복합작용, 그리고 방사선 이외의 화학물질간의 복합작용을 평가하는데 적용함으로써 모델의 타당성 및 적용성을 검증하였다.

제 4 절 상대국과의 공동연구 역할 분담 체계

고등식물 체세포돌연변이 검정법에 사용된 연구용 식물체는 폴란드핵물리연구소에서 이전 공동연구 과정 중에 입수한 식물체를 한국원자력연구원의 표준조건 하에서 재배하여 사용하였다. 실험에 앞서 이들에 대한 유전적 순수성 검증과정을 거친후 공시재료로 사용하였다. 고등동물 조직에 대한 면역조직화학적 분석 및 분자생물학적 분석을 위해서는 Fisher 344 수컷 쥐를 사용하였다.

연구에 이용되는 기반시설로서의 방사선 조사장치의 경우 INP는 자체 보유시설인 X-선 발생장치 (Phillips Co.)를 주로 방사선 조사에 이용하였다. 또한 공동실험용 감마선

조사에는 한국원자력연구원 본원 및 정읍 방사선과학연구소의 저준위 및 고준위 조사시설 (Panoramic Irradiator, AECL)의 ^{60}Co 방사선원을 이용하였다.

폴란드핵물리연구소는 공동연계실험의 경우 돌연변이 분석 및 세포 유전물질 손상 평가를 위한 실험에 5인의 연구인력을 실험 필수기간 중 투입하였다. 연계 공동실험에 소요되는 소모성 경비는 실험연구의 work scope 별로 나누어 각 기관이 부담하였다.

제 5 절 공동연구기관 및 공동연구책임자의 연구수행능력

1. 공동연구기관

○ 기관명 : 헨릭니보드니찬스키 핵물리연구소
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○ 개 관

폴란드 남부 지방에 위치한 고도인 크라쿠프에 위치하고 있는 폴란드핵물리연구소는 1955년 전 폴란드원자력청 (PAEA) 장관의 부친인 Prof. Henryk Niewodniczanski에 의해 설립되었다. 공식 명칭은 설립자의 이름을 따라 헨릭니보드니찬스키 핵물리연구소 (The Henryk Niewodniczanski Institute of Nuclear Physics)로 불린다. 이 연구소는 2003년 말까지는 폴란드원자력청 산하기관이었으나 그 이후 폴란드과학아카데미 (Polish Academy of Sciences)로 소속을 옮겼으며 연구소원의 숫자도 기존 400여명에서 1/4 이하로 줄어들어 규모가 축소되었으며 2008년 3월 현재 연구소 시설 및 설비 전체에 대한 remodelling이 진행 중이다.

공동연구의 상대책임자인 Prof. Antonina Cebulska-Wasilewska는 INP 방사선환경생물연구실 (DREB) 실장인 동시에 폴란드 야길론 대학교 (Jagiellonian University) 예방의학과 교수직을 겸하고 있다. DREB는 방사선생물학 연구에 필요한 각종 연구시설을 갖추고 있다. 연구용 식물체 유지 및 증식에 필요한 대형 자동화 유리온실과 각종 환경조건의 인위적 조절이 가능한 대형 배양설비 (growth facility), 동물세포 배양시설 등은 본 연구에 있어 활용도가 높았던 시설들이다. X-ray 조사시설은 물론 미세 화상분석 설비, 실험동물 사육시설, 현미경실, 세포유전분석실, 초저온설비 등을 갖추고 있다.

2. 공동연구책임자의 연구수행능력

공동연구책임자는 현재 폴란드핵물리연구소의 학술위원회 (Scientific Committee)의 위원인 동시에 다국적 과제인 EU project의 평가위원을 맡고 있으며, 폴란드 원자력/방사선 분야의 가장 권위 있는 학술지인 뉴클레오니카 (*Nukleonika*) 학술 및 편집위원을 맡고 있다. 또한 우리나라 학술진흥재단 등재지인 한국환경생물학회지 (Korean Journal of Environmental Biology)의 편집위원 그리고 정읍 방사선과학연구소가 발간하고 있는 방사선산업학회지 (Journal of Radiation Industry)의 편집위원을 맡고 있어 한국-폴란드 간의 과학기술 교류의 대표적 인물로 꼽히고 있다.

INP 연구소에서 25년 이상에 달하는 방사선생물학연구 수행을 통하여 습득한 선진연구 기법들을 보유하고 있으며 특히 방사선지표식물 연구에 있어서는 전 세계적인 중심 역할을 수행하여 오고 있다. 미국의 BNL 연구소와 일리노이 대학교, 네덜란드 라이든대학교, Netherlands Energy Research Foundation, 오스트리아 빈대학교 암종양연구소, 독일 움대학교 분자생물연구실 등과 오랜 기술교류 및 공동연구를 수행하고 있다는 사실은 연구책임자가 원자력 선진 각국으로부터 그 연구능력을 인정받고 있다는 것을 뜻한다.

빈 면

제2장 국내외 기술개발 현황

빈 면

제 2 장 국내외 기술개발 현황

제 1 절 생물검정 기술의 특성 및 기술개발 현황

한국원자력연구원과 폴란드핵물리연구소가 각각 개발 또는 보유하고 있는 강점기술을 상호전수 및 공유하고 공동으로 발전시키며 상호보완적 실험연구를 수행하여 그 결과를 공동으로 활용하는데 본 공동연구의 목적이 있다. 특히 이온화 방사선과 유해 화학물질이 생물체에 동시에 작용하였을 때 나타나는 복합 생체영향을 평가하고 이를 해석하기 위하여 사용된 평가 기술은 다음과 같다.

고등식물 체세포돌연변이 검정법은 자주달개비의 분열중인 세포들은 이온화 방사선, 돌연변이원, 독성물질 등에 매우 민감하게 반응한다. 분열중인 체세포에 나타나는 유전자 돌연변이 및 치사돌연변이를 생물종말점으로 이온화 방사선과 화학물질의 복합작용을 분석한다. 한국원자력연구원과 폴란드핵물리연구소의 상호검증 공동실험을 통하여 이 기법을 지속적으로 개선하여 염화수은과 감마선의 복합영향을 정량화하는 기술이다. 한편, 면역조직화학 검정법은 특정한 물질과의 면역반응을 이용한 조직화학적 염색법을 이용하여 고등동물 조직을 염색함으로써 necrotic cell, apoptotic cell 등을 판별하는 한편, 조직의 구조적, 형태적 이상 여부를 시각적으로 판별하는 기술로서 외부자극에 민감한 조직에 대한 방사선과 수은화합물의 복합영향을 규명하는 데 적절히 이용될 수 있다. 고등식물의 체세포 돌연변이를 이용하여 이온화 방사선의 영향을 연구한 사례는 다음과 같다.

▷ Cebulska-Wasilewska, A, Schneider, K, and Kim, JK. Relative biological efficiency of californium-252 neutrons in the induction of gene and lethal mutations in Trad-SH normal and enriched with boron-10 cells, Mutation Research - Fundamentals and Molecular Basis of Mutagenesis, 474: 57-70 (2001).

▷ Cebulska-Wasilewska, A. *Tradescantia* stamen-hair mutation bioassay on the mutagenicity of radioisotope-contaminated air following the Chernobyl nuclear accident and one year later. Mut. Res., 270: 23-29 (1992).

▷ Shin HS and Kim JK. Biological effects of mercury compound and radiation evaluated by *Tradescantia* pink mutaions, *The Journal of Disaster-Prevention,* 7(1), 3-13 (2004).

▷ 박희전, 이영엽, 김진규. 방사선과 수은에 의한 자주달개비 체세포 돌연변이. 방사선산업학회지, 1(3): 149-153 (2007)

▷ Kim JK, Shin HS, Lee JH and Cebulska-Wasilewska A. Detection of

environmental mutagen with Tradescantia micronucleus assay and chemical analysis, *In*: (2003) J Maluszynska and M Plewa eds., "BIOASSAYS IN PLANT CELLS" (Proceedings of the EC Workshop on Bioassays in Plant Cells for Improvement of Ecosystem and Human Health, pp. 61-74, May 12-16, 2003, Katowice, Poland (2003).

제 2 절 복합 상승작용에 관한 연구개발 현황

산업 및 의료 분야에 있어서 방사선의 긍정적 이용이 증가되고 있으며 다양한 용도로 사용되는 화학물질 또한 질적 양적 팽창을 거듭하고 있다. 방사선에 민감한 생물지표 또는 생물종말점 중에서 사람 림프구와 TSH 세포는 돌연변이 반응성면에서 많은 유사도를 가지고 있기 때문에 두 실험계를 병립적 또는 복합적으로 이용함으로써 다양한 유해 화학물질과 이온화 방사선의 복합작용에 의한 영향을 정량적으로 평가할 수 있음이 연구개발 결과로 나타나고 있다. 또한 방사선 감수성이 서로 다른 여러 균주가 가용한 효모는 배양이 비교적 용이하기 때문에 입체적 실험에 적합한 공시재료로 각광 받고 있다.

현대 사회는 생물 위해요인이 복합적으로 공존하기 때문에 생체영향의 평가를 위한 다면평가기법이 필요하다. 여러 가지 요인들이 동시에 작용하여 나타나는 복합작용(combined interaction)을 해석하기 위해서는 기존의 평면적 실험으로는 적절한 데이터를 얻기 어렵다. 복합 상승작용을 나타내는 실험결과는 어렵지 않게 접할 수 있으나 이를 각 요인 별 기여도, 상승작용이 차지하는 분율 등으로 구분 해석하거나 더 나아가 복합 상승작용을 일으키는 요인변 강도[농도] 범위 등을 예측하는 것은 매우 어려운 과제이다.

러시아의료방사선연구센터 (MRRC RAMS)과 한국원자력연구원은 각종 실험결과로부터 나타나는 다요인 복합작용의 공통적 규칙성을 인출하고 복합상승작용 유발기작을 표현할 수 이론모델에 관한 연구를 수행해 오고 있다. Synergistic interaction에 관한 연구에 있어서 MRRC는 뛰어난 연구업적을 가지고 있는 기관이다. 연구대상 생물로는 단순한 eukaryotic cell system인 효모 세포를 주로 이용하여, 이온화 방사선은 물론 고온, 자외선, 초음파, 마이크로파 등의 비이온화 방사선 (non-ionizing radiation)에 이르는 폭넓은 분야의 물리·화학적 요인들 간 복합작용을 연구하고 있다. 한국원자력연구원 연구진 또한 복합작용에 관한 연구개발 분야에 있어서는 세계적으로 인정받고 있다.

▷ Kim JK and Petin VG. Theoretical conception of synergistic interactions, Kor. J. Environ. Biol., 20(4): 277-286 (2002).

▷ Kim JK, Petin VG and Tkhabisimova MD. Survival and recovery of yeast cells

after simultaneous treatment of UV light radiation and heat, Photochemistry and Photobiology, 79(4): 349-355 (2004).

▷ Petin VG and Kim JK. Survival and recovery of yeast cells after combined treatments with ionizing radiation and heat, Radiation Research, 161(1): 56-63 (2004).

▷ Kim JK, Petin VG and Zhurakovskaya GP. Exposure rate as a determinant of synergistic interaction of heat combined with ionizing or ultraviolet radiations in cell killing, Journal of Radiation Research, 42(4):361-369 (2001).

▷ Komarova LN, Kim JK and Petin VG. Mathematical description and prognosis of cell recovery after thermoradiation action. Kor. J. Environ. Biol. 26(1): 1-7 (2008).

▷ Kim JK, Belkina SA and Petin VG. Mathematical description and prognosis of synergistic interaction of radon and tobacco smoking, Iranian Journal of Radiation Research, 4(4), 169-174 (2007).

▷ Kim JK, Komarova LN, Zhurakovskaya GP and Petin VG. The fluence rate determines the synergistic interaction of UV radiation and heat for mitotic recombination and cell inactivation in yeasts, Photochemistry and Photobiology, 82(4), 1053-1057 (2006).

▷ Kim JK, Shin HS, Petin VG and Lee YY. Experimental analysis on regularities of synergistic interaction of temperature with physico-chemical environmental factors. *Kor. J. Environ. Biol.* 20(2): 165-172 (2002).

빈 면

제3장 연구개발 수행내용 및 결과

빈 면

제 3 장 연구개발 수행내용 및 결과

제 1 절 방사선과 화학물질 동시처리에 따른 방사선유발 손상 회복의 억제

1. Introduction

It is believed that DNA repair is one of the most important factors in determining cellular sensitivity to ionizing radiation and some other cytotoxic agents [1]. Many tumours are known to become resistant to ionizing radiation due to the increased efficiency of DNA repair [2]. Therefore, the impairment of the cell ability to recover from radiation damage would be of great relevance in cancer treatment. A lot of chemicals are known to enhance the inactivation effect of ionizing radiation on various cellular systems. It seems generally accepted now that the enhancing effects may be due to both direct drug toxicity and to the enhancement of the cellular radiosensitivity [3,4]. It is assumed that drug radiosensitization may be brought about by the inhibition of repair on a cellular level including the recovery from potentially lethal radiation damage. This type of recovery was demonstrated in the cells of various origins and may play a role in the treatment of tumours with ionizing radiation [5]. The inhibition of cell recovery [6-9] and DNA double strand breaks repair [10-12] by chemicals is expressed both as a retarded recovery rate and a lesser extent of recovery. It is obvious that these observations may be caused by the following reasons: (i) the damage or inhibition of the recovery process itself, (ii) the increase in the portion of irreversible damage, (iii) both of these reasons. In our recent publications it was shown that the inhibition of the recovery from potentially lethal damage in yeast cells exposed to hyperthermia and ionizing radiation [13] or hyperthermia and UV light [14] was realized only through the enhanced yield of the irreversible damage whereas the recovery capacity itself was not damaged orimpaired. Although there are considerable interests in combining ionizing radiation with chemicals in order to improve tumor treatment, there are few reports in the literature on a separate estimation of the chemicals influence on the process of recovery itself and the production of irreversible damage. Therefore, it would be of particular interest biophysically to implement such a study. In this paper, a quantitative approach

describing the recovery of a cell from potentially lethal damage as a decrease in the effective dose will be used to estimate separately the probability of recovery per unit time and the fraction of irreversible damage. As an example, this approach will be applied to experimental data published by others [6,7] and related with the inhibition of recovery from potentially lethal damage by chemicals (pyruvate, novobiocin, lactate, nalidixic acid and 3-aminobenzamide) in Chinese hamster V79 cells.

2. Material and methods

Experimental procedures. Experimental data published by others [6,7] have been used for this study. Nevertheless some important points should be mentioned. To determine the time course of the inhibition of recovery from potentially lethal damage, immediately after X-irradiation the stationary phase Chinese hamster cells were incubated with 0, 10, 20 mM of pyruvate, lactate or 3-aminobenzamide, 0, 5, 10, 20 M of novobiocin or nalidixic acid during 6, 12, and 24 h before they were plated without chemicals to determine their survival by colony-forming ability. Besides, cells were treated with 20 mM of pyruvate and lactate and 20 M of novobiocin and nalidixic acid starting 1 h after irradiation for 6, 12, and 24 h and then replated for colony formation without chemicals. Chemicals were added following irradiation in order to limit their effect to the recovery period only. For details of protocol see the initially published papers [6,7].

Estimation of the recovery parameters. During the recovery process the survival of the irradiated cell increases, which means the decrease in the effectiveness of the initial dose D_1 takes place. Then a certain survival $S(t)$ and the corresponding effective dose $D_{eff}(t)$ can be indicated following the recovery during t hours. In other words, the effective dose is equal to the dose in which the cells should initially be irradiated to attain $S(t)$. It was demonstrated [15,16] that duringthe recovery of yeast cells from potentially lethal radiation damage, the decrease in the effective dose could be described by an equation of the form

$$D_{eff}(t) = D_1[K + (1 - K)\, e^{-\beta t}], \quad (1)$$

where D_1 is the initial radiation dose; e is the basis of the natural logarithm, β is the recovery constant that characterizes the probability of recovery from radiation damage per unit time, and K is an irreversible component of the radiation damage expressed as a fraction of the initial irradiation dose by

$K = K(\infty) = D_{eff}(\infty) / D_1,$ (2)

where $K(\infty)$ and $D_{eff}(\infty)$ are determined for mammalian cells at t = 24 h when the recovery curves reach a plateau (conditionally $t = \infty$) and the capability of the cells to recover is saturated or exhausted. The ratio $D_{eff}(\infty) / D_1$ can be considered as an irreversible component of the radiation damage. The cells are believed to be incapable to recover from this part of damage even if they are incubated for a long time at the conditions promoting the recovery. Then the change of this component during the recovery

$K(t) = D_{eff}(t) / D_1$ (3)

reflects the relative part of the initial radiation dose or the primary radiation damage, both reparable and irreversible, which has not been repaired during thours of recovery. Taking into account Eqs. (1) and (2), we have

$e^{-t} = [D_{eff}(t) - D_{eff}(\infty)] / [D1 - D_{eff}(\infty)].$ (4)

The right part of this Equation reflects the relative part of the reparable damage that has not been repaired after t hours of recovery. Designating this part through $A(t)$, we can write

$\beta = -[lnA(t)] / t.$ (5)

Thus, by knowing the dependence of the cell survival for a radiation dose and the recovery pattern after exposure to ionizing radiation applied alone or combined with various chemicals, one can calculate $D_{eff}(t)$ and $D_{eff}(\infty)$. It enables us to calculate $K(t)$ for the dependency of the recovery time t (Eq. 3) and evaluate the irreversible component K (Eq. 2). Having calculated the dependency of $lnA(t)$ for the recovery time t, one can estimate the recovery constant β (Eq. 5). It is worth noting that very similar approach was used before to describe the biological effects after whole body gamma irradiation [17], the recovery of yeast cells irradiated with ionizing radiation [15,16] or exposed to heat combined with ionizing radiation [13] and UV light [14]. So far its applicability to mammalian cells irradiated alone or combined with chemicals has never been tested.

3. Results

Fig. 1 shows the survival (curve 1) and recovery (curve 2) curves of the stationary phase cells of Chinese hamster V79 cells irradiated (300 kV X-rays, dose rate being 1.25 Gy/min) and recovered without chemical treatments. Arrows indicate

an example of the effective dose estimation. Both these curves were obtained by averaging the four dose-effect and four time-effect curves published by other authors [6,7]. Kinetics of the recovery from potentially lethal radiation damage showed [6,7] that the survival increase due to recovery observed in the controls was gradually reduced asthe chemical concentration increased, i.e. the inhibition of the recovery was drug concentration dependent. Using these results and the data presented in Fig. 1, we calculated (Eq. 3) the dependency of the relative fraction of the irreversible damage $K(t) = D_{eff}(t) / D_1$ on the duration of the recovery time of the Chinese hamster V79 cells recovering after irradiation without chemicals and in the presence of various chemical inhibitors of the cell recovery. The results are shown in Fig. 2. It can be noted that the untreated cells subjected to post-irradiation recovery showed an appreciable decrease in $K(t)$ whereas this effect became gradually worse as the chemical concentration increased. It appears that the inhibition of the recovery depends on the drug concentration and is almost complete with 20 mM of pyruvate and lactate and 20 M of novobiocin and nalidixic acid. For instance, the limited values of $K(t)$, i.e. the values of the irreversible component $K = K(\infty)$, *are* equal to 0.60, 0.75, and 0.92 for the cells recovering from radiation damage without a drug and in the presence of 10 and 20 mM pyruvate, respectively. Qualitatively similar results were obtained for other chemicals. Fig. 3 shows the calculated the irreversible component of radiation damage, $K(\infty)$, in Chinese hamster V79 cells recovering from potentially lethal damage with and without various inhibitors of recovery. The obvious increase in the irreversible component with drug concentration should certainly lead to a decrease in the recovery rate because of the decrease in the number of cells capable of recovery.

To clarify whether or not this decrease could explain the observable deceleration of the recovery rate, we estimated the probability of recovery for various conditions of the recovery. The experimental data make it possible to calculate the function $A(t) = [D_{eff}(t) - D_{eff}(\infty)] / [D_1 - D_{eff}(\infty)]$ for the dependency of the recovery time of the Chinese hamster V79 cells recovering after irradiation without chemicals and in the presence of various chemical inhibitors. The results are shown in Fig. 4. One can see that this function declines exponentially with the recovery time independently of whether or not the recovery took place without chemicals or with an increasing concentration of various drugs.

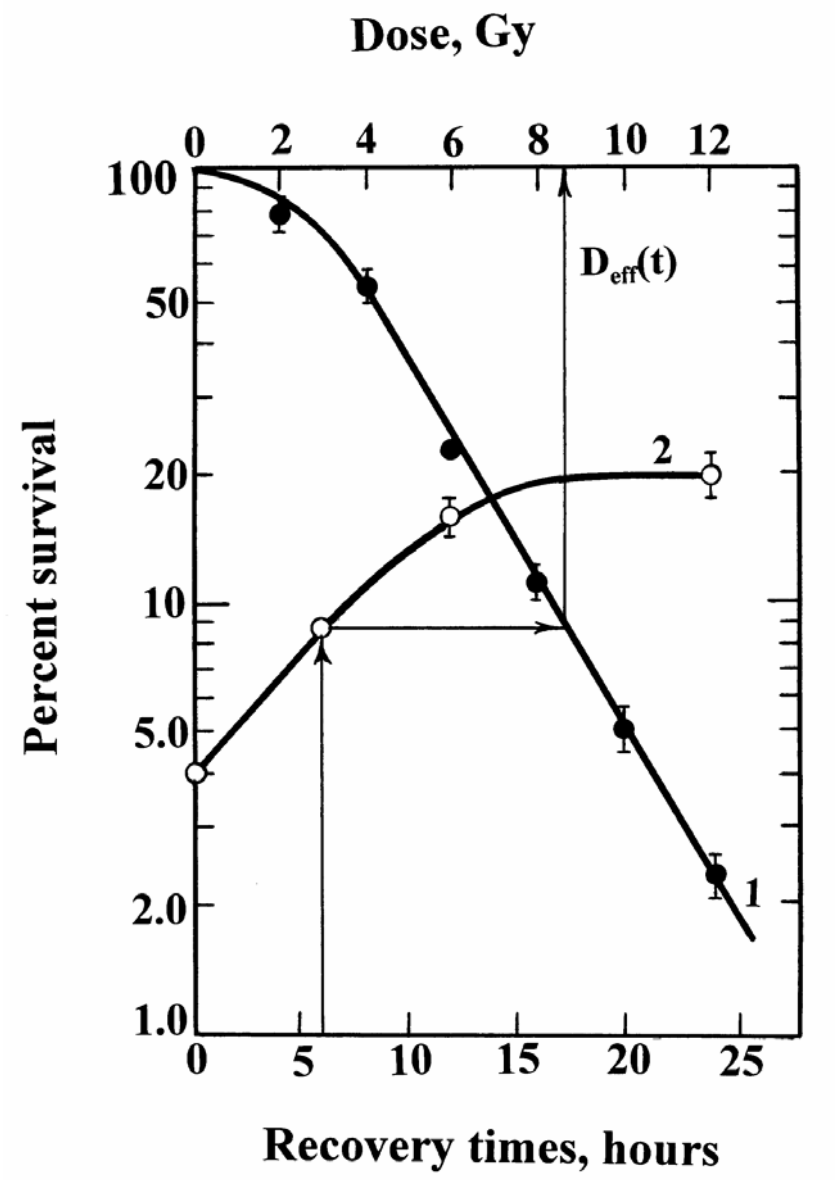

Fig. 1. Survival of Chinese hamster V79 cells in the dependence of X-ray dose (curve 1) and the duration of recovery from potentially lethal damage (curve 2). Cells were irradiated and recovered without chemicals. The results were obtained by averaging of six dose-effect and four time-effect curves published by other authors (6). Arrows indicate an example of the initial dose D_1 and the effective dose $D_{eff}(t)$ determination. *Points*, mean; *bars*, SE.

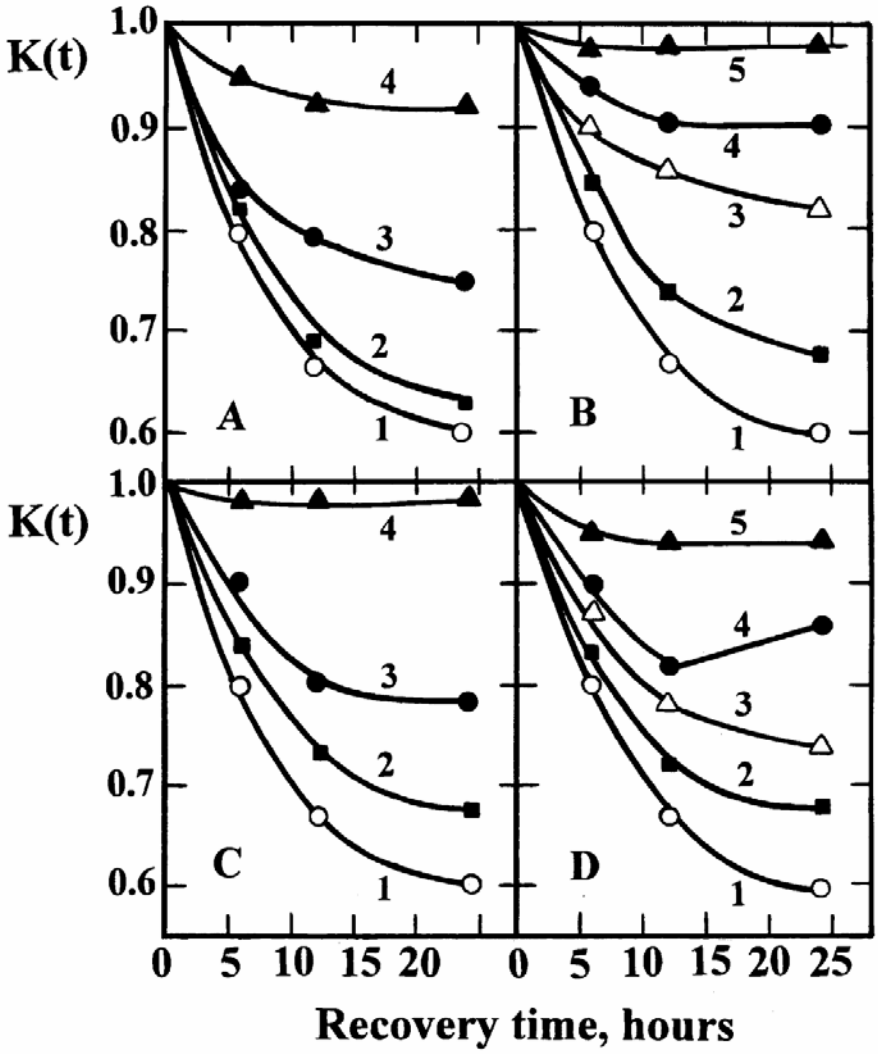

Fig. 2. The dependence of the relative fraction of irreversible damage $K(t) = D_{eff}(t) / D_1$ on the duration of recovery time of Chinese hamster V79 cells recovering after irradiation without chemicals (curves 1) and in the presence of chemical inhibitors of cell recovery. A, pyruvate: 20 mM 1h after irradiation (curve 2), 10 and 20 mM immediately after irradiation (curves 3 and 4). B, novobiocin: 20 M 1h after irradiation (curve 2), 5, 10 and 20 M immediately after irradiation (curves 3, 4 and 5). C, lactate: 20 mM 1h after irradiation (curve 2), 10 and 20 mM immediately after irradiation (curves 3 and 4). D, nalidixic acid: 20 M 1h after irradiation (curve 2), 5, 10 and 20 M immediately after irradiation (curves 3, 4 and 5).

By taking Eq. (5) and the results shown in Fig. 4, we calculated the recovery constant β for all the recovery conditions can be evaluated. The total set of parameters describing the recovery of the Chinese hamster cells under different postirradiation conditions are summarized in Table 1. This Table also includes the parameters describing the recovery of the Chinese hamster cells in the presence of different concentrations of 3-aminobenzamide estimated on the basis of the experimental data published by others [7] but not presented here in Figures. It can be seen that in all the cases the recovery constant was independent of the recovery conditions (β = 0.15 0.01 hour^{-1}) whereas the irreversible component was gradually enhanced as the chemical concentration increased.

To illustrate the correctness of the approach used here, Fig. 5 exhibits the survival of the Chinese hamster V79 cells for dependencyof the duration of recovery from potentially lethal damage recovering after irradiation without chemicals and in the presence of some chemical inhibitors of the cell recovery. Experimental points were taken from (Kumar et al. 1985a). Solid lines were calculated in the following way. The effective dose $D_{eff}(t)$ was computed in accordance with Eq. 1 and the parameters of the recovery obtained in this study (Table 1). Then the corresponding survival $S(t)$ was estimated using the example shown in Fig. 1 by the arrow. One can see a close fit of the predicted curves to the experimental data. Thereby the applicability of the mathematical approach to describe the recovery from potentially lethal damage was demonstrated.

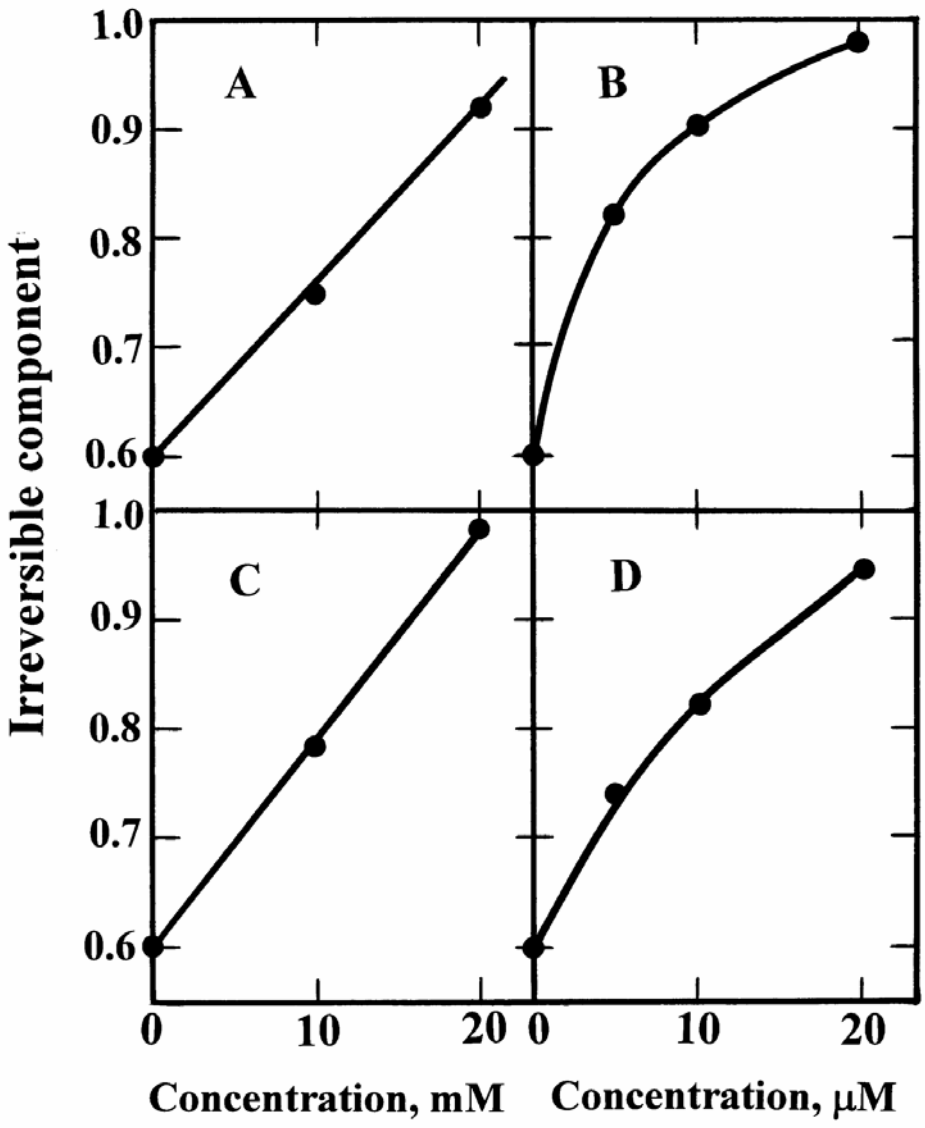

Fig. 3. Irreversible component $K(\infty)$ of radiation damage in Chinese hamster V79 cells recovering from potentially lethal damage in the presence of various concentrations of chemicals: pyruvate (A), novobiocin (B), lactate (C), and nalidixic acid (D).

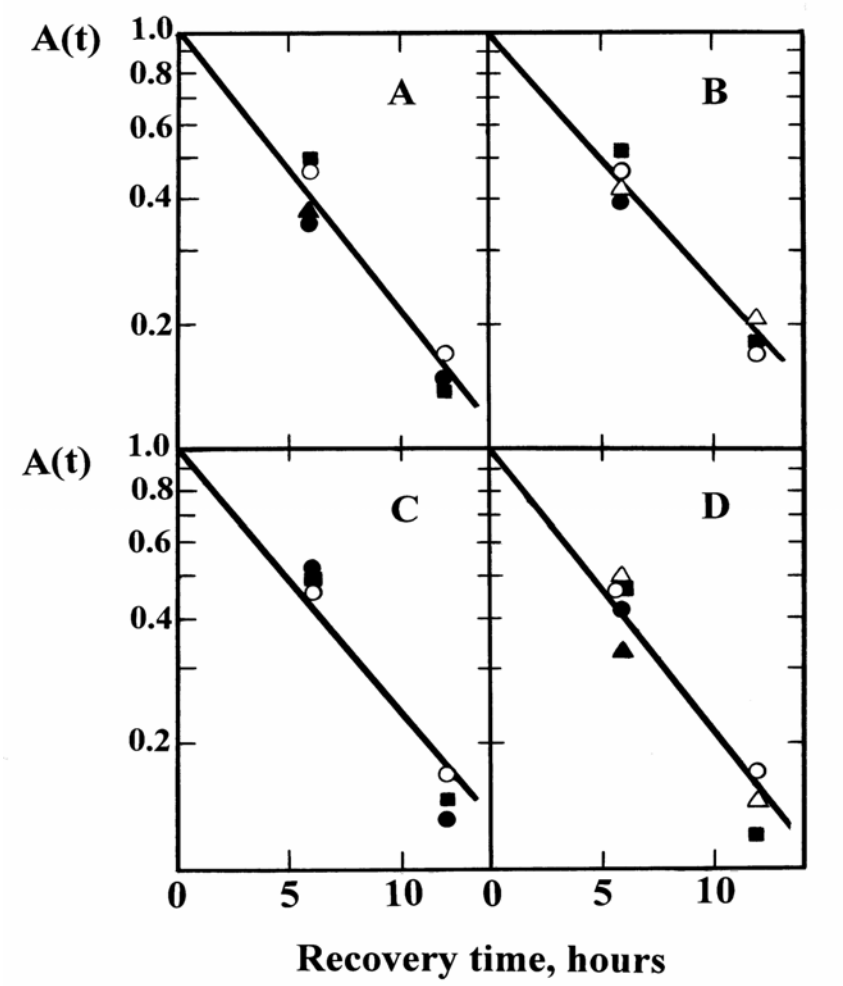

Fig. 4. The dependence of $A(t) = [D_{eff}(t) - D_{eff}(\infty)] / [D_1 - D_{eff}(\infty)]$ on the duration of recovery time of Chinese hamster V79 cells recovering after irradiation without chemicals (open circles)and in the presence of chemical inhibitors of cell recovery. A, pyruvate: 20 mM 1h after irradiation (closed squares), 10 and 20 mM immediately after irradiation (closed circles and triangles, respectively). B, novobiocin: 20 M 1h after irradiation (closed squares), 5, 10 and 20 M immediately after irradiation (open triangles, closed circles and triangles, respectively). C, lactate: 20 mM 1h after irradiation (closed squares), 10 and 20 mM immediately after irradiation (closed circles and triangles, respectively). D, nalidixic acid: 20 M 1h after irradiation (closed squares), 5, 10 and 20 M immediately after irradiation (open triangles, closed circles and triangles, respectively).

Table 1. Radiobiological parameters of Chinese hamster cells recovery

Chemicals	Conditions of recovery	Irreversible component K	Recovery constant β , hr^{-1}
Without chemicals	Without chemicals	0.60	0.15
Pyruvate	20 mM 1h after irradiation	0.63	0.16
	10 mM immediately after irradiation	0.75	0.16
	20 mM immediately after irradiation	*0.92*	*0.16*
Novobiocin	*20 M 1h after irradiation*	*0.68*	*0.14*
	5 M immediately after irradiation	*0.82*	*0.14*
	10 M immediately after irradiation	*0.90*	*0.14*
	20 M immediately after irradiation	*0.98*	-
Lactate	*20 mM 1h after irradiation*	*0.67*	*0.14*
	10 mM immediately after irradiation	*0.78*	*0.14*
	20 mM immediately after irradiation	*0.99*	-
Nalidixic acid	*20 M 1h after irradiation*	*0.68*	*0.15*
	5 M immediately after irradiation	*0.74*	*0.15*
	10 M immediately after irradiation	*0.82*	*0.15*
	20 M immediately after irradiation	*0.94*	-
3-aminobenzamide	*20 mM 3h after irradiation*	*0.68*	*0.15*
	10 mM 1 h before + after irradiation	0.71	0.15
	20 mM 1 h before + after irradiation	0.95	-

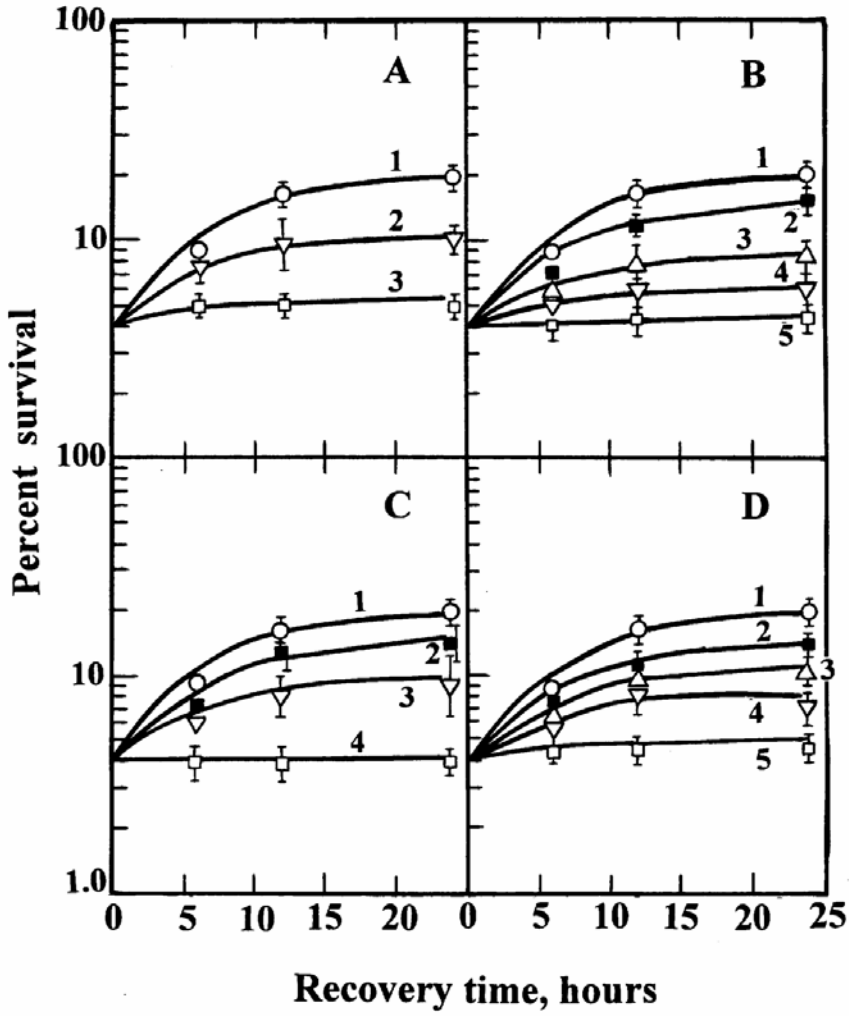

Fig. 5. Survival of Chinese hamster V79 cells in dependence of the duration of recovery from potentially lethal damage recovering after irradiation without chemicals (curves 1) and in the presence of chemical inhibitors of cell recovery. A, pyruvate: 10 and 20 mM immediately after irradiation (curves 2 and 3). B, novobiocin: 20 M 1h after irradiation (curve 2), 5, 10 and 20 M immediately after irradiation (curves 3, 4 and 5). C, lactate: 20 mM 1h after irradiation (curve 2), 10 and 20 mM immediately after irradiation (curves 3 and 4). D, nalidixic acid: 20 M 1h after irradiation (curve 2), 5, 10 and 20 M immediately after irradiation (curves 3, 4 and 5). Experimental points were taken from (6). Solid lines were calculated in accordance with Eq. 1 and parameters of the recovery estimated in this study.

4. Discussion

The aim of this study was to determine whether the inhibition of recovery from potentially lethal damage by chemicals in Chinese hamster cells [6,7] mightbe causally related to the impairment of the recovery capacity itself or to the production of the irreversible damage, which cannot be repaired. It was shown that the basic effect ofthe chemicals tested at a concentration sufficient to inhibit recovery from potentially lethal damage appeared to be a reduction in the number of cells capable of recoveryowing to the increase in the irreversibly damaged cells. The findings revealed that(i) the irreversible component of radiation damage was gradually enhanced as the chemicals concentration increased and (ii) the probability of recovery was independent of whether the process of recovery happened with or without chemicals sensitizing the radiation effect. It is not excluded that the first inference may be explained by a conversion by the drugs of radiation induced repairable damage so that the enzymes could then not deal with the lesions [3,4]. It would seem probable also that chemicals could interfere with the synthesis of the requisite enzymes [10,12]. The second inference would imply that the same portion of the repairable damage is eliminated for a unit time independently of the recovery conditions investigated. This result strongly suggests that the analysed chemicals don't damage the repair enzymes responsible for recovery. Hence, the observed inhibition of the recovery occurred without the damage of the recovery processing *per se*but due to the decrease in the number of damage which cell is capable of recovery. Similar results have been obtained for diploid yeast cells exposed to hyperthermia and ionizing radiation [13] or hyperthermia and UV light [14]. It follows that some general mechanism of radiosensitization or the synergistic effects may underlie the interaction of heat and some chemicals with ionizing radiation. The mechanism should imply the failure of a direct interference with the repair process itself and favor a role of hyperthermia and chemicals analysed in the decreasing rate and the extent of the repair by facilitating either the production of irreversible damage or an early radiation damage fixation before the recovery processes are over or occur.

Detailed analysis of the molecular mechanisms involved is beyond the main aim of this study. However, it can be noted that many mechanisms have been discussed by different authors [6,7,9,18-21]. Only as examples, the following possibilities can be mentioned. Because of the critical role of the DNA topoisomerases in the synthesis and the conformation of DNA, and well-known information that ionizing radiation

inhibits replicative DNA synthesis, there is a possibility that inhibitors of these enzymes might influence the radiation lethality [9]. It can be admitted that a decrease in a quantity of these enzymes wouldn't interfere with the probability of recovery but result in a greater portion of irreversible damage. A similar situation may be realized with insufficient energy metabolism. For instance, novobiocin and nalidixic acid have been shown to inhibit DNA, RNA, and protein synthesis in several mammalian cell lines [6,9,18], their activity may also be expressed through the interference with the function of topoisomerase II in an early stage of DNA repair [6,20]. It was presumed that the inhibition of recovery from potentially lethal damage by lactate and pyruvate may be due to severe metabolic changes such as a decrease in the intracellular ATP concentration [6]. These authors postulated that the recovery inhibition might have occurred due to the raising of the lactate and pyruvate levels complicating the repair of DNA damage. This view is strengthened by the data showing that specific inhibitors of poly (ADP-R) synthesis enhance the cell killing and inhibit the DNA strand break rejoining induced by ionizing radiation [21]. 3-Aminobenzamide has been shown to be a putative specific inhibitor of poly (ADP-R) synthetase [19]. The results obtained by Kumar et al. [7] favour a possible role of the chemical in preventing repair by facilitating an early damage fixation before repair can occur, simultaneously reducing the G_2-arrest. All these observations are consistent with the results obtained in this study.

In conclusion, the results of this paper provide opportunity for searching agents, selectively or simultaneously acting on the probability of recovery and the yieldof irreversible radiation damage. The recognition that specific inhibitors of recovery may exist, such as an inhibitor of the recovery process itself and that resulting in the increased yield of irreversible damage, would provide both a possibility to analyze the mechanism of a drug and ionizing radiation interaction from this point of view and an expectation that useful regimens in cancer research may be devised to make use of these inhibitors.

제 2 절 방사선과 타 요인 복합작용에 관한 이론모델 수립

1. Introduction

Organisms in their natural environment are always simultaneously exposed to a number of chemical and physical agents such as heat, UV light, ionizing radiation, nonionizing electromagnetic radiation, natural and man made electrical and magnetic fields. That high temperature can enhance radiation sensitivity of a wide variety of biological systems both *in vitro* and *in vivo* can no longer be doubted. Heating at temperatures above 40℃ are now used to increase the cellular radiosensitivity, the greatest synergistic effect being observed when heat and irradiation are given simultaneously [22,23]. It has so far generally been assumed that thermal radiosensitization results from the heat-induced inhibition of the repair of radiation damage. This was observed both for the repair of single- and double-strand breaks [24-26] and for cellular recovery [13,27-29].

This inhibition was revealed in a decrease of both the rate and extent of recovery which can be interpreted as being due to either the impairment of the recovery process itself (inactivation of repair enzymes, for instance) or to the production of more severe irreversible lethal damage which can not be repaired. The latter case was proved to be realized for the combined treatment of heat and ionizing radiation on yeast [13] and cultured mammalian cells [30,31] as well as for the exposure of yeast cells to heat and UV light [14]. These novel observations provide insight into the mechanisms underlying the effects of combined exposure and should be taken into account for interpretation and mathematical modeling of synergistic interaction of the combined exposures of various environmental agents. A simple mathematical approach has been earlier proposed to predict and optimize the synergistic interaction of hyperthermia and ionizing radiation [32,33]. This model suggests that the synergistic interaction of ionizing radiation and hyperthermia is expected to result from an additional effective damage arising from the interaction of sublesions induced by both agents. These sublesions are considered non-effective after each agent taken alone. The model was found to predict the dependence of synergistic interaction of the ratio of lethal events produced by every agent used, the greatest synergistic effect and the condition at which the maximal interactive effect can be achieved. However, this model does not take into consideration novel data indicating that additional damage responsible for the synergy is irreversible. Thus, the main goals of this studywere (a)

to develop a mathematical model of synergistic interaction taking into account the production of irrepairable damage; (b) to adopt this model for prognosis of the portion of irreversible damage produced after combined exposures; (c) to test the validity of the model to describe experimental results.

2. Mathematical Approach

Suppose that the additional effective damages, responsible for the synergy, are arisen from the interaction of sublesions induced by both agents and these sublesions are ineffective when each agent is applied separately. It would be reasonable to assume that one sublesion produced by an agent applied with heat interacts with one sublesion from heat to produce one additional effective damage. This assumption is not crucial since we do not define their nature concretely. It seems also plausible to suppose that the number of sublesions is directly proportional to the number of the effective damages. To follow to our previous publications [32-35], we use here index 1 for the agent applied together with heat (index 2). Let p_1 and p_2 be the numbers of sublesions that occur for one effective damage and N_1 and N_2 be the mean numbers of the effective damages in a cell produced by these agents. According to these assumptions, the synergistic effect is attributed to the formation of some additional effective damage N_3. Then the synergistic enhancement ratio k may be expressed as

$$k = \frac{N_1 + N_2 + N_3}{N_1 + N_2} \quad . \qquad (1)$$

A number of additional damages N_3 arising from the interaction of sublesions produced by the two agents may be given as

$$N_3 = \begin{cases} p_1 N_1, & if \quad p_1 N_1 \le p_2 N_2 \\ p_2 N_2, & if \quad p_1 N_1 \ge p_2 N_2 \end{cases} . \qquad (2)$$

In fact, this equation shows that the additional effective damage is a minimal value from two variable quantities: $p_1 N_1$ and $p_2 N_2$, which are the mean number of sublesions produced by the corresponding agent. Taking into account Eq. 2, the expression for synergistic effect (Eq. 1) can be written as

$$k = \begin{cases} 1 + \dfrac{p_1}{1 + N_2 / N_1}, & if \quad p_1 N_1 \le p_2 N_2 \\ 1 + \dfrac{p_2 N_2 / N_1}{1 + N_2 / N_1}, & if \quad p_1 N_1 \ge p_2 N_2 \end{cases} \qquad (3)$$

This equation has two parameters (p_1 and p_2) to be estimated from experimental data. It is evident from here that the effectiveness of the synergistic interaction will be determined by the least value from the two functions: $f_1 = 1 + \dfrac{p_1}{1 + N_2 / N_1}$ and $f_2 = 1 + \dfrac{p_2 N_2 / N_1}{1 + N_2 / N_1}$. This relationship (Eq. 3) between the synergistic enhancement ratio and the ratio of N_2 / N_1 shows an increase in the synergistic enhancement ratio with N_2 / N_1 to a maximum, followed by a subsequent decrease at higher N_2 / N_1. The absolute value of this maximum as well as the ratio of N_2 / N_1 at which it is attained are obviously of great interest not only in thermobiology but for environmental protection consideration as well. Since f_1 decreases while f_2 increases with increasing N_2 / N_1, the greatest synergistic effect will be obtained when $f_1 = f_2$, i.e.

$$\left[1 + \frac{p_1}{1 + N_2 / N_1}\right] = \left[1 + \frac{p_2 N_2 / N_1}{1 + N_2 / N_1}\right]. \qquad (4)$$

It enables the determination of the condition of the highest synergistic interaction achievement:

$$N_2 / N_1 = p_1 / p_2 . \qquad (5)$$

It means that the highest synergistic interaction occurred when both agents produce equal numbers of interacting sublesions: $p_1 N_1 = p_2 N_2$. For a particular case $p_1 \cong p_2$, the highest synergistic interaction occurred when the both agents produce almost equal number of effective damages, i.e. $N_1 \cong N_2$. Combining equations (3) and (5), one can deduce the value of the highest synergistic enhancement ratio which can be given by

$$k_{\max} = 1 + \frac{p_1 p_2}{p_1 + p_2} . \qquad (6)$$

It is evident that the value of the highest synergistic effect doesn't depend on the ratio of N_2 / N_1 and is completely determined by the basic parameters of the model.

It is curiously but the model considered doesn't demand a knowledge of absolute values of N_1 and N_2 but only their dimensionless ratio, N_2 / N_1, which can be derived for cell inactivation (the main end point used in this study) from the ratio of slopes of the corresponding curves

$$N_2 / N_1 = D_{o1} / D_{o2} , \qquad (7)$$

where D_o being a measure of the time for survival to be reduced to $1/e$ on the exponential portion of survival curve.

If irradiated cells are held in a liquid non-nutrient media at 30℃ before plating on to a growth medium, their survival is increased reflecting the process of LHR [38,39]. When samples were plated at different times after the treatment, the number of viable cells increased due to the LHR as a function of time, reaching a plateau after about 2-3 days. The availability of the plateau reflects the existence of the irreversible component of radiation damage which cell is incapable to recover from. In accordance with the definition [40], the irreversible component can be quantitatively estimated as the ratio of the number of irreversible damage to the total number of damage produced. The ratio shows the portion of damage which can not be repaired. Each type of the effective damage (N_1, N_2, N_3) can be characterized by its own value of irreversible component (K_1, K_2, K_3, respectively). Then for the synergistic interaction of two agents the irreversible component (K) might be presented as

$$K = \frac{K_1 N_1 + K_2 N_2 + K_3 N_3}{N_1 + N_2 + N_3} . \qquad (8)$$

Taking into account Eq. (2) and the basic postulates of the model, Eq. (8) can be rewritten as

$$K = \begin{cases} \dfrac{K_1 N_1 + K_2 N_2 + K_3 p_1 N_1}{N_1 + N_2 + p_1 N_1}, & if \quad p_1 N_1 \le p_2 N_2 \\ \dfrac{K_1 N_1 + K_2 N_2 + K_3 p_2 N_2}{N_1 + N_2 + p_2 N_2}, & if \quad p_1 N_1 \ge p_2 N_2 \end{cases}, \qquad (9)$$

where K_1 is the irreversible component after separate exposure to the agent combined with heat, K_2 is the irreversible component after thermal treatment applied alone and K_3 is the irreversible component of additional damages responsible for the synergy. As was mention above, this additional damage is irreversible, i.e. K_3 = 1. For convenience calculation ability this equation may be given by

$$K = \begin{cases} \dfrac{K_1 + K_2 N_2 / N_1 + K_3 p_1}{1 + N_2 / N_1 + p_1}, & if \quad p_1 N_1 \le p_2 N_2 \\ \dfrac{K_1 + K_2 N_2 / N_1 + K_3 p_2 N_2 / N_1}{1 + N_2 / N_1 + p_2 N_2 / N_1}, & if \quad p_1 N_1 \ge p_2 N_2 \end{cases}. \qquad (10)$$

It is plausible to assume that biological damage produced by each acting agent (N_1 and N_2) during combined action can be repaired in the same extent and with the same probability as they were observed after application of these agents individually. One can estimate K_1 and K_2 values from experiments with separate action of each agent used in combination.

3. Validity of the Model

If the observed biological effect is largely induced by heat $(p_1 N_1 < p_2 N_2)$ then taking into account Eq. 3, the parameter p_1 can be expressed as

$$p_1 = (k_1 - 1)(1 + N_2 / N_1), \qquad (11)$$

where k_1 is the value of the synergistic enhancement ratio observed in experiments performed in this condition. On the contrary, if the observed biological effect is mainly induced by another agent applied with heat $(p_1 N_1 > p_2 N_2)$, we have

$$p_2 = (k_2 - 1)(1 + N_1 / N_2), \qquad (12)$$

where k_2 is the experimental value of the synergistic enhancement ratio observed for the condition $p_1N_1 > p_2N_2$. Knowing experimental values of k_1 and k_2 derived from two real experiments in which the cell inactivation was due to the preferential contribution of one of the two agents applied, we can make an empirical estimation of the basic model parameters p_1 (Eq. 11) and p_2 (Eq. 12). To provide more precise description of experimental data, a direct estimation of these parameters from the entire set of data regarding to a given biological object might also be possible [32]. Knowing p_1 and p_2, one can predict the value of the synergistic enhancement ratio for any N_2/N_1 (Eq. 3), the greatest value of the synergistic enhancement ratio (Eq. 6) and the condition under which it can be achieved (Eq. 5).

We tested the applicability of the model for quantitative description, prediction and optimization of the synergistic interaction observed for various biological objects and test systems. Our most numerous findings have been obtained with diploid yeasts - the simplest specimen of eukaryote cells. As was shown previously [13,30,32,38], in diploid yeast cells thermally enhanced sensitivity to ionizing radiation may due to both the inhibition of repair from potentaially lethal damage and/or the enhancement of expression of lethal damage. The reduction of shoulder suggested that hyperthermia also interferes with repair of sublethal damage. Having used these published results, we estimated here the fraction of irreversibly damaged cells (irreversible component), which was determined as a value inversely proportional to the dose-modifying factor that is as the ratio of the D_o after delayed and immediate plating. The calculation showed that the fraction the irreversible component was equal 0.34 at 20 and 40℃ 0.50 at 45℃ 0.77 at 50℃0.86 at 52.5℃ and 0.94 at 55℃ (Table 2). It is apparent that the cell ability to recover radiation damage is inversely proportional to the temperature at which the exposure was delivered. Yeast cells exposed to ionizing radiation at 55℃ almost completely fail to show recovery on delayed plating. It would be of tempting to test the ability of the model considered to predict the extent of the recovery after the combined action of heat and ionizing radiation (Eq. 10). We can reasonably believe that the irreversible component induced by ionizing radiation alone K_1 = 0.34 (irradiation at room temperature). Since in all our experiments with yeast cells no LHR was observed after hyperthermia alone, we consider that K_2 = 1.0. It means that all thermal damages are irreversible. Using

these results and Eq. 10, we calculated the irreversible component K expected after simultaneous action of ionizing radiation and hyperthermia for 3 various values of K_3 - irreversible portion of damage responsible for the synergy. Averaging the results published before [32,38], we considered that for the strain used in these experiments p_1 = 2.5 and p_2 = 3.4.

The results of calculation together with experimental data of the proportion of irreversible damage after simultaneous action of heat and ionizing radiation are collected in Table 2. It can be seen that the values predicted on the basis of Eq. 10 for K_3 = 1.0 show the best correspondence with experimental findings. On the contrary, the values predicted for K_3 = 0.5 and K_3 = 0 are not consistent with experimental data.

Similar calculations of the irreversible component K have been fulfilled for the same diploid yeast cells exposed to ultraviolet light (254 nm) at different temperatures. Experimental values of K (Table 3) have been taken from our recently published work [14]. The calculation showed that the fraction the irreversible component was equal 0.56 at 20℃ 0.60 at 53℃ 0.69 at 54℃ 0.77 at 55℃ 0.92 at 56℃ and 1.00 at 57℃ (Table 3). It is apparent that the cell ability to recover UV light damage is also inversely proportional to the temperature at which the exposure was delivered. It may be considered that the irreversible component induced by UV light alone K_1 = 0.56 (irradiation at room temperature) and K_2 = 1.0.

Using these results and Eq. 10, we calculated the irreversible component K expected after simultaneous action of UV light and hyperthermia for 3 various values of K_3 - irreversible portion of damage responsible for the synergy. The basic parameters of the model for the strain used in these experiments were as follows: p_1 = 1.6 p_2 = 3.5. The results of our calculation together with experimental data of the proportion of irreversible damage after simultaneous action of UV light and hyperthermia are collected in Table 3. It can be seen that the values predicted on the basis of Eq. 10 for K_3 = 1.0 show the best correspondence with experimental findings. On the contrary, the values predicted on the basis of Eq. 10 for K_3 = 0.5 and K_3 = 0.0 are in most cases not consistent with experimental data.

Table 2. The irreversible component of radiation damage (K) after simultaneous action of ionizing radiation (γ-ray of ^{60}Co) and hyperthermia on diploid yeast cells of *Saccharomyces ellipsoideus* (strain Megri 139-B)

Temperature (℃)	**20**	**40**	**45**	**50**	**52.5**	**55**
N_2 / N_1	0	0	0.16	0.53	1.4	7.3
K, experiment	0.34±0.03	0.34±0.03	0.50±0.05	0.77±0.06	0.84±0.07	0.94±0.05
K, predicted for: $K_3 = 1.0$	0.34	0.34	0.61	0.80	0.86	0.94
$K_3 = 0.5$	0.34	0.34	0.45	0.53	0.46	0.82
$K_3 = 0.0$	0.34	0.34	0.29	0.26	0.36	0.71

Table 3. The irreversible component K after simultaneous action of ultraviolet radiation (254 nm) and hyperthermia on diploid yeast cells *Saccharomyces cerevisiae* (strain XS800)

Temperature (℃)	20	53	54	55	56	57
N_2 / N_1	0	0.13	0.18	0.25	1.38	6.45
K, experiment	0.56±0.04	0.60±0.06	0.69±0.06	0.77±.05	0.92±0.07	1.00±0.05
K, predicted for: $K_3 = 1.0$	0.56	0.72	0.76	.79	0.88	0.95
$K_3 = 0.5$	0.56	0.57	0.58	0.59	0.69	0.86
$K_3 = 0.0$	0.56	0.43	0.42	0.38	0.49	0.77

4. Discussion

Analysis of numerous data has shown [22,23] that synergistic interaction of ionizing radiation with other environmental chemical and physical agents results in the inhibition of some cellular repair mechanisms. The inhibition was revealed in a decrease of both the rate and extent of recovery. Two hypotheses could be envisaged. Such lesions, resulting from the simultaneous application of the two insults, might not be reparable. Alternatively, the damage might be similar, but the repair enzymes might be modified so that their ability of repair is changed. The most likely explanation for these results is that heating after irradiation modified the damage so that enzymes could then not deal with the lesions.

The synergistic effects of the combined action of heat with ionizing radiation or UV light interpreted [13,14,30,31] as being due to more severe or more complex damage produced during the combined treatment. In the current study, a novel mathematical approach was developed for quantitative description and prognosis of the synergistic effect and cell recovery after the combined exposures of environmental agents. The range of the experimental radiation doses in the described effect of interaction between heat and radiation was 100 - 1000 Gy. This means that we considered and put into the mathematical model process called as radiosensitization or enhancement of deterministic effects produced by ionizing radiation. The model was based on the supposition that the synergism originated from the production of additional effective damage due to the interaction of some kind of sublesions induced by both agents. The idea of sublesions is widely used in theoretical radiobiology [39-41]. Avery elegant mathematical description of synergistic interaction was presented in molecular theory of radiation biology [42].Some papers showed the clear experimental proofs for a synergy on the various cellular effects (survival and mutation) described by the molecular model of interaction [43,44].

In the model presented here sublesions are supposed to be non-effective after each agent taken alone while the additional damage arising from the interaction and responsible for the synergy is irreversible which the cells are incapable to recover from. The main novel contribution of this model concerns the possibility to predict the irreversible component of radiation damage after the combined exposures of different environmental agents.

To test the approach, the results of experimental research of diploid yeasts cells survival after simultaneous action of heat combined with ^{60}Co ionizing radiation [13]

or 254 nm UV light [14] have been used here. It was shown that the cell ability to the liquid holding recovery decreased with an increase in the temperature, at which the exposure was occurred. A good correspondence between experimental results and model prediction was demonstrated only if the initial supposition about the irreversible character of additional damage holds. Otherwise, the values of irreversible component predicted on the basis of the model developed were in most cases not consistent with experimental data.

The molecular mode of the repair inhibition is still unclear. There is nowabundant evidence pointing that hyperthermic inhibition of the rate and/or extent of repair of radiation-induced DNA damage which was observed with all the assays currently used for DNA damage assessment may reflect the production of irreversible damage rather than the impairment of recovery mechanisms themselves after the combined exposures. In fact, evidence has accumulated that hyperthermic radiosensitization might be due to the nuclear protein aggregation that damaged DNA becomes less accessible for the DNA repair machinery and this (rather than repair enzyme inactivation *per se*) is responsible for the observed thermal inhibition of DNA repair [45-48]. Thermal repair inhibition is generally described as reduced repair rates leading to an increased number of residual strand breaks [49]. It was previously shown by Mills and Meyn (1981)[50] that for a combined treatment with X-irradiation and heat, the decrease in cell survival correlated with the increase in the number of non-rejoined strand breaks. Thecombined treatment appeared to affect the repair rates but also lead to the formation of additional strand breaks [51]. Residual additional strand breaks then can result in additional chromosome aberrations [52,53] finally producing the enhanced cell killing. Considering all these data as a whole, we can conclude that they don't contradict the results obtained in this study. However, the analysis of the molecular mechanism of synergistic interaction and production of irreversible damage is beyond the main aim of this paper. Moreover, it can be envisaged that they might be different for various biological objects, test effects and acting agents. The major points to be inferred from the data presented here are thatthe mathematical model basing on the minimum initial postulates describes in general form the dependence of the synergistic effect on the ratio of the effective damages produced by agents used in combination, predicts the greatest synergistic interaction, condition under which it can be achieved and the portion of the irreversibly damage resulted from the combined exposures.

In summary, it can be concluded that the mechanism of the synergistic interaction of hyperthermia with ionizing radiation or UV light after their simultaneous treatment in yeast cells is not related to the impairment of the recovery capacity itself and it may be attributed to an increased yield of irreversible damage.

제 3 절 상승작용 이론모델을 적용한 라돈과 흡연의 복합작용 해석

1. Introduction

A certain level of background exposure to ionizing radiation and natural or man-made chemicals is always presented in the environment. Thus, all assessments of toxicity, carcinogenicity or mutagenicity of chemical, physical and biological agents should be performed as studies on their combined effects. For example, people are exposed to an indoor radon concentration combined at most time with other agents including tobacco smoking. It is well known from epidemiological and toxicological studies that the kind of interaction which occurs between tobacco smoking and radon may be synergistic, i.e. can lead to the effects, the sum of which is greater than that expected from the addition of the effects from separate exposures to the individual agents [54-56]. This problem is especially important for high natural background areas [57]. A new concept of the synergistic interaction and a simple mathematical model based on this conception has been developed earlier to predict and optimize the synergy when hyperthermia was simultaneously applied together with any of the following modalities: ionizing radiation, ultrasound, ultraviolet light, microwaves and some chemical agents [32,33,38]. The model suggests that the synergism is expected from some additional effective lesions arising from the interaction of sublesions induced by both agents. These sublesions are considered ineffective after each agent is taken alone. The model predicts the dependence of the synergistic interaction on the ratio of effective lesions produced by each agent applied, the greatest value of the synergy as well as the condition under which it can be achieved. However, the model was mainly tested for inactivation of cells differing by their origin. Therefore, it would be of interest to accommodate this model for the description, optimization and prognosis of synergistic effects between tobacco smoking and radon. Thus, this study was designed to implement two purposes: (a) to adjust a mathematical model to describe the synergistic interaction between radon and tobacco smoking (b) to test the validity of the proposed model.

2. Mathematical Model

In the estimation of the synergistic effect as regard to carcinogenic action, the synergistic enhancement ratio (k) can be defined as the ratio of the number of

cancer cases registered due to the combined action of two carcinogenic factors (N_{comb}), to the sum of the number of the carcinogenic events resulting from each agent considered separately:

$$k = \frac{N_{comb}}{N_1 + N_2}, \qquad (1)$$

where N_1 and N_2 are the numbers of cancer cases induced by radon and tobacco smoking, respectively. We notethat the number of cancer cases is in direct proportion to hypothetical effective damages finally responsible for the carcinogenic events. Then the synergistic enhancement ratio defined by equation (1) reflects the ratio of the corresponding effective damages induced by the combined action of both agents (N_{comb})to the sum of the effective damages produced by radon (N_1) and tobacco smoking (N_2).

The model does not apply such a value as a dose from an individual agent, since it is designed for the description of the combined effects of agents which can be completely different by their nature and action mechanism. However, this model can deal with different factors, as it operates with non-dimensional ratio of the effective damages produced by the agents under consideration. The model suggests that carcinogenic agents, in addition to the effective damages resulting from the carcinogenic events, produce some precarcinogenic sublesions. Let us assume that p_1 and p_2 are the numbers of sublesions that occur per a single effective damage induced by ionizing radiation and tobacco smoking, respectively. It should be pointed out at this stage, that the applied model does not postulate or define the nature or type of neither effective damage nor sublesions which occur. Thus this can be of any type. What the model does offer is an empirical mean for their quantitative estimation. These precarcinogenic sublesions are considered to be non-effective,when each agent is applied separately. Additional carcinogenic effective damages responsible for the synergy are assumed to arise from the interaction of precarcinogenic sublesions. As the first step of modelling, let us assume that one sublesion resulting from one individual agent reacts with one sublesion produced by another agent, thus producing an additional effective damage. In other words, the additional carcinogenic damage is produced during the combined action of agents, as a resultof the

interaction of these precarcinogenic sublesions. Thus, in terms of the model presented by equation (1), the synergistic enhancement ratio, k, may be given by:

$$k = \frac{N_1 + N_2 + \min\{p_1 N_1; p_2 N_2\}}{N_1 + N_2} = 1 + \frac{\min\{p_1; p_2 N_2 / N_1\}}{1 + N_2 / N_1}. \qquad (2)$$

Here, the term $\min\{p_1 N_1; p_2 N_2\}$ is a minimal value from two variable quantities, $p_1 N_1$ and $p_2 N_2$, which determine the numbers of additional effective damages arising due to the interaction between precarcinogenic sublesions produced by radon ($p_1 N_1$) and tobacco smoking ($p_2 N_2$). It can be easily demonstrated that, if the cancer induction under combined action of agents is mainly produced by tobacco smoking, or in other words $p_1 N_1 < p_2 N_2$, then the parameter p_1 may be represented as follows:

$$p_1 = (k-1)(1 + N_2 / N_1). \qquad (3)$$

In contrast, if the cancer events are mainly induced by the action of ionizing radiation $(p_1 N_1 > p_2 N_2)$, the parameter p_2 can be determined by the following equation:

$$p_2 = (k-1)(1 + N_1 / N_2). \qquad (4)$$

It can be demonstrated in a purely mathematical means that under condition

$$p_1 N_1 = p_2 N_2 \qquad (5)$$

the synergistic interaction should be characterized by the greatest value and can be calculated as follows:

$$k_{\max} = 1 + \frac{p_1 p_2}{p_1 + p_2}. \qquad (6)$$

Thus, using equations (3) and (4), one can estimate, on the basis of real experiments, the basic model parameters p_1 and p_2. Then the synergistic

enhancement ratio for any ratio of N_2/N_1, its greatest value, and the condition under which this is yielded can be derived from equations (2), (6), and (5), respectively.

3. Results

In order to apply the model to experimental data, it is necessary to define two basic parameters p_1 and p_2. Each of these is calculated for such experimental conditions, when one of the agents induces more precarcinogenic sublesions then another. Two sets of epidemiological data are available for a definition of the interactions between radon and tobaccosmoke interaction - miner and residential study data. The first set of data, i.e. miner data, describes a situation in which the subjects were exposed to high radon concentrations, and here the impact of the action of radon on the lung cancer induction rate is greater than that of the smoke inhalation component. Knowledge of such a condition is necessary in order to define the model parameter p_2. In this case, $\min\{p_1N_1; p_2N_2\} = p_2N_2$ and the synergistic enhancement ratio is calculated according to the following equation:

$$k = 1 + \frac{p_2 N_2 / N_1}{1 + N_2 / N_1} = 1 + \frac{p_2}{1 + \frac{1}{N_2 / N_1}}. \qquad (7)$$

According to this Equation, the value for k is increased in relation to the increase in that for N_2/N_1. In other words, the model predicts that the synergistic enhancement ratio increases with an increase in the ratio of N_2/N_1 until the inequality $p_1N_1 > p_2N_2$ holds.

In order to test this prognosis, we used a survey consisting of periodic sputum cytology evaluation and which was performed among 249 underground uranium miners and 123 controls [54]. Cytological sputum samples yielding moderate atypia, marked atypia, or the presence of cancer cells were classified as being abnormal. To estimate the synergistic enhancement ratio, data on both the combined action of the agents and on the separate action of each individual agent is required. Band et al. [54] presented data for the combined action of agents and the effectsof tobacco smoke inhalation on lung cancer induction in miners for a range of radon concentrations (from less than 2.6 kBq/m^3 to more than 15.7 kBq/m^3). However, if the real

epidemiological results for the combined action of radon and tobacco smoke in cancer induction (N_{comb} values) were listed for all 5 conditions investigated (<2.6, 2.7-5.3, 5.4-10.4, 10.5-15.7 and >15.7 kBq/m^3),the data for radon action alone (not smoking miners) was presented only for two concentration levels, and one of them was obtained for the minimal radon concentration when there was no synergistic interaction at all. The residuary point (5.4-10.4 kBq/m^3) may be characterized as follows. In accordance with published results [54], the frequencies of abnormal cytology were 5% after radon exposure alone (N_1), 7% after cigarette smoking alone (N_2) and 28% after combined action of these modalities (N_{comb}). Then the expected frequency should be 12% for independent action of these agents ($N_1 + N_2$) and in terms of the model discussed, the ratio $N_2 / N_1 =$ 1.4 and the synergistic enhancement ratio $k =$ 2.3. This value was calculated by using equation (1). This point is depicted on the left-hand part of the curve presented in Fig. 6A and 6B. Having used this result and equation (4), we have p_2 = 2.3. Taking into consideration this value and equation (7), that would correct for the condition $p_1 N_1 > p_2 N_2$, the theoretically predicted dependence of the synergistic enhancement ratio on the ratio of N_2 / N_1 was calculated. The result is presented in Fig. 6A and 6B by the left-hand part of the solid curve.

The residential study data represent situations in which people are exposed to relatively low radon concentrations, as compared to those of the affected miners. It may be supposed that in this case, tobacco smoke is the main factor responsible for the lung cancer induction. This hypothesis is supported by the data published by Pershagen et al. [56]. In this case, $\min\{p_1 N_1; p_2 N_2\} = p_1 N_1$, i.e. tobacco smoke induces a larger amount of precarcinogenic sublesions. Such parameter conditions are necessary to define the model parameter p_1 on the basis of equation (3). The synergistic enhancement ratio in this case can be calculated as

$$k = 1 + \frac{p_1}{1 + N_2 / N_1} . \qquad (8)$$

According to this Equation, the value for k is decreased in relation to the increase in that for N_2/N_1. In other words, the model predicts that the synergistic enhancement ratio decreases with the increase in the ratio of N_2/N_1 for which the inequality $p_1N_1 < p_2N_2$ holds. In order to test this prediction, we used the results published by Perschagen et al. [56]. Because of relatively highscattering of the original data [56], we employed two manners to evaluate the parameter of p_1. For the first one, we used two concentrations of radon: 51-80 and >400 Bq/m^3 at the least radon concentration, the synergistic effect was minimal, while for the high radon concentration it had the greatest value. In accordance with the original data [56] for radon concentration >400 Bq/m^3, the relative risk (except for the background risk) was 0.2 after exposure to radon alone (N_1), 11.6 after tobacco smoking alone (N_2) and 31.5 after combined action of both agents (N_{comb}). In this case we assumed the relative risk to be proportional to the number of effective damages responsible for the carcinogenic effects. Under such a supposition all the equations derived above can be applied. Then the expected number of the effective damage should be 11.8 for independent action of these agents (N_1+N_2), the ratio N_2/N_1= 58 and in accord with equation (1), the synergistic enhancement ratio k= 2.7. This data point is depicted on the right-hand part of the curve presented in Fig. 6A. Having used this result and equation (3), we have p_1 = 100. Taking into consideration this value and equation (8) that would correct for the condition $p_1N_1 < p_2N_2$, the theoretically predicted dependence of the synergistic enhancement ratio on the ratio of N_2/N_1 was calculated. The result is presented in Fig. 6A by the right-hand part of the solid curve. The second data point at this part of the curve was evaluated by a similar way for radon concentration of 51-80 Bq/m^3. It is clear that this result does not correspond to the theoretically predicted curve.

The originally published results [54] clearly display a linear dose-effect dependence but also reveal some deviations from the general trend. To avoid this we applied a linear extrapolation to the data reflecting lung cancer incidence rate among the residents who were exposed only to radon. Table 4 summarizes the results of such a calculation. The obtained results are depicted by points on the right-hand part of the curve presented in Fig. 6B. The value of the parameter p_1 of the model can

be estimated from any of the data points obtained. For example, by using equation (3) we have p_1 = 39.5 for a radon concentration of 140-400 Bq/m^3. Taking into consideration this value and equation (8) that would correct for the condition $p_1 N_1 < p_2 N_2$, the theoretically predicted dependence of the synergistic enhancement ratio on the ratio of N_2 / N_1 was calculated. The result is shown in Fig. 6B by the right-hand part of the solid curve. Taking the data presented in Fig. 6B as a whole, one can conclude that the theoretically predicted curves correspond well with the data points regarding to the dependence of the synergistic enhancement ratio on the ratio of the effective damage produced by tobacco smoking and radon.

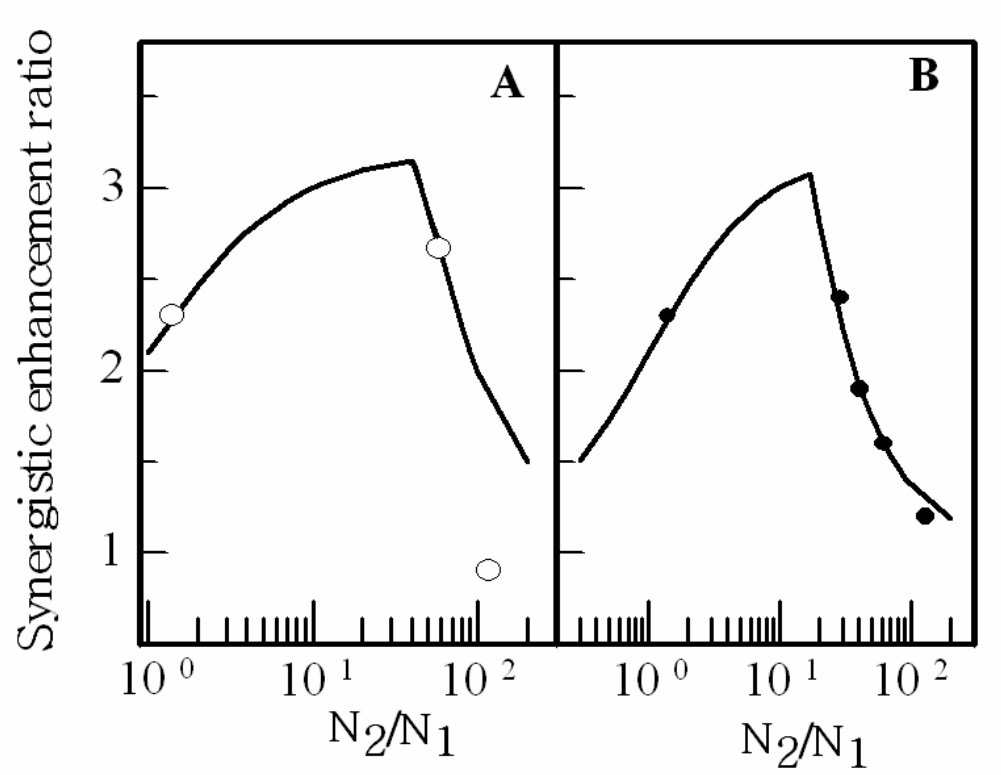

Fig. 6. The dependence of the synergisticenhancement ratio on the ratio of the effective damages produced by tobacco smoking (N_2) and radon (N_1).

Table 4. Model parameters and experimental values of the synergistic enhancement ratio

Radon concentration, Bq/m^3	**Relative risk(except for the background risk)**				k_{exp}	N_2/N_1
	Radon N_1	Tobacco smoking N_2	N_{comb}	$N_1 + N_2$		
<50		9.00				
51-80	0.07		11.3	9.07	1.25	129
81-140	0.15		14.3	9.15	1.56	60
141-400	0.22		17.9	9.22	1.94	41
>400	0.31		22.5	9.31	2.42	29

4. Discussion

Thus, the data presented here indicatedthat the mathematical model for synergism which was previously proposed and tested mainly for the description of cell inactivation, can also be used to describe the carcinogenic effects of the combined action of radon exposure and tobacco smoke inhalation. The main difference in the proposed model when compared to the existing ones [58,59] is the assumption that synergism can be accounted for in terms of the interaction between putative precarcinogenic sublesions, with the frequency of the occurrence of these lesions being proportional to the number of cancer cases. It's important, however, that no attempt is made in the model to apply an assumption on the molecular nature of the precarcinogenic sublesionsand the mechanism of their interaction. Nevertheless, the model enables us to obtainsome non-trivial results - it predicts the synergistic enhancement ratio for any ratio of the effective damages produced by tobacco smoking and by radon as well as the greatest value of the synergistic effect and the condition under which this is yielded.

제 4 절 상승작용 이론모델을 적용한 화학물질 복합작용 해석

1. Introduction

The combined action of various chemical agents is a distinguishing feature of modern life. An interaction between two agents is considered as synergistic when the effect produced is greater than the sum of the two single responses. Many physical and chemical environmental factors can synergistically interact with each other [44,60-62]. The possibility to predict the synergistic effect and its highest value appears to be important for the establishment of safe level of agents after combined exposures [3,63]. The utilization of synergistic interaction in medicine enables to reduce the concentration of medicine or thedose of physical agent to provide the same medical effect or under the same value of agents to reach more effective response [64,65]. In particular, both fluoride and xylitol are known to be widely used as cariostatic agents and the synergistic effect of fluoride and xylitol on glycolysis by mutans sreptococci was established [66-68]. The authors pointed out that percent inhibition by combination of fluoride and xylitol was larger than the sum of percent inhibition by fluoride alone and percent inhibition by xylitol alone, indicating that the combined effect was synergistic. However, the authors did not make an attempt to estimate this effect quantitatively. The prospects of providing a reasonably useful application of this phenomenon seem to lie in the possibility to predict the highest synergisticeffect and condition under which it can be obtained. A new concept of the synergistic interaction and a simple mathematical model based on this conception has been developed to predict and optimize the synergy when hyperthermia was simultaneously applied together with any of the following modalities: ionizing radiation, ultrasound, ultraviolet light, microwaves and some chemical agents [62,32]. The model was tested for inactivation of cells differing by their origin. The model predicted the dependence of the synergistic interaction on the ratio of the effective lesions produced by each ofthe agent applied, the greatest value of the synergy as well as the condition under which it can be achieved. Therefore, it would be of interest to accommodate this model for the description, optimization and prognosis of the synergistic effects between two chemical agents.

2. Mathematical model

In accordance with the model [32,33] which is supposed to be adjusted to describe and predict the interaction of two chemical agents, the synergistic enhancement ratio (k) can be defined as the ratio of theeffective damages produced by the combined action of these agents (N_{comb}), to the sum of the effective damages produced separately. As well as this model is planned to be tested for synergistic interaction of fluoride and xylitol on glycolysis, we designate an amount of some hypothetical effective damage produced by fluoride through N_1 and that produced by xylitol as N_2. Then we can write

$$k = \frac{N_{comb}}{N_1 + N_2} = \frac{N_1 + N_2 + N_3}{N_1 + N_2} . \qquad (1)$$

It follows that the synergism is expected from some additional effective damages (N_3). One can suppose that the additional damages are arising from the interaction of sublesions induced by both agents. These sublesions are considered ineffective after each agent is taken alone. Let us suppose that p_1 and p_2 are the numbers of sublesions that occur per a single effective damage induced by fluoride and xylitol, respectively. We assume that one sublesion resulting from one individual agent reacts with one sublesion produced by another agent, thus producing an additional effective damage. Then, the synergistic enhancement ratio, k, may be given by

$$k = \frac{N_1 + N_2 + \min\{p_1 N_1; p_2 N_2\}}{N_1 + N_2}. \qquad (2)$$

Here, the term $\min\{p_1 N_1; p_2 N_2\}$ is a minimal value from two variable quantities, $p_1 N_1$ and $p_2 N_2$, which determine the numbers of additional effective damages arising due to the interaction between sublesions produced by fluoride ($p_1 N_1$) and xylitol ($p_2 N_2$). An actual amount of real damage and sublesion is thought to be difficult to determine quantitatively in particular because of their molecular nature still should be elucidated. However equation (2) can be rewritten as

$$k = \frac{N_1 + N_2 + \min(p_1; p_2 N_2 / N_1)}{N_2 / N_1} \quad (3)$$

It means the model can operate with non-dimensional ratio of the effective damages (N_2 / N_1) produced by the agents under considerations which can be estimated from experimental results. The extent of the inhibition of acid production by oral bacteria may be supposed to be in the direct proportional to some hypothetical effective damages responsible for inactivating of the cariogenic bacteria. In accordance with this supposition,the extent of inhibition of acid production (% control) produced by fluoride (F) and xylitol (X) may be presented as

$$F = cN_1 \quad (4)$$

and

$$X = cN_2 . \quad (5)$$

Here c is a constant of proportionality. Then the expected additive effect can be calculated as

$$(F + X)_{add} = c(N_1 + N_2) , \quad (6)$$

while the experimentally observed results may be given as

$$(F + X)_{\exp} = c(N_1 + N_2 + N_3) . \quad (7)$$

The ratio of N_2 / N_1 can be determined on the basis of experimental data as

$$N_2 / N_1 = X / F . \quad (8)$$

It can be easily demonstrated that, if the inhibition of acid production under combined action of agents is mainly produced by xylitol, or in other words $p_1N_1 < p_2N_2$, then the parameter p_1 may be presented as follows

$$p_1 = (k-1)(1+N_2/N_1). \quad (9)$$

In contrast, if the inhibition of acid production is mainly induced by the action of fluoride $(p_1N_1 > p_2N_2)$, the parameter p_2 can be determined by the following equation

$$p_2 = (k-1)(1+N_1/N_2). \quad (10)$$

It can be demonstrated in a purely mathematical means that under condition

$$p_1N_1 = p_2N_2, \quad (11)$$

or

$$N_2/N_1 = p_1/p_2 \quad (12)$$

the greatest synergistic interaction can be calculated as follows:

$$k_{\max} = 1 + \frac{p_1p_2}{p_1+p_2}. \quad (13)$$

Thus, using equations (7) and (8), one can estimate, on the basis of real experiments, the basic model parameters p_1 and p_2. Then the synergistic enhancement ratio for any ratio of N_2/N_1, its greatest value, and the condition under which this is yielded can be derived from equations (3), (11), and (10), respectively. All these parameters are very important for environmental toxicology and risk assessment. To test the validity of the model for chemical agents, one needs a great number of experimental data, obtained after action of two toxic agents applied alone and combined together. The prediction of the model presented here will be verified by

a comparison with extensive experimental data on a simultaneous action of fluoride and xylitol on glycolysis published by others [68].

3. The validity of the model

The extensive quantitative study of synergistic inhibition by combination of fluoride and xylitol on glycolysis by mutans streptococci clearly showed [68] that the acid production from glucose by both bacterial strains tested (*Streptococus mutans* NCTC10449 and *Streptococus sobrinus* 6715) was inhibited in the presence of fluoride. The decrease of the acid production was dose-dependent and the effect was synergistically enhanced after simultaneous action of fluoride and xylitol. We used these results to evaluate some model parameters and experimental values of the synergistic enhancement ratio for various conditions of the combined action of fluoride and xylitol on the acid production by *S. mutans* (Table 5) and *S. sobrinus* (Table 6). The both tables contain the following data: fluoride concentration, pH, the extent of inhibition of acid production (% control) produced by fluoride (F) and xylitol (X) applied individually, the expected additive effect $(F + X)_{add}$, the actually observed inhibition in experiment $(F + X)_{exp,}$ the ratio N_2 / N_1 [Equation (4)], and the experimentally observed synergistic enhancement ratio [Equation (1)]. These results clearly indicate that for a constant xylitol concentration an optimal concentration of fluoride may exist providing the highest synergistic effect.Just such dependence should be expected qualitatively from the mathematical model presented above. Hence, it would be of interest to apply the described mathematical model to these results.

In order to apply this model quantitatively to the experimental data, it is necessary to define two basic parameters p_1 and p_2. Each of these should be calculated for such experimental condition, when one of the agents applied induces more sublesions than another [Equations (7) and (8)]. Thus estimated set of these parameters is collected in Table 7. Having used this set of the basic parameters, the synergistic enhancement ratio for any ratio of N_2 / N_1, its greatest value, and the condition under which this is yielded can be derived from equations (3), (11), and (10), respectively.

Table 5. The dependence of some model parameters and experimental values of the synergistic enhancement ratio for the combined effect of fluoride and xylitol on the acid production by the cells of *S. mutans* NCTC19449 on the condition of treatment. The original results have been published by others [68].

Fluoride, m*M*	pH	Fluoride $F = cN_1$	Xylitol $X = cN_2$	$(F + X)_{add} = c(N_1 + N_2)$	$(F + X)_{exp} = c(N_1 + N_2 + N_3)$	N_2/N_1	k*(exp)*
0	7.0	0	9	9	9	–	–
0.8	7.0	3	0	12	20	3.0	1.7
1.3*	7.0	15	9	24	50	0.6	2.1
1.6	7.0	22	9	31	59	0.41	1.9
2.0*	7.0	38	9	47	73	0.24	1.55
2.3	7.0	56	9	65	87	0.16	1.34
0	5.5	0	9	9	9	–	–
0.1	5.5	2	9	11	15	4.5	1.36
0.15*	5.5	4	9	13	29	2.25	2.23
0.20	5.5	6	9	15	42	1.5	2.8
0.25*	5.5	11	9	20	63	0.82	3.15
0.30	5.5	17	9	26	80	0.53	3.1
0.35*	5.5	50	9	59	90	0.18	1.52
0,40	5.5	75	9	84	98	0.12	1.17

*data obtained by extrapolation

Table 6. The dependence of some model parameters and experimental values of the synergistic enhancement ratio for the combined effect of fluoride and xylitol on the acid production by the cells of *S.* sobrinus 6715 on the condition of treatment. The original results have been published by others [68].

Fluoride, m*M*	pH	Fluoride $F = cN_1$	Xylitol $X = cN_2$	$(F + X)_{add} = c(N_1 + N_2)$	$(F + X)_{exp} = c(N_1 + N_2 + N_3)$	N_2/N_1	k*(exp)*
0	7.0	0	5	5	5	–	–
0.8	7.0	5	5	10	15	1.0	1.5
1.25*	7.0	10	5	15	23	0.5	1.53
1.6	7.0	15	5	20	31	0.33	1.55
2.4	7.0	28	5	33	58	0.18	1.76
2.9*	7.0	40	5	45	70	0.125	1.56
3.25	7.0	52	5	57	79	0.096	1.39
3.5	7.0	60	5	65	82	0.083	1.26
4.0	7.0	78	5	83	92	0.064	1.11
0	5.5	0	8	8	8	–	–
0.1	5.5	5	8	13	15	1.6	1.15
0.15*	5.5	10	8	18	30	0.8	1.67
0.2	5.5	20	8	28	48	0.4	1.71
0.225*	5.5	28	8	36	55	0.29	1.53
0.25*	5.5	40	8	48	62	0.2	1.29
0.3	5.5	60	8	68	80	0.133	1.18
0.35*	5.5	78	8	86	90	0.103	1.05

*data obtained by extrapolation

In Fig. 7, the synergistic enhancement ratio k of a simultaneous action of fluoride and xylitol on the inhibition of acid productionby mutans streptococci from glucose is shown as a function of the ratio of effective damages produced by these agents (N_2/N_1). One can see that the theoretically predicted interaction (solid lines)corresponds fairly well with the experimental data (circles). It can be inferred that the synergy occurred only within a definite ratio of the effects (N_2/N_1) produced by each agent. It is also apparent that there is an optimal ratio of N_2/N_1 which results in the greatest synergistic interaction. Table 7 includes the highest synergistic effect that should be expected [Eq. (11)] and the ratio N_2/N_1, at which it can be achieved (Eq. 10). Fig. 7 demonstrates that a good agreement exists between these predicted values and the experimental data. It means that the model presented here can be used for mathematical description, optimization and prediction of synergistic interaction of fluoride and xylitol on glycolysis.

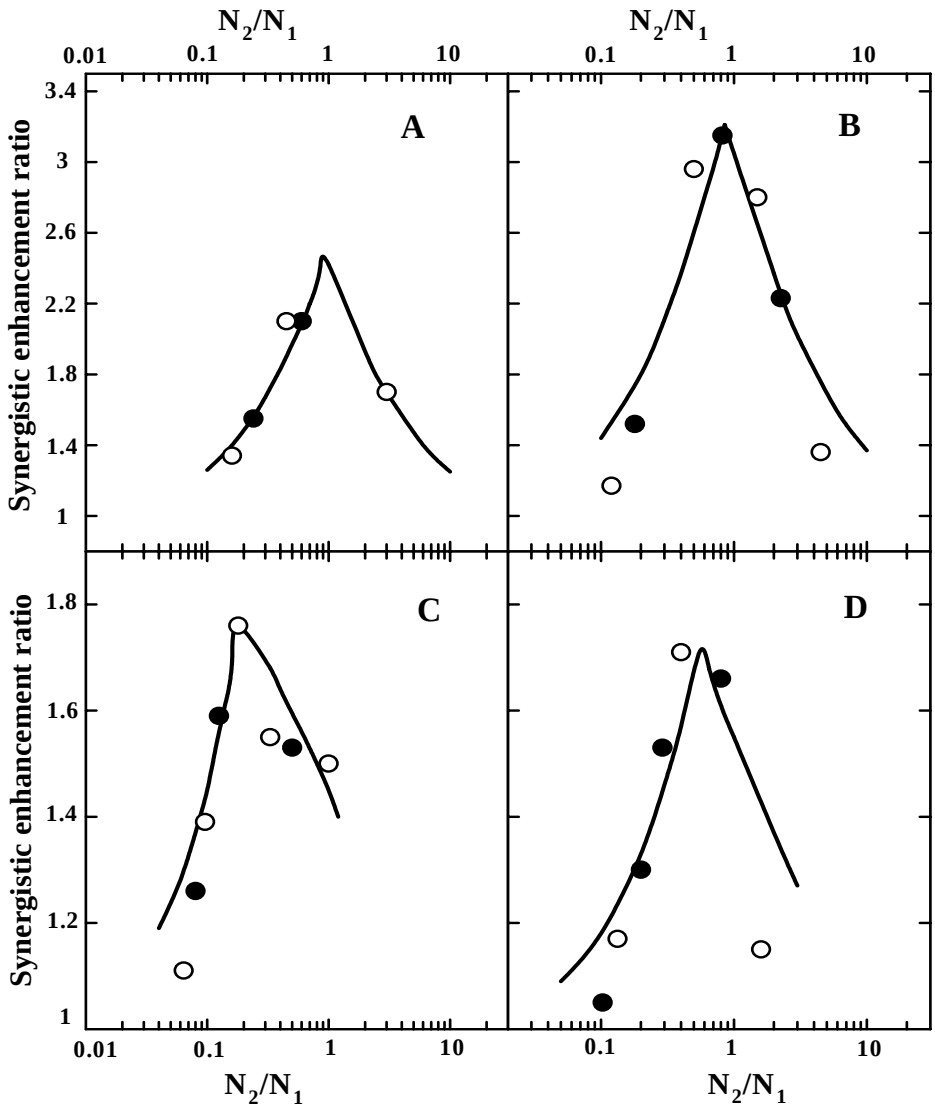

Fig. 7. The theoretically predicted (solid lines) and experimentally observed dependences of the synergistic enhancement ratio on the ratio of the effective damages produced by 60 mM xylitol (N_2) and various concentration of fluoride (N_1) after their combined action on the acid production for 10 min from 10mM glucose by the cells of *S. mutans* 10449 (A - pH 7.0; B - pH 5.5) and *S. sobrinus* 6715 (C - pH 7.0; D - pH 5.5). Closed circles - experimental data, open circles - the data estimated by the extrapolation.

Table 7. The dependence of the basic model parameters (p_1 and p_2), the predicted greatest values of the synergistic enhancement ratio (k_{max}) and the ratio of $(N_2/N_1)_{max}$ at which it can be achieved after the combined effect of fluoride and xylitol on the acid production by the cells of *Streptococus mutans* and *Streptococus sobrinus* on the condition of treatment

Strain	pH	p_1	p_2	$(N_2/N_1)_{max}$	k_{max}
Streptococus mutans NCTC10449	7.0	2.8	2.9	0.96	2.42
Streptococus mutans NCTC10449	5.5	4.1	4.8	0.85	3.21
Streptococus sobrinus 6715	7.0	0.9	5.0	0.18	1.77
Streptococus sobrinus 6715	5.5	1.1	2.0	0.55	1.71

4. Summary

A simple mathematical model was suggested for the optimization and prognosis of the synergistic interaction of fluoride and xylitol on acid production by mutans streptococci. The model is based on the assumption that synergism is conditioned by additional effective damage arising from an interaction of the sublesions induced by both agents. These sublesions are noneffective when each agent is applied alone. The predictions of the model were verified by comparison with experimental data published by other researchers [68]. In the proposed model, the synergistic interaction is given by $\{p_1N_1; p_2N_2\}$. This means that one sublesion caused by fluoride interacts with one sublesion produced by xylitol. This process is assumed to proceed until the very last sublesion of the less frequent type is used up. However, it does not mean that the model requires some kind of unreal long-range interaction. To estimate the basic parameters of p_1 and p_2 we have used the experimental values of the synergistic enhancement ratios [Eqs. (9) and (10)]. It means that the model takesinto consideration only the real interaction. The model proposed here is not concerned with the molecular nature or type of effective damages or sublesions which occur, and the mechanism of their interaction remains to be elucidated. What the model does offer is an empirical mean for their quantitative estimation. The model appears to be appropriate and the predictions are valid. Indeed, it was shown that the model describesthe experimental data, predicts the greatest value of the synergistic effect and the condition under which it can be achieved. The synergistic effect appeared to decline with any deviation from the optimal value of the ratio of carcinogenic effective damages produced by each agent alone. From the model standpoint, the highest synergy can be obtained for a specific condition when an equal number of sublesions are produced by each of the agents applied in combination with each other.

As far as the basic model postulates do not concern the real biochemical mechanism, it is expected the model can be useful for description of synergistic effect of other chemical agents combined with each other, providing the methodology of optimization and prediction of some non trivial results of synergistic interaction.

제 5 절 카페인과 아스코브산의 방사선손상 방어에 관한 면역조직 화학적 평가

1. Introduction

A requirement of radioprotectors has been rising because of the scattered possibility of exposure to radiation; accidental external or internal exposure, chronic exposure of the lungs to environmental alpha-particles, cosmic radiation exposure during a manned space flight and working over the long haul in an airplane, and therapeutic exposures to radiation. The oxygen effect in radiation biology is well known. Radiation injury occurs through the formation of reactive oxygen species. Since oxygen also enhances radiation-induced biological damage, antioxidants should be radioprotectors. Numerous antioxidants have proven beneficial to radioprotection, among which well known are cysteine, cysteamine, glutathione, WR-compounds, tempace, vitamine A, E, and C, bioactive lipids (dimethylprostaglandin E2, platelet activating factor, and leukotriene C4), and immunomodulators (glucan, synthetic trehalose dicorynomycolate, and interleukin-1) [69,70]. Ascorbic acid is a well-known antioxidant and an essential component in the diet of humans and a small range of other mammals. Ascorbic acid is known to react with free radicals [71]. Because ionizing radiation leads to the production of the activated oxygen species, increased glutathione levels in tissues by the vitamins may contribute to scavenging free radicals generated from irradiation and *in vivo* protection against radiation injury [72]. Radioprotective effects of ascorbic acid have been demonstrated in certain cells and animals, which would result from scavenging free radicals [73,74]. Because the vitamins do not modify tumor growth delay induced by irradiation and concomitantly exhibit radioprotective effects in normal tissues [75], these agents have great therapeutic potential as radioprotectants.

Caffeine (1,3,7-trimethylxanthine) is one of the most extensively studied ingredients in the food supply. Caffeine exists in diet from a variety of sources - primarily coffee, tea, chocolate, drinks, and both prescription and nonprescription drugs. Recently, caffeine in a cell cycle has shown to override the cell cycle checkpoint in particular a G_2 delay. Caffeine inhibits ATM and ATR in a dose-dependent fashion. The concentration that inhibits ATM by 50% *in vitro* is 1 mM and the 50% inhibitory concentration for ATR is about 3 mM. Studies by George *et al.* (1999) showed that a dose of 80 mg/kg administered 60 min before a whole-body exposure

to 7.5 Gy was optimal for a maximal radioprotection [76]. Caffeine and its xanthine metabolites had an oxygen radical absorbing capacity comparable with that of ascorbic acid or uric acid [77,78]. Moreover, Lu *et al.* (2000) [79] demonstrated that caffeine had an inhibitory effect on UV-induced carcinogenesis. Knowledge of the clear function and side effects of caffeine in the irradiated individuals has been poor. Thus the present studies are to examine the effects of caffeine treatment before whole-body irradiation in male mice. According to the previous investigations, the testis is now recognized as one of the most radiosensitive organs of the body. Interestingly, differential sensitivity of irradiation depends on cell types in the testes [80]. The male mouse gonad contains both radiosensitive (premeiotic germ cells) and radioresistant (somatic) cells. Indeed, irradiation (5 Gy) of the male gonad selectively destroys premeiotic (differentiating spermatogonia) germ cells, whereas other cells, i.e., somatic (Leydig and Sertoli cell) cells and postmeiotic (spermatids) germ cells are unaffected [81]. However, it has been reported that fetal irradiation is associated with an increase of hCG binding and testosterone production per Leydig cell, and a decrease in the number of these Leydig cells per testis [82,83]. The previous data suggest that somatic cells may be biochemically and physiologically radiosensitive. Therefore, the present study is designed to investigate and compare the radioprotective effects of caffeine and ascorbic acid on spermatogenesis of mice after whole-body gamma irradiation.

2. Materials and Methods

Animals and irradiation

The animal was obtained from Daehan Biolink (Chungbuk, Korea). C57BL/6N mice (male, 8-week-old) were used throughout the investigations. All the mice were maintained under the following conditions; temperature (23°C) and lighting (14 hr light: 10 hr dark) and allowed free access to food and water. Irradiated groups were exposed to g-irradiation using a ^{60}Co source with a total dose of 6.5 Gy, and a dose rate of 12.8 Gy/hr [84].

Treatments of caffeine and ascorbic acid

The caffeine treated group was administrated with 80 mg/ kg body weight by *i.p*

injection, a single treatment, one hour before irradiation [76]. The ascorbic acid-treated group was administered with 330 mg/liter in drinking water during experiment [85]. The remaining mice were kept as sham controls. According to the experiment schedules, mice were sacrificed by cervical dislocation 2 weeks after whole-body irradiation. The body weight (BW) and organ weight (OW) were recorded and the indices were calculated with the equation; OW×100 / BW.

Immunohistochemical analysis for Bcl2 and Bax in testis

Testes were fixed overnight at room temperature with 10% neutralized buffered formalin (NBF), dehydrated, and embedded in paraffin. Sections (6 μm) were prepared and mounted on slides. The slides were then hematoxylin and eosin (H-E) stained for morphological observations.

For the immunohistochemical analysis, a rabbit polyclonal IgG antibody against Bcl-2 (N-10) and a mouse monoclonal IgG26 antibody (B-9) from Santa Cruz Biotechnology (Santa Cruz, CA) were used. Sections (5 m) were mounted onto poly-lysine-coated slides. After rehydration, the slides were washed with distilled water (DW) each followed by the antigen retrieval at 121°Cor 15 min in 10 mM citrate solution (pH 6.0). After the washes with DW, enough drops of 0.05 ml of peroxide blocking solution (0.3% H_2O_2 in methanol) were applied to each section and incubated for 10 min. After washing the slides, enough drops of block solution (1% BSA in phosphate buffer) to minimize the non-specific binding of the antibodies were placed at each section and incubated for 5 min. After blocking, a primary antibody (1:400 diluted in phosphate buffered saline) was applied to each section and incubated at 4°C overnight. Incubation with a secondary antibody and visualization was performed using InnoGenexTM IHC kit (InnoGenex, CA) according to the mansufacturer's instructions. The slides were subsequently counterstained with Mayer's hematoxylin (Sigma).

Measurement of Testosterone Levels by Radioimmunoassay (RIA)

The concentration (ng/ml) of serum testosteone was measured by radioimmunoassay kits (Diagnostic Systems Laboratories, USA) with a sensitivity of 0.08 ng/ml. The intra- and interassay coefficients of variations were < 3 % and < 7 %, respectively. All experimental groups were assayed in duplicate. The 50 ㎕ of serum or standards was mixed with Testosterone [I-125] reagents. After gentle mixing of the test tube

rack by hand, all tubes were incubated in a water bath at 37 ± 2°C for 60 to 70 min. Then, all tubes except the total count tubes were decanted. Radioactivity was measured by a liquid scintillation counter (LS 6500S, Beckman Instruments Int., Fullerton, CA).

Statistical Analyze

Statistical analysis was performed by Student's *t* test for a simple comparison of the two groups using Sigma Plot software (Jandel Scientific, Germany). They are expressed as mean SEM and P<0.05 was considered significant.

3. Results and Discussions

Effect of body and organ weights in irradiated mice

The present studies were designed to investigate the effect of caffeine and ascorbic acid before whole-body irradiation. The dosage and treatment route of caffeine and ascorbic acid used in this experiment referred to the previous reports [76,85]. The rate of increase in the body weight during the experimental period was presented in Fig. 8. Two week after whole-body irradiation, the mean body weight of the irradiated control (RC) group showed a significant decrease (-4.08 g, $P<0.02$) compared with those of the control group. The decrease in the body weights of the caffeine-treated irradiated group (AR) was not marked. In the ascorbic acid-treated irradiated (AR) group, the decrease was higher than the RC group. While the unirradiated groups (CT, A, and B) showed increases in the body weights, the irradiated groups (RC, AR, and BR) resulted in decreases from the mean body weights at the irradiation point on the whole. It is noteworthy that the treatment of caffeine before whole-body irradiation improved the radiation-induced body weight decreases and that the effect of caffeine was superior to that of ascorbic acid.

To investigate the organ-sensitive defects corresponding to the reduction of the body weights of each group, we were calculated the organ indices of liver, kidney, spleen, and testis. The indices for the experimental groups were shown in Table 8. The index (%) was calculated from the organ weights divided by the body weights as mentioned before. Liver indices in Table 8 did not show difference among the experimental groups. Because kidney indices were affected by gamma irradiation, the

value of the RC group was reduced to 87% of those in the control group.

The values of spleen and testis in the RC group decreased significantly by 50% compared with those of the control group ($P<0.05$). However, the values of the AR group reflected a marked recovery, especially in spleen indices, compared with those of the RC group. While spleen indices of the AR group showed a similar level to the control group, the values of the BR group was not improved into the level of the RC group. Because spleen and testis were the most sensitive to radiation, pretreatments of caffeine and ascorbic acid played important roles in the organ-specific influence on the irradiated groups. In case of testis indices (*ie*, gonad indices), both of the AR and BR group did not differ from the values of the RC group. Though testis indices of the AR and BR group were not better than the RC group, the spleen indices of the AR group indicated that caffeine protected the organ against whole-body irradiation.

Concentration of the circulating testosterone on irradiated mice

As a functional parameter of radiation-induced testicular defects, we investigated concentration of the circulating testosterone. The level (ng/ml) of testosterone in serum was determined by a radioimmunoassay with a sensitivity of 0.08 ng/ml (Fig. 9). In the RC group, the concentration of testosterone was significantly reduced, compared with the value of the CT group (<36.2, $P<0.02$). The caffeine pretreated groupshowed a similar level to the caffeine only group. However, the level of the AR group did not show a difference from the value of the RC group. We have previously shown that the concentration of circulating testosterone in all the irradiated groups was significantly reduced irrespective of caffeine pretreatmentsix hours after irradiation [86]. The results obtained in the present study lead to supposition that caffeine pretreatment was associated with protection or recovery of the androgen productive system. As a result of the function of caffeine against γ-ray, the concentration of testosterone two weeks after irradiation showed a similar level compared with those of the A group. Moreover, ascorbic acids did not play a role like the action of caffeine in the steroidogenic system in the present study.

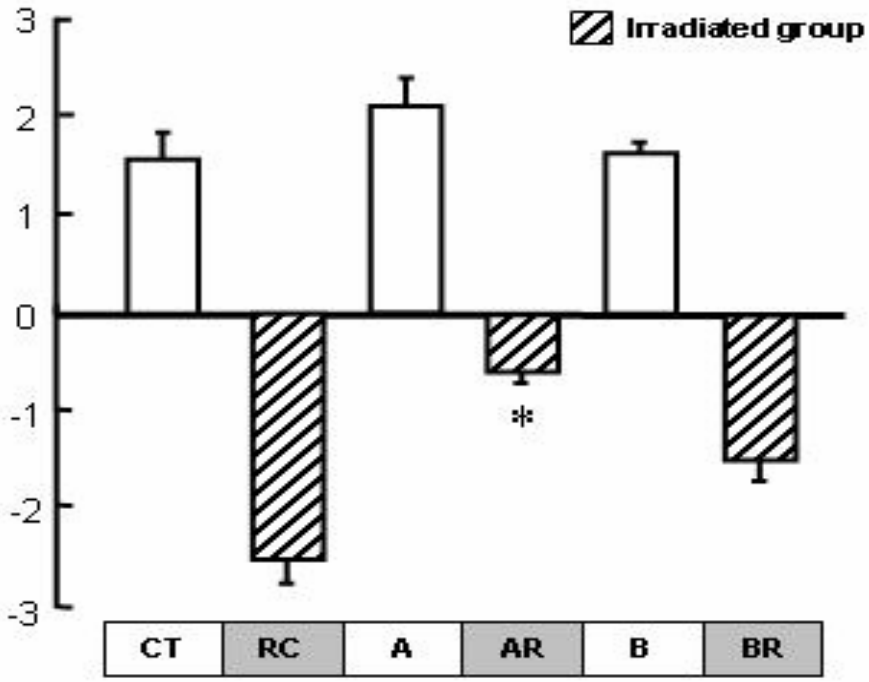

Fig. 8. Increased ratio of the body weights (g) during the experiments. Zero value indicated the mean of the body weights of the experimental groups at the irradiation time. Each bar showed an increase or decrease in body weights 2 weeks after whole-body irradiation. Abbreviations: CT, the control group; RC, the irradiated control group; A, the caffeine-treated group; AR, the caffeine treated before irradiation; B, the ascorbic acid-treated group; and BR, the ascorbic acid-treated before irradiation. *, significantly different from the irradiated control group ($P<0.05$).

Table 8. Calculated organ indices of the experimental groups 2 weeks after whole-body irradiation

	Organ indices after whole-body irradiation			
	Liver	Kidney	Spleen	Testis
CT	3.45 0.04 (1.00)	0.62 0.01 (1.00)	0.27 0.002 (1.00)	0.38 0.04 (1.00)
RC	3.83 0.01 (1.11)	0.54 0.02 (0.87)	0.11 0.001* (0.41)	0.20 0.02 (0.53)
A	4.22 0.01 (1.23)	0.61 0.01 (0.98)	0.24 0.001 (0.89)	0.34 0.02 (0.89)
AR	4.09 0.01 (1.18)	0.50 0.01* (0.81)	0.28 0.001* (1.03)	0.20 0.01 (0.53)
B	3.82 0.02 (1.10)	0.62 0.08 (1.00)	0.20 0.002* (0.74)	0.36 0.03 (0.95)
BR	3.60 0.02 (1.04)	0.58 0.02* (0.93)	0.10 0.001 (0.37)	0.23 0.02* (0.61)

*, significantly different from the value of the irradiated control group.

Each value was calculated as described in 'Materials and methods' and represented the mean SEM of data from 5 mice/group. A parenthesized value showed a relative potency compared with those of the control group.

Abbreviations in this table are the same as in Fig. 8.

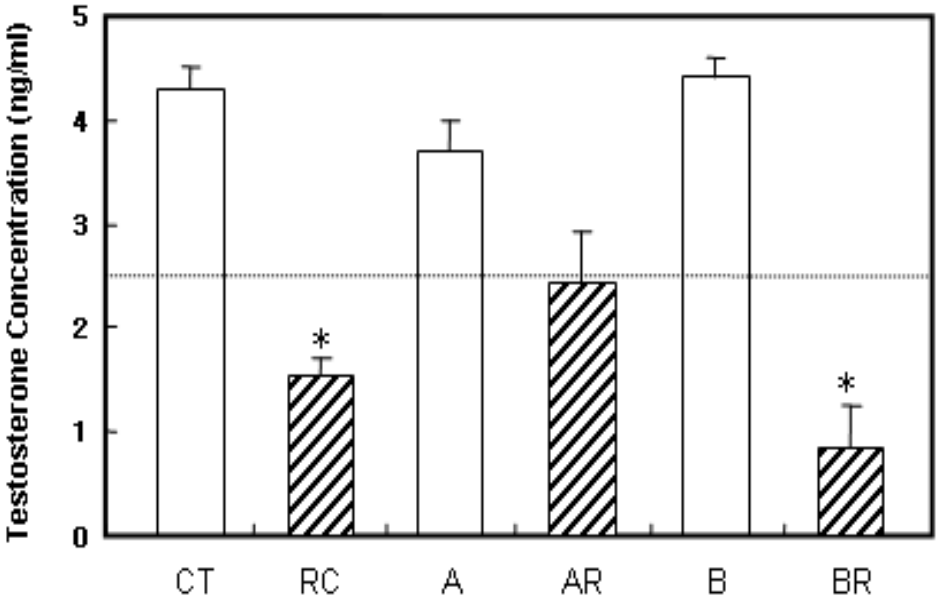

Fig. 9. Changes of the testosterone concentration (ng/ml) in serum of C57BL/6N mice after whole-body irradiation. Mice were treated with caffeine (80 mg/kg) in saline by *i.p* injection or ascorbic acid (330 mg/kg) in drinking water and sacrificed 2 weeks after irradiation. Samples were measured by radioimmunoassay kits with a sensitivity of 0.08 ng/ml. Striped bars correspond to the irradiated groups. Abbreviations are the same in Fig. 8. *, $P<0.05$.

Histological Observation

Many studies have been performed by qualitative or quantitative examination to detect the testicular impairments via histological investigations [87]. Qualitative evaluation involves the Leydig cells and their subcellular features, blood vessels, intertubular fluid build-up, cell infiltration, basement membranes, multinucleated giant cells, Sertoli cell morphology, delayed spermiation, missing germ cells, abnormalities of germ cells and germ cell development, absence of the tubular lumen, and disruption of the Sertoli cell barrier. Quantitative evaluation includes testis, fertility, tubular diameter, determination of sperm production, cell ratios, and cell numbers by morphometry.

Irradiation of male reproductive system causeda loss of spermatocytes, missing germ cells, and absence tubular lumen in seminiferous tubules [88]. Results of the morphological assessment using H-E staining 2 weeks after whole-body irradiation showed that, compared with the control mice, the seminiferous tubules of the irradiated group significantly decreased in their diameters and increased a complete atrophy and vacuolation inside the tubules (Fig. 10). While vacuoles of the AR group also remained in quantities, they were not observed inside the tubules of the BR group. However, the BR group showed a reduction in the size of seminiferous tubules and disturbance of the spermatogenesis process. The earlier studies demonstrated that pretreatment with ascorbic acid resulted in a significant reduction of radiation-induced delay in wound healing such as increased collagen contents and fibroblast and vascular densities [89]. In the present investigations, ascorbic acid may provide an assistance to regeneration of defects by reducing the vacuole and upkeep the spermatogenesis.

Immunohistochemistry using an antibody raised against Bcl-2 and Bax showed their presence in the seminiferous tubules although the staining did not gave rise to a particularly intense spot. The Bcl-2 family of proteins isa widely recognized group of regulation of apoptosis. This family consists both of pro- (Bax, Bad, Bak, Bik, Bok, Diva, Hrk, and Bid) and anti-apoptotic (Bcl-2, Bcl-xL, Mcl-1, Blc-w, and Bfl-1/A1) proteins that modulate the execution phase of the cell death [90]. Numerous studies have indicated an important role of Bcl-2 familyfor functional spermatogenesis. Bax-deficient male mice are infertile due to an inappropriate accumulation of premeiotic germ cells [91]. Transgenic mice that over-express Bcl-2 are infertile and show a disorganization of the cells of the seminiferous tubules [81].

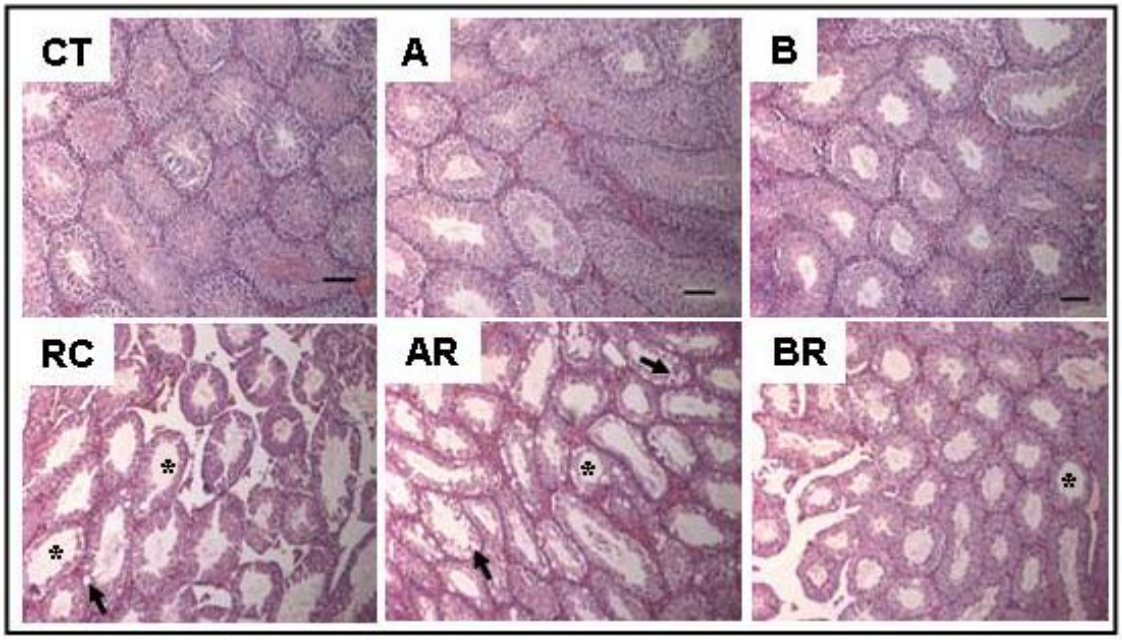

Fig. 10. Photomicrographs of the hematoxylin-eosin staining of the seminiferous tubules from the experimental group two weeks after whole-body irradiation. Abbreviations are the same in Fig. 8. An asterisk or arrow indicatesa complete atrophy and vacuolation in seminiferous tubules. A scale bar indicates 100 ㎛.

Two weeks after irradiation, Bax in all the irradiated groups was strongly expressed in the nuclei of the round spermatids and a few primary spermatocytes, but it was also stained in the cytoplasm of the seminiferous tubules and the interstitium (Fig. 11-B). Expression of Bcl-2 was weak in the tubules of all the experimental groups. In case of the CT and BR group, Bcl-2 expression was localized in the cytoplasm of the differentiating spermatids close to the luminal surface (Fig. 11-A). These data were not sufficient to identify the differential expression of regulators among the irradiated groups (RC, AR, and BR group), but further researches for qualitative analysis and isolation upon the cell type in the seminiferous tubules will give the answer to the question whether the treatment of caffeine or ascorbic acid before γ-irradiation affects regulatory proteins which act executively in radiation-induced alteration.

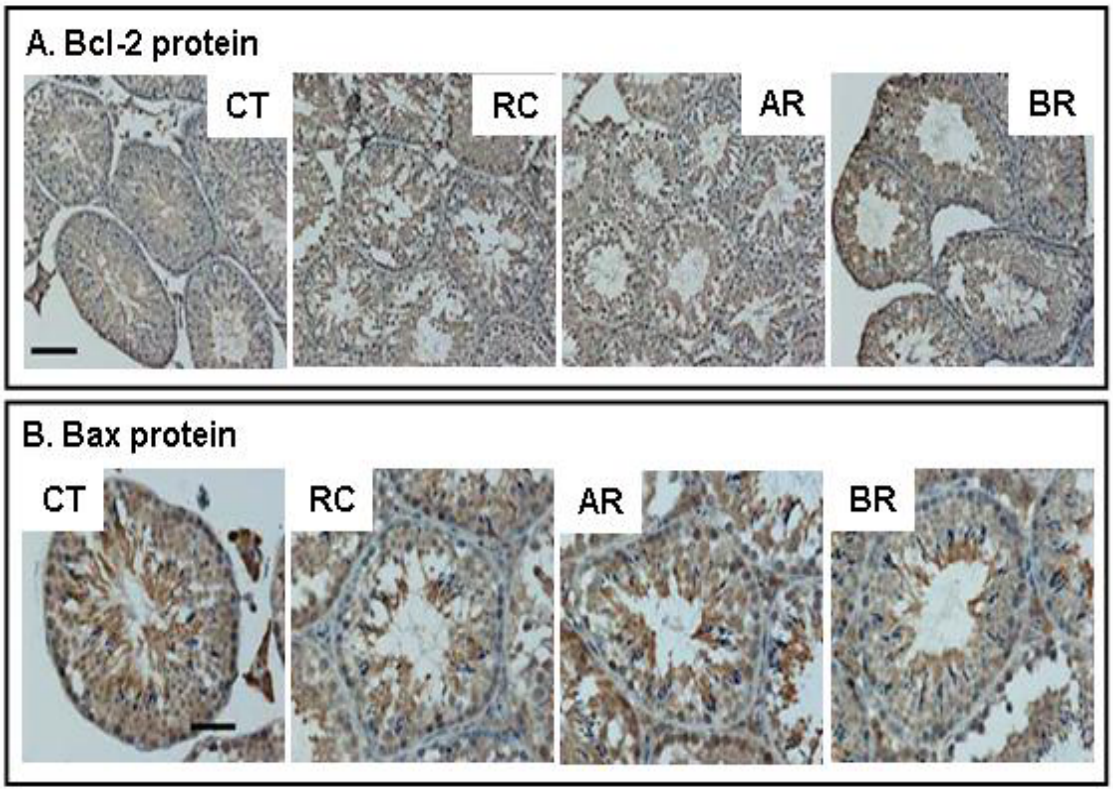

Fig. 11. Bcl-2 (upper panel) and Bax (lower panel) immunohistochemistry in the testis of C57Bl/6N mice two weeks after whole-body gamma irradiation. Abbreviations are the same in Fig. 8. A scale bar indicates 50 ㎛.

4. Conclusions

As radiation-induced defects were concomitant with the oxidative stress, antioxidants should be radioprotectors. Caffeine and ascorbic acid in the present study were essential components in the diets in human and a small range of other mammals. The antioxidative ability of caffeine and ascorbic acid was evaluated in numerous reports, but there was no quantitative approach to their radioprotection role *in vivo*. Therefore, the present study investigated thefunctional radioprotection of caffeine and ascorbic acidagainst gamma radiation in irradiated C57BL/6N mice. The caffeine treated mice before irradiation showed higher body weights, spleen indices, and concentrations of the circulating testosterone than those of the other irradiated groups. Ascorbic acid treatment before irradiation exhibited high gonad indices, diameters of the seminiferous tubules, and decrease of vacuoles in cytoplasm of the Sertoli cells. In conclusion, the several defects related with male reproduction caused by γ ray in mice were improved by pretreatment of caffeine or ascorbic acid. Especially, the ascorbic acid treated group was distinguished in structural improvement compared with those of the other irradiated groups and the caffeine treated group showed the excellent steroidogenesis in testis.

제 6 절 이온화 방사선 및 저농도 염화수은 처리에 따른 영향

1. 서론

자연계에서 수은의 분포는 다양하게 나타나며, 토양의 경우 약 10~150 ppb, 대기 중에 0.01~0.02 μgm^{-3}, 담수의 경우 평균 0.03 ppb, 해수에는 0.1 ppb 정도 존재하는 것으로 보고된 바 있다. 우리나라 논토양에서 자연 함유량을 조사한 결과는 평균 0.09 ppm 이였다 [92]. 수은을 통한 오염은 대기 오염을 통한 빗물이나 강물의 오염 등으로 생물을 비롯한 인간에서 영향을 줄 수 있으며, 수은을 포함한 천연연료의 연소, 자동차 매연, 쓰레기 소각, 그리고 공업화와 산업화에 따른 방출에 의해 생태계는 지속적으로 오염되고 있다. 이러한 수은에 대한 독성 연구도 다양하게 이루어지고 있으며, 수은에 화학적 형상에 따라 다각적으로 접근되고 있다. 무기수은, 메틸수은, 염화수은 등의 화학적 분류에 따라 그 증상도 다양하게 나타나고 있으며, 주로 신경장애, 신장 및 간 기능의 손상 등을 대표적으로 일으킨다. 호흡기로 흡수되는 수은 증기는 대부분 체내에서 빠른 속도로 2가의 수은으로 산화되어 혈청 알부민, 적혈구의 헤모글로빈과 결합하게 되며, 그 분포 및 배설양식은 경구로 흡수된 수은과 같게 된다. 그러나 일부는 금속 수은 상태로 혈중을 흐르다가 뇌혈액 관문을 통과하게 되어 뇌에 치명적인 손상을 일으키게 된다. 무기수은의 장관흡수율은 5% 정도로 낮은 편이나 전신에서의 생물학적 반감기는 29~60일이며, 신장에서는 반감기가 더 길다. 그러나 메틸수은의 경우는 장관흡수율이 90%이상이며 거의 대부분 흡수되며, 흡수된 메틸수은의 10%는 뇌에 축적된다고 알려져 있다. 이미 일본이나 이란에서는 수은에 의한 오염이 인체로 전달되어, 큰 문제를 일으킨 적이 있다 [93].

수은에 의한 동물 실험은 고농도의 독성학적 접근 위주로 이루어져 다양하게 진행 되었으나, 염화수은을 경구 투여 하였을 때 생쥐에서 반수치사량 (LD_{50}) 농도는 30~35 mg/kg 으로 환경적으로 인간이 일상적으로 접하는 농도와는 상당한 차이를 보인다 [94].

따라서 수은의 환경오염이 증가 뿐 아니라 특정분야 근로자들의 직업적 노출이 증가되고 있는 시점에서 저농도 수은의 경구노출에 따른 생물학적 손상의 특성파악과 이온화 방사선에 의한 생체 손상과의 비교 자료를 얻기 위해서 본 연구를 수행하였다.

2. 재료 및 방법

실험재료 및 처리

본 실험에 사용된 동물, 처리, 시료의 채취 등은 앞서 기보고 된 논문을 바탕으로 수행하였다 [95]. 생후 4주령의 수컷 F344 흰쥐 (Daehan Biolink)를 사용하였다. 한국원자력연구소 내의 사육실에서 고형 사료와 물을 충분히 공급한 상태로 10/14 시간 (명/암)의 조명 조건 하에서 1주일간 적응시킨 후 실험에 사용하였다.

이온화 방사선 처리군은 한국원자력연구소의 감마선 발생장치를 이용하여 조사하였다 [86]. 조사선량은 선량-반응식을 기준으로 단기간 내에 손상이 확인된 선량을 조사하였다 [96]. ^{60}Co 선원의 강도는 약 150 TBq, 선량율은 12.82 Gy/hour 이며, 30분간 조사하였다. 수은 처리군의 경우에는 음용수에 1 mg/kg의 농도로 염화수은(II)을 2주간 처리하였다. 2주간의 처리 후 주요 기관 내 신호전달에 관여하는 물질의 발현량을 비교하기 위해, 혈액과 조직을 채취 하였다.

혈액내 주요 인자의 수준 확인

실험 동물 혈액내 red blood cell (RBC), white blood cells (WBC), hemoglobin (HB), hematocrit (HCT) 등의 혈액 성분의 변화를 측정하기 위해 채혈 즉시 항응고제 (EDTA)를 처리하였다. 처리 후 시료를 전문 분석기관 (녹십자)에 의뢰하여 분석을 시행하였다.

혈액내 cortisol 농도 분석

혈액내 cortisol의 농도를 분석하기 위해, 채혈 후 일부를 상온에서 30분간 응고 시켰다. 직후 4℃에서 3,000 ×g에서 원심분리 하여, 혈청을 얻었다. 각 혈청 중 25 ㎕를 취하여, 호르몬 정량을 수행하였다 (DSL system, US). 분석을 위한 시료의 측정은 gamma counter (DELFIA, US)를 이용하였으며, 정량 분석을 위해 KC-junior 프로그램을 이용하여 계산하였다.

Total RNA 추출

정소, 신장의 total RNA를 추출하기 위해 각 조직 0.1 g 당 Trizol (GibcoBRL) 1 ml을 넣고 homogenizer로 균질화 시켰다. 균질화 된 현탁액에 0.2 ml의 chloroform을 넣어 혼합시킨 후 원심분리기를 이용하여 12,000×g 로 15분간 4℃를 유지하면서 원심분리 하였다. 원심분리 후 핵산을 보유하고 있는 상층부의 무색 수용액을 취하여 동량의 isopropanol로 침전시켰다. 영하 20℃에서 12시간 침전시킨 후 12,000×g, 15분간 4℃에서 원심분리 하였다. 상층액을 버린 후 75% ethanol을 이용하여 세척하여 7,500×g, 5분간 원심분리 하였다. 세척 후 진공상태에서 10분간 건조시킨 후 diethyl pyrocarbonate (DEPC, Sigma-Aldrich)를 처리한 증류수나 nuclease free water에 용해시켜, 55℃에서 20분간 녹인 후, 일부를 spectrophotometer를 이용하여 RNA를 정량 하였다. 이 후 단계 수행까지 초저온 냉동고에 보관하였다.

세포자연사 중재 분자의 역전사 중합효소 연쇄 반응

세포자연사에 관여하는 여러 분자들 중에서 미토콘드리아에 관련한 Bcl-2/Bax 분자와 실질적인 세포자연사 실행자인 caspase 3 유전자 발현 변화를 알아보기 위해 역전사 중합

효소 연쇄 반응 (reverse transcription - polymerase chain reaction, RT-PCR)을 수행하였다. complementary DNA library를 만들기 위해서 각각의 RNA 1 ㎍을 10 pM oligodT 15 primer (Promega, USA), RNase inhibitor (40 U/㎕, Promega, USA), 2.5 mM dNTP (Takara, Japan), AMV RT reaction buffer (5×, Promega, USA), AMV reverse-transcriptase (10 U/㎕, Promega, USA)를 포함하는 반응액 속에서 70분 동안 42℃에서 반응시켰다 (Thermal cycler 480, Takara). 제조가 끝난 cDNA는 다음 PCR을 수행할 때까지 -20℃에 보관하였다.

RNA 추출 및 역전사가 제대로 수행되었는지를 확인하기 위해서 house keeping gene으로 알려진 glyceraldehydes 3-phosphate dehydrogenase (GAPDH)를 먼저 PCR로 증폭하였다. GAPDH의 forward primer sequence는 5'-CCC TCA AGA TTG TCA GCA ATG C-3', reverse primer sequence는 5'-GTC CTC AGT GTA GCC CAG GAT-3'을 제조하여 사용하였고, PCR 산물의 크기는 410 bp 이다 [108]. Thin wall tube에 동일 양의 cDNA, $MgCl_2$, 10X PCR buffer, dNTPs, 그리고 각각의 제조된 primer를 Taq DNA polymerase (QIAGEN)과 중합반응 시켰다. PCR 반응 조건은 94℃에서 5분간 predenaturation 시킨 후, 94℃에서 1분간 denaturation, 각 primer에 따라 58～62℃의 온도에서 30초～1분간 annealing, 72℃에서 1분간 extention을 총 28～35회 실시하였다. 마지막으로 72℃에서 5분간 postelongation 시켜 충분히 PCR 산물을 얻어내어 전기영동을 실시하기 전까지 4℃에서 보관하였다.

전기영동은 6X loading dye를 7 ㎕의 PCR 산물과 혼합 후 2% agarose gel에 loading 하여 100 V로 30분간 전기영동 하였다. Ethidium bromide (EtBr)로 10분간 염색한 후 Image analyzer (VILER LOURMAT) 하에서 관찰하고 촬영하였다. GAPDH의 발현 양에 비례하여 각 유전자의 발현 양의 변화를 비교하였다. 각 반응은 3회 이상 수행하여 통계처리 하였다.

통계학적 분석

통계학적 유의성 검정은 일원분산분석 (one-way ANOVA test)을 사용하였고, p 값이 0.05 보다 작은 경우를 유의하다고 판정하였다.

3. 결 과

체중 및 기관 무게의 변화

방사선 전신 조사 및 염화수은(II)의 음수 처리 2주후의 체중 증가치를 실험군간 비교하였다 (Fig. 12). 대조군에 비하여 염화수은(II)을 처리한 군은 체중 증가가 유사한 수준으로 나타났으나, 방사선을 전신 조사 후 2주가 지난 실험군의 경우에는 유의한 감소를 보였다. 이 방사선 조사군의 경우는 지속적인 감소로 5주 경과 시에는 대조군에 비하여

약 40% 정도의 체중 감소를 보였다. Table 9의 경우는 조직의 채취 당시의 습무게 (wet weights)를 측정하여 산출한 기관지수 (organ indices)의 차이를 통계적 유의성으로 구분하여 정리하였다. 방사선을 조사한 실험군에서는 대조군과 비교하여 체중뿐만 아니라 방사선에 민감한 면역기관인 비장과 생식기관인 정소의 기관지수 감소가 뚜렷이 나타났다. 수은을 처리한 실험군에서는 체중의 차이는 보이지 않았으나, 신장의 무게가 증가하는 것으로 나타났다.

혈액내 지표 인자의 변화

실험동물의 혈액학적 지표로 이용되는 적혈구, 백혈구, 헤모글로빈, 헤마토크리트의 수준을 분석하였다 (Fig. 13). 방사선을 조사한 실험군에서는 대조군에 비해 조사 표지인자가 모두 감소를 보였고, 특히 백혈구의 수치는 약 80%의 감소를 보였다. 수은 조사군의 실험 결과에서는 적혈구 증가, 백혈구 감소, 헤모글로빈 증가 등을 보였으나, 유의한 차이를 보이지는 않았다.

혈청내 cortisol 농도의 변화

스트레스 호르몬이라 불리는 cortisol의 혈청내 변화를 측정해 본 결과 대조군에 비해 두 실험군 모두 증가하는 것을 보였다 (Fig. 14). 염화수은 처리군에 비해서는 방사선 조사군이 높은 증가를 보였다.

기관내 세포자연사 중재 인자의 발현 변화

세포자연사를 매개하는 세포내 분자 중 미토콘드리아와 관련한 세포자연사 신호전달을 중재하는 bax와 bcl-2에 대한 mRNA 발현 양상을 정소와 신장 기관에서 조사하였다. 각 분자의 발현은 생체내 house keeping gene인 GAPDH에 대한 발현양에 대해 그 비율을 이미지 분석을 통해 비교・확인하였다. 정소에서 방사선을 조사했을 경우에 bax의 발현은 대조군에 비해 현저하게 증가(약 220%) 하였으며, 염화수은을 처리한 경우에는 방사선을 조사한 경우보다는 낮으나 대조군 수준 이상의 발현 (약 180%)을 보였다. 그러나 정소내 bcl-2의 경우에는 방사선 조사군은 대조군에 비해 유의하게 증가된 발현을 보인 반면 염화수은의 경우에는 대조군보다 낮아지는 경향 (25% 감소)을 확인하였다. 신장에서 보이는 bax의 발현은 방사선을 조사한 경우와 같이 급격한 증가를 보이지는 않았으나 유의하게 증가하였으며, 두 실험군에서 증가를 보였다. bcl-2의 경우, 정소에서는 증가하고 신장에서는 대조군보다 낮은 발현을 보였다.

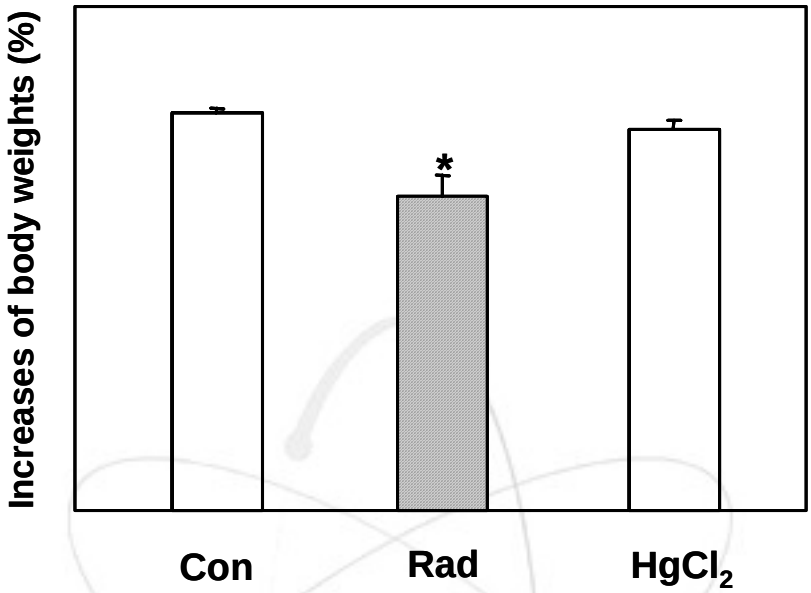

Fig. 12. The rate of the increase in the body weight during experimental period. Abbreviations; CON, control group; Rad, irradiated group; $HgCl_2$, mercury-exposed group. *, $p<0.05$.

Table 9. Statistical distintion of body weight and organ indices between experimental groups

	Control	Irradiation	$HgCl_2$ (II)
Body wt	−	+	−
Liver	−	−	−
Kidney	−	−	+
Spleen	−	+	−
Testis	−	+	−

The '+' represents a case which is statistically significant ($p<0.05$).

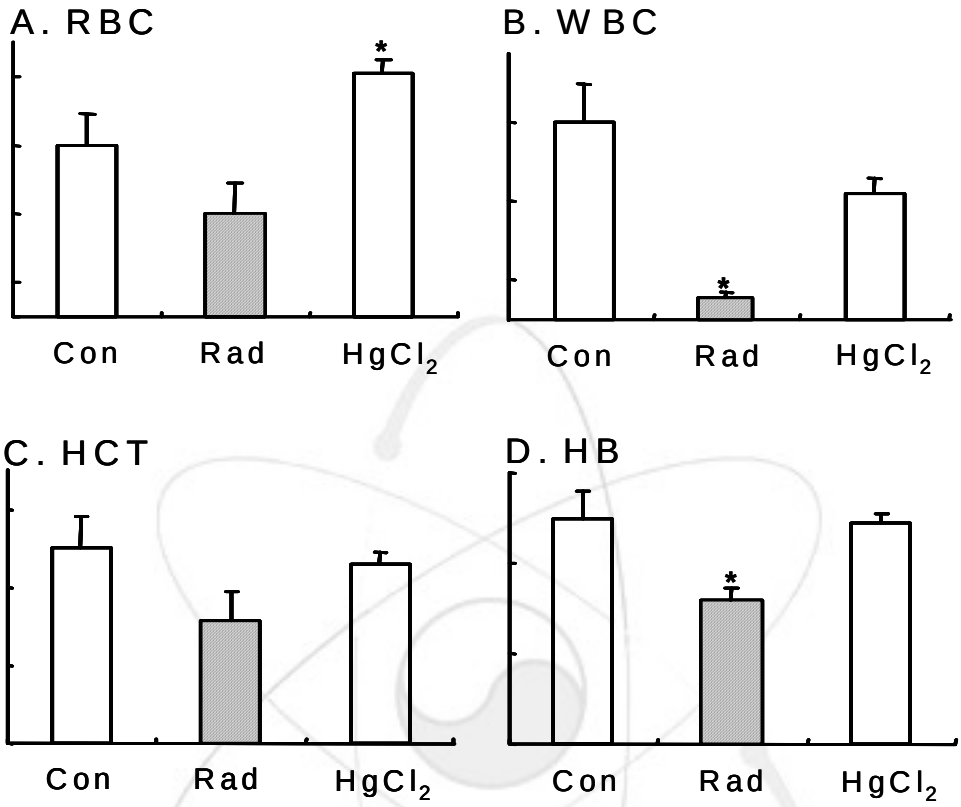

Fig. 13. Changes in hematological parameters of the experimental animals. Abbreviations; Con, control group; Rad, irradiated group; $HgCl_2$, mercury-exposed group; RBC, red blood cells; WBC, white blood cells; HCT, hematocrit; HB, hemoglobin. *, $p<0.05$.

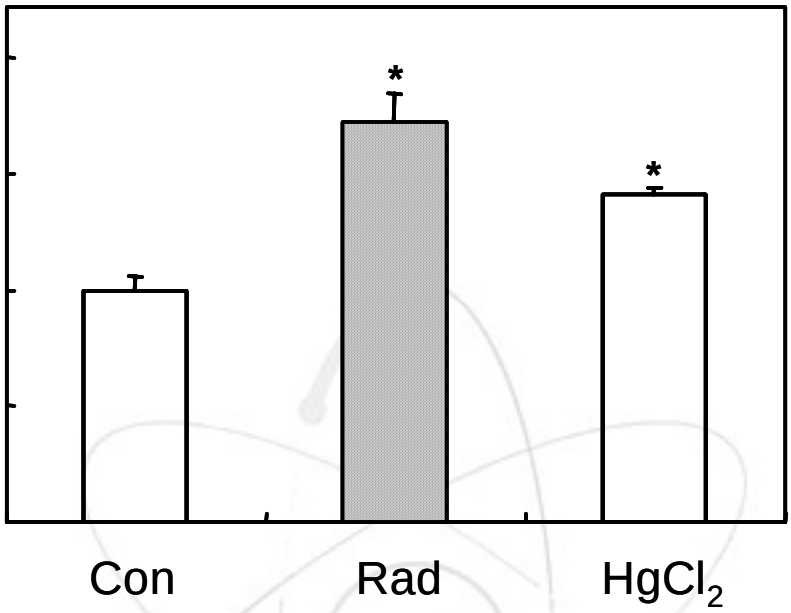

Fig. 14. Changed levels of cortisol in serum of experimental animals.

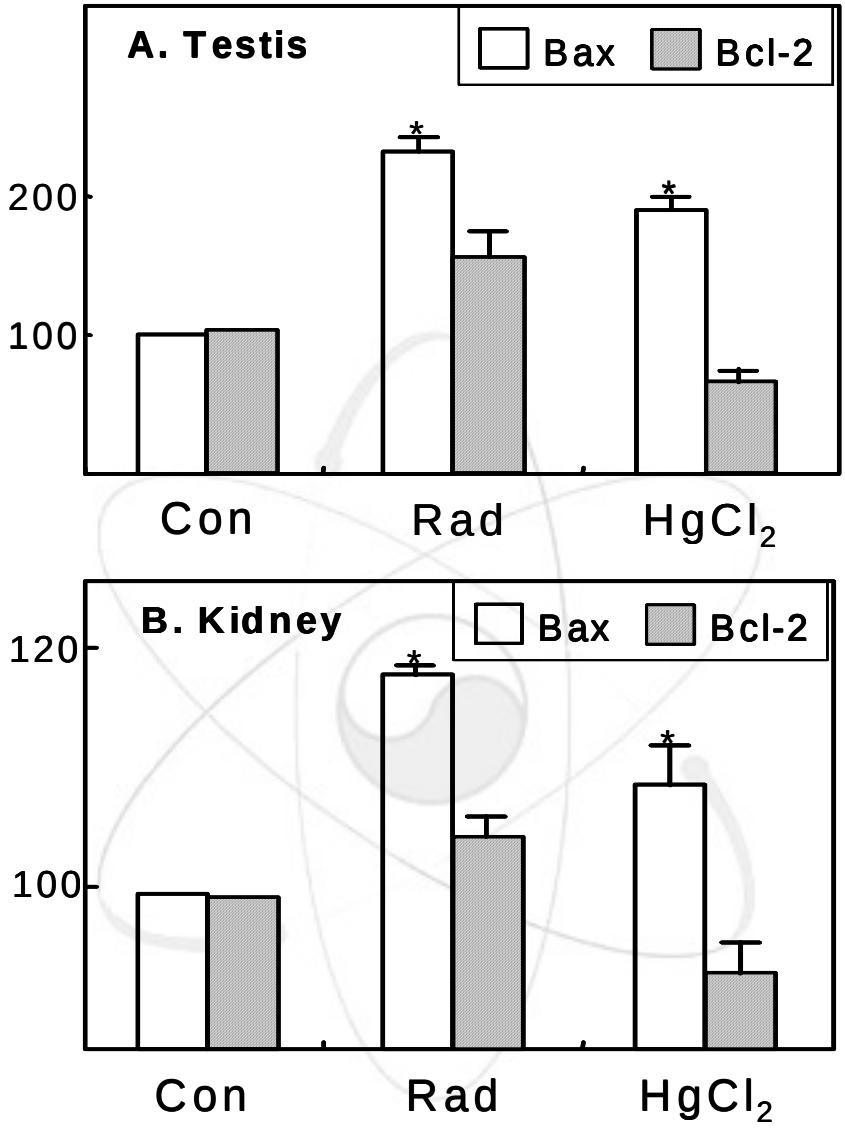

Fig. 15. Comparison of mRNA expression levels of bax and bcl-2 in testis and kindey of the experimental animals.

4. 고찰

급격한 산업 문명의 발달 및 인간 활동의 증가로 인한 생태계 오염은 매우 다양한 형태로 나타나고 있다. 중금속의 생태계 오염이 심각한 유해인자로 간주되고 있으나 이들 중금속의 생물학적 위해성에 관해서는 분명하게 밝혀져 있지 않은 경우가 많다. 특히 수은의 경우 그 화학적 형태의 다양성과 산업상의 유용성으로 인해 대기 오염을 포함한 다방면의 오염을 유발하고 있다. 세계 여러 국가에서 화학적 오염원에 대한 모니터링 및 관리감독 및 제재가 적극적으로 이뤄지고 있다. 수은과 같은 유독성 화학물질의 생태계 유입은 오염의 파급효과가 매우 빠르게 나타나고 있으며 일본 등에서는 그 피해가 심각하게 나타난 바 있다. 수은의 생물학적 영향에 대한 연구는 대부분 높은 농도이 수은을 처리하여 수행하는 실험연구이기 때문에 일반적으로 노출될 수 있는 환경 수준에서의 위해성을 파악하기는 어렵다. 따라서 본 연구에서는 기존 수은에 대한 동물 실험의 농도를 낮추어, 이온화 방사선의 생물학적 효과와 비교함으로써 서로 다른 인자의 영향을 수평적으로 비교하였다.

실험기간 동안 염화수은을 음수에 녹여 투여하였으며, 방사선을 전신조사한 실험 동물간 분석치를 비교하였다. 실험의 시작점에서 종료까지의 체중의 변화는 수은 처리한 실험군에서는 약간 증가하는 경향을 보였으나 통계적으로 유의하지 않았으며, 방사선 조사에 따른 체중 감소는 보이지 않았다. 이는 본 실험에서 처리한 수은의 농도가 급성 독성을 일으키는 수준이 아니기 때문에 체중의 변화를 크게 야기하지 않은 것으로 보인다. 그러나 각 기관의 기관지수를 산출한 결과 신장을 제외한 간장, 비장 및 정소의 지수는 대조군과 차이를 보이지 않았으나, 신장의 경우에는 증가하는 양상을 보였다. 이것은 음수로 처리된 염화수은의 영향이 신장을 주대상기관 (target organ)으로 작용하였음을 의미한다. 방사선을 조사한 실험군의 경우에는 간장과 신장은 대조군과 유사한 지수 수치를 보였으나, 비장 및 정소의 지수는 현저하게 감소하였다. 이 감소된 지수 수치는 실제적인 무게값으로는 다른 실험군과 매우 큰 차이를 보였으나, 기관 지수의 산출과정에서 체중에 대비한 값으로 환산함으로써 차이가 감소하였다. 비장과 정소는 방사선을 전신조사하였을 때 가장 뚜렷한 변화를 나타냄으로써 이 두 기관이 실질적으로 방사선에 민감한 기관임을 확인되었다.

혈액내 몇 가지 구성물의 수준 변화를 확인한 결과에 따르면, 방사선에 의해서는 백혈구가 급격하게 감소하는 것과 적혈구를 비롯한 모든 조사 인자의 감소를 확인하였으나 수은에 의해서는 백혈구의 미미한 감소와 적혈구 및 헤모글로빈 등이 증가하는 경향성을 볼 수 있었다. 이 증가는 수은이 순환계에서 적혈구내 헤모글로빈 등과 결합하는 대사적 특성에 의한 것으로 판단되며, 수은은 이러한 결합을 통해 체내 주요기관의 손상을 야기하게 된다. 신장에 있어서는 수은의 체류 기간이 다른 기관에 비해 길기 때문에 수은 영향의 주대상기관이 되고 있는 것으로 사료된다. 방사선 전신조사에 의해 비장의 손상이

야기되고 이는 이차적인 면역계 및 전체 생체시스템의 문제를 야기하는 것으로 보여, 수은 처리시 나타나는 수치와 차이가 있는 것으로 사료된다.

스트레스 호르몬으로 알려져 있는 cortisol의 혈청내 농도 변화는 두 실험군 모두 증가하는 것으로 나타났으며, 방사선과 마찬가지로 염화수은의 경우는 신장 대사와 관련한 부신의 손상이 이러한 증가의 원인인 것으로 보인다. 혈청내 cortisol의 증가는 방사선 전신조사에 의한 체중 감소 등에서 보이는 것과 같이 기본적인 생체 시스템의 손상에 따르는 것으로 보이나 염화수은의 경우에는 기관 및 체중의 변화와 같은 큰 손상이 야기된 것은 아니나 미네랄 대사와 관련한 신장기능의 이상 [98]이 그 증가의 원인으로 사료된다.

외부 자극에 민감한 생식소인 정소와 염화수은의 주표적기관인 신장에서 세포자연사를 매개하는 분자에 대한 mRNA 발현 수준을 비교 하였다. 이 결과 정소에서는 두 배 이상의 bax 발현이 방사선에 의해 증가되는 것을 확인하였으며, 염화수은에 의한 증가율의 큰 차이를 확인할 수 있었다. 그러나 bcl-2 발현을 비교해 본 결과 방사선에 의한 증가와는 달리 염화수은을 음용한 실험군에서는 대조군 이하의 발현을 보여 방사선에 의해 야기되는 세포자연사와는 그 기전이 다른 것을 확인 할 수 있었다 [99]. 신장에서 확인한 두 분자의 발현도 정소의 경우와 유사하게 나타났으나, 방사선을 조사한 경우에 신장에서의 그 증가율이 정소처럼 급격하게 나타나지 않는 것을 알 수 있었다. 그러나 두 분자의 발현 양상은 정소에서와 유사하여 결과적으로 일어나는 세포자연사 기전이 두 외인성 요인에 다르게 나타나는 것을 확인할 수 있다. 이것은 세포자연사 관련 분자의 추가적인 발현 양상을 조사하여 비교해 보아야 할 것으로 여겨진다.

본 실험의 결과들을 통해 염화수은과 방사선의 조사에 따른 손상의 차이가 명확히 다른 것으로 보여지며, 그 기전 또한 확연히 다른 것을 확인 할 수 있었다. 그러나 부가적인 실험을 통해 실제 환경준위와 유사한 노출과 복합적인 영향에 대한 것을 확인할 필요가 있다. 이는 단일 요인에 의한 손상이 다발적 외인성 자극과 동시에 일어났을 때 어떠한 양상으로 변화되는 지에 관한 실질적인 정보를 제공할 것이다.

제 7 절 이온화 방사선과 염화수은에 의한 고등식물 체세포돌연변이

1. 서 론

이온화 방사선은 생물체에 대하여 다양한 반응을 유발하며 그 반응의 정도는 선량에 따라 다르다. 생물체를 이루는 다양한 세포들 중에는 방사선에 대한 민감도가 높아 특히 영향이 잘 나타나는 것들이 있다. 자주달개비 속 (genus *Tradescantia*)에는 많은 수의 자연종이 속해 있으며 또한 이들 종의 생물학적 속성을 특이하게 변화시킨 인위적 종류들이 속해있다. 일부 클론들은 그들이 지니고 있는 생물학적 특성 때문에 이온화 방사선을 포함한 돌연변이 유발원에 노출될 경우 쉽게 체세포 돌연변이를 일으킨다. *Tradescantia BNL 4430* (T-4430) 클론은 남색 꽃을 피우는 종인 *T . hirsutiflora*와 분홍색 꽃을 피우는 종인 *T . subacaulis*를 인위적으로 교잡시켜 만든 종간 잡종으로 꽃색에 있어서 유전적 이형성을 띄며 정상적으로 우성인 남색 꽃이 핀다 [100].

T-4430의 수술털은 꽃색의 이형성에 의하여 실제로 사람이 있어서 환경에서 접하게 되는 낮은 준위의 이온화 방사선과 다양한 화학 물질의 유전적 효과를 감지하는데 가장 적합한 실험재료로 사용되고 있다. 1960년대 후반에 개발된 자주달개비 수술털 분석법과 1970년대 후반에 개발된 자주달개비 미세핵 분석법)은 현장실험과 실험실 실험에 폭넓게 사용되고 있다 [101,102]. 자주달개비 수술털의 선단 세포는 분열능력이 있어서 발생 또는 분열과정 중 돌연변이원에 노출되면 쉽게 체세포 돌연변이를 일으킨다 [103]. 돌연변이원이나 발암원에 의해 꽃색의 우성인자가 존재하는 염색체 부분의 결실이 일어날 경우 상동 염색체 상에 존재하는 열성형질이 발현되어 꽃잎과 수술털의 전체 또는 일부분이 분홍빛을 띈다. 한편, 상동염색체의 양쪽 모두가 결실되어 색소발현능력이 소실되면 무색의 수술털 세포가 생기기도 하며 때로는 선단세포의 분열능력 상실로 인한 수술털 세포수 감소로 인해 수술털이 비정상적으로 짧아지는 경우에도 나타난다 [104].

자주달개비 꽃 속에 있는 6개의 수술은 각각 약 25개의 세포로 이뤄진 70개 정도의 털을 가지고 있으므로 하나의 꽃은 10,000개 이상의 수술털 세포를 가지고 있는 경우가 대부분이다. 수술털은 처음 한 개의 세포가 연속적으로 분열하는 과정에서 자연적으로 발생하는 돌연변이에 의해 10,000 세포 당 1~2건의 분홍 돌연변이 세포가 나타나기도 하는데 방사선과 화학적 돌연변이원 같은 외부 자극을 부가적으로 받게 되면 분홍 돌연변이의 발생이 더욱 늘어나게 되며, 특히 꽃이 만들어 지는 초기에 돌연변이가 일어날 경우는 꽃 전체의 색깔이 분홍으로 바뀌기도 한다. 이러한 특성이 자주달개비를 방사선 지표식물로 불리게 하는 과학적 근거가 되었고 수많은 방사선생물학적 연구 특히 저준위 방사선의 생물학적 반응연구에 자주달개비가 효과적으로 이용되어 왔다. 자주달개비 꽃과 수술털 세포의 돌연변이를 이용하여 방사선 반응을 검출하는 경우 방사선 측정기를 사용하여 방사선량을 계측하는 것과 같이 적은 양의 방사선까지 정밀한 수치로서 표현해 낼 수는 없다. 그러나 자주달개비의 분

홍 돌연변이 분석을 통해 아주 낮은 방사선량까지 감지 할 수 있다. 자주달개비의 자연발생적 체세포 돌연변이 빈도는 사람 림프구의 돌연변이 자발빈도인 10^{-3}~10^{-7}과 같은 수준이다 [105]. 한편 낮은 방사선량율과 관련한 보고도 있다. Mericle *et al.* (1965) 은 자주달개비 클론 02를 이용하여 250 μRh^{-1} 의 선량률로 2주간 조사시 돌연변이율이 증가한 결과를 보고하였다. 한국원자력연구원 금곡농장의 감마선장에서 수행된 T-4430 분홍 돌연변이 실험에서 150 μRh^{-1}의 공간 방사선량률에 대해서도 유의한 수준의 분홍 돌연변이율 증가를 나타냈다 [105]. 이러한 자주달개비 수술털 세포의 돌연변이는 환경내의 모든 요인의 영향을 반영한 결과이기 때문에 포괄적인 의미에서의 생물학적 안전성을 밝히는 데는 기계적, 물리적 방법을 능가하는 장점이 있다. 실제로 일본, 폴란드 등지에서는 자주달개비를 이용한 환경방사선 감시사례가 보고된 바 있다. Ichikawa (1981) 는 일본의 여러 원자력 발전소 주변에서 자주달개비의 분홍 돌연변이율을 이용하여 환경방사선의 준위변동을 발전소 가동기간과 연계하여 감시하였다 [106]. 한편 Cebulska-Wasilewska (1992) 는 소련 체르노빌 원자력 사고가 발생한 직후 폴란드 크라쿠프 지역의 방사능오염 공기의 생물학적 영향을 자주달개비 돌연변이율로 조사하기 시작하여 사고 1년 경과시의 돌연변이율과 비교하여 공기 중 환경방사선의 준위변동을 감시하였다.

국내에서는 자주달개비 T-4430 클론을 이용하여 수술털 체세포 돌연변이에 있어서 중성자의 선량-반응과 생물학적 효과비 검증, 방사선과 환경인자에 의한 체세포 돌연변이 빈도 및 환경방사선 감시 등의 연구가 수행된 바 있다 [107]. 따라서 본 연구에서는 이온화 방사선과 염화수은의 복합처리에 따른 생물학적 영향을 평가하기 위하여 T-4430을 실험재료로 이용하여 수술털 세포에 나타나는 체세포 돌연변이 빈도 변화를 분석하였다.

재료 및 방법

1. 재료

실험에 사용되는 식물체는 방사선에 민감하게 반응하면서도 자발돌연변이율이 낮은 종간 잡종 T-4430 클론을 사용하였다. T-4430 식물체의 꽃차례를 15 cm 길이로 절취하여 실험실에서 24시간 순치시킨 다음 14시간 명기, 10시간 암기의 조건으로 인공조광을 실시하고 온도는 24℃로 유지하였다. 평균 15개씩의 화서를 하나의 실험군으로 하여 분석 가능한 화아의 수를 200개 이상으로 하였다.

2. 방사선 조사

한국원자력연구원 ^{60}Co 저준위 조사시설 (Panoramic Irradiaitor, AECL)의 ^{60}Co 선원을 이용하여 감마선을 조사하였다. 선원강도는 111 TBq였으며 선량률의 측정은 Friche 선량계를 이용하였다. 상온, 공기 중에서 실험군별로 0, 0.3, 0.5, 1.0, 1.5 및 2.0 Gy의 감마선을 조사하였다.

3. 염화수은의 처리

$HgCl_2$ (Sigma)를 희석하여 수은 농도를 각기 0, 0.2, 0.25, 0.3 mM 로 맞춘 Hyponex® 1,000 배 희석 용액에서 배양하고, 배양용기에는 기포발생기를 각각 설치하여 폭기를 실시하였으며 1일 간격으로 동일한 농도의 양액을 교체・공급하였다.

4. 돌연변이 검경・계수

만개상태의 화기(化器)를 실험군별로, 채화하고 6개의 수술 모두를 떼어내서 파라핀유가 도말된 슬라이드 상에 펴서 검경용 프레파라트를 제작하였다. 그 다음 실체현미경 (Nikon, 배율 x100)을 이용하여 돌연변이를 계수하여 100 개의 수술털 당 돌연변이 건수로서 돌연변이 빈도를 산정하였다. 한 개 또는 다수의 연속된 변이세포군 각각을 하나의 돌연변이 건수로 계수하였으며 한 개의 정상세포로 분리되어 있는 분홍 세포군의 경우 정상세포 양쪽을 각각 건수로, 한 수술털 전체가 분홍 또는 무색 세포로 이뤄진 경우이거나 수술 전부 또는 꽃 전체가 분홍 또는 무색 세포로 이뤄진 경우도 한 건의 돌연변이로 계수하였다. 또한 수술털을 이루고 있는 세포의 수가 16개 이하이면 이를 하나의 치사돌연변이 건수로 계수하였다.

결과 및 고찰

1. 수은 처리에 의한 생육 영향

수은 처리가 실험 생물체인 자주달개비의 생육에 영향을 미칠 경우 돌연변이 빈도 분석 결과에도 큰 영향을 미칠 수 있다.

염화수은 농도가 0, 0.2, 0,25 및 0.3 mM 네 개의 실험군에 대하여 꽃당 평균 수술털 수를 계수하였다. 염화수은 0, 0.2 및 0.25 mM 처리 실험군은 평균 수술털 수가 40~50개를 유지하였으나, 0.3 mM 처리 실험군에서는 시간이 경과함에 따라 수술털 수가 계속 감소하였고 처리 9일 째부터는 개화한 꽃을 볼 수가 없었다 (Fig. 16). 염화수은 농도 0.25 mM 이하를

처리한 실험군에서 11~13일까지 수술털 수가 약간 감소하는 경향을 나타내긴 했지만 분석 결과에 영향을 미칠 정도의 생육저해는 나타나지 않았다. 실험 식물체인 자주달개비의 생육에 저해를 주지 않고 실험기간 중 돌연변이 값을 얻을 수 있는 적정 염화수은 농도는 0.25 mM 이하라는 것을 확인되었다.

2. 분홍 돌연변이

자주달개비 수술털은 꽃색의 이형성에 의하여 실제로 사람에게 있어서 환경에서 접하게 되는 낮은 준위의 이온화 방사선과 다양한 화학 물질의 유전적 효과를 감지하는데 가장 적합한 실험재료 중의 하나이다. 방사선에 의하여 유발되는 돌연변이는 일반적으로 방사선 조사 후 5일 경부터 증가하기 시작하여 최대 값에 이른 다음 다시 감소하여 자발돌연변이율 수준까지 낮아지는 경향을 보인다. 본 실험의 경우 돌연변이 고조기간은 방사선 조사 후 7일에서 11일 사이에 나타났다. 염화수은을 함유하지 않은 양액에서 배양한 방사선 조사 실험군의 돌연변이율 변화를 그림에 나타내었다 (Fig. 17). 본 실험의 경우 분홍 돌연변이율은 방사선 조사후 6일 경부터 큰 폭으로 증가하기 시작하였으며 7일 이후 최대 값에 도달하였다.

동일한 방사선량을 조사하고, 양액중 염화수은 농도를 다르게 하여 처리한 실험군에서도 방사선량의 증가에 따라 돌연변이율이 증가하는 경향은 뚜렷하였다. 염화수은을 처리하지 않은 실험군과 0.2 mM 에서는 분홍 돌연변이 빈도가 비슷하였으나 양액의 수은 농도가 0.25 mM인 실험군에서는 분홍돌연변이의 빈도가 7.5로 방사선과 수은 복합작용에 의한 영향이 뚜렷하게 나타났다 (Fig. 18).

3. 무색 돌연변이

방사선 조사 후 수은을 처리하지 않은 실험군과 0.2 mM 및 0.25 mM 수은처리 심험군에 대한 분석을 실시하였다 (Table 10). 염화수은을 처리하지 않은 실험군의 경우 방사선량 0~2.0 Gy 범위에서 무색 돌연변이 빈도는 0.08~1.37이었다. 염화수은의 농도가 0.2 mM 일 때 방사선량 0~2.0 Gy 범위에서 무색 돌연변이 빈도는 최저 0.25에서 최고 1.12였다. 이와 같은 결과로 볼 때 0.2 mM 이하의 염화수은은 방사선 유발 무색 돌연변이에 미치는 영향은 미미한 것으로 생각된다. 그러나 0.25 mM 이상의 수은과 방사선을 복합 처리하였을 때의 무색 돌연변이율은 최저 0.35에서 최고 6.53을 나타냄으로써 수은에 의한 영향이 큰 것으로 평가되었다.

4. 치사 돌연변이

방사선 2 Gy 조사 실험군의 분석결과를 기준으로 볼 때 염화수은을 함유하지 않은 양액에서 배양한 경우 고조기간 중 치사 돌연변이 빈도 최대값은 3.9였으나 (Fig. 19), 염화수은 0.25 mM 처리군에서의 고조기간 중 최대 치사 돌연변이 빈도는 47.2였다 (Fig. 20). 이와 같은 결과로 볼 때 염화수은 농도가 0.2 mM 이하인 경우 방사선과의 복합 작용에 의한 효과는 크지 않았으나 염화수은 농도가 0.25 mM일 때는 방사선과의 복합작용은 치사돌연변이를 큰 폭으로 증가시키는 것을 알 수 있다.

결 론

실제로 저선량 영역에 있어서 방사선에 의한 자주달개비 수술털 세포의 분홍돌연변이 유발은 선형적 일차함수를 이용한 선량-반응 관계식으로 해석하는 것이 정당함은 이미 보고된 바 있다. 방사선과 수은을 복합 처리한 실험군의 돌연변이 고조기간인 6~11일 사이의 통합데이터로부터 산출한 선량-반응은 일차함수로 확인되었다. 염화수은 농도 0.2 mM까지는 방사선 유발 돌연변이에 별 영향을 주지 않았으나 수은 농도가 0.25 mM 일 때는 복합작용에 의해 돌연변이 빈도가 큰 폭으로 증가되었다. 또한 수은 농도가 0.3 mM일 때는 자주달개비의 생육 자체가 저해되므로 돌연변이 계수를 통한 생물학적 영향 평가가 불가능하거나 무의미 한 것이 확인되었다. 자주달개비의 생육을 저해하지 않는 염화수은의 농도는 0.25 mM 이하인 것으로 판단된다. 유해 화학물질인 염화수은과 방사선의 복합 작용에 의한 생물학적 영향을 평가하기 위한 실험 모델로서 종간잡종 클론인 T-4430 식물체가 효과적으로 이용될 수 있음이 본 연구를 통해 확인되었다.

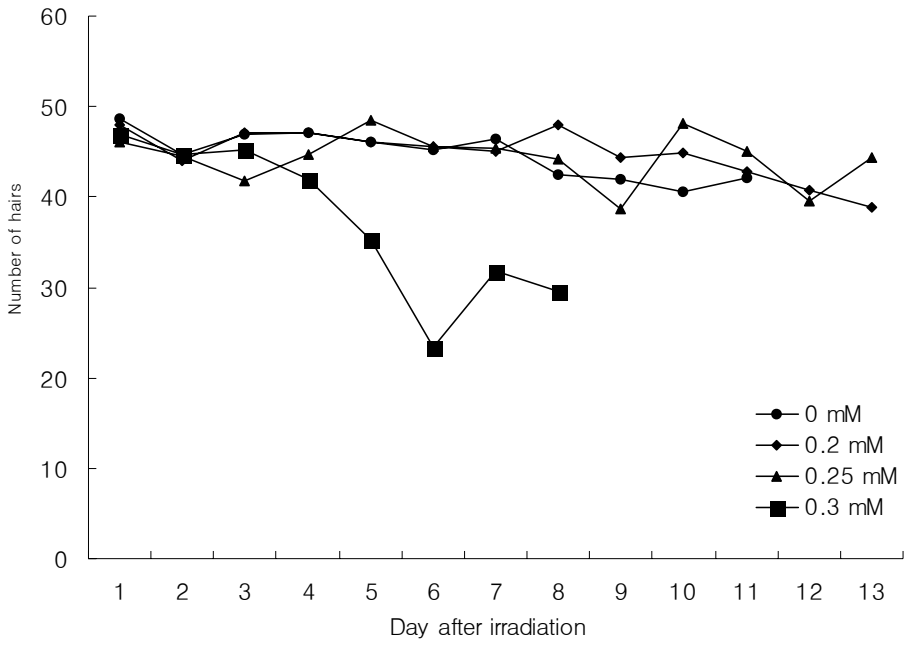

Fig. 16. Average number of hairs in flowers of T-4430 treated with different concentrations of mercury chloride.

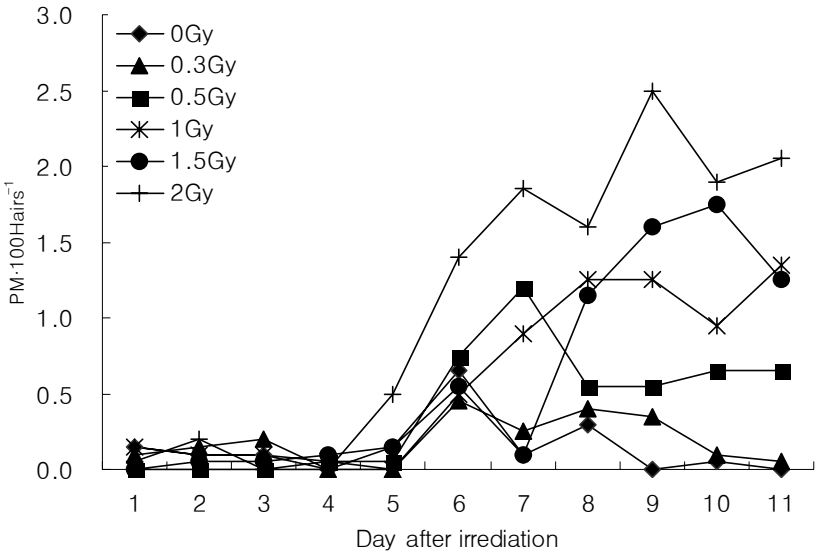

Fig. 17. Changes of pink mutation frequencies in the stamen hair cells of T-4430 irradiated with gamma rays.

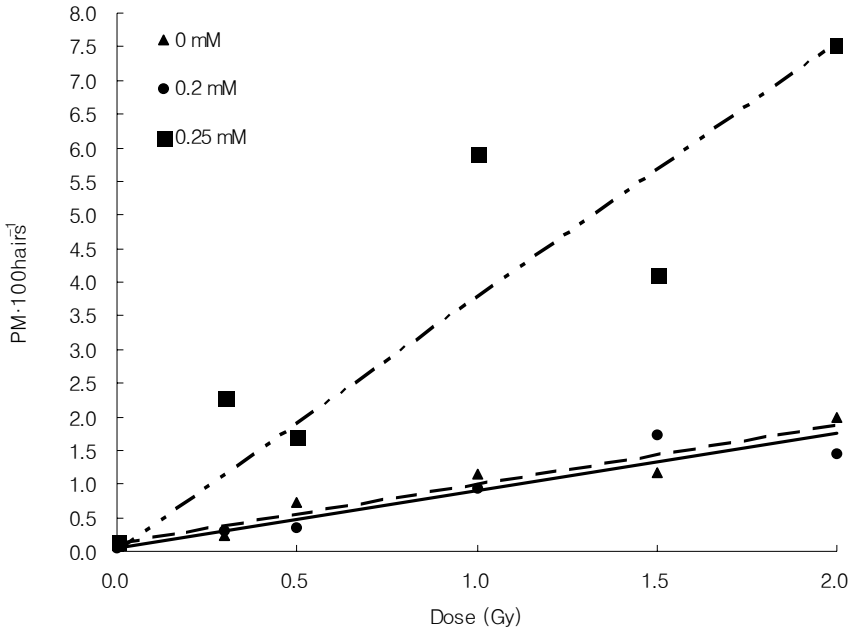

Fig. 18. Dose-response curves of pink mutation frequencies during the peak interval in T-4430 treated with different concentrations of mercury chloride.

Table 10. Frequency of colorless mutations in T-4430 treated with 0, 0.2 and 0.25 mM $HgCl_2$

$HgCl_2$ (mM) / Radiation dose (Gy)	0	0.2	0.25
0	0.08	0.88	0.36
0.3	0.22	0.35	0.35
0.5	0.42	0.25	1.82
1.0	0.53	0.77	5.26
1.5	0.41	1.12	4.08
2.0	1.37	0.95	6.53

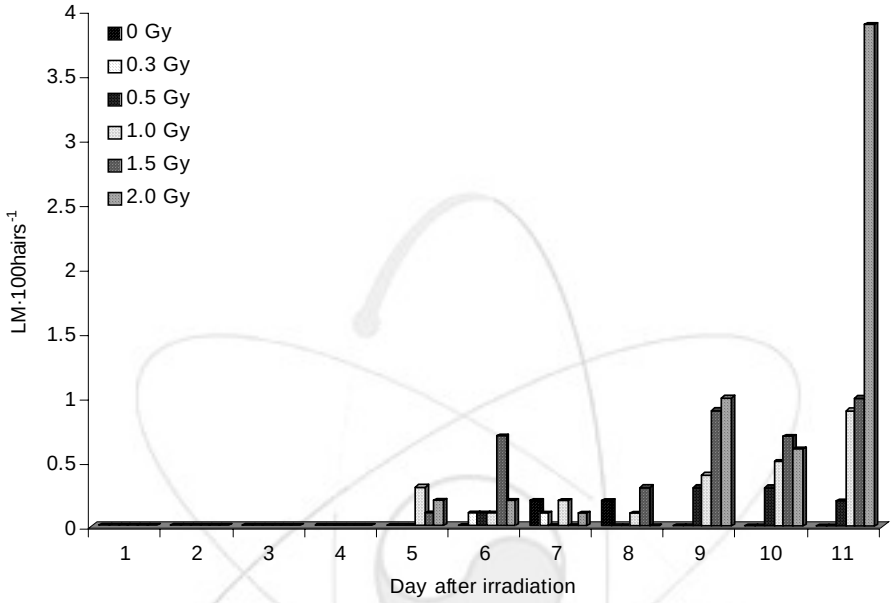

Fig 19. Changes of lethal mutation frequencies in the stamen hair cells of T-4430 irradiated with gamma rays.

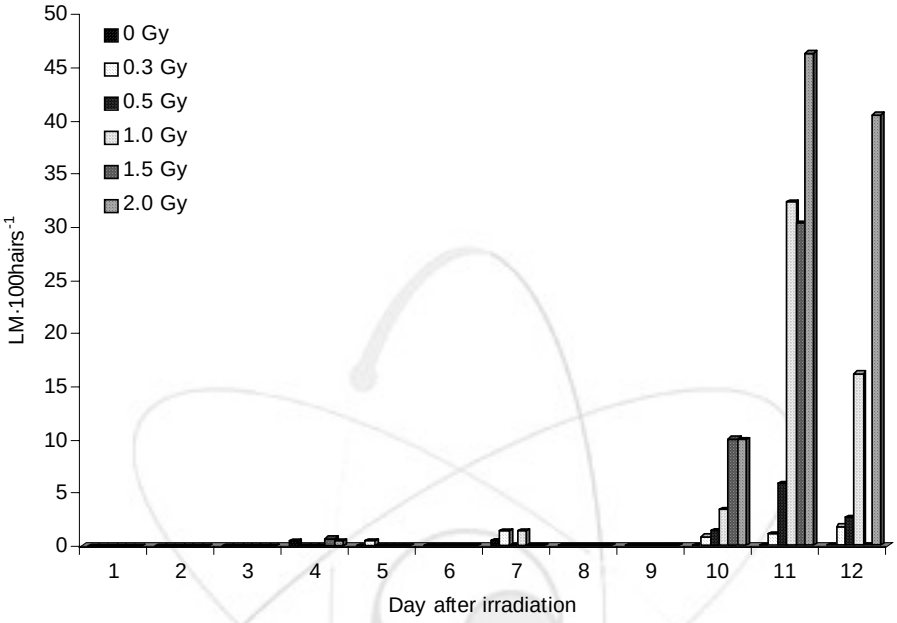

Fig 20. Changes of lethal mutation in T-4430 irradiated with gamma-rays and treated with 0.25 mM mercury chloride.

빈 면

제4장 목표달성도 및 관련분야에의 기여도

빈 면

제 4 장 목표달성도 및 관련분야에의 기여도

2005년 5월 1일부터 2008년 3월 31일까지 총 35개월에 걸친 연구기간 중 연도별로 설정한 세부연구목표 및 가중치는 다음과 같다.

구 분	연 구 개 발 목 표	가중치(%)
1차년도 (05.05~06.03)	- 고등동물 세포에 대한 방사선 및 수은 처리후 생존률 평가 - 방사선과 수은 복합작용에 따른 DNA 손상도 정량화 - 국내외 학술발표 1건	30 30 40
2차년도 (06.04~07.03)	- 방사선민감 조직에 대한 감마선 조사후 조직 손상 및 세포자사 평가 - 민감 조직에 대한 수은 처리후 조직손상 및 자사 평가 - 감마선과 수은이온 복합작용에 따른 손상 및 세포사멸 정량화 - SCI 논문 1편, 국내학술지 논문 1편, 국내외학술발표 1건	20 20 30 30
3차년도 (07.03~08.03)	- 방사선과 수은에 의한 세포 손상 유발 및 변화 평가 - 방사선과 수은 등의 물리화학요인의 복합작용 평가 이론의 적용 및 개선 - 방사선과 수은 등 두요인 복합생체작용에 관한 학술논문 작성	40 30 30

본 연구의 일차년도에 설정한 연구목표는 세포실험을 통하여 달성하였다. 감마선과 수은화합물이 세포주 DNA에 미치는 영향을 실험적으로 평가하였으며 사람 림프구에 대한 방사선과 수은이온의 복합영향을 평가한 결과를 국제학술회의에 한-폴 공동논문으로 발표하였다. 이차년도에는 Fisher 344 숫컷 쥐를 이용한 동물실험을 통하여 방사선과 수은이 고등동물 민감조직에 미치는 영향을 규명하였다. 조직손상, 형태적 변화 및 세포자사 등을 면역조직화학 검정법을 통해 분석하였다. 삼차년도는 일차년도 및 이차년도에 수행하여온 실험결과 등을 바탕으로 복합작용에 관한 이론을 수립하고 이를 검증 및 적용하는 연구를 수행하였다.

연구개발 기간 중 본 과제와 관련하여 과학기술단체총연합회에서 주관하는 브레인풀 사업의 지원을 받아 러시아의료방사선연구센터의 Vladislav G. Petin 박사를 초빙하여 과제수행에 활용하였다.

설정한 연차별 연구목표의 달성을 통하여 연구개발의 최종목표인 “이온화 방사선과 유해물질 복합영향의 평가기술 개발 - 복합영향에 의한 생체손상 및 회복기작 해석기법 확립″을 이룩하였으며, 연구성과물 산출목표 (SCI학술지논문 2편 및 국내학술지논문 2편 이상)를 모두 달성하였다. 연구개발을 통해 절차가 개선되었을 뿐 아니라 적용성이 확립된 고등식물 체세포돌연변이 검정법과 면역조직화학 검정법은 방사선과 화학물질의 복합영향 평가는 물론 환경에 존재하는 유해인자들 간의 복합영향을 검정하는 기술로 활용될 수 있다. 따라서 본 과제의 성과는 방사선생명과학 분야, 환경 분야는 물론 관련된 학문 분야의 응용기술로 적용이 가능할 것으로 판단된다.

연구기간 중 과제수행과 관련하여 국내외 학술지 또는 서적에 게재된 연구실적물은 논문게재 8편 (SCI 2편, 해외학술지 2편, 국내학술지 4편) 및 학술발표 14편 (국제학술회의 논문발표 9건, 국내학술회의 논문발표 5건)을 이룩하였다.

제5장 연구개발결과의 활용계획

빈 면

제 5 장 연구개발결과의 활용계획

본 과제의 연구범위는 통상적으로 방사선생물학적 연구내용으로 분류될 수 있으나 그 결과 및 기술의 실제 활용분야는 폭이 매우 넓어서 방사선생물학, 핵의학, 환경생물학, 분자역학 분야 등 다양한 분야에 적용가능한 기술을 포함하고 있다. KAERI-INP 공동연구는 두 기관이 보유한 강점기술을 공유함은 물론 각 기관의 장점을 최대로 활용할 수 있었다. 또한 동일한 공시재료 및 분석절차를 이용한 공동실험 또는 병립적 실험을 통해 한국원자력연구원 보유기술의 수준을 검증하고 개선할 수 있었다. 본 공동연구를 통해 획득된 모든 연구 결과들은 이미 국내외 저명학술지에 논문으로 게재되었거나 가까운 장래에 공동논문으로 게재함으로써 연구결과에 대한 학술적 검증을 받음은 물론 한국·폴란드 간의 과학기술 분야 관계증진에 기토록 할 것이다.

고등식물체를 이용한 체세포돌연변이 검정법, 면역조직화학적 검정법 및 복합작용 해석 모델링 및 적용기술 등은 국제적 경쟁력을 갖춘 기술이며 개선 및 응용분야 확장을 통해 EU, 아세안 등 다자간/다국간 협력을 위한 그거기술로 활용할 것이다.

○ 이온화 방사선과 유해 화학물질이 고등동물 조직, 사람 림프구 및 세포주 세포에 미치는 복합영향의 평가기법은 활용성이 큰 기술로서 방사선생명과학, 의·약학 및 환경감시, human monitoring, molecular epidemiology 분야 등 필요성이 제기되는 분야에 활용할 것이다.

○ 기관간 연계 공동실험을 통하여 이원적 연계실험의 설계 및 공시재료의 통일, 공시재료의 장기 보존 및 장거리 운송조치 기술을 확보하였으며 이러한 기술은 향후 다국간/다자간 공동실험 참여시 생물체 이송과 관련한 귀중한 기반기술로 이용될 수 있다.

○ 공동연구를 통해 확보된 인적 교류망을 활성화하여 유명학술지의 학술, 편집, 심사위원으로 교환 참여하는 등 국내과학자의 해외인지도 고양 및 국내 과학기술 수준의 세계화에 기여토록 한다.

○ 연구개발 결과를 국제적으로 인지도가 높은 SCI 등재지에 학술논문으로 게재함으로써 우리나라 과학기술 경쟁력의 위상을 제고하는데 기여할 것이며, 다년간의 공동연구 수행경험을 바탕으로 국가간의 상호 협력과 이해의 폭을 넓히는 데 일익을 담당하게 될 것이며 특히 과학기술분야의 호혜적 동반자관계 발전을 위한 선험사례로 활용될 수 있도록 할 것이다.

빈 면

제6장 연구개발과정에서 수집한 해외과학기술정보

빈 면

제 6 장 연구개발과정에서 수집한 해외과학기술정보

연구개발 과정에서 수집한 해외과학기술 정보는 주요학술지에 게재된 학술논문 (journal papers), 워크샵이나 심포지엄의 보문집 (proceedings) 그리고 관련 연구기관의 소개책자 (brochure) 및 단행본 (monograph) 등으로 구분할 수 있다. 인터넷 및 학술지를 통해 입수 가능한 공개 논문의 경우 대부분 연구 수행과정에서 참고자료로 활용되었으며 본 연구보고서의 참고문헌으로 인용되어 있기 때문에 중복을 피하기 위하여 본 항의 해외 수집자료 목록에 포함시키지 않았다.

학술회의 발표논문을 제한된 부수로 생산한 보문집, 학술회의 개최기관 또는 방문기관의 소개책자 (Brochure), 관련 연구기관의 연구연보 (Annual Report) 등 해외에서 입수한 자료들의 목록은 다음과 같다.

▷ Report on Research Activities 2005-2006 (ISSN: 1425-3763), The Henryk Niewodniczanski Institute of Nuclear Physics, Polish Academy of Sciences, Krakow, Poland (Jan. 2007). (CD-ROM included)

▷ International Journal of Low Radiation (ISSN 1477-6545), Vol. 4, No. 3, Inderscience (2007)

▷ International Journal of Low Radiation (ISSN 1477-6545), Vol. 4, No. 2, Inderscience (2007)

▷ Iranian Journal of Radiation Research (ISSN 1728-4554), Vol. 5, No. 1 (2007)

▷ Iranian Journal of Radiation Research (ISSN 1728-4554), Vol. 4, No. 4 (2007)

▷ Iranian Journal of Radiation Research (ISSN 1728-4554), Vol. 4, No. 3 (2007)

▷ Iranian Journal of Radiation Research (ISSN 1728-4554), Vol. 4, No. 2 (2007)

▷ Indian Journal of Radiation Research (ISSN 0973-0168), Vol. 3, No. 4 (2007)

▷ Proceedings of the 6th LOWRAD International Conference on Low Dose Radiation Effects on Human Health and Environment, Oct. 17-20, 2007, Budapest, Hungary (2007)

▷ Book of Abstract, The Second Asian and Oceanic Congress for Radiation Protection (AOCRP-2), Oct. 9-13, 2006, Beijing, China (2006)

빈 면

제7장 참고문헌

빈 면

제 7 장 참고문헌

1. Alper T. Cellular Radiobiology. Cambridge University Press. 1979.
2. Burt RK, MC Porier, CJ Jr Link and VA Bohr. Antineoplastic drug resistance and DNA repair. Ann. Oncol. 2: 325-334. 1991.
3. Streffer C and WU Müller. Radiation risk from combined exposures to ionizing radiation and chemicals. Adv. Radiat. Biol. 11: 173-210. 1984.
4. Hill BT and AS Bellamy. An overview of experimental investigations of interaction between certain antitumor drugs and X-irradiation *in vitro.* Adv. Radiat. Biol. 11: 211-267. 1984.
5. Weichselbaum RR. Radioresistant and repair proficient cells may determine radiocurability in human tumors. Int. J. Radiat. Oncol. Biol. Phys. 12: 637-639. 1986.
6. Kumar A, J Kiefer, E Schneider and NEA Crompton. Inhibition of recovery from potentially lethal damage by chemicals in Chinese hamster V79 A cells. Radiat. Environ. Biophys. 24: 89 - 98. 1985a.
7. Kumar A, J Kiefer, E Schneider and NEA Crompton. Enhanced cell killing, inhibition of recovery from potentially lethal damage and increased mutation frequency by 3-aminobenzamide in Chinese hamster V79 cells exposed to X-rays. Int. J. Radiat. Biol. 47: 103-112. 1985b.
8. Little JD, AN Ueno and WK Dahlberg. Differential response of human and rodent cell lines to chemical inhibition of the repair of potentially lethal damage. Radiat. Environ. Biophys. 28: 193-202. 1989.
9. Utsumi H, ML Shibuya and MM Elkind. Novobiocin inhibits the repair of potentially lethal damage but not the repair of sublethal damage. Radiat. Res. 123: 55-60. 1990.
10. Boothman DA, DK Trask and AB Pardee. Inhibition of potentially lethal DNA damage repair in human tumor cells by -lapachone, an activator of topoispomerase I. Cancer Res. 49: 605-612. 1989.
11. Yang L, EB Douple, JA O'Hara and H Wang. Carboplatin enhances the production and persistence of radiation-induced DNA single-strand breaks. Radiat.Res. 143: 302-308. 1995.

12. Takahashi S, E Takeda, Y Kubota and R Okayasu. Inhibition of repair of radiation-induced DNA double-strand breaks by nickel and arsenite. Radiat. Res.154: 686-691. 2000.
13. Petin VG and JK Kim. Survival and recovery of yeast cells after combined treatments with ionizing radiation and heat. Radiat. Res.161: 56-63. 2004.
14. JK Kim, Petin VG, and Tkhabisimova MD. Survival and recovery of yeast cells after simultaneous treatment of UV light radiation and heat. Photochem Photobiol. 79: 349-355. 2004.
15. Korogodin VI, YuG Kapultcevich, MN Myasnik, AF Mosin and VV Gridnev. Cellular repair processes: survival of irradiated yeast, bacteria, and phages under different postradiation conditions. Adv. Biol. Med. Physics. 12: 253-274. 1968.
16. Korogodin VI. The study of post-irradiation recovery of yeast: the 'premolecular period'. Mutation Res. 289: 17-26. 1993.
17. Davidson HO. Biological Effects of Whole Body Gamma Radiation on Human Beings. Johns Hopkins University Press. Baltimore. 1957.
18. Mattern MRand RB Painter. Dependence of mammalian DNA replication of DNA supercoiling. II. Effects of novobiocin. Biochim. Biophys. Acta.553: 306-312. 1979.
19. Purnell MR and WJD Whish. Novel inhibitors of poly (ADP-ribose) synthetase. Biochem.J. 185: 775-777. 1980.
20. Cleaver JE. Specificity and completeness of inhibition of DNA repair by novobiocin and aphidicolin. Carcinogenesis. 3: 1171-1174. 1982.
21. Ben-Hur E and MM Elkind. Poly (ADP-ribose) metabolism in X-irradiated Chinese hamster cells: Its relation to repair of potentially lethal damage. Int.J. Radiat Biol. 45: 515-523. 1984.
22. Hahn GM. Hyperthermia and Cancer. Plenum Press, New York. 1982.
23. Streffer C, P. Vaupel and G. Hahn. Biological Basis of Oncologic Thermotherapy. Springer, Berlin. 1990.
24. Jorritsma JBM and AWT Konnings. Inhibition of repair of radiation-induced strand breaks by hyperthermia, and its relationship to cell survival after hyperthermia alone. Int. J. Radiat. Biol. 43:505-516. 1983.
25. Iliakis G, R Seaner and R Okayasu. Effects of hyperthermia on the repair of radiation-induced DNA single- and double-strand breaks in DNA double-strand break repair-deficient and repair-proficient cell lines. Int. J. Hyperthermia 6:813-833. 1990.

26. Dahm-Daphi J, I Brammer and E Dikomey. Heat effects on the repair of DNA double-strand breaks in CHO cells. Int. J. Radiat. Biol. 72:171-179. 1997.
27. Ben-Hur E, MM Elkind and BV Bronk. Thermally enhanced radioresponse of cultured Chinese hamster cells: inhibition of repair of sublethal damage and enhancement of lethal damage. Radiat. Res. 58: 38-51. 1974.
28. Miyakoshi J, M Furukawa and E. Kano. Recovery kinetics from radiation and hyperthermia damage to cultured Chinese hamster V79 cells. Nat. Cancer Inst. Monograph 61:263-266. 1982.
29. Raaphorst GP, MM Feeley, CE Danjoux, V Da Silva and LH Gerig. Hyperthermia enhancement of radiation response and inhibition of recovery from radiation damage in human glioma cells. Int. J. Hyperthermia. 7:629-641. 1991.
30. Petin VG and JK Kim. Recovery inhibitors: Clue for understanding of the mechanisms involved. pp. 423-431. In Proceedings of the 19thKorea Atomic Industrial Forum and Korean Nuclear Society Annual Conference. Seoul, April 25-27. 2004b.
31. Kim JK, LN Komarova, MD Tkhabisimova and VG Petin. Inhibition of recovery from potentially lethal damage by chemicals in Chinese hamster cells is realized through the production of irreversible damage. Korean J. Environ. Biol. 23:390-397. 2005.
32. Petin VG and VP Komarov. Mathematical description of synergistic interaction of hyperthermia and ionizing radiation. Mathem. Biosci. 146:115-130. 1997.
33. Kim JK and VG Petin. Theoretical conception of synergistic interactions. Korean J. Environ. Biol. 20:277-286. 2002.
34. Petin VG, GP Zhurakovskaya and LNKomarova. Mathematical description of combined action of ultrasound and hyperthermia on yeast cells. Ultrasonics 37:79-83. 1999.
35. Petin, VG, JK Kim, GP Zhurakovskaya and AV Rassokhina. Mathematical description of synergistic interaction of UV-light and hyperthermia for yeast cell. J. Photochem. Photobiol. B: Biol. 55:74-79. 2000.
36. Korogodin VI. Some regularity of the post-irradiation changes in resting yeast cells. Biofizika 3:703-710. 1958.
37. Korogodin VI. Problems of Postirradiation Recovery, Energoatomizdat, Moscow. 1966.

38. Petin VG, GP Zhurakovskaya and JK Kim. Synergetic effects of different pollutants and equidosimetry. pp. 207-222. In Equidosimetry - Ecological Standardization and Equidosimetry for Radioecology and Environmental Ecology (Bréchignac F and G Desmet eds.). Springer, Dordrecht. 2005.
39. Leenhouts HP and KH Chadwick. An analysis of synergistic sensitization. Br. J. Cancer 37 (Suppl. 3):198-201. 1978.
40. Murthy MSS, VV Deorukhakar and BS Rao. Hyperthermic inactivation of diploid yeast and interaction of damage caused by hyperthermia and ionizing radiation. Int. J. Radiat. Biol. 35:333-341. 1979.
41. Zaider M and HH Rossi. The synergistic effects of different radiations. Radiat. Res. 83: 732-739. 1980.
42. Chadwick KH. and HP Leenhouts. The Molecular Theory of Radiation Biology. Springer Verlag, Berlin - Heidelberg - New York. 1981.
43. Leenhouts HP, KH Chadwick and A. Cebulska-Wasilewska. An analytical approach to the comparison of chemical and radiation hazards to man. Proc. Internat. Cong. Rad. Protection, IRPA, Israel, Vol. 2:1155. 1980.
44. Cebulska-Wasilewska, A., Leenhouts, H.P., Chadwick, K.H., Synergism between EMS and X-rays for the induction of somatic mutations in Tradescantia. Int. J. Radiat. Biol. 40, 163-173. 1981.
45. Wynstra JH, WD Wright and JL Roti Roti. Repair of radiation-induced DNA damage in thermotolerant and nonthermotolerant HeLa cells. Radiat. Res. 85:85-89. 1990.
46. Sakkers RJ, AR Filon, JF Brunsting, HH Kampinga, AW Konings and LH Mullenders. Selective inhibition of repair of active genes by hyperthermia is due to inhibition of global and transcription coupled repair pathways. Carcinogenesis 16:743-748. 1995.
47. Kampinga HH. Cell biological effects of hyperthermia alone or combined with radiation or drugs: a short introduction to newcomers in the field. Int. J. Hyperthermia 22:191-196. 2006.
48. Kampinga HH, JR Dynlacht and E Dikomey. Mechanism of radiosensitization by hyperthermia (≥ 43 degrees C) as derived from studies with DNA repair defective mutant cell lines. Int. J. Hyperthermia 20:131-139. 2004.
49. Warters RL. Persistence of radiation-induced double-strand breaks in the DNA of heated CHO cells. Int. J. Radiat. Biol. 64:669-676. 1993.

50. Mills MD and RE Meyn. Effects of hyperthermia on repair of radiation-induced DNA strand breaks. Radiat. Res. 87:314-328. 1981.
51. Dikomey E and H Jung. Correlation between thermal radiosensitization and slowly rejoined DNA strand breaks in CHO cells. Int. J. Radiat. Biol. 68:227-233. 1995.
52. Dewey WC, SA Sapareto and DA Betten. Hyperthermic radiosensitization of synchromous Chinese hamster cells: Relationship between lethality and chromosomal aberrations. Radiat. Res. 76:48-59. 1978.
53. Weissenborn U and G Obe. Modification of X-ray induced chromosome aberration frequency by pre- and postirradiation hyperthermia of human peripheral lymphocytes. Int. J. Radiat. Biol. 59:973-984. 1991.
54. Band P, Feldstein M, Saccomanno G, Watson L, King G. Potentiation of cigarette smoking and radiation: evidence from a sputum cytology survey among uranium miners and controls. *Cancer 45: 1273-1277*. 1980.
55. Chameaud J, Perraud R, Chretien J, Masse R, Lafuma J. Lung carcinogenesis during in vivo cigarette smoking and radon daughter exposure in rats. *Recent Results in Cancer Res 82: 11-20*. 1982.
56. Pershagen G, Åkerblom G, Axelson O, Clavensjö B, Damber L et al. Residental radon exposure and lung cancer in Sweden. *N Engl J Med 330:159-164*. 1994.
57. Monfared AS, Jalali F, Sedaghat S, Mansoorizade E, Jarrahi AM, Hajiahmadi M, Samavat H. High natural background radiation areas, Iran: can inhabitants feel safe? *Int J Low Radiat* 3: 171-177. 2006.
58. Moolgavkar SH and Knudson AG, Jr. Mutation and cancer: a model for human carcinogenesis. *J Natl Cancer Inst 66: 1037-1052*. 1981.
59. Chadwick KH, Leenhouts HP, Brugmans MJ. Implications of the analysis of epidemiological data using a two-mutation carcinogenesis model for radiation risks. *Radiat Prot Dosimetry 99: 265-268*. 2002.
60. Hoerter, J., Eisenstark, A., Synergistic killing of bacteria and phage by polystyrene and ultraviolet radiation. Environ. Molec. Mutagen. 12, 261-264. 1988.
61. Little, J.B., Low-dose radiation effects: interactions and synergism. Health Phys. 59, 49-55. 1990.
62. Petin, V.G., Kim J.K., Zhurakovskaya G.P., Dergacheva I.P., Some general regularities of synergistic interaction of hyperthermia with various physical and chemical inactivating agents. Int. J. Hyperthermia 18, 40-49. 2002.

63. Streffer, C., Bücker, J., Cansier,A., Cansier, D., Gethmann, C.F., Guderian, R., Hanekamp, G., Henschler, D., Pöch, G., Rehbinder, E., Renn, O., Slesina, M, Wuttke, K., Environmental Standards. Combined Exposures and Their Effects on Human Beings and Their Environment. Springer-Verlag, Berlin-Heidelberg. 2003.
64. Matsumoto, M., Takagi, H., Yoshimura, N., Synergistic suppression of retinal pigment epithelial cell proliferation in culture by radiation and hyperthermia. Invest. Opthalmol. Visual Sci. 34, 2068-2073. 1993.
65. Chang, P.C., Li, H.Y., Tang, H.J., Liu, J.W., Wang, J.J., Chuang, Y.C., In vitro synergy of baicelein and gentamicin against vancomycin-resistant Eneterococcus. J. Microbiol. Immunol. Infect. 40, 56-61. 2007.
66. Scheie, A.A., Assev, S., Rölla. G. Combined effect of xylitol, NaF and $ZnCl_2$ on growth and metabolism of *Streptococcus sobrinus* OMZ 176. APMIS 96, 761-767. 1988.
67. Rogers, A.H., Bert, A.G., Effects of xylitol and fluoride on the response to glucose pulses of *Streptococcus mutans* T8 growing in continuous culture. Oral Microbiol. Immunol. 7, 124-126. 1992.
68. Maehara, H., Iwami, Y., Mayanagi, H., Takahafhi, N. Synergistic inhibition by combination of fluoride and xylitol on glycolysis by mutants streptococci and its biochemical mechanism. Caries Res. 39, 521-528. 2005.
69. Goa KL and CM Spencer. Amifostine. A review of its pharmacodynamoc and pharmacokinetic protperties, and therapeutic potential as a radioprotector and cuytotoxic chemoprotector. Drugs. 50: 1001-1031. 1995.
70. Nair CKK, DK Parida, and T Nomura. Radioprotection in radiotherapy. J. Radiate. Res. 42: 21-37. 2001.
71. Singh VN and SK Gaby. Premalignant lesions: role ofantioxidant vitamins and beta-carotene in risk reduction and prevention of malignant transformation. Am. J. Clin. Nutr.53(1 Suppl): 386S. 1991.
72. Tubiana M, J Dutreix, and A Wambersie. Introduction to radiobiology, Taylor & Francis, New York. 1990.
73. O'Connor MK, JF Malone, M Moriarity, and S Mulgrew. A radioprotective effect of vitamin C observed in Chinese hamster ovary cells. Br. J. Radiol. 50: 587-591. 1977.
74. Stratford MRL, RJ Hodgkiss and RR Watfa. Studies on the role of antioxidants in radioprotection. Pharmacol. Therap. 39: 389-391. 1988.

75. Okunieff P and HD Suit. Toxicity, radiation sensitivity modification, and combined drug effects of ascorbic acid with misonidazole in vivo on FSaII murine fibrosarcomas. J. Natl. Cancer Inst. 79: 377-381. 1987.
76. George KC, SA Hebbar, SP Kale, and PC Kesavan. Caffeine protects mice against whole-body lethal dose of gamma-irradiation. J. Radiol. Prot. 19: 171-176. 1999.
77. Chul L. Antioxidant ability of caffeine and its metabolites based on the study of oxygen radical absorbing capacity and inhibition of LDL peroxidation. Clin. Chim. Acta. 295: 141-154. 2000.
78. Azam S, N Hadi, NU Khan, and SM Hadi. Antioxidant and prooxidant properties of caffeine, theobromine and xanthine. Med. Sci. Monit. 9: BR-325-30. 2003.
79. Lu YP, Lou YP, Li XH, Xie JG, Brash D, Huang MT, Conney AH. Stimulatory effect of oral administration of green tea or caffeine on ultraviolet light-induced increases in epidermal wild-type p53, p21(WAF1/CIP1), and apoptotic sunburn cells in SKH-1 mice. Cancer Res. 60: 4785-4791. 2000.
80. Inano H, K Suzuki, H Ishii-Ohba, Y Imada, R Kumagai, S Kurihara, and A Sato. Steroid hormone production in testis, ovary, and adrenal gland of immature rats irradiated in utero with ^{60}Co. Radiat. Res. 117: 293-303. 1989.
81. Rodriguez I, C Ody, K Araki, I Garcia, and P Vassalli. An early and massive wave of germinal cell apoptosis is required for the development of functional spermatogenesis. EMBO J. 16: 2262-2270. 1997.
82. Jansz GF and DK Pomerantz. Fetal irradiation increases androgen production by dispersed leydig cells of the rat. J. Androl. 5: 344-350. 1984.
83. Pinon-Lateillade G, MC Viguier-Martinez, AM Touzalin, J Maas, and B Jegou. Effect of an acute exposure of rat testes to gamma rays on germ cells and on Sertoli and Leydig cell functions. Reprod. Nutr. Dev. 31: 617-629. 1991.
84. Kim JK, CJ Lee, KW Song, BR Do, YD Yoon. γ-Radiation accelerates ovarian follicular atresia in immature mice. In Vivo. 13: 21-24. 1999.
85. Maeda N, H Hagihara, Y Nakata, S Hiller, J Wilder, and R Reddick. Aortic wall damage in mice unable to synthesize ascorbic acid. Proc. Natl. Acad. Sci. USA. 97: 841-846. 2000.
86. Kim JK, JH Kim, and YD Yoon. Evaluation of caffeine as a radioprotector in whold-body irradiated male mice. In Vivo. 17: 197-200. 2003.
87. Russell LD, RA Etllin, APS AHikim, and ED Cleff. Histological and histopathological evaluation of the testis. Cache River Press, Florida. 1990.

88. Muller WU, C Streffer, A Woicik, and F Niedereichholz. Radiation-induced malformations after exposure germ cells in various stages of spermatogenesis. Mutat. Res. 425: 99-106. 1999.
89. Jagetia GC, GK Rajanikant, and SK Rao. Evaluation of the effect of ascorbic acid treatment on wound healing in mice exposed to different doses of fractionated gamma radiation. Radiat. Res. 159: 371-380. 2003.
90. Richburg JH. The relevance of spontaneous- and chemically-induced alterations in testicular germ cell apoptosis to toxicology. Toxicol. Lett. 112-113: 79-86. 2000.
91. Kundson CM, KSK Tung, WG Tourtellotte, GAJ Brown, and SJ Korsmeyer.Bax-deficient mice with lymphoid hyperplasia and male germ cell death. Science. 270: 96-99. 1995.
92. 국립환경연구원, 우리나라 논토양 및 현미 중 중금속 자연함유량에 관한 조사 연구. 1988.
93. Gochfeld M. Cases of mercury exposure, bioavailability, and absorption. Ecotoxic. Environ. Safety. 56:174-179. 2003.
94. Emanuelli T, JB Rocha, ME Pereira, LO Porciuncula, VM Morsch, AF Martins, DO Souza. Effect of mercuric chloride intoxication and dimercaprol treatment on delta-aminolevulinate dehydratase from brain, liver and kidney of adult mice. Pharmacol. Toxicol. 79(3):136-143. 1996.
95. Kim JH, JK Kim, BH Lee, HJ Kim, KB Park, KB Park. Alteration of intracellular signaling following KW water administration in apoptosis-induced animal models. J. KASBIR. 4(1):189-195. 2004.
96. Lee CJ, HH Park, BR Do, YD Yoon, JK Kim. Natural and radiation-induced degeneration of primordial and primary follicles in mouse ovary. Animal Reprod Sci, 59:109-117. 2000.
97. Horikoshi T and M Sakakibara. Quantification of relative mRNA expression in the rat brain using RT-PCR and ethidium bromide staining. J. Neuroscience Methods. 99:45-51. 2000.
98. Leblond VS and A Hontela. Effects of in vitro exposures to cadmium, mercury, zinc, and 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane on steroidogenesis by dispersed interrenal cells of rainbow trout (*Oncorhynchus mykiss*). Toxicol. Appl. Pharmacol. 157(1): 16-22. 1999.
99. Nath KA, AJ Croatt, S Likely, TW Behrens, D Warden. Renal oxidant injury and oxidant response induced by mercury. Kidney Int. 50(3):1032-1043. 1996.

100. 김진규, 천기정, 이동우, 지표생물을 이용한 방사선의 방재적 감시연구, 방재연구, 제 3권 제 1호, 12월. 2000.
101. 김진규, 신해식, 이병헌, 송희섭, 자주달개비 미세핵분석법 (Trad-MCN)을 이용한 잠재적 생물재해요인의 평가, 방재연구, 5(1): 27-36. 2002.
102. Wang S, Wang X, The *Tradescantia*-micronucleus test on the genotoxicity of UV-B radiation, *Mutation Research* 426 151-153. 1999.
103. Arutyunyan RM, Pogosyan VS, Simonyan EH., Atoyants AL, Djigardjian EM, In situ monitoring of the ambient air around the chloroprene rubber industrial plant using the *Tradescantia*-stamen-hair mutation assay, *Mutation Research* 426 117-120. 1999.
104. 허귀석, 이재환, 신해식, 김진규, 이영엽, 이대운, 이진홍, 화학공장 실내 작업장에서의 유해물질 평가를 위한 VOC 분석법과 자주달개비 미세핵 분석법의 비교, *Analytical Science & Technology, Vol.* 16, No.1, 1-11. 2003.
105. Kim JK, Kim WR, Kim JS, Shin HS and Lee JJ, Effects Diurnal temperature difference and gamma radiation on the frequency of somatic cell mutations in the stamen hairs of *Tradescantia* 4430, *Korean J. Environ. Biol*, 16(3):253-262. 1998.
106. Ichikawa. S. Insitu monitoring with Tradescantia around Nuclear power plants. Envitonmental Health Perspectires, 37 : 145-164. 1981.
107. 김진규, 김원록, 이창주, 장화형, 이영근, 자주달개비 수술털에서 방사선에 의해 유발되는 세포손상에 대한 살충제의 방어효과 Korean J. Environ. Biol. 17(1): 21-26. 1999.

빈 면

부 록

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2. 연구개발결과・활용보고서
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4. 기술실시계약보고서 (해당시)
5. 연구성과 증빙자료 사본

빈 면

자체평가의견서

1. 과제현황

		과제코드			
사업구분	국제공동연구사업				
연구분야	대분류코드 기입	중분류코드 기입		과제구분	단위
사 업 명	양자간공동연구사업				주관
총괄과제				총괄책임자	김 진 규
과 제 명	이온화 방사선과 화학물질의 복합작용해석기술 개발			과제유형	응용
연구기관	한국원자력연구원			연구책임자	김진규
연구기간 연 구 비 (천원)	연차	기간	정부	민간	계
	1차년도	'05.05-'06.03	90,000		90,000
	2차년도	'06.04-'07.03	90,000		90,000
	3차년도	'07.04-'08.03	45,000		45,000
	4차년도				
	5차년도				
	계		225,000		225,000
참여기업					
상 대 국	폴란드		상대국연구기관	헨릭니보드니찬스키 핵물리연구소	

2. 평가일 :

3. 평가자(연구책임자) :

소속	직위	성명	서명
한국원자력연구원	책임연구원	김 진 규	

4. 평가자(연구책임자) 확인 :

본인은 평가대상 과제에 대한 연구결과에 대하여 객관적으로 기술하였으며, 공정하게 평가하였음을 확약하며, 본자료가 전문가 및 전문기관 평가시에 기초자료로 활용되기를 바랍니다.

확 약	

Ⅰ. 연구개발실적

※ 다음 각 평가항목에 따라 자체평가한 등급 및 실적을 간략하게 기술(200자 이내)

1. 연구개발결과의 우수성/창의성

■ 등급 : (S)

- 이온화 방사선과 화학물질 (염화수은 등)이 생물체에 동시에 작용할 때 나타나는 생체영향을 다양한 생물종말점을 대상으로 정량화한 연구로서 실용적 측면과 학술적 측면에서의 중요성이 모두 강조된 연구임.
- 연구개발 결과를 SCI를 포함한 전문 학술지에 다수 게재하였으며 국제학술대회에서 발표함으로써 연구개발 결과의 객관성, 창의성 및 우수성을 인정받음
- 다요인 복합작용에 관한 이론모델을 이용한 상승작용 해석기술을 개발하여 이 분야 기술개발의 세계적 선도위치를 점함

2. 연구개발결과의 파급효과

■ 등급 : (A)

- 본 연구과정에서 확립한 고등식물 체세포돌연변이 검정법, 면역조직화학 검정법, 홀몬 검정법 등의 평가기술은 생명과학, 의약학, 환경독성학 등 광범위한 응용확장성을 가진 실험기법임
- 모델 분석을 통한 다요인 복합생체영향 해석기술 및 관련 논문은 Norwegian Radiation Protection Authority, Spain Dawnesh Research Institute, Indian Society of Biophysics 등 많은 연구기관으로부터 요청이 잇따르는 등 국제적 파급효과가 상당함
- 한국·폴란드간 과학기술협력의 근간을 이룰 수 있는 가시적 성과물을 생산하였으며 이로서 양국간 호혜적 협력 관계를 증진시키는 데 크게 기여함

3. 연구개발결과에 대한 활용가능성

■ 등급 : (A)

- 본 연구에서 수립된 검정기술은 생명과학, 의약학, 환경학, 독성학, 위해성평가, human monitoring, molecular epidemiology 등 폭 넓은 분야의 분석평가 기술로 활용가능함
- 연구개발결과를 학술지 논문으로 게재하는 한편 국내외 학술발표회의 발표를 통하여 결과를 공표함으로써 관련 분야 연구에 실용적 기술로 활용될 수 있도록 함
- 개발된 이론모델은 후속연구 및 지속적 보완·개선을 거쳐 생체영향 다요인 복합영향 입체 평가법으로 활용될 수 있음

4. 연구개발 수행노력의 성실도

■ 등급 : (S)

- 계획에 비해 연구비가 많이 감액 조정되었으며 특히 삼년차 연구비가 대폭 삭감에 따른 실험 및 인력투입에 어려움에도 불구하고 복합영향 평가기법을 수립하여 국제적으로 인정받은 것은 연구수행 노력의 증거임
- SCI 학술지 2편의 논문을 포함하여 다수의 논문을 게재하였고 학술발표 등 결과의 공표 및 홍보노력을 기울임으로써 연구수행의 성실도를 입증함

5. 공개발표된 연구개발성과(논문, 지적소유권, 발표회 개최 등)

■ 등급 : (A)

- 논문게재 8편 (SCI 2, 비SCI 국제 2, 국내 4편)
  SCI : *Chemosphere* (1), *JPhotochem. Photobiol.* (1)
  비SCI (국외) : *Iran. J. Radiat. Res..* (1), *Phys. Particles Nuclei Lett.* (1)
  (국내) : 한국환경생물학회지 (4)
- 논문발표 14 편 (국외 9편, 국내 5편)

Ⅱ. 연구목표 달성도

번호	세부연구목표 (연구계획서상에 기술된 연구목표)	달성내용	달성도 (%)
1	고등식물 세포에 대한 방사선 및 수은 처리후 생체영향 평가	TSH 검정법을 적용하여 감마선과 염화수은의 복합영향을 평가하여 목표를 달성	100
2	고등동물세포에 대한방사선과 수은 복합작용에 따른 DNA 손상도 정량화	세포주를 이용하여 DNA 손상도를 평가하고 그 결과를 국내논문으로 게재함	100
3	민감 조직에 대한 감마선 조사 및 수은 처리에 따른 조직손상 및 세포자사 평가	면역조직화학적검정법 및 홀몬분석법을 통하여 조직손상 및 세포사멸 평가달성	100
4	방사선과 수은 등의 물리화학요인의 복합작용 평가이론의 적용 및 개선	복합작용 해석 이론모델 수립 및 응용	100
5	SCI학술지 논문 2편, 국내학술지 논문 2편 이상	SCI학술논문2, 국외2편, 국내논문4편	>100

Ⅲ. 종합의견

1. 연구개발결과에 대한 종합의견

- 설정한 연구목표를 100% 달성하였음 - 노력의 성실도가 인정되며 연구의 창의성과 논문발표 실적이 아주 우수함 - 공동연구의 경험 및 결과는 한국·폴란드 양국의 협력관계 증진에 기여할 것임

2. 평가시 고려할 사항 또는 요구사항

- 각국이 이온화 방사선과 물리화학적 유해요인의 복합작용에 관한 연구에 많은 노력을 기울이고 있음. - 본 과제 수행을 통해 수립한 복합작용 이론모델 및 해석기술은 국제적으로 인정받음 - 이온화 방사선의 산업적, 의학적, 학술적 이용이 증가하고 있는 현실을 감안하여 실용적 측면과 학술적 측면을 균형 있게 다룬 연구였음

3. 연구결과의 활용방안 및 향후조치에 대한 의견

- 복합작용 평가기법은 방사선생물학, 핵의학, 환경생물학, 유해성평가 및 재해예방 분야의 핵심기술로 활용될 수 있음 - KAERI-INP 공동연구는 양국간 과학기술 협력의 기반을 공고히 하는 역할을 해왔으며 특히 양국간 호혜적 협력관계 발전에 기여할 것임 - 한·폴 공동연구 및 다른 나라와의 공동연구 후속과제를 도출하기 위해 노력할 것임

Ⅳ. 보안성 검토

o 특정연구개발사업보안관리지침에서 정하는 바에 따라 작성 o 연구책임자의 보안성 검토의견, 연구기관 자체의 보안성 검토결과를 기재함

1. 연구책임자의 의견

- 본 연구개발의 결과는 연구계, 학계 및 산업계 등에 공개하여 다양한 분야에 활용토록 하는 것이 필요하며 보안을 유지해야할 사유는 없다고 판단됨 (공개 가능)

2. 연구기관 자체의 검토결과

- 연구책임자의 의견과 일치함 (공개 가능)

연구개발 결과·활용보고서

①과제번호						
②사업구분	중사업명	국제공동연구				
	세부사업명	양자간공동연구사업				
③기술분야		방사선이용				
④연구과제명		이온화 방사선과 화학물질의 복합작용해석기술 개발		⑤ 연구책임자	김 진 규	
⑥ 연구기관명		한국원자력연구소		⑦ 참여기업명	(없음)	
⑧연구기간 및 연구개발비 (단위 : 천원)		연 구 기 간 (년 월 일)	정부출연금	정부이외출연금	기업부담금	총연구비
	1차	2005.05.01-2006.03.31	90,000			90,000
	2차	2006.04.01-2007.03.31	90,000			90,000
	3차	2007.04.01-2008.03.31	45,000			45,000
		합계	225,000			225,000

⑨ 연구개발성과의 활용 (해당란에 "○"표시)					
기업화 완 료	기업화 추진중	2단계 연구추진	선행 및 기초 연 구 활 용	기타목적 활용	연구성과 활용중단
			○	O	
기타목적 활용실적을 선택한 경우 구체적 활용실적을 서술	Norwegian Radiation Protection Authorities와의 협력연구 추진 (중)				
⑩ 기업화(기업화 완료 또는 추진중인 경우, 반드시 기재)					
업 체 명		기업화 완료( ) 기업화 예정( )		년 월 일	
제 품 명		제 품 용 도			
기업화이용유형중 택일(해당란에"∨표시)	신제품개발( ), 기존제품 개선( ), 신공정개발( ), 기존공정개선( ), 기타( )				
⑪ 기술료 징수(기업화 완료이거나 기술실시계약을 체결한 경우 기재)					
징 수 조 건		징 수 현 황		천원(전년도말 현재)	
⑫ 산업재산권(발명특허, 실용신안, 의장, 상표, 규격 등으로 구분하고, 산업재산권명을 세부적으로 전부(건별로)기록,국외인 경우 반드시 국명을 기록)					

구 분	산업재산권명칭 (총 건수)	국명	출원			등 록			기 타
			출원인	출원일	출원번호	등록인	등록일	등록번호	

	⑬ 국내외 전문학술지 논문게재(전문학술지명을 세부적으로 전부(건별로)기록하고, 국외인 경우 반드시 국명을 기록)-본 연구과제 수행결과로서 발표하거나 게재한 것만 기재할 것						
	학술지 명칭 (총 8 건)	제목	년도	호	발 행 기 관	국 명	SCI여부
	(별지 목록 참조)						
	⑭ 학 술 회 의 논문발표(학술회의명을 세부적으로 전부(건별로)기록하고, 국외인 경우 반드시 국명을 기록)-본 연구과제 수행결과로서 발표한 것만 기재할 것						
	학술회의 명칭 (총 14 건)	제목	년도	호	장 소	국 명	
	(별지 목록 참조)						

	⑮ 실적(1년동안)					
	수입대체효과 (백만원/년)	수출증대효과 (백만원/년)	매출증대효과 (백만원/년)	생산성향상 효과 (백만원/년)	고용창출효과 (인력양성인원수)	기타
	⑯ 향후 기대효과					
	수입대체효과 (백만원/년)	수출증대효과 (백만원/년)	매출증대효과 (백만원/년)	생산성향상 효과 (백만원/년)	고용창출효과 (인력양성인원수)	기타

	⑰ 타 연구개발사업에의 활용			
	과제발주체(부처)	사업명	과제명	책임자
	⑱ 연구성과활용중단 사유			

※ 각 항목별 기재내용의 분량이 많을 경우, 별지 사용

[별 지]

⑬ 국내외 전문학술지 게재논문

○ VG Petin, JK Kim, RO Kritsky, Mathematical description, oprimization and prediction of synergistic interaction of fluoride and xylitol on glycolysis. *Chemosphere* (in press) (2008) - Elsevier 발행, SCI

○ JK Kim, LN Komarova, GP Zhurakovskaya, VG Petin, The fluence rate determines the synergistic interaction of UV radiation and heat for mitotic recombination and cell inactivation in yeasts, *Photochemistry and Photobiology*, 82(4), 1053-1057 (2006) - American Society for Photobiology 발행, SCI

○ JK Kim, SA Belkina, VG Petin, Mathematical description and prognosis of synergistic interaction of radon and tobacco smoking, *Iranian Journal of Radiation Research*, 4(4), 169-174 (2007) - 이란방사선학회 발행, 비SCI

○ VG Petin, NM Kabakova, VI Wrigglesworth, JK Kim, RBE of α particles and postradiation recovery in a temperature conditional mutant of diploid yeast for various criteria of cell death, *Physics of Particles and Nuclei Letters*, 3(4), 241-245 (2006) - 러시아물리학회 발행, 비SCI

○ LN Komarova, JK Kim, VG Petin, Mathematical description and prognosis of cell recovery after thermoradiation action. *Korean Journal of Environmental Biology,* 26(1): 1-7 (2008) - 한국환경생물학회 발행, 비SCI

○ HJ Woo, JH Kim, A Cebulska-Wasilewska, JK Kim, Evaluation of DNA damage by mercury chloride (II) and ionizing radiation in HeLa cells. *Korean Journal of Environmental Biology,* 24(1): 46-52 (2006) - 한국환경생물학회 발행, 비SCI

○ JK Kim, LN Komarova, MD Tkhabisimova, VG Petin, Inhibition of recovery from potentially lethal damage by chemicals in Chinese hamster cells is realized through the production of irreversible damage, *Korean Journal of Environmental Biology,* 23(4): 390-397 (2005) - 한국환경생물학회 발행, 비SCI

○ JH Kim, KJ Chun, YD Yoon, JK Kim, Differential functions of caffeine and ascorbic acid in γ-irradiated male mice, *Korean Journal of Environmental Biology,* 23(4): 398-404 (2005) - 한국환경생물학회 발행, 비SCI

[별 지}

⑭ 학술회의 논문발표

<국제학술회의발표 9건>

○ SH Kim, JK Kim, Cell survival and gene expressions of *Saccharomyces cervisiae* treated with ionizing radiation and mercury chloride. *Proceedings of 2007 International Meeting of the Federation of Korean Microbiological Societies*, p.219, Oc.t. 11-12, 2007, Seoul, Korea (2007)

○ JK Kim, VG Petin, KP Mishra, Developing a theoretical predictive model for cellular response to combined actions of low radiation and hyperthermia. *Proceedings of 6th LOWRAD International Conference on Low Dose Radiation Effects on Human Health and Environment*, p.70. Oct. 17-20, 2007, Budapest, Hungary (2007)

○ JK Kim, VG Petin, SV Belkina, Mathematical description of synergistic interaction between radon and smoking, *Proceedings of 6th LOWRAD International Conference on Low Dose Radiation Effects on Human Health and Environment*, p.69. Oct. 17-20, 2007, Budapest, Hungary (2007)

○ JK Kim, MJ Plewa, In vivo and in vitro assays for radioprotectant and antioxidant activity of wild plants, *Indian Journal of Radiation Research vol.3, no.4, p.180, International Conference on Radiation Biology and 5th LOWRAD (Low Radiation) International Conference on Low Dose Radiation Effects on Human Health and Environment*, November 20-22, 2006, Varanasi, India (2006)

○ JK Kim, JH Kim, A Cebulska-Wasilewska, Biological damage and molecular changes in rats exposed to gamma radiation and mercury chloride, *Indian Journal of Radiation Research vol.3, no.4, p.181, International Conference on Radiation Biology and 5th LOWRAD (Low Radiation) International Conference on Low Dose Radiation Effects on Human Health and Environment*, November 20-22, 2006, Varanasi, India (2006)

○ A Panek, JK Kim, A Cebulska-Wasilewska, Influence of mercury ions on repair of the DNA damage induced by X-rays, *Indian Journal of Radiation Research vol.3, no.4, p.260, International Conference on Radiation Biology and 5th LOWRAD (Low Radiation) International Conference on Low Dose Radiation Effects on Human Health and Environment*, November 20-22, 2006, Varanasi, India (2006)

○ JK Kim, YJ Lee, JW lee, LN Komarova, VG Petin, The order of synergistic interaction of ionizing radiation with the other agents among the chaos of the effects observed, *Indian Journal of Radiation Research vol.3, no.4, p.182, International Conference on Radiation Biology and 5th LOWRAD (Low Radiation) International Conference on Low Dose Radiation Effects on Human Health and Environment*, November 20-22, 2006, Varanasi, India (2006)

○ JK Kim, YJ Lee, JW Lee, LN Komarova, VG Petin, Exploitable mechanism, optimization and prediction of the biological responses to radiation combined with the other agent, Book of Abstract, p.308, *The Second Asian and Oceanic Congress for Radiation Protection (AOCRP-2)*, Oct. 9-13, 2006, Beijing, China (2006)

○ A Panek, A Cebulska-Wasilewska, JK Kim, Mercury ions influence on repair of the radiation induced DNA damage, *Proceedings of the symposium on "Nuclear Techniques in Industry, Medicine, Agriculture and Environmental Protection (ISBN-83-921064-2-3)", pp. 296-301, Krakow, Dec. 7-9, 2005 (Poland)*

<국내학술회의발표 5건>

○ SH Kim, JK Kim, Cell viability and gene expressions of *Saccharomyces cervisiae* after radiation and mercury treatment, 한국환경생물학회 2007년 추계 학술대회, 초록집 p.00, 2007. 11. 16-17, 제주 하이테크산업진흥원 (2007)

○ 박희전, 이영엽, 김수현, 김진규, 방사선량 및 수은농도에 따른 자주달개비 분홍돌연변이 빈도 변화, 대한방사선방어학회 논문집 p.164-165, 2007. 11. 1-2, 제주도 한화리조트(2007)

○ 김진규, 이병헌, A. Cebulska-Wasilewska, 도전선량 검정법을 이용한 흡연 및 직업군에 따른 유전물질 손상 수선능력의 차이 평가, 한국방사선산업학회 2007년 학술발표대회 초록집 p.74, 2007. 8. 24-25, 무주리조트 (2007)

○ 김진규, 최대성, A. Panek, A. Cebulska-Wasilewska, 방사선유발 DNA 손상의 복구능력에 미치는 환경 유해화학물질의 영향, 한국환경생물학회 2007 춘계학술대회 초록집 p.31, 2007. 6. 8-9, 한국생명공학연구원 (2007)

○ JK Kim, YJ Lee, DS Choi, JH Kim, A Cebulska-Wasilewska, Hematological changes induced by mercury ions and ionizing radiation in experimental animals, Book of Abstract, p.74, *2006 Autumn Meeting of the Korean Nuclear Society*, November 2-3, 2006, Kyungju (2006)

참여기업 의견서

1. 과제현황

		과제코드			
사업구분	국제공동연구사업				
연구분야	대분류코드 기입	중분류코드 기입		과제구분	
사 업 명	양자/다자간 공동연구 사업중 해당사업				
총괄과제				총괄책임자	
과 제 명				과제유형	
연구기관				연구책임자	
연구기간 연 구 비 (천원)	연차	기간	정부	민간	계
	1차년도				
	2차년도				
	3차년도				
	4차년도				
	5차년도				
	계				
참여기업					
상 대 국		상대국연구기관			

2. 제출자

가. 제출일 :

나. 참여기업명 :

다. 대표이사(제출자) 성명 : (서명)

1. 연구개발 목표의 달성도는?

☐ 우 수 ☐ 보 통 ☐ 미흡

2. 연구성과가 참여기업의 기술력 향상에 도움이 되었는가?

☐ 충 분 ☐ 보 통 ☐ 불충분

3. 연구성과가 기업의 시장성 및 경제성에 도움이 되었는가?

☐ 충 분 ☐ 보 통 ☐ 불충분

[4, 5, 6번 항은 연차별 진도관리시에 해당]

4. 연구수행과정은 기업의 요청을 충분히 반영하였는가?

☐ 충 분 ☐ 보 통 ☐ 불충분

5. 향후 계속 참여 의사는?

☐ 충분 ☐ 고려중 ☐ 중단

6. 계속 참여 혹은 고려중인 연구개발비의 투자규모(전년도 대비)는?

☐ 확 대 ☐ 동 일 ☐ 축 소

[7, 8번 항은 최종(단계)평가시에 해당]

7. 연구개발결과의 기업화 여부는?

☐ 즉시 기업화 가능 ☐ 수년내 기업화 가능 ☐ 기업화 불가능

8. 기업화가 불가능한 경우 그 이유는?

※ 기타 종합의견(본과제에 대한 의견, 전문기관에 대한 요구사항 등)

참여기업입장에서 본 연구결과의 만족도	S	A	B	C	D

기술실시계약보고서

① 과제번호						
② 중사업명	과학기술국제화사업					
③ 세부사업명	양자/다자간 공동연구 사업 중 해당사업 기재					
④기술실시계약명/기술분야						
⑤ 연구과제명						
⑥ 연구기관명					⑦ 참여기업명	
⑧ 연구책임자						
⑨ 연구기간 및 연구개발비 (단위 : 천원)	⑩연 구기간		⑪ 정부출연금	⑫ 정부이외출연금	⑬ 기업부담금	⑭ 총연구비
	1차					
	2차					
	3차					
	4차					
	합계					
⑮ 실시기관	⑯ 기관명				⑰ 기관유형	
	⑱ 담당부서(담당자)				⑲ 전화번호	
	⑳ 법인소재지				㉑ 팩스번호	
㉒ 기술실시계약일					㉓ 실시기간	
㉔ 기술료산정근거						
㉕ 기술료총예정금액	기술료 총예정금액이 확정되어 있지 않다면, 정부출연금을 기술료로 추정기록					
㉖ 기술료징수조건 (가로안에 ∨표시) (단위 : 원)	㉗ 정액기술료 ( )		㉙ 연차별 기술료 징수계획			
			㉚ 징수일	㉛ 징수금액	㉜ 전문기관 납부금액	
			. .			
			. .			
			…			
			㉝ 총 계			
	㉘ 변동기술료 ( )		㉞ 선급기술료			
			㉟ 경상기술료			
			㊱ 최저기술료			
㊲ 기타특기사항						

특정연구개발사업처리규정 제39조 4항에 따라 상기와 같이 연구개발성과에 대한 기술실시계약이 체결되었음을 보고합니다.

첨부 : 1. 기술실시계약서 사본 2부
2. 중소기업임을 증명하는 서류 1부(실시기관이 중소기업으로서 특정연구개발사업처리규정 제40조1항에 의거 정부출연금의 70%를 감면받기로 계약한 경우)

200 년 월 일

주관연구기관장 : (직인)

연구성과 증빙자료

(연구논문에 한함)

1. CHEM_9067.pdf : Petin VG, Kim JK and Kritsky RO. Mathematical description, oprimization and prediction of synergistic interaction of fluoride and xylitol on glycolysis. *Chemosphere* (in press) (2008)
2. PnP_82(4)_1053-1057.pdf : Kim JK, Komarova LN, Zhurakovskaya GP and Petin VG. The fluence rate determines the synergistic interaction of UV radiation and heat for mitotic recombination and cell inactivation in yeasts, *Photochemistry and Photobiology*, 82(4), 1053-1057 (2006)
3. IJRR_4(4)_169-174.pdf : Kim JK, Belkina SA and Petin VG. Mathematical description and prognosis of synergistic interaction of radon and tobacco smoking, *Iranian Journal of Radiation Research*, 4(4), 169-174 (2007)
4. PPNL_3(4)_241-245.pdf : Petin VG, Kabakova NM, Wrigglesworth VI and Kim JK. RBE of α particles and postradiation recovery in a temperature conditional mutant of diploid yeast for various criteria of cell death, *Physics of Particles and Nuclei Letters*, 3(4), 241-245 (2006)
5. KJEB_26(1)_1-7.pdf : Komarova LN, Kim JK and Petin VG. Mathematical description and prognosis of cell recovery after thermoradiation action. *Korean Journal of Environmental Biology,* 26(1): 1-7 (2008)
6. KJEB_24(1)_46-52.pdf : Woo HJ, Kim JH, Cebulska-Wasilewska A and Kim JK. Evaluation of DNA damage by mercury chloride (II) and ionizing radiation in HeLa cells. *Korean Journal of Environmental Biology,* 24(1): 46-52 (2006)
7. KJEB_23(4)_390-39752.pdf : Kim JK, Komarova LN, Tkhabisimova MD and Petin VG. Inhibition of recovery from potentially lethal damage by chemicals in Chinese hamster cells is realized through the production of irreversible damage, *Korean Journal of Environmental Biology,* 23(4): 390-397 (2005)
8. KJEB_23(4)_398-404.pdf : Kim JH, Chun KJ, Yoon YD and Kim JK. Differential functions of caffeine and ascorbic acid in γ-irradiated male mice, *Korean Journal of Environmental Biology,* 23(4): 398-404 (2005)

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Mathematical description, optimization and prediction of synergistic interaction of fluoride and xylitol

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Abstract

The potential ability of various physical or chemical agents to enhance their effect when they are applied simultaneously with each other is well-known. The purpose of this study was to adjust a simple mathematical model to describe, optimize and predict a synergistic interaction between fluoride and xylitol on acid production by mutans streptococci. The model suggests that the synergism is caused by the additional effective damage arising from an interaction of sublesions induced by each agent. These sublesions are considered to be ineffective when each agent is used individually. The predictions of the model were verified by comparison with experimental data published by other researchers. It was shown that the model describes the experimental data, predicts the greatest value of the synergistic effect and the condition under which it can be achieved. The synergistic effect appeared to decline with any deviation from the optimal value of the ratio of effective damages produced by each agent alone.
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Keywords: Synergistic interaction; Mathematical model; Fluoride; Xylitol; Glycolysis

1. Introduction

The combined action of various chemical agents is a distinguishing feature of modern life. An interaction between two agents is considered as synergistic when the effect produced is greater than the sum of the two single responses. Many physical and chemical environmental factors can synergistically interact with each other (Cebulska-Wasilewska et al., 1981; Hoerter and Eisenstark, 1988; Little, 1990; Petin et al., 2002). The possibility to predict the synergistic effect and its highest value appears to be important for the establishment of safe level of agents after combined exposures (Streffer and Müllier, 1984; Streffer et al., 2003). The utilization of synergistic interaction in medicine enables to reduce the concentration of medicine or the dose of physical agent to provide the same medical effect or under the same value of agents to reach more effective response (Matsumoto et al., 1993; Chang et al., 2007). In particular, both fluoride and xylitol are known to be widely used as cariostatic agents and the synergistic effect of fluoride and xylitol on glycolysis by mutans streptococci was established ~~(Scheie et al., 1988; Maehara et al., 2005).~~ The authors pointed out that percent inhibition by combination of fluoride and xylitol was larger than the sum of percent inhibition by fluoride alone and percent inhibition by xylitol alone, indicating that the combined effect was synergistic. However, the authors did not make an attempt to estimate this effect quantitatively. The prospects of providing a reasonably useful application of this phenomenon seem to lie in the possibility to predict the highest synergistic effect and condition under which it can be obtained. A new concept of the synergistic interaction and a simple mathematical model based on this conception has been developed to predict and optimize the synergy when hyperthermia was simultaneously applied together with any of the following modalities: ionizing radiation, ultrasound,

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ultraviolet light, microwaves and some chemical agents (Petin and Komarov, 1997; Petin et al., 2002). The model was tested for inactivation of cells differing by their origin. The model predicted the dependence of the synergistic interaction on the ratio of the effective lesions produced by each of the agent applied, the greatest value of the synergy as well as the condition under which it can be achieved. Therefore, it would be of interest to accommodate this model for the description, optimization and prognosis of the synergistic effects between two chemical agents.

2. Mathematical model

In accordance with the model ~~(Petin and Komarov, 1997; Petin et al., 2002)~~ which is supposed to be adjusted to describe and predict the interaction of two chemical agents, the synergistic enhancement ratio (k) can be defined as the ratio of the effective damages produced by the combined action of these agents (N_{comb}), to the sum of the effective damages produced separately. As well as this model is planned to be tested for synergistic interaction of fluoride and xylitol on glycolysis, we designate an amount of some hypothetical effective damage produced by fluoride through N_1 and that produced by xylitol as N_2. Then we can write

$$k = \frac{N_{comb}}{N_1 + N_2} = \frac{N_1 + N_2 + N_3}{N_1 + N_2}. \quad (1)$$

It follows that the synergism is expected from some additional effective damages N_3. One can suppose that the additional damages are arising from the interaction of sublesions induced by both agents. These sublesions are considered ineffective after each agent is taken alone. The idea of sublesion interaction is widely used in quantitative chemistry (Cebulska-Wasilewska et al., 1981; Streffer and Müllier, 1984) and radiobiology (Zaider and Rossi, 1980; Little, 1990; Harder et al., 1994; Petin and Komarov, 1997). Let us suppose that p_1 and p_2 are the numbers of sublesions that occur per a single effective damage induced by fluoride and xylitol, respectively. We assume that one sublesion resulting from one individual agent reacts with one sublesion produced by another agent, thus producing an additional effective damage. Then, the synergistic enhancement ratio, k, may be given by

$$k = \frac{N_1 + N_2 + \min\{p_1N_1; p_2N_2\}}{N_1 + N_2}. \quad (2)$$

Here, the term $\min\{p_1N_1; p_2N_2\}$ is a minimal value from two variable quantities, p_1N_1 and p_2N_2, which determine the numbers of additional effective damages arising due to the interaction between sublesions produced by fluoride (p_1N_1) and xylitol (p_2N_2). An actual amount of real damage and sublesion is thought to be difficult to determine quantitatively in particular because of their molecular nature still should be elucidated. However Eq. (2) can be rewritten as

$$k = \frac{N_1 + N_2 + \min\{p_1; p_2\frac{N_2}{N_1}\}}{\frac{N_2}{N_1}}. \quad (3)$$

It means the model can operate with non-dimensional ratio of the effective damages $\frac{N_2}{N_1}$ produced by the agents under considerations which can be estimated from experimental results. The extent of the inhibition of acid production by oral bacteria may be supposed to be in the direct proportional to some hypothetical effective damages responsible for inactivating of the cariogenic bacteria. In accordance with this supposition, the extent of inhibition of acid production (% control) produced by fluoride (F) and xylitol (X) may be presented as

$$F = cN_1 \quad (4)$$

and

$$X = cN_2. \quad (5)$$

Here c is a constant of proportionality. Then the expected additive effect can be calculated as

$$(F + X)_{add} = c(N_1 + N_2), \quad (6)$$

while the experimentally observed results may be given as

$$(F + X)_{exp} = c(N_1 + N_2 + N_3). \quad (7)$$

The ratio of $\frac{N_2}{N_1}$ can be determined on the basis of experimental data as

$$\frac{N_2}{N_1} = \frac{X}{F}. \quad (8)$$

It can be easily demonstrated that, if the inhibition of acid production under combined action of agents is mainly produced by xylitol, or in other words $p_1N_1 < p_2N_2$, then the parameter p_1 may be presented as follows:

$$p_1 = (k - 1)\left(1 + \frac{N_2}{N_1}\right). \quad (9)$$

In contrast, if the inhibition of acid production is mainly induced by the action of fluoride ($p_1N_1 > p_2N_2$), the parameter p_2 can be determined by the following equation:

$$p_2 = (k - 1)\left(1 + \frac{N_1}{N_2}\right). \quad (10)$$

It can be demonstrated in a purely mathematical means that under condition

$$p_1N_1 = p_2N_2, \quad (11)$$

or

$$\frac{N_2}{N_1} = \frac{p_1}{p_2} \quad (12)$$

the greatest synergistic interaction can be calculated as follows:

$$k_{max} = 1 + \frac{p_1p_2}{p_1 + p_2}. \quad (13)$$

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Thus, using Eqs. (7) and (8), one can estimate, on the basis of real experiments, the basic model parameters p_1 and p_2. Then the synergistic enhancement ratio for any ratio of $\frac{N_2}{N_1}$, its greatest value, and the condition under which this is yielded can be derived from Eqs. (3), (11) and (10), respectively. All these parameters are very important for environmental toxicology and risk assessment. To test the validity of the model for chemical agents, one needs a great number of experimental data, obtained after action of two toxic agents applied alone and combined together. The prediction of the model presented here will be verified by a comparison with extensive experimental data on a simultaneous action of fluoride and xylitol on glycolysis published by others (Maehara et al., 2005).

3. The validity of the model

The extensive quantitative study of synergistic inhibition by combination of fluoride and xylitol on glycolysis by mutans streptococci clearly showed (Maehara et al., 2005) that the acid production from glucose by both bacterial strains tested (*Streptococcus mutans* NCTC10449 and *Streptococcus sobrinus* 6715) was inhibited in the presence of fluoride. The decrease of the acid production was dose-dependent and the effect was synergistically enhanced after simultaneous action of fluoride and xylitol. We used these results to evaluate some model parameters and experimental values of the synergistic enhancement ratio for various conditions of the combined action of fluoride and

Table 1
The dependence of some model parameters and experimental values of the synergistic enhancement ratio for the combined effect of fluoride and xylitol on the acid production by the cells of *S. mutans* NCTC19449 on the condition of treatment

Fluoride, mmol	pH	Fluoride $F = cN_1$	Xylitol $X = cN_2$	$(F+X)_{add} = c(N_1+N_2)$	$(F+X)_{exp} = c(N_1+N_2+N_3)$	N_2/N_1	k(exp)
0	7.0	0	9	9	9	–	–
0.8	7.0	3	0	12	20	3.0	1.7
1.3[a]	7.0	15	9	24	50	0.6	2.1
1.6	7.0	22	9	31	59	0.41	1.9
2.0[a]	7.0	38	9	47	73	0.24	1.55
2.3	7.0	56	9	65	87	0.16	1.34
0	5.5	0	9	9	9	–	–
0.1	5.5	2	9	11	15	4.5	1.36
0.15[a]	5.5	4	9	13	29	2.25	2.23
0.20	5.5	6	9	15	42	1.5	2.8
0.25[a]	5.5	11	9	20	63	0.82	3.15
0.30	5.5	17	9	26	80	0.53	3.1
0.35[a]	5.5	50	9	59	90	0.18	1.52
0.40	5.5	75	9	84	98	0.12	1.17

The original results have been published by others (Maehara et al., 2005).
[a] Data obtained by extrapolation.

Table 2
The dependence of some model parameters and experimental values of the synergistic enhancement ratio for the combined effect of fluoride and xylitol on the acid production by the cells of *S. sobrinus* 6715 on the condition of treatment

Fluoride, mmol	pH	Fluoride $F = cN_1$	Xylitol $X = cN_2$	$(F+X)_{add} = c(N_1+N_2)$	$(F+X)_{exp} = c(N_1+N_2+N_3)$	N_2/N_1	k(exp)
0	7.0	0	5	5	5	–	–
0.8	7.0	5	5	10	15	1.0	1.5
1.25[a]	7.0	10	5	15	23	0.5	1.53
1.6	7.0	15	5	20	31	0.33	1.55
2.4	7.0	28	5	33	58	0.18	1.76
2.9[a]	7.0	40	5	45	70	0.125	1.56
3.25	7.0	52	5	57	79	0.096	1.39
3.5	7.0	60	5	65	82	0.083	1.26
4.0	7.0	78	5	83	92	0.064	1.11
0	5.5	0	8	8	8	–	–
0.1	5.5	5	8	13	15	1.6	1.15
0.15[a]	5.5	10	8	18	30	0.8	1.67
0.2	5.5	20	8	28	48	0.4	1.71
0.225[a]	5.5	28	8	36	55	0.29	1.53
0.25[a]	5.5	40	8	48	62	0.2	1.29
0.3	5.5	60	8	68	80	0.133	1.18
0.35[a]	5.5	78	8	86	90	0.103	1.05

The original results have been published by others (Maehara et al., 2005).
[a] Data obtained by extrapolation.

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xylitol on the acid production by *S. mutans* (Table 1) and *S. sobrinus* (Table 2). The extrapolated data represented by the white dots on the figures as well as some values in Tables 1 and 2 were estimated by the authors with a great accuracy using only the original experimental data having a very little scattering. The errors of extrapolated points have been taken as a middle value between errors of neighbour experimental points.

Table 3
The dependence of the basic model parameters (p_1 and p_2), the predicted greatest values of the synergistic enhancement ratio (k_{max}) and the ratio of $(\frac{N_2}{N_1})$max at which it can be achieved after the combined effect of fluoride and xylitol on the acid production by the cells of *Streptococcus mutans* and *Streptococcus sobrinus* on the condition of treatment

Strain	pH	p_1	p_2	$(\frac{N_2}{N_1})$max	k_{max}
Streptococcus mutans NCTC10449	7.0	2.8	2.9	0.96	2.42
Streptococcus mutans NCTC10449	5.5	4.1	4.8	0.85	3.21
Streptococcus sobrinus 6715	7.0	0.9	5.0	0.18	1.77
Streptococcus sobrinus 6715	5.5	1.1	2.0	0.55	1.71

Both the tables contain the following data: fluoride concentration, pH, the extent of inhibition of acid production (% control) produced by fluoride (F) and xylitol (X) applied individually, the expected additive effect $(F + X)_{add}$, the actually observed inhibition in experiment $(F + X)_{exp,}$ the ratio $\frac{N_2}{N_1}$ (Eq. (4)), and the experimentally observed synergistic enhancement ratio (Eq. (1)). These results clearly indicate that for a constant xylitol concentration an optimal concentration of fluoride may exist providing the highest synergistic effect. Just such dependence should be expected qualitatively from the mathematical model presented above. Hence, it would be of interest to apply the described mathematical model to these results.

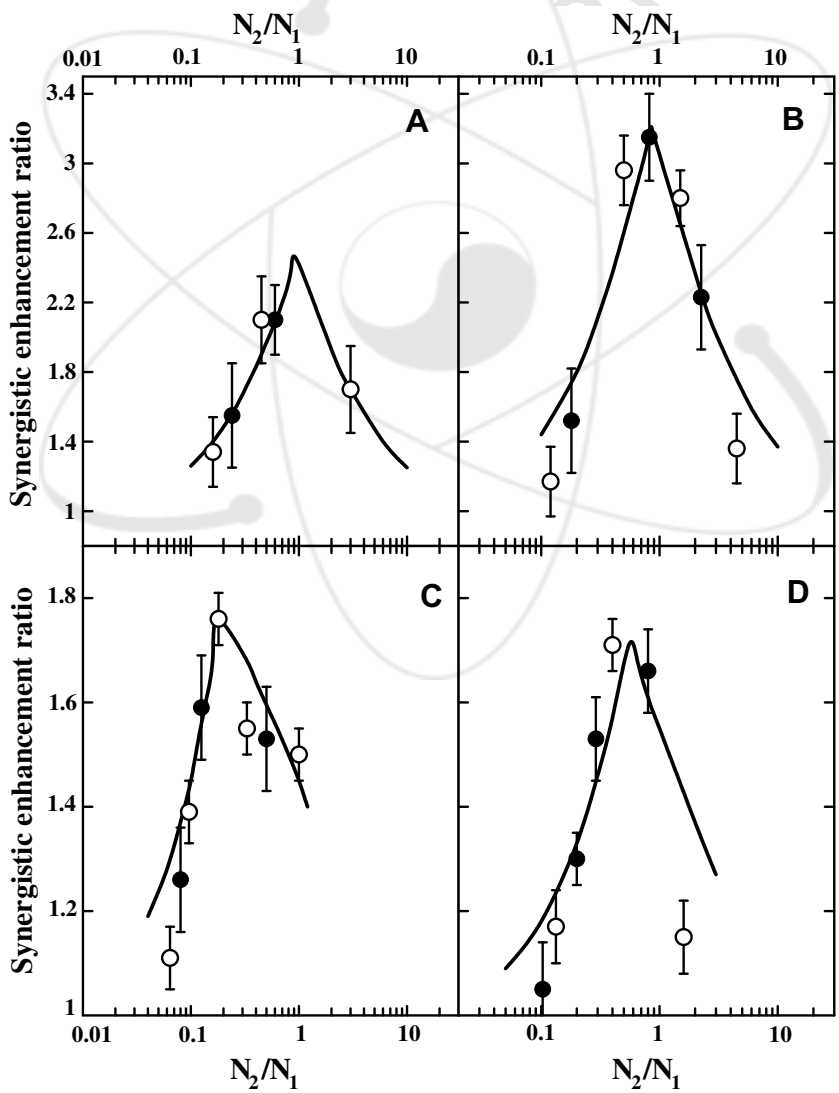

Fig. 1. The theoretically predicted (solid lines) and experimentally observed dependences of the synergistic enhancement ratio on the ratio of the effective damages produced by 60 mmol xylitol (N_2) and various concentration of fluoride (N_1) after their combined action on the acid production for 10 min from 10 mmol glucose by the cells of *S. mutans* 10449 (A–pH 7.0; B–pH 5.5) and *S. sobrinus* 6715 (C–pH 7.0; D–pH 5.5). Closed circles, experimental data; open circles, the data estimated by the extrapolation.

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In order to apply this model quantitatively to the experimental data, it is necessary to define two basic parameters p_1 and p_2. Each of these should be calculated for such experimental condition, when one of the agents applied induces more sublesions than another (Eqs. (7) and (8)). Thus estimated set of these parameters is collected in Table 3. Having used this set of the basic parameters, the synergistic enhancement ratio for any ratio of $\frac{N_2}{N_1}$, its greatest value, and the condition under which this is yielded can be derived from Eqs. (3), (11) and (10), respectively.

In Fig. 1, the synergistic enhancement ratio k of a simultaneous action of fluoride and xylitol on the inhibition of acid production by mutans streptococci from glucose is shown as a function of the ratio of effective damages produced by these agents $\frac{N_2}{N_1}$. One can see that the theoretically predicted interaction (solid lines) corresponds fairly well with the experimental data (circles). It can be inferred that the synergy occurred only within a definite ratio of the effects $\frac{N_2}{N_1}$ produced by each agent. It is also apparent that there is an optimal ratio of $\frac{N_2}{N_1}$ which results in the greatest synergistic interaction. Table 3 includes the highest synergistic effect that should be expected (Eq. (11)) and the ratio $\frac{N_2}{N_1}$, at which it can be achieved (Eq. (10)). Fig. 1 demonstrates that a good agreement exists between these predicted values and the experimental data. It means that the model presented here can be used for mathematical description, optimization and prediction of synergistic interaction of fluoride and xylitol on glycolysis.

4. Summary

A simple mathematical model was suggested for the optimization and prognosis of the synergistic interaction of fluoride and xylitol on acid production by mutans streptococci. The model is based on the assumption that synergism is conditioned by additional effective damage arising from an interaction of the sublesions induced by both agents. These sublesions are non-effective when each agent is applied alone. The predictions of the model were verified by comparison with experimental data published by other researchers (Maehara et al., 2005). In the proposed model, the synergistic interaction is given by $\{p_1N_1; p_2N_2\}$. This means that one sublesion caused by fluoride interacts with one sublesion produced by xylitol. This process is assumed to proceed until the very last sublesion of the less frequently type is used up. However, it does not mean that the model requires some kind of unreal long-range interaction. To estimate the basic parameters of p_1 and p_2 we have used the experimental values of the synergistic enhancement ratios (Eqs. (9) and (10)). It means that the model takes into consideration only the real interaction. The model proposed here is not concerned with the molecular nature or type of effective damages or sublesions, which occur, and the mechanism of their interaction remains to be elucidated. Moreover the nature of sublesions and their interactions may be different for various chemical agents. What the model does offer is an empirical mean for their quantitative estimation. The model appears to be appropriate and the predictions are valid. Indeed, it was shown that the model describes the experimental data, predicts the greatest value of the synergistic effect and the condition under which it can be achieved. The synergistic effect appeared to decline with any deviation from the optimal value of the ratio of carcinogenic effective damages produced by each agent alone. From the model standpoint, the highest synergy can be obtained for a specific condition when an equal number of sublesions are produced by each of the agents applied in combination with each other.

The basic model postulates do not concern the real biochemical mechanism, so it can be expected the model can be useful for description of synergistic effect of other chemical agents combined with each other. This model has already been tested for different chemical and physical agents (Petin and Berdnikova, 1979; Cebulska-Wasilewska et al., 1981; Petin et al., 2005).

5. Uncited references

Kim and Petin (2002) and Rogers and Bert (1992). Q1

Acknowledgements

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References

Cebulska-Wasilewska, A., Leenhouts, H.P., Chadwick, K.H., 1981. Synergism between EMS and X-rays for the induction of somatic mutations in *Tradescantia*. Int. J. Radiat. Biol. 40, 163–173.

Chang, P.C., Li, H.Y., Tang, H.J., Liu, J.W., Wang, J.J., Chuang, Y.C., 2007. In vitro synergy of baicelein and gentamicin against vancomycin-resistant *Eneterococcus*. J. Microbiol. Immunol. Infect. 40, 56–61.

Harder, D., Virsik-Peuckert, R.P., Bartels, E.R., 1994. Theory of intratrack pairwise lesion interaction. Radiat. Prot. Dosimetry 52, 13–16.

Hoerter, J., Eisenstark, A., 1988. Synergistic killing of bacteria and phage by polystyrene and ultraviolet radiation. Environ. Mol. Mutagen. 12, 261–264.

Kim, J.K., Petin, V.G., 2002. Theoretical conception of synergistic interactions. Korean J. Environ. Biol. 20, 277–286.

Little, J.B., 1990. Low-dose radiation effects: interactions and synergism. Health Phys. 59, 49–55.

Maehara, H., Iwami, Y., Mayanagi, H., Takahafhi, N., 2005. Synergistic inhibition by combination of fluoride and xylitol on glycolysis by mutants streptococci and its biochemical mechanism. Caries Res. 39, 521–528.

Matsumoto, M., Takagi, H., Yoshimura, N., 1993. Synergistic suppression of retinal pigment epithelial cell proliferation in culture by radiation and hyperthermia. Invest. Ophthalmol. Vis. Sci. 34, 2068–2073.

Petin, V.G., Berdnikova, I.P., 1979. Effect of elevated temperatures on the radiation sensitivity of yeast cells of different species. Radiat. Environ. Bioph. 16, 49–61.

Petin, V.G., Kim, J.K., Zhurakovskaya, G.P., Dergacheva, I.P., 2002. Some general regularities of synergistic interaction of hyperthermia

with various physical and chemical inactivating agents. Int. J. Hyperthermia 18, 40–49.

Petin, V.G., Komarov, V.P., 1997. Mathematical description of synergistic interaction of hyperthermia and ionizing radiation. Math. Biosci. 14, 115–130.

Petin, V.G., Zhurakovskaya, G.P., Kim, J.K., 2005. Synergetic effects of different pollutants and Eq.uidosimetry. In: Bréchignac, F., Desmet, G. (Eds.), "Eq.uidosimetry – Ecological Standardization and Eq.uidosimetry for Radioecology and Environmental Ecology". Springer, pp. 207–222.

Rogers, A.H., Bert, A.G., 1992. Effects of xylitol and fluoride on the response to glucose pulses of *Streptococcus mutans* T8 growing in continuous culture. Oral Microbiol. Immun. 7, 124–126.

Scheie, A.A., Assev, S., Rölla, G., 1988. Combined effect of xylitol, NaF and $ZnCl_2$ on growth and metabolism of *Streptococcus sobrinus* OMZ 176. APMIS 96, 761–767.

Streffer, C., Müllier, W.U., 1984. Radiation risk from combined exposures to ionizing radiation and chemicals. Adv. Radiat. Biol. 11, 173–210.

Streffer, C., Bücker, J., Cansier, A., Cansier, D., Gethmann, C.F., Guderian, R., Hanekamp, G., Henschler, D., Pöch, G., Rehbinder, E., Renn, O., Slesina, M., Wuttke, K., 2003. Environmental standards. In: Combined Exposures and their Effects on Human Beings and their Environment. Springer-Verlag, Berlin-Heidelberg.

Zaider, M., Rossi, H.H., 1980. The synergistic effects of different radiations. Radiat. Res. 83, 732–739.

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The Fluence Rate Determines the Synergistic Interaction of UV Radiation and Heat for Mitotic Recombination and Cell Inactivation in Yeasts

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ABSTRACT

The significance of the UV fluence rate for the synergistic interaction of UV light (254 nm) and heat was demonstrated for the frequency of mitotic recombination in a wild-type diploid yeast of *Saccharomyces cerevisiae* (strain T1) and for cell inactivation of two wild-type diploid yeast of *S. cerevisiae* (strains T1, XS800). It was shown for mitotic recombination that a decrease in the intensity of UV exposure results in the necessity of decreasing the temperature at which UV irradiation occurs to provide the same value of the synergistic enhancement ratio. For cell inactivation, there was a specific temperature maximizing the synergistic effect for any constant fluence rate and the temperature range, synergistically increasing the inactivation effect of UV radiation, should be shifted to lower temperatures with a decrease in the fluence rate. To interpret the results observed, a simple mathematical model of the synergistic interaction was applied. The model suggests that the synergistic interaction of UV light and hyperthermia is expected to result from some additional effective damages arising from the interaction of some sublesions induced by both agents. On the basis of data obtained, it was supposed that the synergistic interaction of these factors might take place at small intensities of UV light and temperatures existing in the biosphere. In other words, for a long duration of interaction, which is important for problems of UV light protection and health effects, one can expect a synergistic interaction of this factor with environmental heat or physiological temperatures and thereby an increase in their inactivating and genetic consequences.

INTRODUCTION

The whole biota on the Earth, including human beings, is constantly exposed to UV light as a part of natural solar electromagnetic radiations. The potential hazards of UV radiation have risen due to the depletion of protective ozone layer in the stratosphere and the formation of ozone holes. Moreover, the effect of UV radiation may be greatly increased due to synergistic interaction of UV light with other environmental factors. For example, several lines of evidence show that UV light exhibits a synergistic interaction with ionizing radiation (1,2), various chemical agents (3,4) and hyperthermia (5,6). But these data have been obtained mainly at relatively high fluence rates. Fluence rate is known to constitute a very important parameter in photobiology. While it is generally accepted that lowering the UV light fluence rate results in a decrease of the cell killing or mutagenesis efficiency per unit dose (7), the matter is still unclear with regards to the synergistic interaction of UV light and another environmental agent. It is of great interest to investigate whether or not the synergistic interaction can take place at low intensities of inactivating and/or mutagenic factors. Since such experiments are very difficult to perform at the real intensities observed in the biosphere, a feasible approach to this problem is to analyze the dependence of the synergistic interaction on the intensity of the agents applied. Several hypothetical possibilities may be realized in principle. If the level of synergism is reduced progressively with a decrease in the fluence rate, it would mean that the synergistic interaction will hardly be of any importance in terms of UV light safety. This conclusion would be also true for the case when a decrease in the fluence rate is accompanied by the increased intensity of another factor to provide the same level of synergism. The only possibility important for the problem of low doses and UV light safety is the case when a decreased fluence rate must be accompanied by a decrease in the intensity of another factor used in combination to provide the same level of synergism. Appropriate data of a combined action of ionizing radiation and heat have revealed that the dose rate can be considered a determinant of synergy (8,9). The decrease in the dose rate of the ionizing radiation required a decrease in the temperature at which the irradiation was carried out to preserve a definite effect of a simultaneous thermoradiation action on T4 bacteriophage (10), dry *Bacillus subtilis* spores (11), attenuated poliovirus (12), mammalian (13) and yeast (14) cells. In earlier investigations of yeast cell inactivation by a simultaneous treatment with UV light and hyperthermia (15,16), the correlation between the optimal temperature that maximized the synergy and the UV light fluence rate was estimated: this temperature shifted towards the lower temperature values with a decreasing fluence rate. However all the above mentioned results supporting this conclusion were obtained for the inactivation of various unicellular organisms. There have been no studies for the similar effects on the genetic level, though the evaluation of the genetic risks is necessary for any potentially hazardous environmental factors. Moreover, UV radiation is considered the most important initiator and cofactor for carcinogenesis

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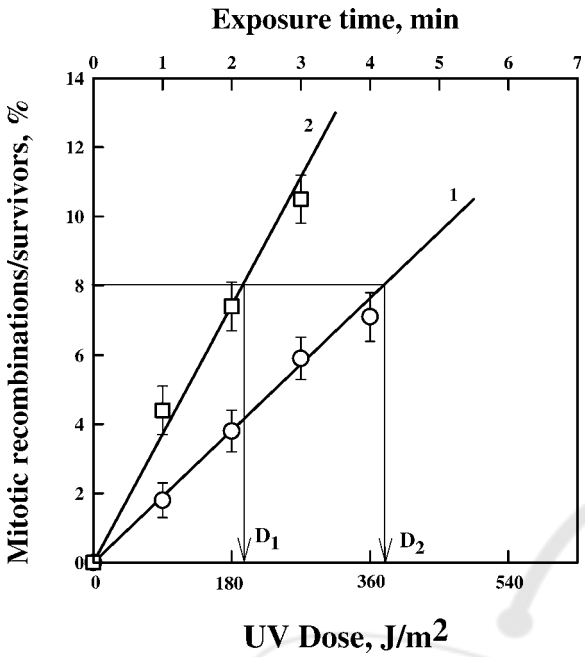

Figure 1. The dependence of the yield of mitotic recombination on UV dose for diploid yeast cells (*S. cerevisiae*, strain T1). Curve 1 presents the yield of mitotic recombination after UV light radiation (254 nm, 1.5 W m^{-2}) applied at room temperature. Curve 2 shows the yield of mitotic recombination after simultaneous treatment with UV light and heat (57.5°C).

of human skin exposed to UV radiation (17). In this study, we used the mitotic recombination of yeast cells as a genetic test often used for this purpose (18–20). As a factor synergistically increasing the effects of UV radiation we used high temperature. Heat is known to be an important modifier of UV radiation sensitivity (5,6,8,15). Moreover, UV exposure of skin is often encountered at hot ambient temperatures. Therefore, the elucidation of new fundamental aspects of the simultaneous action of UV radiation and heat is the actual goal. Thus, the purpose of the present work was to establish whether the UV fluence rate alters the synergistic interaction between hyperthermia and UV light for the mitotic recombinogenic effects in yeast. The same yeast strain and another one were used to test several exposure rates and confirm the dependence of the synergistic effect on the UV light intensity for cell inactivation. These data are expected to be valuable in providing additional information on the synergistic interaction of UV light with heat and other environmental agents on cellular and genetic levels.

MATERIALS AND METHODS

Strain and culture conditions. Two wild-type diploid yeasts of *S. cerevisiae*, strain XS800 and T1 (PG-154), were used in this study. Strain T1 has been specifically designed for the screening of the recombinogenic effects (21). This strain is heterozygous for the gene *ADE2* (*ade2-192*/*ade2-45*), which makes it possible to detect recombinogenic events (19–21). The alleles differ in their extent of colony pigmentation and complement each other so that the diploid is prototroph and produces white colonies. Mutation of the *ade2-192* locus produces red colonies while the *ade2-45* produces pink colonies. The cells were grown before irradiation up to stationary stage of growth for 3–5 days at 30°C on a complete growth medium of the following composition (in water): peptone, 20 g L^{-1}; yeast extract, 1 g L^{-1}; agar, 25 g L^{-1}; glucose, 20 g L^{-1}. In order to determine the mitotic recombination and cell survival after the treatments with UV and hyperthermia the following complete nutrient medium was used: glucose, 20 g L^{-1}; agar, 20 g L^{-1}; yeast extract, 1 g L^{-1}; KH_2PO_4, 2 g L^{-1}; $MgSO_4$, 1 g L^{-1}; $(NH_4)_2SO_4$, 1 g L^{-1}.

Exposure treatments. Cell suspension (1.5 mL, 10^6 cells mL^{-1}) in a quartz vessel was irradiated with germicidal lamp that emitted predominantly UV light of a wavelength 254 nm (far-UV radiation) at fluence rates of 0.033, 0.15, 0.25 and 1.5 W m^{-2} at different temperatures (47.5–60.0°C). The temperatures were thermostatically controlled to be within 0.2°C. The source of far-UV light at 254 nm was a low pressure mercury vapor lamp (a General Electric germicidal lamp, G15T8) with an integral filter that provided more than 95% of the emitted radiation at 254 nm. Variation of the intensity was achieved by means of calibrated metal wire nets. The UV light source was calibrated by potassium ferrioxalate actinometry (7), and the fluence rates were checked with a germicidal meter (General Electric Co., Fairfield, CT).

The following heating method was used: 0.1 mL of cell suspension at room temperature (about 1.5×10^6 cells) was placed into 1.4 mL of sterile water preheated to a required temperature in a water bath. Thus, the final volume and the concentration of the treated cells were 1.5 mL and 10^6 cells mL^{-1}, respectively. For the simultaneous thermal and UV light treatment, the time interval between the introduction of the cells into the preheated water and the beginning of the exposure was about 0.1 min, which was significantly less than the total treatment time. At the end of the treatment, the samples were rapidly cooled to room temperature. This means that the length of the UV light and heat exposure was identical. To avoid photoreactivation, the UV exposure, dilution and other procedures were performed under a red ambient light, while the postirradiation incubation was carried out under dark conditions.

Mitotic crossing over measure. The induction of mitotic crossing over was measured by determining the twin-sectored red/pink colonies together with other sector or complete monochrome colony coloration. A recombination of cells was determined by counting colonies on the 10th day after irradiation. The frequency of recombination was expressed as a percentage of the aberrant colonies to the total number of colonies produced after the exposure. Between 1000 and 2000 colonies were scored to determine the recombinant frequency for each experimental point. The survival response was determined by the colony counts on the 5th day of incubation. Each data point represents an average value from three to five independent experiments pooled together. Error bars in all figures show interexperimental errors. Other details were as described previously (15,16,20,22).

RESULTS

Synergism and mitotic recombination

Figure 1 shows a typical example of the basic experimental data observed in this study for the recombinant frequency of yeast cells (*S. cerevisiae*, strain T1) as a function of UV dose or exposure time. Curve 1 presents the yield of mitotic recombination after UV light radiation (254 nm, 1.5 W m^{-2}) applied at room temperature. Curve 2 shows the yield of mitotic recombination after simultaneous treatment with UV light and heat (57.5°C). Similar curves were obtained in this study for a wide range of exposure temperatures (47.5–60°C) and two fluence rates (0.15 and 1.5 W m^{-2}). Some of these data have been partially published (20) but they have not been used before to evaluate the synergistic interaction. A whole set of these data is used in this study for the first time to establish the significance of the fluence rate for the synergistic interaction of UV light and heat at a recombinogenic level.

To estimate quantitatively the sensitization action of hyperthermia, we used the synergistic enhancement ratio (k). This coefficient, in accord with the definition, was calculated by the ratio of UV dose which resulted in the same effect after an additive summation of the effects produced by each agent (D_2) to that observed in the experiments (D_1) under a simultaneous action of both modalities, *i.e.* $k = D_2/D_1$. An example of D_1 and D_2 estimation is given in Fig. 1. The value of this coefficient does not

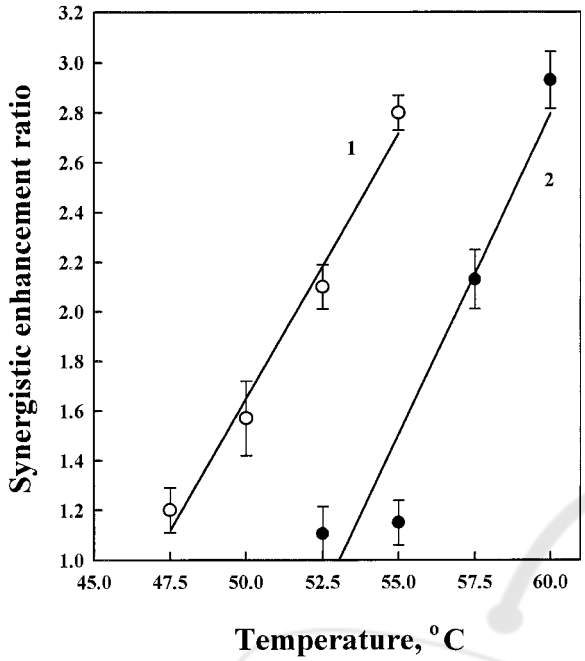

Figure 2. The dependence of the synergistic enhancement ratio on the temperature at which the UV irradiation (254 nm) occurred on the frequency of the recombinants in the diploid yeast cells (*S. cerevisiae*, strain T1). Curve 1 exhibits this dependence for UV radiation delivered at 0.15 W m^{-2}. Curve 2 presents this dependence for UV fluence rate of 1.5 W m^{-2}.

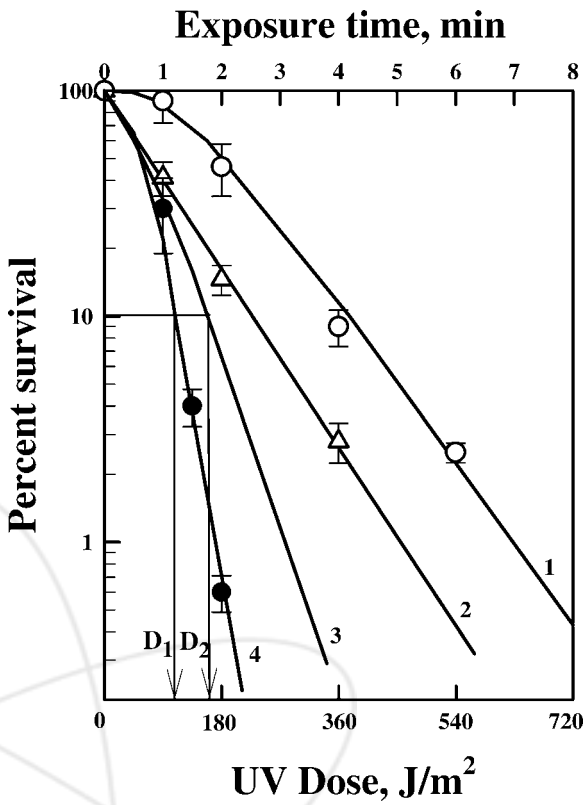

Figure 3. Survival curves of *S. cerevisiae* diploid yeast cells (strain T1): curve 1, UV light irradiation (254 nm, 1.5 W m^{-2}) alone (room temperature); curve 2, heat treatment alone (57.5°C); curve 3, calculated curve for independent action of UV light and high temperature; curve 4, experimental curve obtained after simultaneous action of both agents.

depend on the level of recombination for which it is calculated due to the linear relationship between the recombinant frequency of the yeast cells and the duration of the exposure. Note that the curves of the recombinant frequency for an additive summation and for UV light alone are identical since hyperthermia alone does not affect the recombinant yield. Therefore, here we consider the sensitizing action as a particular case of a synergistic interaction.

Figure 2 presents the dependence of the synergistic enhancement ratio on the temperature at which the irradiation occurred in the case of a simultaneous action of UV radiation and hyperthermia on the frequency of the recombinants in the diploid yeast cells. Curve 1 shows this dependence for UV radiation delivered at 0.15 W m^{-2}, while curve 2 presents this dependence for UV fluence rate of 1.5 W m^{-2}. These data have been calculated based on a whole set of dose–effect curves. It is seen that for a fixed intensity a rise in temperature is accompanied by an increase of the synergistic enhancement ratio over the range of temperatures. It is also evident that a decrease in the intensity of UV exposure results in the necessity of decreasing the temperature at which irradiation occurs to provide the same value of the synergistic enhancement ratio.

Synergism and cell inactivation

Figure 3 exhibits a typical example of the basic experimental data obtained in this study for cell inactivation. Here, the survival curves were obtained for *S. cerevisiae* diploid yeast cells (strain T1) after UV light irradiation alone (at room temperature, 1.5 W m^{-2}, curve 1), heat treatment alone (57.5°C, curve 2), the theoretical survival curve expected for an independent action of these agents (curve 3) and the experimental survival curve obtained after a simultaneous action of both modalities (curve 4). These types of survival curves were obtained for every temperature and fluence rate investigated for two diploid *S. cerevisiae* wild-type yeast strains (T1 and XS800).

To quantify the synergistic effect, we again used the synergistic enhancement ratio (k). For cell inactivation, this coefficient was estimated as the ratio of the expected UV light dose (assuming an independent effect of UV light and hyperthermia) to that obtained from the experimental survival curve after a simultaneous action of both agents at a fixed level of survival, *i.e.* for a 10% survival, $k = D_2/D_1$ (Fig. 3). Figure 4 (A,B) summarizes the results of a simultaneous action of UV light and high temperature on the inactivation of diploid *S. cerevisiae* wild-type yeast cells strains T1. Figure 5 (A,B) presents similar results for diploid *S. cerevisiae* wild-type yeast cells strain XS800. Here, Figs. 4A and 5A show the synergistic enhancement ratio as a functions of the exposure temperature and fluence rate. Figures 4B and 5B represent the relationship between the exposure temperature and UV light fluence rate providing the highest synergistic enhancement ratio. These data were calculated on the basis of a whole set of dose–effect curves. It is worth noting that here (Figs. 4 and 5) we present the ultimate findings based on the most complete set of our experimental data on this subject, part of which was published before (9,15). It is evident from these data that, for a given intensity, there is a specific temperature maximizing the synergistic effect. From these findings, it can be concluded that the temperature at which the UV light is delivered should be reduced to obtain the highest synergistic effect with a decrease in the fluence rate. It means also that the temperature range, synergistically

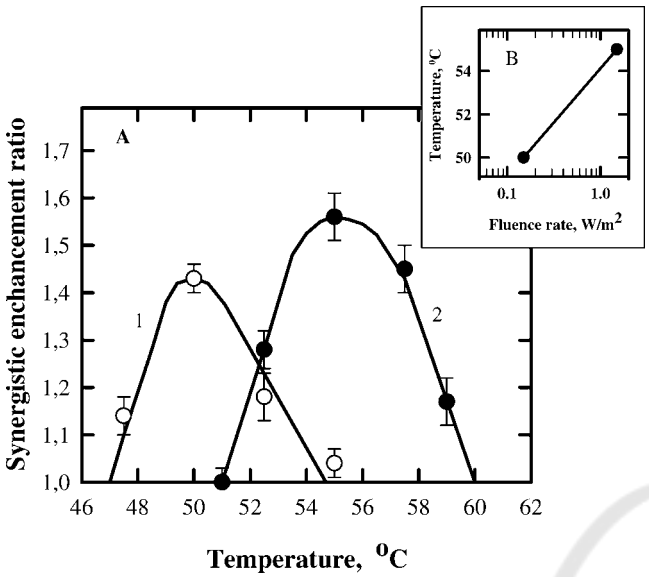

Figure 4. The results of a simultaneous action of UV light (254 nm) and high temperature on the inactivation of diploid *S. cerevisiae* wild-type yeast cells, strain T1. A: The dependence of the synergistic enhancement ratio on the exposure temperature and UV light fluence rate (curve 1, 0.15 W m^{-2}; curve 2, 1.5 W m^{-2}). B: The relationship between exposure temperature and UV light fluence rate providing the highest synergistic enhancement ratio.

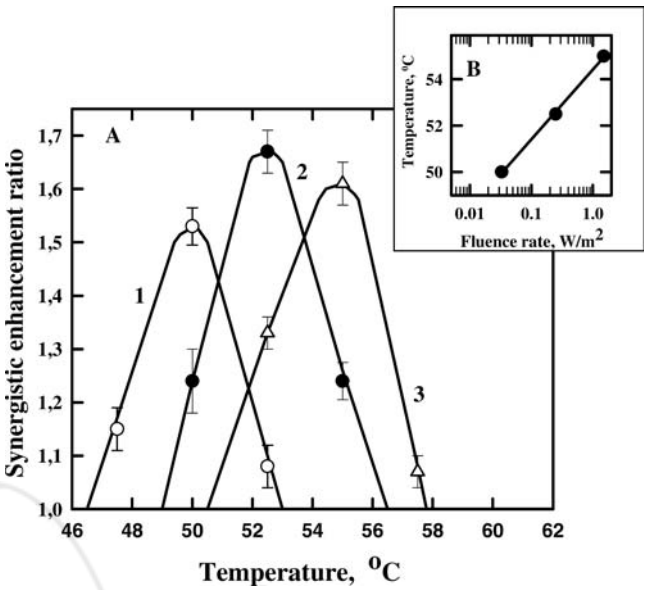

Figure 5. The results of a simultaneous action of UV light (254 nm) and high temperature on the inactivation of diploid *S. cerevisiae* wild-type yeast cells, strain XS800. A: The dependence of the synergistic enhancement ratio on the exposure temperature and UV light fluence rate (curve 1, 0.033 W m^{-2}; curve 2, 0.25 W m^{-2}; curve 3, 1.5 W m^{-2}). B: The relationship between exposure temperature and UV light fluence rate providing the highest synergistic enhancement ratio.

increasing the inactivation effect of UV radiation, should be shifted to lower temperatures with a decrease in the fluence rate.

DISCUSSION

To interpret the results obtained in this study, a simple mathematical model of the synergistic interaction for lethal effects produced by a simultaneous combined action of UV radiation and hyperthermia (16) may be engaged. The model suggests that the synergistic interaction of UV light and hyperthermia is expected to result from some additional lethal damages arising from the interaction of some sublesions induced by both agents. These sublesions are considered ineffective when each agent is taken alone. One of the main conclusions of this model is the dependence of the synergistic effect on the ratio of lethal damages (N_2/N_1) produced by UV light (N_1) and hyperthermia (N_2) applied alone (16). In accordance with the model's consequences, within a definite range of N_2/N_1, the synergistic enhancement ratio increases, reaches its highest value and then decreases with the N_2/N_1 increasing. It could be expected that, for any constant exposure time and temperature, the decrease in the fluence rate would result in the reduction of N_1 under an unchanged N_2. To preserve the same effectiveness of the synergistic interaction, the number of thermal damages, N_2, *i.e.* the acting temperature should be decreased. Therefore, the decrease in the fluence rate of the UV radiation would result in a shifting of the temperature range, synergistically increasing the cell inactivation, towards the more lower temperatures. Exactly such a dependence was obtained here for cell inactivation (Figs. 4 and 5).

For the recombinogenic test, in contrast to cell inactivation, the synergistic enhancement ratio did not pass through a maximum value and was linearly related with the temperature. It can be explained by the fact that the mitotic crossing-over frequency would have increased to a peak at moderate exposure doses and then decreased rapidly (19). That is why in our investigation we tested only relatively low doses of UV exposure within which the frequency of the recombination and the synergistic enhancement ratio were linear functions of the UV dose and exposure temperature. For the recombinant yield the temperature range synergistically increasing the effect of UV radiation was shifted to lower temperatures with a decrease in the fluence rate (Fig. 2).

The assumptions underlying the model discussed have an abstract pattern and are not related to a particular cytotoxic modality, either the nature of its effective damage or sublesion, so the model may have an application for the prediction of the synergistic interaction of various inactivating or mutagenic factors (16,23). We can adapt this model to explain the data observed for the recombinogenic effects. According to the model, additional recombinogenic damage would result from the interaction of some pre-recombinogenic sublesions, which are ineffective when each agent is applied separately. It is worth noting that the nature of the supposed sublesions and the mechanism of their interaction may be different for the lethal and recombinogenic effects and still remain to be elucidated. Let N_1 and N_2 be the numbers of recombinogenic events induced by the UV light and high temperature, respectively. We believe that even for the case when $N_2 < 1$ (ineffectiveness of hyperthermia to induce mitotic recombination) there are some sublesions produced by hyperthermia to provide the synergistic effects observed. Then the following predictions of the synergy modification should be expected. First, an increase in the synergistic enhancement ratio with an increase in the exposure temperature for any constant exposure time and fluence rate of UV light (the increasing of N_2 at the constant of N_1) should be observed. Just such a dependence of the synergistic enhancement ratio as a function of the exposure temperature was observed in this study (Fig. 2). For the same exposure time and temperature, the decrease in the fluence rate resulted in a reduction of the effective

damages produced by UV light (N_1). Secondly, to maintain the same synergistic effectiveness with a decreasing UV light intensity the temperature of the hyperthermia treatment should be lowered to decrease the amount of thermal lesions (N_2). Exactly such results were observed in this study (Fig. 2).

Therefore, for a long duration of interaction, which is important for problems of UV light protection and health effects, one would expect a synergistic interaction of this factor with ambient temperatures. From here, the biological meaning and significance of the results obtained in this study are readily apparent. Despite the fact that all of the data were received for yeast cells and temperatures far beyond the physiological range, the data may, in principle, serve as an indicator that low UV light intensities existing in the biosphere may synergistically interact with environmental heat or physiological temperatures of homoiothermal animals and men and thereby increase their inactivating and genetic consequences.

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REFERENCES

1. Martignoni, K. D. and K. C. Smith (1973) The synergistic action of ultraviolet- and X-radiation on mutants of *Escherichia coli* K-12. *Photochem. Photobiol.* **18**, 1–8.
2. Holmberg, M. and R. Strausmains (1983) The repair of chromosome aberrations in human lymphocytes after combined irradiation with UV-radiation (254 nm) and X-rays. *Mutat. Res.* **120**, 45–50.
3. Ansari, A. S. and R. Ali (1984) Synergistic action of ultraviolet radiation and hydrogen peroxide on citrulline. *J. Radiat. Res.* **25**, 283–289.
4. Hoerter, J. and A. Eisenstark (1988) Synergistic killing of bacteria and phage by polystyrene and ultraviolet radiation. *Environ. Mol. Mutagen.* **12**, 261–264.
5. Tyrrell, R. M. (1976) Synergistic lethal action of ultraviolet-radiations and mild heat in *Esherichia coli*. *Photochem. Photobiol.* **24**, 345–351.
6. Joshi, D. S., J. Haveman and G. W. Barendsen (1984) Influence of hyperthermia on the effectiveness of UV-radiation for induction of reproductive death of cultured mammalian cells. *Indian J. Exp. Biol.* **22**, 248–250.
7. Jagger, J. (1967) *Introduction to Research in Ultraviolet Photobiology*. Prentice-Hall, Englewood Cliffs, NJ.
8. Kim, J. K., V. G. Petin and G. P. Zhurakovskaya (2001) Exposure rate as a determinant of synergistic interaction of heat combined with ionizing or ultraviolet radiations in cell killing. *J. Radiat. Res.* **42**, 361–365.
9. Petin, V. G., J. K. Kim, G. P. Zhurakovskaya and I. P. Dergacheva (2002) Some general regularities of synergistic interaction of hyperthermia with various physical and chemical inactivating agents. *Int. J. Hyperth.* **18**, 40–49.
10. Trujillo, R. and V. L. Dugan (1972) Synergistic inactivation of viruses by heat and ionizing radiation. *Biophys. J.* **12**, 92–113.
11. Reynolds, M. C. and J. P. Brannen (1973) Thermal enhancement of radiosterilization. In *Radiation Preservation of Food*, pp. 165–176. International Atomic Energy Agency, Vienna.
12. Dugan, V. L. and R. Trujillo (1975) Heat-accelerated radioinactivation of attenuated poliovirus. *Radiat. Environ. Biophys.* **12**, 187–195.
13. Ben-Hur, E. (1976) Mechanisms of the synergistic interactions between hyperthermia and radiation in cultured mammalian cells. *J. Radiat. Res.* **17**, 92–98.
14. Petin, V. G. and G. P. Zhurakovskaya (1995) The peculiarities of the interaction of radiation and hyperthermia in *Saccharomyces cerevisiae* irradiated with various dose rates. *Yeast* **11**, 549–554.
15. Petin, V. G., G. P. Zhurakovskaya and L. N. Komarova (1997) Fluence rate as a determinant of synergistic interaction under simultaneous action of UV light and mild heat in *Saccharomyces cerevisiae*. *J. Photochem. Photobiol. B: Biol.* **38**, 123–128.
16. Petin, V. G., J. K. Kim, G. P. Zhurakovskaya and A. V. Rassokhina (2000) Mathematical description of synergistic interaction of UV light and hyperthermia for yeast cells. *J. Photochem. Photobiol. B: Biol.* **55**, 74–79.
17. Shore, R. E. (1990) Overview of radiation-induced skin cancer in humans. *Int. J. Radiat. Biol.* **57**, 809–827.
18. Kiefer, J., J. Müller and J. Götzen (1988) Mitotic recombination in continuously γ-irradiated diploid yeast. *Radiat. Res.* **113**, 71–78.
19. Pakhomova, O. N. and T. S. Tsyb (1994) Mutagenous effect and the mitotic crossing-over induction in yeasts under combined exposure to alpha-particles and gamma-rays. In *Molecular Mechanisms in Radiation Mutagenesis and Carcinogenesis*, (Edited by K. H. Chadwick, R. Cox, H. P. Leenhouts and J. Thacker), pp. 203–206. European Commission, Brussels.
20. Petin, V. G., J. K. Kim, A. V. Rassokhina and G. P. Zhurakovskaya (2001) Mitotic recombination and inactivation in *Saccharomyces cerevisiae* induced by (254 nm) radiation and hyperthermia depend on UV fluence rate. *Mutat. Res* **478**, 169–176.
21. Zacharov, I. A., S. V. Kovaltsova and S. V. Marfin (1979) Strains of yeast *Saccharomyces cerevisiae* for studying induced mutagenesis and recombination. *Genetics* **15**, 41–48. [In Russian]
22. Kim, J. K., V. G. Petin and M. D. Tkhabisimova (2004) Survival and recovery of yeast cells after simultaneous treatment of UV light radiation and heat. *Photochem. Photobiol.* **79**, 349–355.
23. Golovach, I. N., L. N. Komarova, G. P. Zhurakovskaya and V. G. Petin (1998) Prediction and optimization of synergism in the combined action of ultrasound and hyperthermia on yeast cells. *Acoustical Phys.* **44**, 297–300.

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Mathematical description and prognosis of synergistic interaction of radon and tobacco smoking

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Background: Radon and its short-lived decay products are considered as the important sources of public exposure to natural radioactivity. The synergistic interaction between tobacco smoking and radon is known to be an actual problem. This study has provided a mathematical description and prognosis of the carcinogenic effects after combined action of radon with smoking. ***Materials and Methods:*** A simple mathematical model was adjusted for the optimization and prognosis of the synergistic interaction of radon with smoking. The model postulates that the occurrence of synergism is to be expected as a result of additional carcinogenic damage arising from the interaction of sublesions induced by the two agents under consideration. ***Results:*** The predictions of the model were verified by comparison with experimental data published by other researchers. The model appears to be appropriate and the predictions valid. ***Conclusion:*** The suggested mathematical model predicts the greatest level of synergistic effect and condition under which this level is reached. The synergistic effect appeared to decline with any deviation from the optimal value of the ratio of carcinogenic effective damages produced by each agent alone. **Iran. J. Radiat. Res., 2007; 4 (4): 169-174**

Keywords: *Radon, smoking, synergistic interaction, mathematical model, carcinogenesis.*

INTRODUCTION

A certain level of background exposure to ionizing radiation and natural or man-made chemicals is always presented in the environment. Thus, all assessments of toxicity, carcinogenicity or mutagenicity of chemical, physical and biological agents should be performed as studies on their combined effects. For example, people are exposed to a indoor radon concentration combined at most time with other agents including tobacco smoking. It is well known from epidemiological and toxicological studies that the kind of interaction which occurs between tobacco smoking and radon may be synergistic, i.e. can lead to the effects, the sum of which is greater than that expected from the addition of the effects from separate exposures to the individual agents [1-3]. This problem is especially important for high natural background areas [4]. A new concept of the synergistic interaction and a simple mathematical model based on this conception has been developed earlier to predict and optimize the synergy when hyperthermia was simultaneously applied together with any of the following modalities: ionizing radiation, ultrasound, ultraviolet light, microwaves and some chemical agents [5-7]. The model suggests that the synergism is expected from some additional effective lesions arising from the interaction of sublesions induced by both agents. These sublesions are considered ineffective after each agent is taken alone. The model predicts the dependence of the synergistic interaction on the ratio of effective lesions produced by each agent applied the greatest value of the synergy as well as the condition under which it can be achieved. However, the model was mainly tested for inactivation of cells differing by their origin. Therefore, it would be of interest to accommodate this model for the description, optimization and prognosis of synergistic effects between tobacco smoking and radon. Thus, this study was designed to implement two purposes: (a) to adjust a

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mathematical model to describe the synergistic interaction between radon and tobacco smoking; (b) to test the validity of the proposed model.

MATERIALS AND METHODS

In the estimation of the synergistic effect as regard to carcinogenic action, the synergistic enhancement ratio (k) can be defined as the ratio of the number of cancer cases registered due to the combined action of two carcinogenic factors (N_{comb}), to the sum of the number of the carcinogenic events resulting from each agent considered separately:

$$k = \frac{N_{comb}}{N_1 + N_2} \qquad (1)$$

where N_1 and N_2 are the numbers of cancer cases induced by radon and tobacco smoking, respectively. We note that the number of cancer cases is in direct proportion to hypothetical effective damages finally responsible for the carcinogenic events. Then the synergistic enhancement ratio defined by equation (1) reflects the ratio of the corresponding effective damages induced by the combined action of both agents (N_{comb}) to the sum of the effective damages produced by radon (N_1) and tobacco smoking (N_2).

The model does not apply such a value as a dose from an individual agent, since it is designed for the description of the combined effects of agents which can be completely different by their nature and action mechanism. However, this model can deal with different factors, as it operates with non-dimensional ratio of the effective damages produced by the agents under consideration. The model suggests that carcinogenic agents, in addition to the effective damages resulting from the carcinogenic events, produce some precarcinogenic sublesions. Let us assume that p_1 and p_2 are the numbers of sublesions that occur per a single effective damage induced by ionizing radiation and tobacco smoking, respectively. It should be pointed out at this stage, that the applied model does not postulate or define the nature or type of neither effective damage nor sublesions which occur. Thus this can be of any type. What the model does offer is an empirical mean for their quantitative estimation. These precarcinogenic sublesions are considered to be non-effective, when each agent is applied separately. Additional carcinogenic effective damages responsible for the synergy are assumed to arise from the interaction of precarcinogenic sublesions. As the first step of modelling, let us assume that one sublesion resulting from one individual agent reacts with one sublesion produced by another agent, thus producing an additional effective damage. In other words, the additional carcinogenic damage is produced during the combined action of agents, as a result of the interaction of these precarcinogenic sublesions. Thus, in terms of the model presented by equation (1), the synergistic enhancement ratio, k, may be given by:

$$k = \frac{N_1 + N_2 + \min\{p_1 N_1 ; p_2 N_2\}}{N_1 + N_2} = 1 + \frac{\min\{p_1 ; p_2 N_2 / N_1\}}{1 + N_2 / N_1} \qquad (2)$$

Here, the term $\min\{p_1N_1;p_2N_2\}$ is a minimal value from two variable quantities, p_1N_1 and p_2N_2, which determine the numbers of additional effective damages arising due to the interaction between precarcinogenic sublesions produced by radon (p_1N_1) and tobacco smoking (p_2N_2). It can be easily demonstrated that, if the cancer induction under combined action of agents is mainly produced by tobacco smoking, or in other words $p_1N_1<p_2N_2$, then the parameter p_1 may be represented as follows:

$$p_1 = (k-1)(1 + N_2 / N_1) \qquad (3)$$

In contrast, if the cancer events are mainly induced by the action of ionizing radiation $p_1N_1>p_2N_2$, the parameter p_2 can be determined by the following equation:

$$p_2 = (k-1)(1 + N_1 / N_2) \qquad (4)$$

It can be demonstrated in a purely

mathematical means that under condition

$$p_1N_1 = p_2N_2 \qquad (5)$$

the synergistic interaction should be characterized by the greatest value and can be calculated as follows:

$$k_{max} = 1 + \frac{p_1p_2}{p_1 + p_2} \qquad (6)$$

Thus, using equations (3) and (4), one can estimate, on the basis of real experiments, the basic model parameters p_1 and p_2. Then the synergistic enhancement ratio for any ratio of N_2/N_1, its greatest value, and the condition under which this is yielded can be derived from equations (2), (6), and (5), respectively.

RESULTS

In order to apply the model to experimental data, it is necessary to define two basic parameters p_1 and p_2. Each of these is calculated for such experimental conditions, when one of the agents induces more precarcinogenic sublesions then another. Two sets of epidemiological data are available for a definition of the interactions between radon and tobacco smoke interaction-miner and residential study data. The first set of data, i.e. miner data, describes a situation in which the subjects were exposed to high radon concentrations, and here the impact of the action of radon on the lung cancer induction rate is greater than that of the smoke inhalation component. Knowledge of such a condition is necessary in order to define the model parameter p_2. In this case, $\min\{p_1N_1;p_2N_2\}=p_2N_2$ and the synergistic enhancement ratio is calculated according to the following equation:

$$k = 1 + \frac{p_2N_2/N_1}{1 + N_2/N_1} = 1 + \frac{p_2}{1 + \frac{1}{N_2/N_1}} \qquad (7)$$

According to this Equation, the value for k is increased in relation to the increase in that for N_2/N_1. In other words, the model predicts that the synergistic enhancement ratio increases with an increase in the ratio of N_2/N_1 until the inequality $p_1N_1>p_2N_2$ holds.

In order to test this prognosis, we used a survey consisting of periodic sputum cytology evaluation and which was performed among 249 underground uranium miners and 123 controls [1]. Cytological sputum samples yielding moderate atypia, marked atypia, or the presence of cancer cells were classified as being abnormal. To estimate the synergistic enhancement ratio, data on both the combined action of the agents and on the separate action of each individual agent is required. Band *et al.* [1] presented data for the combined action of agents and the effects of tobacco smoke inhalation on lung cancer induction in miners for a range of radon concentrations (from less than 2.6 kBq/m^3 to more than 15.7 kBq/m^3). However, if the real epidemiological results for the combined action of radon and tobacco smoke in cancer induction (N_{comb} values) were listed for all 5 conditions investigated (<2.6, 2.7-5.3, 5.4-10.4, 10.5-15.7 and >15.7 kBq/m^3), the data for radon action alone (not smoking miners) was presented only for two concentration levels, and one of them was obtained for the minimal radon concentration when there was no synergistic interaction at all. The residuary point (5.4-10.4 kBq/m^3) may be characterized as follows. In accordance with published results [1], the frequencies of abnormal cytology were 5% after radon exposure alone (N_1), 7% after cigarette smoking alone (N_2) and 28% after combined action of these modalities (N_{comb}). Then the expected frequency should be 12% for independent action of these agents (N_1+N_2) and in terms of the model discussed, the ratio $N_2/N_1=1.4$ and the synergistic enhancement ratio k=2.3. This value was calculated by using equation (1). This point is depicted on the left-hand part of the curve presented in figures 1A and 1B. Having used this result and equation (4), we have $p_2= 2.3$. Taking into consideration this value and equation (7) that would correct for the condition $p_1N_1>p_2N_2$, the theoretically predicted dependence of the synergistic enhancement ratio on the ratio of N_2/N_1 was calculated. The result is presented

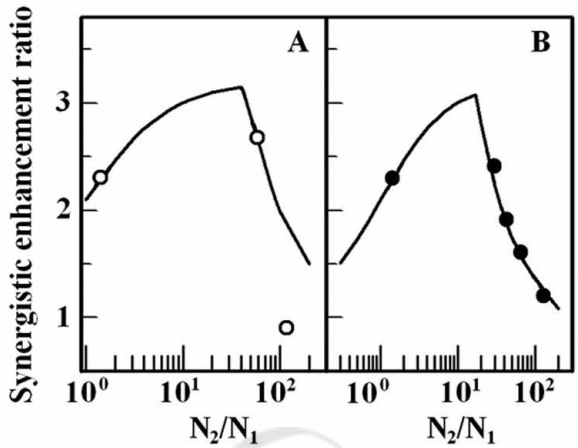

Figure 1. The dependence of the synergistic enhancement ratio on the ratio of the effective damages produced by tobacco smoking (N_2) and radon (N_1).

in figures 1A and 1B by the left-hand part of the solid curve.

The residential study data represent situations in which people are exposed to relatively low radon concentrations, as compared to those of the affected miners. It may be supposed that in this case, tobacco smoke is the main factor responsible for the lung cancer induction. This hypothesis is supported by the data published by Pershagen *et al.* [3]. In this case, $\min\{p_1N_1;p_2N_2\}=p_1N_1$, i.e. tobacco smoke induces a larger amount of precarcinogenic sublesions. Such parameter conditions are necessary to define the model parameter p_1 on the basis of equation (3). The synergistic enhancement ratio in this case can be calculated as

$$k = 1 + \frac{p_1}{1 + N_2/N_1} \qquad (8)$$

According to this Equation, the value for k is decreased in relation to the increase in that for N_2/N_1. In other words, the model predicts that the synergistic enhancement ratio decreases with the increase in the ratio of N_2/N_1 for which the inequality $p_1N_1<p_2N_2$ holds. In order to test this prediction, we used the results published by Pershagen *et al.* [3]. Because of relatively high scattering of the original data [3], we employed two manners to evaluate the parameter of p_1. For the first one, we used two concentrations of radon: 51-80 and >400 Bq/m^3; at the least radon concentration, the synergistic effect was minimal, while for the high radon concentration it had the greatest value. In accordance with the original data [3] for radon concentration >400 Bq/m^3, the relative risk (except for the background risk) was 0.2 after exposure to radon alone (N_1), 11.6 after tobacco smoking alone (N_2) and 31.5 after combined action of both agents (N_{comb}). In this case we assumed the relative risk to be proportional to the number of effective damages responsible for the carcinogenic effects. Under such a supposition all the equations derived above can be applied. Then the expected number of the effective damage should be 11.8 for independent action of these agents (N_1+N_2), the ratio N_2/N_1= 58 and in accord with equation (1), the synergistic enhancement ratio k= 2.7. This data point is depicted on the right-hand part of the curve presented in figure 1A. Having used this result and equation (3), we have p_1=100. Taking into consideration this value and equation (8) that would correct for the condition $p_1N_1<p_2N_2$, the theoretically predicted dependence of the synergistic enhancement ratio on the ratio of N_2/N_1 was calculated. The result is presented in figure 1A by the right-hand part of the solid curve. The second data point at this part of the curve was evaluated by a similar way for radon concentration of 51-80 Bq/m^3. It is

clear that this result does not correspond to the theoretically predicted curve.

The originally published results [3] clearly display linear dose-effect dependence but also reveal some deviations from the general trend. To avoid this we applied a linear extrapolation to the data reflecting lung cancer incidence rate among the residents who were exposed only to radon. Table 1 summarizes the results of such a calculation. The obtained results are depicted by points on the right-hand part of the curve presented in figure 1B. The value of the parameter p_1 of the model can be estimated from any of the data points obtained. For example, by using equation (3) we have p_1=39.5 for a radon concentration of 140-400 Bq/m^3. Taking into consideration this value and equation (8) that would correct for the condition $p_1N_1<p_2N_2$, the theoretically predicted dependence of the synergistic enhancement ratio on the ratio of N_2/N_1 was calculated. The result is shown in figure 1B by the right-hand part of the solid curve. Taking the data presented in figure 1B as a whole, one can conclude that the theoretically predicted curves correspond well with the data points regarding to the dependence of the synergistic enhancement ratio on the ratio of the effective damage produced by tobacco smoking and radon.

Table 1. Model parameters and experimental values of the synergistic enhancement ratio.

Radon concentration, Bq/m^3	Relative risk (except for the background risk)				k_{exp}	N_2/N_1
	Radon	Tobacco smoking	N_{comb}	$N_1 + N_2$		
	N_1	N_2				
<50		9.00				
51-80	0.07		11.3	9.07	1.25	129
81-140	0.15		14.3	9.15	1.56	60
141-400	0.22		17.9	9.22	1.94	41
>400	0.31		22.5	9.31	2.42	29

DISCUSSION

Thus, the data presented here indicated that the mathematical model for synergism which was previously proposed and tested mainly for the description of cell inactivation, can also be used to describe the carcinogenic effects of the combined action of radon exposure and tobacco smoke inhalation. The main difference in the proposed model when compared to the existing ones [8, 9] is the assumption that synergism can be accounted for in terms of the interaction between putative precarcinogenic sublesions, with the frequency of the occurrence of these lesions being proportional to the number of cancer cases. It's important, however, that no attempt is made in the model to apply an assumption on the molecular nature of the precarcinogenic sublesions and the mechanism of their interaction. Nevertheless, the model enables us to obtain some non-trivial results-it predicts the synergistic enhancement ratio for any ratio of the effective damages produced by a combination of tobacco smoking and radon as well as the greatest value of the synergistic effect and the condition under which this is yielded. .

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REFERENCES

1. Band P, Feldstein M, Saccomanno G, Watson L, King G (1980) Potentiation of cigarette smoking and radiation: evidence from a sputum cytology survey among uranium miners and controls. *Cancer,* ***45****: 1273-1277.*
2. Chameaud J, Perraud R, Chretien J, Masse R, Lafuma J (1982) Lung carcinogenesis during in vivo cigarette smoking and radon daughter exposure in rats. *Recent Results in Cancer Res,* ***82****: 11-20.*
3. Pershagen G, Åkerblom G, Axelson O, Clavensjö B, Damber L *et al.* (1994) Residental radon exposure and lung cancer in Sweden. *N Engl J Med,* ***330****: 159-164.*
4. Monfared AS, Jalali F, Sedaghat S, Mansoorizade E, Jarrahi AM, Hajiahmadi M, Samavat H (2006) High natural background radiation areas, Iran: can inhabitants feel safe? *Int J Low Radiat,* ***3****: 171-177.*
5. Petin VG and Komarov VP (1997) Mathematical description of synergistic interaction of hyperthermia and ionizing radiation. *Mathem Biosci,* ***14****: 115-130.*
6. Kim JK and Petin VG (2002) Theoretical conception of synergistic interactions. *Kor J Environ Biol,* ***20****: 277-286.*
7. Petin VG, Zhurakovskaya GP, Kim JK (2005) Synergetic effects of different pollutants and equidosimetry. In: *Equidosimetry-Ecological Standardization and Equidosimetry for Radioecology and Environmental Ecology,* (Bréchignac F and Desmet G, eds.), Springer, Dordrecht, The Netherlands, pp. 207-222.
8. Moolgavkar SH and Knudson AG, Jr (1981) Mutation and cancer: a model for human carcinogenesis. *J Natl Cancer Inst,* ***66****: 1037-1052.*
9. Chadwick KH, Leenhouts HP, Brugmans MJ (2002) Implications of the analysis of epidemiological data using a two-mutation carcinogenesis model for radiation risks. *Radiat Prot Dosimetry,* ***99****: 265-268.*

방사선과 수은에 의한 자주달개비 체세포 돌연변이

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Somatic Mutations of *Tradescantia* 4430 Treated with Radiation and Mercury

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Abstract − *Tradescantia* 4430 clone is an interspecific hybrid made by artificially crossing *T. hirsutiflora* with *T. subacaulis*. It has hereditary heteromorphy in its flower color, blue dominant and pink recessive. The hybrid clone is exttremely sensitive to ionizing radiation and chemical mutagens. Groups of inflorescence cuttings were irradiated with 0.3, 0.5, 1.0, 1.5 and 2.0 Gy. After irradiation, the cuttings were maintained at 24°C under a controlled light:dark (14 : 10) cycle. Five days after irradiation, mutations started to increase rapidly and reached a maximum rate during 8～10 days after irradiation. Mutation frequencies increased with radiation dose and with mercury concentration. In conclusion irradiation as well as mercury caused a dose- or concentration-response relationship in the somatic mutation frequencies of *Tradescantia* 4430. *Tradescantia* 4430 proved to be a good material for studying the combined effect of radiation and mercury.

Key words : Radiation, Mercury, *Tradescantia* bioassay, Dose-response relationship

서 론

이온화 방사선은 생물체에 대하여 다양한 반응을 유발하며 그 반응의 정도는 선량에 따라 다르다. 생물체를 이루는 다양한 세포들 중에는 방사선에 대한 민감도가 높아 특히 영향이 잘 나타나는 것들이 있다. 자주달개비 속 (genus *Tradescantia*)에는 많은 수의 자연종이 속해 있으며 또한 이들 종의 생물학적 속성을 특이하게 변화시킨 인위적 종류들이 속해있다. 일부 클론들은 그들이 지니고 있는 생물학적 특성 때문에 이온화 방사선을 포함한 돌연변이 유발원에 노출될 경우 쉽게 체세포 돌연변이를 일으킨다. *Tradescantia BNL · 4430* (T-4430) 클론은 남색 꽃을 피우는 종인 *T . hirsutiflora*와 분홍색 꽃을 피우는 종인 *T . subacaulis*를 인위적으로 교잡시켜 만든 종간 잡종으로 꽃색에 있어서 유전적 이형성을 띄며 정

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상적으로 우성인 남색 꽃이 핀다(김 등 2000).

T-4430의 수술털은 꽃색의 이형성에 의하여 실제로 사람이 있어서 환경에서 접하게 되는 낮은 준위의 이온화 방사선과 다양한 화학 물질의 유전적 효과를 감지하는데 가장 적합한 실험재료로 사용되고 있다. 1960년대 후반에 개발된 자주달개비 수술털 분석법과 1970년대 후반에 개발된 자주달개비 미세핵 분석법은 현장실험과 실험실 실험에 폭넓게 사용되고 있다(김 등 2002; Wang *et al.* 1999). 자주달개비 수술털의 선단 세포는 분열능력이 있어서 발생 또는 분열과정 중 돌연변이원에 노출되면 쉽게 체세포 돌연변이를 일으킨다(Arutyun- yan *et al.* 1999). 돌연변이원이나 발암원에 의해 꽃색의 우성인자가 존재하는 염색체 부분의 결실이 일어날 경우 상동염색체 상에 존재하는 열성형질이 발현되어 꽃잎과 수술털의 전체 또는 일부분이 분홍빛을 띈다. 한편, 상동염색체의 양쪽 모두가 결실되어 색소발현능력이 소실되면 무색의 수술털 세포가 생기기도 하며 때로는 선단세포의 분열능력 상실로 인한 수술털 세포수 감소로 인해 수술털이 비정상적으로 짧아지는 경우에도 나타난다(허 등 2003; Sadao *et al.* 1996).

자주달개비 꽃 속에 있는 6개의 수술은 각각 약 25개의 세포로 이뤄진 70개 정도의 털을 가지고 있으므로 하나의 꽃은 10,000개 이상의 수술털 세포를 가지고 있는 경우가 대부분이다. 수술털은 처음 한 개의 세포가 연속적으로 분열하는 과정에서 자연적으로 발생하는 돌연변이에 의해 10,000 세포 당 1～2건의 분홍 돌연변이 세포가 나타나기도 하는데 방사선과 화학적 돌연변이원 같은 외부 자극을 부가적으로 받게 되면 분홍 돌연변이의 발생이 더욱 늘어나게 되며, 특히 꽃이 만들어 지는 초기에 돌연변이가 일어날 경우는 꽃 전체의 색깔이 분홍으로 바뀌기도 한다. 이러한 특성이 자주달개비를 방사선 지표식물로 불리게 하는 과학적 근거가 되었고 수많은 방사선생물학적 연구 특히 저준위 방사선의 생물학적 반응연구에 자주달개비가 효과적으로 이용되어 왔다. 자주달개비 꽃과 수술털 세포의 돌연변이를 이용하여 방사선 반응을 검출하는 경우 방사선 측정기를 사용하여 방사선량을 계측하는 것과 같이 적은 양의 방사선까지 정밀한 수치로서 표현해 낼 수는 없다. 그러나 자주달개비의 분홍 돌연변이 분석을 통해 아주 낮은 방사선량까지 감지 할 수 있다. 자주달개비의 자연발생적 체세포 돌연변이 빈도는 사람 림프구의 돌연변이 자발빈도인 10^{-3}～10^{-7}과 같은 수준이다(김 등 1998; Kim *et al.* 1998). 한편 낮은 방사선량율과 관련한 보고도 있다. Mericle *et al.* (1965) 은 자주달개비 클론 02를 이용하여 250 μRh^{-1} 의 선량률로 2주간 조사시 돌연변이율이 증가한 결과를 보고하였다. 한국원자력연구소 금곡농장의 감마선장에서 수행된 T-4430 분홍 돌연변이 실험에서 150 μRh^{-1}의 공간 방사선량률에 대해서도 유의한 수준의 분홍 돌연변이율 증가를 나타냈다(김 등 1998). 이러한 자주달개비 수술털 세포의 돌연변이는 환경내의 모든 요인의 영향을 반영한 결과이기 때문에 포괄적인 의미에서의 생물학적 안전성을 밝히는 데는 기계적, 물리적 방법을 능가하는 장점이 있다. 실제로 일본, 폴란드 등지에서는 자주달개비를 이용한 환경방사선 감시사례가 보고된 바 있다. Ichikawa (1981) 는 일본의 여러 원자력 발전소 주변에서 자주달개비의 분홍 돌연변이율을 이용하여 환경방사선의 준위변동을 발전소 가동기간과 연계하여 감시하였다. 한편 Cebulska-Wasilewska (1992) 는 소련 체르노빌 원자력 사고가 발생한 직후 폴란드 크라쿠프 지역의 방사능오염 공기의 생물학적 영향을 자주달개비 돌연변이율로 조사하기 시작하여 사고 1년 경과시의 돌연변이율과 비교하여 공기 중 환경방사선의 준위변동을 감시하였다.

국내에서는 자주달개비 T-4430 클론을 이용하여 수술털 체세포 돌연변이에 있어서 중성자의 선량-반응과 생물학적 효과비 검증, 방사선과 환경인자에 의한 체세포 돌연변이 빈도 및 환경방사선 감시 등의 연구가 수행된 바 있다(김 등 1999). 따라서 본 연구에서는 이온화 방사선과 염화수은의 복합처리에 따른 생물학적 영향을 평가하기 위하여 T-4430을 실험재료로 이용하여 수술털 세포에 나타나는 체세포 돌연변이 빈도 변화를 분석하였다.

재료 및 방법

1. 재료

실험에 사용되는 식물체는 방사선에 민감하게 반응하면서도 자발돌연변이율이 낮은 종간 잡종 T-4430 클론을 사용하였다. T-4430 식물체의 꽃차례를 15 cm 길이로 절취하여 실험실에서 24시간 순치시킨 다음 14시간 명기, 10시간 암기의 조건으로 인공조광을 실시하고 온도는 24°C로 유지하였다. 평균 15개씩의 화서를 하나의 실험군으로 하여 분석가능한 화아의 수를 200개 이상으로 하였다.

2. 방사선 조사

한국원자력연구원 저준위 조사시설 (Panoramic Irradiaitor, AECL)의 ^{60}Co 선원을 이용하여 감마선을 조사하

였다. 선원강도는 111 TBq였으며 선량률의 측정은 Friche 선량계를 이용하였다. 상온, 공기 중에서 실험군별로 0, 0.3, 0.5, 1.0, 1.5 및 2.0 Gy의 감마선을 조사하였다.

3. 염화수은의 처리

$HgCl_2$ (Sigma)를 희석하여 수은 농도를 각기 0, 0.2, 0.25, 0.3 mM 로 맞춘 Hyponex® 1,000 배 희석 용액에서 배양하고, 배양용기에는 기포발생기를 각각 설치하여 폭기를 실시하였으며 1일 간격으로 동일한 농도의 양액을 교체 · 공급하였다.

4. 돌연변이 검경 · 계수

만개상태의 화기(化器)를 실험군별로, 채화하고 6개의 수술 모두를 떼어내서 파라핀유가 도말된 슬라이드 상에 펴서 검경용 프레파라트를 제작하였다. 그 다음 실체현미경 (Nikon, 배율 ×100)을 이용하여 돌연변이를 계수하여 100개의 수술털 당 돌연변이 건수로서 돌연변이 빈도를 산정하였다. 한 개 또는 다수의 연속된 변이세포군 각각을 하나의 돌연변이 건수로 계수하였으며 한 개의 정상세포로 분리되어 있는 분홍 세포군의 경우 정상세포 양쪽을 각각 건수로, 한 수술털 전체가 분홍 또는 무색 세포로 이뤄진 경우이거나 수술 전부 또는 꽃 전체가 분홍 또는 무색 세포로 이뤄진 경우도 한 건의 돌연변이로 계수하였다. 또한 수술털을 이루고 있는 세포의 수가 16개 이하이면 이를 하나의 치사돌연변이 건수로 계수하였다.

결과 및 고찰

1. 수은 처리에 의한 생육 영향

수은 처리가 실험 생물체인 자주달개비의 생육에 영향을 미칠 경우 돌연변이 빈도 분석 결과에도 큰 영향을 미칠 수 있다.

염화수은 농도가 0, 0.2, 0,25 및 0.3 mM 네 개의 실험군에 대하여 꽃당 평균 수술털 수를 계수하였다. 염화수은 0, 0.2 및 0.25 mM 처리 실험군은 평균 수술털 수가 40～50개를 유지하였으나, 0.3 mM 처리 실험군에서는 시간이 경과함에 따라 수술털 수가 계속 감소하였고 처리 9일 째부터는 개화한 꽃을 볼 수가 없었다 (Fig. 1). 염화수은 농도 0.25 mM 이하를 처리한 실험군에서 11～13일까지 수술털 수가 약간 감소하는 경향을 나타내긴 했지만 분석결과에 영향을 미칠 정도의 생육저해는 나타나지 않았다. 실험 식물체인 자주달개비의 생육에 저해를 주지 않고 실험기간 중 돌연변이 값을 얻을 수 있는 적정 염화수은 농도는 0.25 mM 이하라는 것을 확인되었다.

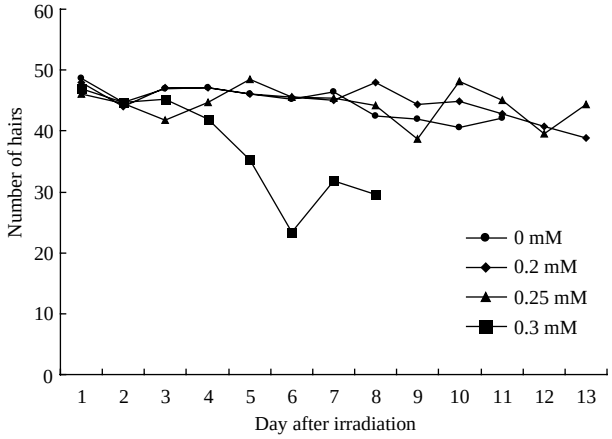

Fig. 1. Average number of hairs in flowers of T-4430 treated with different concentrations of mercury chloride.

2. 분홍 돌연변이

자주달개비 수술털은 꽃색의 이형성에 의하여 실제로 사람에게 있어서 환경에서 접하게 되는 낮은 준위의 이온화 방사선과 다양한 화학 물질의 유전적 효과를 감지하는데 가장 적합한 실험재료 중의 하나이다 (Ichkawa 1999). 방사선에 의하여 유발되는 돌연변이는 일반적으로 방사선 조사 후 5일 경부터 증가하기 시작하여 최대값에 이른 다음 다시 감소하여 자발돌연변이율 수준까지 낮아지는 경향을 보인다. 본 실험의 경우 돌연변이 고조기간은 방사선 조사 후 7일에서 11일 사이에 나타났다. 염화수은을 함유하지 않은 양액에서 배양한 방사선 조사 실험군의 돌연변이율 변화를 Fig. 2에 나타내었다. 본 실험의 경우 분홍 돌연변이율은 방사선 조사후 6일 경부터 큰 폭으로 증가하기 시작하였으며 7일 이후 최대 값에 도달 하였다.

동일한 방사선량을 조사하고, 양액중 염화수은 농도를 다르게 하여 처리한 실험군에서도 방사선량의 증가에 따라 돌연변이율이 증가하는 경향은 뚜렷하였다. 염화수은을 처리하지 않은 실험군과 0.2 mM 에서는 분홍 돌연변이 빈도가 비슷하였으나 양액의 수은 농도가 0.25 mM인 실험군에서는 분홍 돌연변이의 빈도가 7.5로 방사선과 수은 복합작용에 의한 영향이 뚜렷하게 나타났다 (Fig. 3).

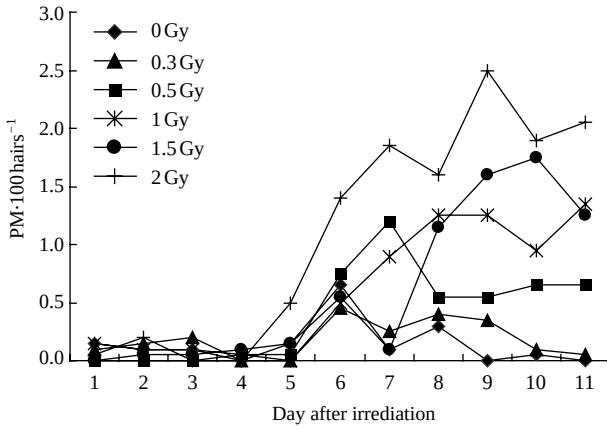

Fig. 2. Changes of pink mutation frequencies in the stamen hair cells of T-4430 irradiated with gamma rays.

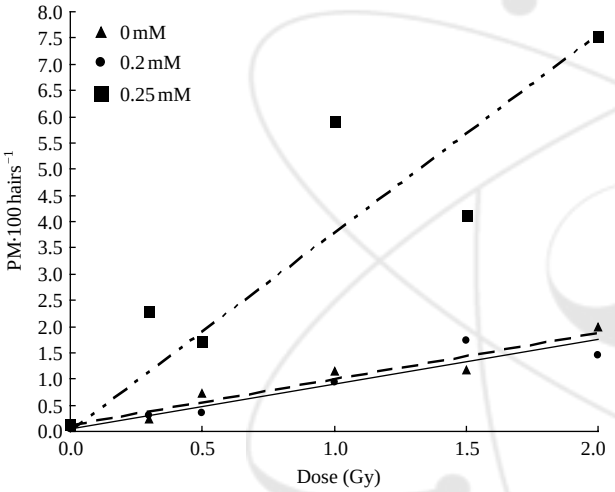

Fig. 3. Dose-response curves of pink mutation frequencies during the peak interval in T-4430 treated with different concentrations of mercury chloride.

3. 무색 돌연변이

방사선 조사 후 수은을 처리하지 않은 실험군과 0.2 mM 및 0.25 mM 수은처리 심험군에 대한 분석을 실시하였다(Table 1). 염화수은을 처리하지 않은 실험군의 경우 방사선량 0～2.0 Gy 범위에서 무색 돌연변이 빈도는 0.08～1.37이었다. 염화수은의 농도가 0.2 mM 일 때 방사선량 0～2.0 Gy 범위에서 무색 돌연변이 빈도는 최저 0.25에서 최고 1.12였다. 이와 같은 결과로 볼 때 0.2 mM 이하의 염화수은은 방사선 유발 무색 돌연변이에 미치는 영향은 미미한 것으로 생각된다. 그러나 0.25 mM 이상의 수은과 방사선을 복합 처리하였을 때의 무색 돌연변이율은 최저 0.35에서 최고 6.53을 나타냄으로써 수은에 의한 영향이 큰 것으로 평가되었다.

Table 1. Frequency of colorless mutations in T-4430 treated with 0, 0.2 and 0.25 mM mercury chloride

Radiation dose (Gy)	$HgCl_2$ (mM)		
	0	0.2	0.25
0	0.08	0.88	0.36
0.3	0.22	0.35	0.35
0.5	0.42	0.25	1.82
1.0	0.53	0.77	5.26
1.5	0.41	1.12	4.08
2.0	1.37	0.95	6.53

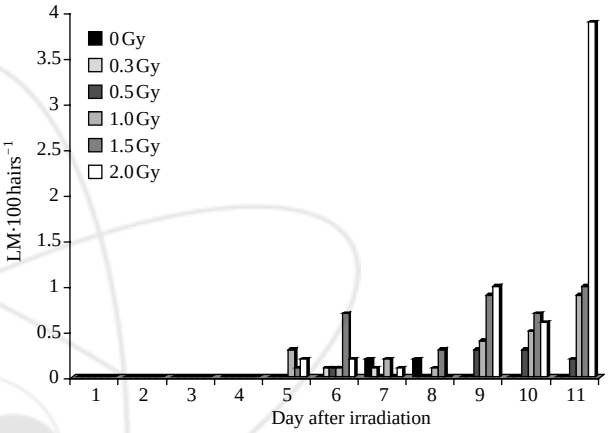

Fig. 4. Changes of lethal mutation frequencies in the stamen hair cells of T-4430 irradiated with gamma rays.

4. 치사 돌연변이

방사선 2 Gy 조사 실험군의 분석결과를 기준으로 볼 때 염화수은을 함유하지 않은 양액에서 배양한 경우 고조기간 중 치사 돌연변이 빈도 최대값은 3.9였으나 (Fig. 4), 염화수은 0.25 mM 처리군에서의 고조기간 중 최대 치사 돌연변이 빈도는 47.2였다(Fig. 5). 이와 같은 결과로 볼 때 염화수은 농도가 0.2 mM 이하인 경우 방사선과의 복합 작용에 의한 효과는 크지 않았으나 염화수은 농도가 0.25 mM일 때는 방사선과의 복합작용은 치사돌연변이를 큰 폭으로 증가시키는 것을 알 수 있다.

결 론

실제로 저선량 영역에 있어서 방사선에 의한 자주달개비 수술털 세포의 분홍돌연변이 유발은 선형적 일차함수를 이용한 선량-반응 관계식으로 해석하는 것이 정

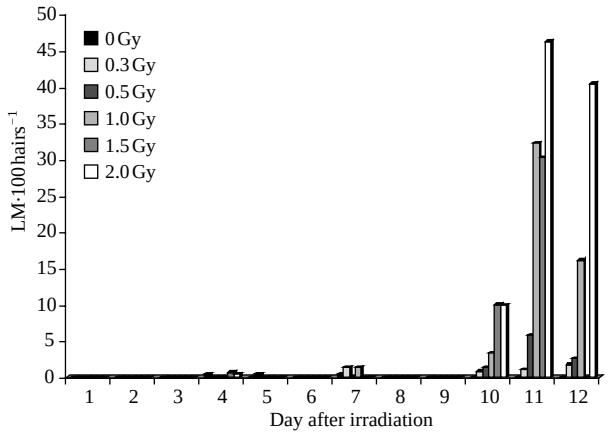

Fig. 5. Changes of lethal mutation in T-4430 irradiated with gamma-rays and treated with 0.25 mM mercury chloride.

당함은 이미 보고된 바 있다. 방사선과 수은을 복합 처리한 실험군의 돌연변이 고조기간인 6～11일 사이의 통합데이터로부터 산출한 선량-반응은 일차함수로 확인되었다. 염화수은 농도 0.2 mM까지는 방사선 유발 돌연변이에 별 영향을 주지 않았으나 수은 농도가 0.25 mM 일 때는 복합작용에 의해 돌연변이 빈도가 큰 폭으로 증가되었다. 또한 수은 농도가 0.3 mM일 때는 자주달개비의 생육 자체가 저해되므로 돌연변이 계수를 통한 생물학적 영향 평가가 불가능하거나 무의미 한 것이 확인되었다. 자주달개비의 생육을 저해하지 않는 염화수은의 농도는 0.25 mM 이하인 것으로 판단된다. 유해 화학물질인 염화수은과 방사선의 복합 작용에 의한 생물학적 영향을 평가하기 위한 실험 모델로서 종간잡종 클론인 T-4430 식물체가 효과적으로 이용될 수 있음이 본 연구를 통해 확인되었다.

사 사

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참 고 문 헌

김진규, 김원록, 김재성, 신해식, 이정주. 1998. 기온일교차와 감마선의 영향에 의한 자주달개비 수술털의 체세포 돌연변이 빈도, 한국환경생물학회지. **16**(3):253-262.

김진규, 김원록, 이창주, 장화형, 이영근. 1999. 자주달개비 수술털에서 방사선에 의해 유발되는 세포손상에 대한 살충제의 방어효과 한국환경생물학회지. **17**(1):21-26.

김진규, 신해식, 이병헌, 송희섭. 2002. 자주달개비 미세핵분석법 (Trad-MCN)을 이용한 잠재적 생물재해요인의 평가, 방재연구, **5**(1):27-36.

김진규, 천기정, 이동우. 2000. 지표생물을 이용한 방사선의 방재적 감시연구, 방재연구, 제 3권 제 1호, 12월.

허귀석, 이재환, 신해식, 김진규, 이영엽, 이대운, 이진홍. 2003. 화학공장 실내 작업장에서의 유해물질 평가를 위한 VOC 분석법과 자주달개비 미세핵 분석법의 비교, 분석과학. **16**(1):1-11.

Arutyunyan RM, Pogosyan VS, Simonyan EH., Atoyants AL and Djigardjian EM. 1999. In situ monitoring of the ambient air around the chloroprene rubber industrial plant using the *Tradescantia*-stamen-hair mutation assay, *Mutat. Res.* **426**:117-120

Kim JK, Kim WR, Kim JS, Shin HS and Lee JJ. 1998. Effects Diurnal temperature difference and gamma radiation on the frequency of somatic cell mutations in the stamen hairs of *Tradescantia* 4430, *Korean J. Environ. Biol.* **16**(3):253-262.

Kim JK, Kim WR, Lee YK, Kim JS, Song HS, Jeong CY, Kim KN, Suh OS and Hong KP. 1998, Monitoring of environmental radiation using *Tradescantia* bioindicator, *Korean J. Environ. Biol.* **16**(2):85-91.

Ichicawa S. 199l. Validity of simplified scoring methods of somatic mutations in *Tradescantia* stamen hairs. *Envir. Exp. Bot.* **31**:247-252.

Ichicawa S, Nakano A, Makoto K, Yamamoto I, Murai M, Takahashi E, Yamaguchi A, Watanabe K, Tomiyama M, Sugiyama K, Yogo A, Yazaki T, Okumura M, Shima N, Satoh M, Yoshimoto M. and Ling Z. 1996, Yearly variation of spontaneous somatic mutation frequency in the stamen hares of *Tradescantia* clone KU9 grown outdoors, which showed a significant increase after the Chernobyl accident, *Mutat. Res.* **349**:249-259.

Wang S, Wang X. 1999. The *Tradescantia*-micronucleus test on the genotoxicity of UV-B radiation, *Mutat. Res.* **426**: 151-153.

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RBE of α Particles and Postradiation Recovery in a Temperature Conditional Mutant of Diploid Yeast for Various Criteria of Cell Death

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Abstract—The influence of linear energy transfer (LET) on relative biological effectiveness (RBE) and the liquid-holding recovery (LHR) ability was studied for a temperature conditional mutant of diploid yeast. Both the RBE and the rate of LHR were much lower for reproductive killing at 36°C and cell killing without division at both 23 and 36°C. However, the probability of recovery was independent of the culture temperature being identical for both low- and high-LET radiations. It was concluded that the LHR process itself is not damaged after densely ionizing radiation, while the enhanced RBE value and the reduced rate and volume of recovery after high-LET radiation may be entirely caused by the increased yield of the irreversible radiation damages.

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1. INTRODUCTION

The unexpected role of specific repair pathways in the RBE values of high-LET radiation was demonstrated for bacterial [1–3], yeast [4–6], and mammalian [7–9] cells. The data presented in these communications demonstrate that (i) the RBE values of densely ionizing radiations were higher for more radioresistant wild-type cells, in comparison with those for radiosensitive mutants defective in some reparation systems, and (ii) the rate of recovery was reduced for high-LET radiation. However, it is still unknown whether high-LET radiation affects the recovery process itself or it only produces a higher number of irreversible damages that cannot be repaired at all. Both these possibilities may be realized simultaneously. This question remains to be determined. It is known that the diploid temperature conditional radiosensitive mutant of *Saccharomyces cerevisiae* (strain g580L, *rad54-3*) is capable of repairing radiation-induced DNA double-strand breaks (DSB), if they are cultured at the permissive temperature of 23°C during the postirradiation period [10]. The RBE of the α particle was higher for this postirradiation temperature, while it was smaller at the restrictive temperature of 36°C, due to a considerable depression of DNA double-stranded break repair [5]. The RBE values of the α particle was higher for reproductive yeast cell killing than for cells killing without any postirradiation division and incapable of recovery [11]. Thus, it would be of interest to get additional evidence of the participation of cell recovery in the RBE of densely ionizing radiation, by comparing the RBE value of α particles for reproductive cell killing and for interphase cell killing without postirradiation division for the temperature conditional radiosensitive mutant cultured after irradiation at 23 and 36°C. The other goals of this study were: (i) to elucidate to what extent reduced recovery after high-LET radiation, due to damage to the recovery mechanisms themselves and/or to the formation of irreversible unrepairable damage, would be; (ii) to estimate whether the reduced cell ability to recover at 36°C is related to the increased yield of irreversible damage or due to the decrease in the probability of recovery. In this study liquid-holding recovery (LHR) will serve as an indicator of cellular repair activity.

2. MATERIAL AND METHODS

The radiosensitive diploid yeast mutant of *Saccharomyces cerevisiae* (strain d580L, *rad54-3*), which is temperature conditional with respect to DSB rejoining cells, was used in this study. This strain was obtained by us from Dr. D. Frankenberg (Germany). The cells were grown before irradiation up to the stationary phase of growth on a solid complete nutrient medium (1% yeast extract, 2% peptone, 2% glucose, and 1.5% agar) for 7 d at 30°C. To separate clumped cells, they were sonicated for 30 s at 30 W. Aliquots from the same cell suspension were irradiated with ^{60}Co γ rays (40 Gy/min) and with ^{239}Pu α particles (25 Gy/min). The LET of a particle reaching the cell monolayer was estimated to

[1] The text was submitted by the authors in English.

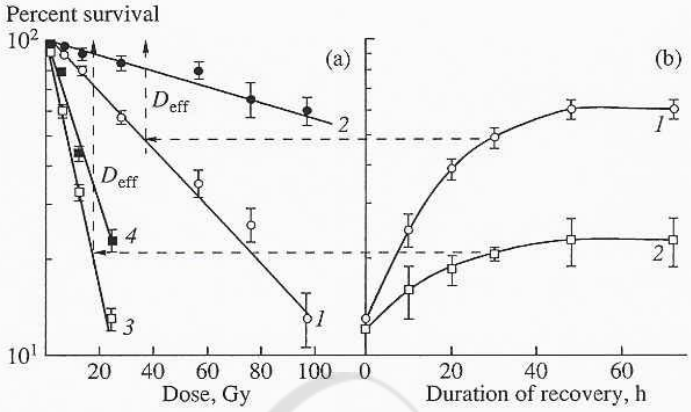

Fig. 1. (a) The dependence of cell survival on the radiation dose for the reproductive killing of diploid yeast cells (*S. cerevisiae*, strain g580L). The cells were exposed to graded doses of γ rays (*1*, *2*), α particles (*3*, *4*), and plated on a nutrient medium immediately after irradiation (*1*, *3*) at 23°C after a holding period for 72 h at the permissive temperature for DSB repair (*2*, *4*). (b) The dependence of cell survival on the duration of postirradiation recovery after exposure to γ rays (*1*), α particles (*2*), and plated on a nutrient medium after various recovery times at 23°C. The control and exposed cells were grown at 23°C during the postirradiation period. The arrows indicate the examples of the effective dose, $D_{eff}(t)$, determination following 30 h of recovery. Data are the mean values calculated from at least three independent experiments. Error bars indicate the standard error of the mean. Curves were fitted to the data points by eye measurements.

be 134 keV/μm. Just at about this LET value the maximum RBE–LET relationship was registered for inactivation of many eukaryotic cell systems. Immediately after irradiation part of the irradiated cells was plated onto a nutrient agar (immediate plating) for an assay of cell survival at 23 and 36°C. Another part of the irradiated cell suspension was placed for the LHR at 23 and 36°C, and cell survival was determined as a function of the incubation time (delayed plating). Plated cells were grown for 36 h on nutrient agar. It is known that dying cells can be inactivated either without any division (interphase killing) or after one or several reproduction cycles (reproductive killing). Cell survival was estimated microscopically using two criteria. First, cells that were capable of producing a microcolony consisting of more than 40 cells were considered to be viable (reproductive death). It is worth noting that the cell survivals estimated with macrocolony and microcolony counting coincided well. Second, the survival cells have to accomplish at least one division (a cell killing without any divisions).

During the LHR process a number of the primary radiation damage is eliminated, resulting in increased cell survival. This can be considered as a reduction of initial dose D_1 to a certain effective dose $D_{eff}(t)$, which is proportional to the mean number of the residual damages, both reparable and irreversible, after recovery for t hours. It has been demonstrated for yeast cells [12, 13] that a decrease in effective dose $D_{eff}(t)$, with recovery time t, could be fitted by an equation of the form

$$D_{eff}(t) = D_1[K + (1-K)e^{-\beta t}], \quad (1)$$

where K is the irreversible component of radiation damage, i.e., the fraction of radiation damage resistant to recovery, and β is the recovery constant that characterizes the probability of the recovery per unit time. The ratio $K(t) = D_{eff}(t)/D_1$ reflects the relative part of the primary radiation damage that has not been repaired during t hours of recovery. If t is sufficiently large (for yeast cells it is about 2–3 d), the recovery curves reach a plateau when the ability of the cells to recover is saturated or exhausted. In this case, we can write

$$K = K(\text{plateau}) = D_{eff}(\text{plateau})/D_1. \quad (2)$$

In this expression D_{eff}(plateau) is the effective dose that corresponds to the plateau of the recovery curve, which is proportional to the mean number of irreversible damages. Knowing the survival and recovery curves after cell exposure to low- and high-LET radiations, one can calculate the corresponding values of $D_{eff}(t)$, D_{eff}(plateau), $K(t)$, K, and β.

3. RESULTS AND DISCUSSION

Figure 1 shows the dependence of cell survival on the radiation dose, (a) and the duration of postirradiation recovery (b) for the reproductive killing of diploid yeast cells (*Saccharomyces cerevisiae*, strain g580L). The cells were exposed to graded doses of γ rays (curves *1*, *2*), α particles (curves *3*, *4*), and plated on a nutrient medium immediately after irradiation (curves *1*, *3*) and after 3 d of storage in conditions promoting the LHR (curves *2*, *4*). The control and exposed cells were grown at 23°C during the postirradiation period. Cells that were incubated at a temperature permissive

RBE of α particles and the parameters of LHR of conditional thermosensitive diploid yeast cell mutants of *Saccharomyces cerevisiae* (strain g580L, *rad54-3*) for various criteria of cell killing. The table includes the temperature at which LHR and postradiation cultivation was carried out

T, °C	The form of cell killing	RBE	$K(\gamma)$	$K(\alpha)$	$B(\gamma)$, h^{-1}	$B(\alpha)$, h^{-1}
23	Reproductive	4.6 ± 0.4	0.28 ± 0.03	0.69 ± 0.05	0.05 ± 0.005	0.05 ± 0.005
	Without division	2.9 ± 0.2	1.00	1.00	0.00	0.00
36	Reproductive	3.0 ± 0.3	0.71 ± 0.06	0.68 ± 0.04	0.05 ± 0.005	0.05 ± 0.005
	Without division	2.5 ± 0.2	1.00	1.00	0.00	0.00

for DSB repair (23°C) [5] yielded shoulder survival curves with a small extrapolation number. The RBE of α particles, defined as the ratio of doses producing 10% survival after low- and high-LET radiations, was found to be 4.6 (see table). This value is close to that observed for wild-type yeast cells [4, 14]. That means that these radiosensitive mutant cells being incubated at 23°C show radiobiological responses similar to those of wild-type cells capable of recovery.

The recovery patterns are presented in Fig. 1b. Here recovery kinetics after low- and high-LET radiations are compared at approximately equal levels of survival (i.e., at equal amounts of lethal lesions). The arrows indicate examples of the effective dose, $D_{eff}(t)$, determination following 30 h of recovery. It can be seen from Fig. 1b that the survival of irradiated cells held before plating in conditions favoring LHR was greatly enhanced in comparison with that obtained after immediate plating for both the low- and high-LET radiations. The number of viable cells increased as a function of time, reaching a plateau after about 2–3 d. Differences in the repair kinetics are also evident, with slower recovery for α-particle irradiation. The probability of recovery per unit time (β) was estimated using the survival and recovery curves after cell exposure to low- and high-LET radiations and Eq. (1). The fraction of irreversible damage (K) was calculated as the ratio of doses producing 10% survival or the mean lethal dose after immediate and delayed plating. Both these parameters are also included in the table after γ and α irradiation. It is evident that the fraction of irreversible damage was markedly greater, i.e., the magnitude of postirradiation recovery was smaller after α irradiation than that observed for γ rays. In contrast, the probability of recovery was identical for both low- and high-LET radiations. Moreover, the recovery probability of these mutant cells was identical with that observed earlier for the wild-type strain (XS800) [15]. Thus, the data presented suggest that recovery decreases with increasing LET of radiation. The decrease becomes apparent in both the rate and volume of recovery. The independence of the probability of recovery from radiation quality means that the process of recovery itself remained unchanged and a slower recovery may reflect a smaller yield of reversible damages produced by high-LET radiation. It can be concluded on this basis that the LHR process itself is not damaged after densely ionizing radiation, and the enhanced RBE of the high-LET radiation may be related to increased yield of the irreversible damage. Strong evidence for a causal relationship between the rejoining of DSB, LHR, and RBE of α particles has been reported [5]. We can suppose on this basis that the identical probability of the LHR observed in this paper may signify the identical probability of DSB rejoining after low- and high-LET radiations, while the slowing down and smaller volume of DSB rejoining may reflect the reduction in a number of DSB, which are capable of rejoining.

The data described above prompted similar types of experiments that used restrictive (36°C) temperature for postirradiation cultivation. Figure 2 exhibits the results of these experiments. It is evident that at the restrictive temperature of 36°C, the *rad54-3* mutant, yields exponential survival curves. One can see that the radiobiological response is quite different from that observed at 23°C, permissive for DSB rejoining. The mutant cells became more sensitive to low-LET radiation, and their ability to undergo LHR also decreased. The decrease is apparent in both the rate and volume of recovery. Here again, it was of interest to elucidate to what extent the reduced recovery was due to damage to the recovery mechanism itself and/or to the formation of irreversible unrepairable damage. We estimated the corresponding parameters, describing the LHR process in these conditions. They are also listed in the table. It is clear that the probability of recovery (β) was identical for low- and high-LET radiation at both the permissive (23°C) and restrictive (36°C) for DSB rejoining temperatures. That means that an identical fraction of the reversible radiation damage is recovered per unit time independent of radiation quality at both temperatures investigated. The irreversible component was enhanced for the restrictive temperature even for low-LET radiation, and was similar to that observed for high-LET radiation. Therefore, the recovery process itself is not damaged after densely ionizing radiation, and the enhanced RBE value of the high-LET radiation as well as the high radiosensitivity of mutant cells at the restrictive temperature may be related to the increased yield of the irreversible radiation damage.

The LHR processes are known to fail for interphase cell killing when they are inactivated immediately after

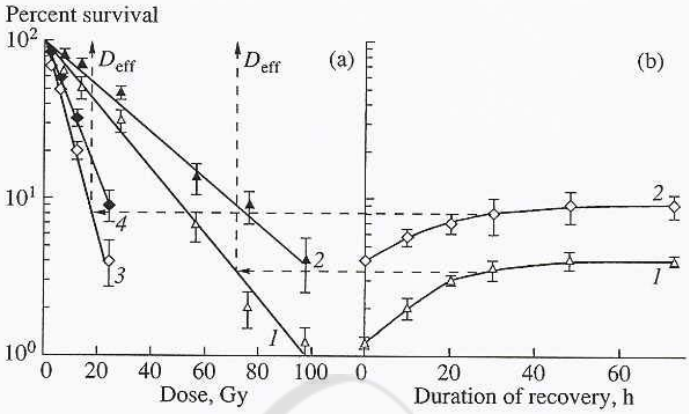

Fig. 2. The same as in Fig. 1, but the cells were recovered and grown during the postirradiation period at a restrictive temperature of 36°C.

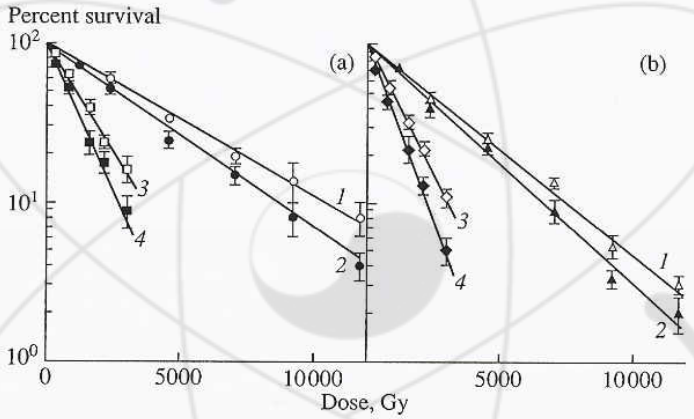

Fig. 3. The dependence of cell survival on the radiation dose for a diploid yeast cell (*S. cerevisiae*, strain g580L), killing without division. The cells were exposed to graded doses of γ rays (*1*, *2*), α particles (*3*, *4*), and plated on a nutrient medium immediately after irradiation (*1*, *3*) and after the LHR procedure for 72 h (*2*, *4*). The cells underwent the LHR and were grown during the postirradiation period at a permissive (23°C (a)) and restrictive (36°C (b)) temperature for DSB repair. Data are the mean values calculated from at least three independent experiments. Error bars indicate the standard error of the mean. Curves were fitted to the data points by eye measurements.

irradiation without any postradiation division [11]. Figure 3 exhibits the survival curves for diploid yeast cells of *S. cerevisiae* (strain d580L) killing without division after γ- and α-exposure. The irradiated cells were kept during the LHR procedure and were then incubated on nutrient media at 23°C (Fig. 3a) and 36°C (Fig. 3b). It can be seen that unusually tremendous radiation doses were used in these experiments after which the degradation of the main cell compounds was observed [12]. Due to this fact, no sort of recovery was recorded. Moreover, in all cases some decrease in the cell survival was observed after the LHR procedure (Fig. 3). This fact can be considered as an indication of a connection failure between interphase cell killing and a cell's ability to recover from radiation damage, and perhaps may be related with unrepairable radiation damages of vitally important cell structure. As the RBE value of densely ionizing radiation correlates with a cell's ability to recover from radiation damage, it can be expected that for cell killing without division the RBE of α particles would be smaller in comparison with that for the reproductive cell killing. This prediction is justified by the data presented in the table and calculated on the basis of Figs. 1–3. It is evident that the RBE of α particles for cell killing without a division was smaller than that for reproductive cell killing, lowering from 4.6 to 2.9 for 23°C and from 3.0 to 2.5 for 36°C. These reduced RBE values are closed to those observed for radiosensitive yeast mutants incapable of recovery [4].

As was mentioned above, the action of ionizing radiation on living cells is determined by both the physical properties of ionizing radiation and the biological ability of cells to recover from potentially lethal radiation damage. The data of this paper can be considered as an indication of the fact that the observed RBE value for the nonrecoverable condition (RBE = 2.5 – 3.0) may be caused by the physical property of high-LET radiation, while a further increase of this value (up to 4.6) may be due to the cell's ability to recover from radiation damage inflicted by low-LET radiation.

In conclusion, the finding that the fraction of irreversible damage was greater for high-LET radiation in comparison with that for low-LET radiation corresponds to a number of experimental data observed at cellular and molecular levels [3–9]. The explanation of these results can be originated from the fact that high-LET radiations are known to produce clustering of ionizations and excitations in DNA [15], which produce more severe and complex damage than low-LET reference radiation. The independence of the probability of recovery from the radiation quality means that the slowing down of a cell's ability to undergo LHR after high-LET radiation is associated with a great number of irreversible unrepairable damages rather than the impairment or damage to recovery process itself.

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REFERENCES

1. R. H. Haynes, "The Interpretation of Microbial Inactivation and Recovery Phenomena," Radiat. Res. Suppl. 1–29 (1966).
2. T. Alper, "Cell Death and Its Modification: the Role of Primary Lesions in Membranes and DNA," in *Biophysical Aspects of Radiation Quality* (Vienna, 1971), pp. 171–184.
3. E. A. Krasavin, *Problems of RBE and DNA Reparation* (Energoatomizdat, Moscow, 1989).
4. V. G. Petin and N. M. Kabakova, "RBE of Densely Ionizing Radiation for Wild-Type and Radiosensitive Mutants of Yeast," Mutat. Res. **82**, 285–294 (1981).
5. M. Frankenberg-Schwager, D. Frankenberg, and R. Harbich, "Repair of DNA Double-Strand Breaks As a Determinant of RBE of Alpha-Particles," Br. J. Cancer Suppl. **49** (VI), 169-173 (1984).
6. V. G. Petin, *Genetic Control of Cell Radiosensitivity Modification* (Energoatomizdat, Moscow, 1987).
7. T. M. Coquerelle and K. F. Weibezahn, "Lu Cke-Huhle C. Rejoining of Double Strand Breaks in Normal Human and Ataxia-Telangiectasia Fibroblasts After Exposure to ^{60}Co Gamma-Rays, ^{241}Am Alpha-Particles Or Bleomycin," Intern. J. Radiat. Biol. **51**, 209–218 (1987).
8. J. D. Shadley, et al., "The Effect of Radon Daughter α-Particle Irradiation in K1 and xrs-5 CHO Cell Lines," Mutat. Res. **248**, 73–83 (1991).
9. W. K. Weyrather, et al., "RBE for Carbon Track-Segment Irradiation in Cell Lines of Differing Repair Capacity," Intern. J. Radiat. Biol. **75**, 1357–1364 (1999).
10. M. Budd and R. K. Mortimer, "Repair of Double-Strand Breaks in a Temperature Conditional Radiation-Sensitive Mutant of Saccharomyces Cerevisiae," Mutat. Res. **103**, 19–24 (1982).
11. V. I. Shvedenko, N. M. Kabakova, and V. G. Petin, "A Comparative Study of RBE of Densely Ionizing Radiation for Various Criteria of Yeast Cell Death," Radiat. Biol. Radioecology **41**, 361–365 (2001).
12. V. I. Korogodin, *Problems of Postirradiation Recovery.* (Energoizdat, Moscow, 1966).
13. V. I. Korogodin, et al., "Cellular Repair Processes: Survival of Irradiated Yeast, Bacteria, and Phages Under Different Postradiation Conditions," Adv. Biol. Med. Phys. **12**, 253–274 (1968).
14. V. G. Petin and J. K. Kim, "Liquid-Holding Recovery Kinetics in Wild-Type and Radiosensitive Mutants of Yeast Cells Exposed To Low- and High-LET Radiations," Mutat. Res. **570**, 1–8 (2005).
15. D. T. Goodhead, "Initial Events in the Cellular Effects of Ionizing Radiations: Clustering Damage in DNA," Intern. J. Radiat. Biol. **65**, 7–17 (1994).

SPELL: OK

Mathematical Description and Prognosis of Cell Recovery after Thermoradiation Action

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Abstract − A mathematical model for the synergistic interaction of physical and chemical environmental agents was suggested for quantitative prediction of irreversibly damaged cells after combined exposures. The model took into account the synergistic interaction of agents and was based on the supposition that additional effective damages responsible for the synergy are irreversible and originated from an interaction of ineffective sublesions. The experimental results regarding the irreversible component of radiation damage of diploid yeast cells simultaneous exposed to heat with ionizing radiation (^{60}Co) or UV light (254 nm) are presented. It was shown that the cell ability of the liquid holding recovery decreased with an increase in the temperature, at which the exposure was occurred. A good correspondence between experimental results and model prediction was demonstrated. The importance of the results obtained for the interpretation of the mechanism of synergistic interaction of various environmental factors is discussed.

Key words : mathematical model, recovery, irreversible damage, ionizing radiation, UV light, heat, yeast cells

INTRODUCTION

Organisms in their natural environment are always simultaneously exposed to a number of chemical and physical agents such as heat, UV light, ionizing radiation, nonionizing electromagnetic radiation, natural and man made electrical and magnetic fields. That high temperature can enhance radiation sensitivity of a wide variety of biological systems both *in vitro* and *in vivo* can no longer be doubted. Heating at temperatures above 40°C are now used to increase the cellular radiosensitivity, the greatest synergistic effect being observed when heat and irradiation are given simultaneously (Hahn 1982; Streffer *et al.* 1990). It has so far generally been assumed that thermal radiosensitization results from the heat-induced inhibition of the repair of radiation damage. This was observed both for the repair of single- and double-strand breaks (Jorritsma and Konings 1983; Iliakis *et al.* 1990; Dahm-Daphi *et al.* 1997) and for cellular recovery (Ben-Hur *et al.* 1974; Miyakoshi *et al.* 1982; Raaphorst *et al.* 1991; Petin and Kim 2004a).

This inhibition was revealed in a decrease of both the rate and extent of recovery which can be interpreted as being due to either the impairment of the recovery process itself (inactivation of repair enzymes, for instance) or to the production of more severe irreversible lethal damage which can not be repaired. The latter case was proved to be realized for the combined treatment of heat and ionizing radiation on yeast (Petin and Kim 2004a) and cultured mammalian cells (Petin and Kim 2004b; Kim *et al.* 2005) as well as for the exposure of yeast cells to heat and UV light (Kim *et al.* 2004). These novel observations provide insight into

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the mechanisms underlying the effects of combined exposure and should be taken into account for interpretation and mathematical modeling of synergistic interaction of the combined exposures of various environmental agents. A simple mathematical approach has been earlier proposed to predict and optimize the synergistic interaction of hyperthermia and ionizing radiation (Petin and Komarov 1997; Kim and Petin 2002). This model suggests that the synergistic interaction of ionizing radiation and hyperthermia is expected to result from an additional effective damage arising from the interaction of sublesions induced by both agents. These sublesions are considered non-effective after each agent taken alone. The model was found to predict the dependence of synergistic interaction of the ratio of lethal events produced by every agent used, the greatest synergistic effect and the condition at which the maximal interactive effect can be achieved. However, this model does not take into consideration novel data indicating that additional damage responsible for the synergy is irreversible. Thus, the main goals of this study were (a) to develop a mathematical model of synergistic interaction taking into account the production of irreparable damage; (b) to adopt this model for prognosis of the portion of irreversible damage produced after combined exposures; (c) to test the validity of the model to describe experimental results.

MATHEMATICAL APPROACH

Suppose that the additional effective damages, responsible for the synergy, are arisen from the interaction of sublesions induced by both agents and these sublesions are ineffective when each agent is applied separately. It would be reasonable to assume that one sublesion produced by an agent applied with heat interacts with one sublesion from heat to produce one additional effective damage. This assumption is not crucial since we do not define their nature concretely. It seems also plausible to suppose that the number of sublesions is directly proportional to the number of the effective damages. To follow to our previous publications (Petin and Komarov 1997; Petin *et al.* 1999, 2000; Kim and Petin 2002), we use here index 1 for the agent applied together with heat (index 2). Let p_1 and p_2 be the numbers of sublesions that occur for one effective damage and N_1 and N_2 be the mean numbers of the effective damages in a cell produced by these agents. According to these assumptions, the synergistic effect is attributed to the formation of some additional effective damage N_3. Then the synergistic enhancement ratio k may be expressed as

$$k=\frac{N_1+N_2+N_3}{N_1+N_2}. \tag{1}$$

A number of additional damages N_3 arising from the interaction of sublesions produced by the two agents may be given as

$$N_3=\begin{cases} p_1 N_1, & if \quad p_1N_1 \le p_2N_2 \\ p_2 N_2, & if \quad p_1N_1 \ge p_2N_2 \end{cases}. \tag{2}$$

In fact, this equation shows that the additional effective damage is a minimal value from two variable quantities: p_1N_1 and p_2N_2, which are the mean number of sublesions produced by the corresponding agent. Taking into account Eq. 2, the expression for synergistic effect (Eq. 1) can be written as

$$k=\begin{cases} 1+\dfrac{p_1}{1+N_2/N_1}, & if \quad p_1N_1 \le p_2N_2 \\ 1+\dfrac{p_2N_2/N_1}{1+N_2/N_1}, & if \quad p_1N_1 \ge p_2N_2 \end{cases}. \tag{3}$$

This equation has two parameters (p_1 and p_2) to be estimated from experimental data. It is evident from here that the effectiveness of the synergistic interaction will be determined by the least value from the two functions: $f_1=1+\dfrac{p_1}{1+N_2/N_1}$ and $f_2=1+\dfrac{p_2N_2/N_1}{1+N_2/N_1}$. This relationship (Eq. 3) between the synergistic enhancement ratio and the ratio of N_2/N_1 shows an increase in the synergistic enhancement ratio with N_2/N_1 to a maximum, followed by a subsequent decrease at higher N_2/N_1. The absolute value of this maximum as well as the ratio of N_2/N_1 at which it is attained are obviously of great interest not only in thermobiology but for environmental protection consideration as well. Since f_1 decreases while f_2 increases with increasing N_2/N_1, the greatest synergistic effect will be obtained when $f_1=f_2$, i.e.

$$\left(1+\frac{p_1}{1+N_2/N_1}\right)=\left(1+\frac{p_2N_2/N_1}{1+N_2/N_1}\right). \tag{4}$$

It enables the determination of the condition of the highest

synergistic interaction achievement:

$$N_2/N_1 = p_1/p_2. \quad (5)$$

It means that the highest synergistic interaction occurred when both agents produce equal numbers of interacting sublesions: $p_1N_1 = p_2N_2$. For a particular case $p_1 \cong p_2$, the highest synergistic interaction occurred when the both agents produce almost equal number of effective damages, i.e. $N_1 \cong N_2$. Combining equations (3) and (5), one can deduce the value of the highest synergistic enhancement ratio which can be given by

$$k_{\max} = 1 + \frac{p_1 p_2}{p_1 + p_2}. \quad (6)$$

It is evident that the value of the highest synergistic effect doesn't depend on the ratio of N_2/N_1 and is completely determined by the basic parameters of the model.

It is curiously but the model considered doesn't demand a knowledge of absolute values of N_1 and N_2 but only their dimensionless ratio, N_2 / N_1, which can be derived for cell inactivation (the main end point used in this study) from the ratio of slopes of the corresponding curves

$$N_2/N_1 = D_{o1}/D_{o2}, \quad (7)$$

where D_o being a measure of the time for survival to be reduced to $1/e$ on the exponential portion of survival curve.

If irradiated cells are held in a liquid non-nutrient media at 30°C before plating on to a growth medium, their survival is increased reflecting the process of LHR (Korogodin 1958, 1993). When samples were plated at different times after the treatment, the number of viable cells increased due to the LHR as a function of time, reaching a plateau after about 2～3 days. The availability of the plateau reflects the existence of the irreversible component of radiation damage which cell is incapable to recover from. In accordance with the definition (Korogodin 1966), the irreversible component can be quantitatively estimated as the ratio of the number of irreversible damage to the total number of damage produced. The ratio shows the portion of damage which can not be repaired. Each type of the effective damage (N_1, N_2, N_3) can be characterized by its own value of irreversible component (K_1, K_2, K_3, respectively). Then for the synergistic interaction of two agents the irreversible component (K) might be presented as

$$K = \frac{K_1N_1 + K_2N_2 + K_3N_3}{N_1 + N_2 + N_3}. \quad (8)$$

Taking into account Eq. (2) and the basic postulates of the model, Eq. (8) can be rewritten as

$$K = \begin{cases} \dfrac{K_1N_1 + K_2N_2 + K_3p_1N_1}{N_1 + N_2 + p_1N_1}, & if \quad p_1N_1 \le p_2N_2 \\ \dfrac{K_1N_1 + K_2N_2 + K_3p_2N_2}{N_1 + N_2 + p_2N_2}, & if \quad p_1N_1 \ge p_2N_2 \end{cases}, \quad (9)$$

where K_1 is the irreversible component after separate exposure to the agent combined with heat, K_2 is the irreversible component after thermal treatment applied alone and K_3 is the irreversible component of additional damages responsible for the synergy. As was mentioned above, this additional damage is irreversible, i.e. $K_3 = 1$. For convenience calculation ability this equation may be given by

$$K = \begin{cases} \dfrac{K_1 + K_2N_2/N_1 + K_3p_1}{1 + N_2/N_1 + p_1}, & if \quad p_1N_1 \le p_2N_2 \\ \dfrac{K_1 + K_2N_2/N_1 + K_3p_2N_2/N_1}{1 + N_2/N_1 + p_2N_2/N_1}, & if \quad p_1N_1 \ge p_2N_2 \end{cases}. \quad (10)$$

It is plausible to assume that biological damage produced by each acting agent (N_1 and N_2) during combined action can be repaired in the same extent and with the same probability as they were observed after application of these agents individually. One can estimate K_1 and K_2 values from experiments with separate action of each agent used in combination.

THE VALIDITY OF THE MODEL

If the observed biological effect is largely induced by heat ($p_1N_1 < p_2N_2$) then taking into account Eq. 3, the parameter p_1 can be expressed as

$$p_1 = (k_1 - 1)(1 + N_2/N_1), \quad (11)$$

where k_1 is the value of the synergistic enhancement ratio observed in experiments performed in this condition. On the contrary, if the observed biological effect is mainly induced by another agent applied with heat ($p_1N_1 > p_2N_2$), we have

$$p_2 = (k_2 - 1)(1 + N_1/N_2), \quad (12)$$

where k_2 is the experimental value of the synergistic enhancement ratio observed for the condition $p_1N_1 > p_2N_2$. Knowing experimental values of k_1 and k_2 derived from two real experiments in which the cell inactivation was due to the preferential contribution of one of the two agents applied, we can make an empirical estimation of the basic model parameters p_1 (Eq. 11) and p_2 (Eq. 12). To provide more precise description of experimental data, a direct estimation of these parameters from the entire set of data regarding to a given biological object might also be possible (Petin and Komarov 1997). Knowing p_1 and p_2, one can predict the value of the synergistic enhancement ratio for any N_2/N_1 (Eq. 3), the greatest value of the synergistic enhancement ratio (Eq. 6) and the condition under which it can be achieved (Eq. 5).

We tested the applicability of the model for quantitative description, prediction and optimization of the synergistic interaction observed for various biological objects and test systems. Our most numerous findings have been obtained with diploid yeasts - the simplest specimen of eukaryote cells. As was shown previously (Petin and Komarov 1997; Petin and Kim 2004; Petin *et al.* 2005), in diploid yeast cells thermally enhanced sensitivity to ionizing radiation may due to both the inhibition of repair from potentaially lethal damage and/or the enhancement of expression of lethal damage. The reduction of shoulder suggested that hyperthermia also interferes with repair of sublethal damage. Having used these published results, we estimated here the fraction of irreversibly damaged cells (irreversible component), which was determined as a value inversely proportional to the dose-modifying factor that is as the ratio of the D_o after delayed and immediate plating. The calculation showed that the fraction the irreversible component was equal 0.34 at 20 and 40℃; 0.50 at 45℃; 0.77 at 50℃; 0.86 at 52.5℃ and 0.94 at 55℃ (Table 1). It is apparent that the cell ability to recover radiation damage is inversely proportional to the temperature at which the exposure was delivered. Yeast cells exposed to ionizing radiation at 55℃ almost completely fail to show recovery on delayed plating. It would be of tempting to test the ability of the model considered to predict the extent of the recovery after the combined action of heat and ionizing radiation (Eq. 10). We can reasonably believe that the irreversible component induced by ionizing radiation alone K_1=0.34 (irradiation at room temperature). Since in all our experiments with yeast cells no LHR was observed after hyperthermia alone, we consider that K_2= 1.0. It means that all thermal damages are irreversible. Using these results and Eq. 10, we calculated the irreversible component K expected after simultaneous action of ionizing radiation and hyperthermia for 3 various values of K_3-irreversible portion of damage responsible for the synergy. Averaging the results published before (Petin and Komarov 1997; Petin *et al.* 2005), we considered that for the strain used in these experiments p_1=2.5 and p_2=3.4.

The results of calculation together with experimental data of the proportion of irreversible damage after simultaneous action of heat and ionizing radiation are collected in Table 1. It can be seen that the values predicted on the basis of Eq. 10 for K_3=1.0 show the best correspondence with experimental findings. On the contrary, the values predicted for K_3=0.5 and K_3=0 are not consistent with experimental data.

Similar calculations of the irreversible component K have been fulfilled for the same diploid yeast cells exposed to ultraviolet light (254 nm) at different temperatures. Experimental values of K (Table 2) have been taken from our recently published work (Kim *et al.* 2004). The calculation showed that the fraction the irreversible component was equal 0.56 at 20℃; 0.60 at 53℃; 0.69 at 54℃; 0.77 at 55℃; 0.92 at 56℃ and 1.00 at 57℃ (Table 2). It is apparent that the cell ability to recover UV light damage is also inversely proportional to the temperature at which the exposure was

Table 1. The irreversible component of radiation damage (K) after simultaneous action of ionizing radiation (γ-ray of ^{60}Co) and hyperthermia on diploid yeast cells of *Saccharomyces ellipsoideus* (strain Megri 139-B)

Temperature, ℃	20	40	45	50	52.5	55
N_2/N_1	0	0	0.16	0.53	1.4	7.3
K, experiment	0.34±0.03	0.34±0.03	0.50±0.05	0.77±0.06	0.84±0.07	0.94±0.05
K, predicted for:						
K_3=1.0	0.34	0.34	0.61	0.80	0.86	0.94
K_3=0.5	0.34	0.34	0.45	0.53	0.46	0.82
K_3=0.0	0.34	0.34	0.29	0.26	0.36	0.71

Table 2. The irreversible component K after simultaneous action of ultraviolet radiation (254 nm) and hyperthermia on diploid yeast cells *Saccharomyces cerevisiae* (strain XS800)

Temperature, °C	20	53	54	55	56	57
N_2/N_1	0	0.13	0.18	0.25	1.38	6.45
K, experiment	0.56±0.04	0.60±0.06	0.69±0.06	0.77±0.05	0.92±0.07	1.00±0.05
K, predicted for:						
K_3=1.0	0.56	0.72	0.76	0.79	0.88	0.95
K_3=0.5	0.56	0.57	0.58	0.59	0.69	0.86
K_3=0.0	0.56	0.43	0.42	0.38	0.49	0.77

delivered. It may be considered that the irreversible component induced by UV light alone K_1=0.56 (irradiation at room temperature) and K_2=1.0.

Using these results and Eq. 10, we calculated the irreversible component K expected after simultaneous action of UV light and hyperthermia for 3 various values of K_3-irreversible portion of damage responsible for the synergy. The basic parameters of the model for the strain used in these experiments were as follows: p_1=1.6 p_2=3.5. The results of our calculation together with experimental data of the proportion of irreversible damage after simultaneous action of UV light and hyperthermia are collected in Table 2. It can be seen that the values predicted on the basis of Eq. 10 for K_3=1.0 show the best correspondence with experimental findings. On the contrary, the values predicted on the basis of Eq. 10 for K_3=0.5 and K_3=0.0 are in most cases not consistent with experimental data.

DISCUSSION

Analysis of numerous data has shown (Hahn 1982; Streffer *et al.* 1990) that synergistic interaction of ionizing radiation with other environmental chemical and physical agents results in the inhibition of some cellular repair mechanisms. The inhibition was revealed in a decrease of both the rate and extent of recovery. Two hypotheses could be envisaged. Such lesions, resulting from the simultaneous application of the two insults, might not be reparable. Alternatively, the damage might be similar, but the repair enzymes might be modified so that their ability of repair is changed. The most likely explanation for these results is that heating after irradiation modified the damage so that enzymes could then not deal with the lesions.

The synergistic effects of the combined action of heat with ionizing radiation or UV light interpreted (Kim *et al.* 2004, 2005; Petin and Kim 2004a, b) as being due to more severe or more complex damage produced during the combined treatment. In the current study, a novel mathematical approach was developed for quantitative description and prognosis of the synergistic effect and cell recovery after the combined exposures of environmental agents. The range of the experimental radiation doses in the described effect of interaction between heat and radiation was 100∼1,000 Gy. This means that we considered and put into the mathematical model process called as radiosensitization or enhancement of deterministic effects produced by ionizing radiation. The model was based on the supposition that the synergism originated from the production of additional effective damage due to the interaction of some kind of sublesions induced by both agents. The idea of sublesions is widely used in theoretical radiobiology (Leenhouts and Chadwick 1978; Murthy *et al.* 1979; Zaider and Rossi 1980). A very elegant mathematical description of synergistic interaction was presented in molecular theory of radiation biology (Chadwick and Leenhouts 1981). Some papers showed the clear experimental proofs for a synergy on the various cellular effects (survival and mutation) described by the molecular model of interaction (Leenhouts *et al.* 1980; Cebulska-Wasilewska *et al.* 1981).

In the model presented here sublesions are supposed to be non-effective after each agent taken alone while the additional damage arising from the interaction and responsible for the synergy is irreversible which the cells are incapable to recover from. The main novel contribution of this model concerns the possibility to predict the irreversible component of radiation damage after the combined exposures of different environmental agents.

To test the approach, the results of experimental research of diploid yeasts cells survival after simultaneous action of heat combined with ^{60}Co ionizing radiation (Petin and Kim 2004a) or 254 nm UV light (Kim *et al.* 2004) have been

used here. It was shown that the cell ability to the liquid holding recovery decreased with an increase in the temperature, at which the exposure was occurred. A good correspondence between experimental results and model prediction was demonstrated only if the initial supposition about the irreversible character of additional damage holds. Otherwise, the values of irreversible component predicted on the basis of the model developed were in most cases not consistent with experimental data.

The molecular mode of the repair inhibition is still unclear. There is now abundant evidence pointing that hyperthermic inhibition of the rate and/or extent of repair of radiation-induced DNA damage which was observed with all the assays currently used for DNA damage assessment may reflect the production of irreversible damage rather than the impairment of recovery mechanisms themselves after the combined exposures. In fact, evidence has accumulated that hyperthermic radiosensitization might be due to the nuclear protein aggregation that damaged DNA becomes less accessible for the DNA repair machinery and this (rather than repair enzyme inactivation *per se*) is responsible for the observed thermal inhibition of DNA repair (Wynstra *et al.* 1990, Sakkers *et al.* 1995; Kampinga *et al.* 2004; Kampinga 2006). Thermal repair inhibition is generally described as reduced repair rates leading to an increased number of residual strand breaks (Warters 1993). It was previously shown by Mills and Meyn (1981) that for a combined treatment with X-irradiation and heat, the decrease in cell survival correlated with the increase in the number of non-rejoined strand breaks. The combined treatment appeared to affect the repair rates but also lead to the formation of additional strand breaks (Dikomey and Jung 1995). Residual additional strand breaks then can result in additional chromosome aberrations (Dewey *et al.* 1978, Weissenborn and Obe 1991) finally producing the enhanced cell killing. Considering all these data as a whole, we can conclude that they don't contradict the results obtained in this study. However, the analysis of the molecular mechanism of synergistic interaction and production of irreversible damage is beyond the main aim of this paper. Moreover, it can be envisaged that they might be different for various biological objects, test effects and acting agents. The major points to be inferred from the data presented here are that the mathematical model basing on the minimum initial postulates describes in general form the dependence of the synergistic effect on the ratio of the effective damages produced by agents used in combination, predicts the greatest synergistic interaction, condition under which it can be achieved and the portion of the irreversibly damage resulted from the combined exposures.

In summary, it can be concluded that the mechanism of the synergistic interaction of hyperthermia with ionizing radiation or UV light after their simultaneous treatment in yeast cells is not related to the impairment of the recovery capacity itself and it may be attributed to an increased yield of irreversible damage.

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REFERENCES

Ben-Hur E, MM Elkind and BV Bronk. 1974. Thermally enhanced radioresponse of cultured Chinese hamster cells: inhibition of repair of sublethal damage and enhancement of lethal damage. Radiat. Res. 58:38-51.

Chadwick KH. and HP Leenhouts. 1981. The Molecular Theory of Radiation Biology. Springer Verlag, Berlin-Heidelberg-New York.

Cebulska-Wasilewska A, HP Leenhouts and KH Chadwick. 1981. Synergism between EMS and Xrays for the induction of somatic mutations in *Tradescantia*. Int. J. Radiat. Biol. 40:163173.

Dahm-Daphi J, I Brammer and E Dikomey. 1997. Heat effects on the repair of DNA double-strand breaks in CHO cells. Int. J. Radiat. Biol. 72:171-179.

Dewey WC, SA Sapareto and DA Betten. 1978. Hyperthermic radiosensitization of synchromous Chinese hamster cells: Relationship between lethality and chromosomal aberrations. Radiat. Res. 76:48-59.

Dikomey E and H Jung. 1995. Correlation between thermal radiosensitization and slowly rejoined DNA strand breaks in CHO cells. Int. J. Radiat. Biol. 68:227-233.

Hahn GM. 1982. Hyperthermia and Cancer. Plenum Press, New York.

Iliakis G, R Seaner and R Okayasu. 1990. Effects of hyperther-

mia on the repair of radiation-induced DNA single- and double-strand breaks in DNA double-strand break repair-deficient and repair-proficient cell lines. Int. J. Hyperthermia 6:813-833.

Jorritsma JBM and AWT Konnings. 1983. Inhibition of repair of radiation-induced strand breaks by hyperthermia, and its relationship to cell survival after hyperthermia alone. Int. J. Radiat. Biol. 43:505-516.

Kampinga HH. 2006. Cell biological effects of hyperthermia alone or combined with radiation or drugs: a short introduction to newcomers in the field. Int. J. Hyperthermia 22: 191-196.

Kampinga HH, JR Dynlacht and E Dikomey. 2004. Mechanism of radiosensitization by hyperthermia (>or =43 degrees C) as derived from studies with DNA repair defective mutant cell lines. Int. J. Hyperthermia 20:131-139.

Kim JK, LN Komarova, MD Tkhabisimova and VG Petin. 2005. Inhibition of recovery from potentially lethal damage by chemicals in Chinese hamster cells is realized through the production of irreversible damage. Korean J. Environ. Biol. 23:390-397.

Kim JK and VG Petin. 2002. Theoretical conception of synergistic interactions. Korean J. Environ. Biol. 20:277-286.

Kim JK, VG Petin and VD Tkhabisimova. 2004. Survival and recovery of yeast cells after simultaneous treatment of UV light radiation and heat. Photochem. Photobiol. 79:349-355.

Korogodin VI. 1958. Some regularity of the post-irradiation changes in resting yeast cells. Biofizika 3:703-710.

Korogodin VI. 1966. Problems of Postirradiation Recovery, Energoatomizdat, Moscow.

Korogodin VI. 1993. The study of post-irradiation recovery: the premolecular period. Mutat. Res. 289:17-26.

Leenhouts HP and KH Chadwick. 1978. An analysis of synergistic sensitization. Br. J. Cancer 37 (Suppl. 3):198-201.

Leenhouts HP, KH Chadwick and A. Cebulska-Wasilewska. 1980. An analytical approach to the comparison of chemical and radiation hazards to man. Proc. Internat. Cong. Rad. Protection, IRPA, Israel, Vol. 2:11551158.

Mills MD and RE Meyn. 1981. Effects of hyperthermia on repair of radiation-induced DNA strand breaks. Radiat. Res. 87:314-328.

Miyakoshi J, M Furukawa and E Kano. 1982. Recovery kinetics from radiation and hyperthermia damage to cultured Chinese hamster V79 cells. Nat. Cancer Inst. Monograph 61:263-266.

Murthy MSS, VV Deorukhakar and BS Rao. 1979. Hyperthermic inactivation of diploid yeast and interaction of damage caused by hyperthermia and ionizing radiation. Int. J. Radiat. Biol. 35:333-341.

Petin VG and JK Kim. 2004. Survival and recovery of yeast cells after combined treatment with ionizing radiation and heat. Radiat. Res. 161:132-139.

Petin VG and JK Kim. 2004. Recovery inhibitors: Clue for understanding of the mechanisms involved. pp. 423-431. In Proceedings of the 19th Korea Atomic Industrial Forum and Korean Nuclear Society Annual Conference. Seoul, April 25-27.

Petin VG, JK Kim, GP Zhurakovskaya and AV Rassokhina. 2000. Mathematical description of synergistic interaction of UV-light and hyperthermia for yeast cell. J. Photochem. Photobiol. B: Biol. 55:74-79.

Petin VG and VP Komarov. 1997. Mathematical description of synergistic interaction of hyperthermia and ionizing radiation. Mathem. Biosci. 146:115-130.

Petin VG, GP Zhurakovskaya and JK Kim. 2005. Synergetic effects of different pollutants and equidosimetry. pp. 207-222. In Equidosimetry-Ecological Standardization and Equidosimetry for Radioecology and Environmental Ecology (Bréchignac F and G Desmet eds.). Springer, Dordrecht.

Petin VG, GP Zhurakovskaya and LN Komarova. 1999. Mathematical description of combined action of ultrasound and hyperthermia on yeast cells. Ultrasonics 37:79-83.

Raaphorst GP, MM Feeley, CE Danjoux, V Da Silva and LH Gerig. 1991. Hyperthermia enhancement of radiation response and inhibition of recovery from radiation damage in human glioma cells. Int. J. Hyperthermia. 7:629-641.

Sakkers RJ, AR Filon, JF Brunsting, HH Kampinga, AW Konings and LH Mullenders. 1995. Selective inhibition of repair of active genes by hyperthermia is due to inhibition of global and transcription coupled repair pathways. Carcinogenesis 16:743-748.

Streffer C, P Vaupel and G Hahn. 1990. Biological Basis of Oncologic Thermotherapy. Springer, Berlin.

Warters RL. 1993. Persistence of radiation-induced double-strand breaks in the DNA of heated CHO cells. Int. J. Radiat. Biol. 64:669-676.

Weissenborn U and G Obe. 1991. Modification of X-ray induced chromosome aberration frequency by pre- and postirradiation hyperthermia of human peripheral lymphocytes. Int. J. Radiat. Biol. 59:973-984.

Wynstra JH, WD Wright and JL Roti Roti. 1990. Repair of radiation-induced DNA damage in thermotolerant and non-thermotolerant HeLa cells. Radiat. Res. 85:85-89.

Zaider M and HH Rossi. 1980. The synergistic effects of different radiations. Radiat. Res. 83: 732-739.

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이온화 방사선 및 염화수은(II)에 의한 자궁경부암 세포의 DNA 손상 평가

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Evaluation of DNA Damage by Mercury Chloride (II) and Ionizing Radiation in HeLa Cells

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Abstract − The mercury is among the most highly bioconcentrated toxic trace metals. Many national and international agencies and organisations have targeted mercury for the possible emission control. The mercury toxicity depends on its chemical form, among which alkylmercury compounds are the most toxic. A human cervix uterus cancer cell line HeLa cells was employed to investigate the effect of the toxic heavy metal mercury (Hg) and ionizing radiation. In the *in vitro* comet assays for the genotoxicity in the HeLa cells, the group of Hg treatment after irradiation showed higher DNA breakage than the other groups. The tail extent moment and olive tail moment of the control group were 4.88±1.00 and 3.50±0.52 while the values of the only Hg treatment group were 26.90±2.67 and 13.16±1.82, respectively. The tail extent moment and olive tail moment of the only 0.001, 0.005, 0.01 Hg group were 12.24±1.82, 8.20±2.15, 20.30±1.30, 12.26±0.52, 40.65±2.94 and 20.38±1.49, respectively. In the case of Hg treatment after irradiation, the tail extent moment and olive tail moment of the 0.001, 0.005, 0.01 Hg group were 56.50±3.93, 32.69±2.48, 62.03±5.14, 31.56±1.97, 72.72±3.70 and 39.44±3.23, respectively. The results showed that Hg induced DNA single-strand breaks or alkali labile sites as assessed by the Comet assay. It is in good agreement with the reported results. The mercury inhibits the repair of DNA. The bacterial formamidopyrimidine-DNA glycosylase (Fpg protein) recognizes and removes some oxidative DNA base modifications. Enzyme inactivation by Hg (II) may therefore be due either to interactions with cysteine residues outside the metal binding domain or to very high-affinity binding of Hg (II) which readily removes Zn (II) from the zinc finger.

Key words : mercury chloride (II), ionizing radiation, HeLa cell, comet assay

서 론

생체 내에 외인성 유기화합물 (xenobiotics)이 섭취되

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면 생체는 이 물질들이 체내에 쌓이지 않도록 각종 방어기구를 가동시켜 체외로 방출할 수 있도록 대사 한다. 이것이 생체의 이물질에 대한 방어 반응이며 흡수, 대사 및 배설을 포함한 해독 작용이라고도 할 수 있다. 이때 그대로는 체외배출이 곤란한 친유성 물질에 대하여 생체는 여러 가지 다양한 효소를 이용하여 그 물질을 산화, 환원, 가수분해 등의 양식에 의해 무독화하기도 하고 더욱 수용성으로 변화시켜 체외로 배출시킨다. 금속은 강한 생리활성을 가지고 있기 때문에 미량으로 인체에 필수 불가결한 것(Fe, Zn, Cu, Co 등)도 있지만 생물체 본래의 구성성분이 아니고 동식물의 생육 과정이나 식품의 가공, 제조 중에 외부에서 오염되어 혼입되는 환경오염성 중금속(environmental heavy metals)도 있다. 인체에 비교적 독성이 강한 As, Cd, Hg, Pb 등은 대표적인 환경오염금속으로 알려져 있다(Sauve *et al*. 2002). 이 중 수은(Hg)은 가장 오래된 중금속 중독의 하나로서 대부분의 경우 증기 상태로 호흡기를 통하여 체내에 노출되지만 피부나 경구를 통해서도 노출될 수 있다(Zalups *et al*. 2000).

수은 중독에 의한 대표적인 사고로는 1950년대 초 일본의 Kyushu의 Minamata만에서 발생한 사건과 1965년의 Niigata 지역에서의 사건, 그리고 1960년대와 1970년대의 이라크에서 발생한 수은중독 곡물사건을 들 수 있는데, 이들 모두 원인물질은 메틸수은(methylmercury)이었다. 일본에서 발생한 미나마타 사건의 원인은 미나마타만 상류의 화학공장에서 촉매로 사용했던 염화 제2수은($HgCl_2$)이 폐수 중에 방출되어 이토의 혐기적 조건하에서 유기수은인 메틸수은으로 전환되었고 메틸수은은 무기수은보다 생체막을 더 쉽게 투과하여 어패류에 축적, 사람이 이들 어패류를 섭취함으로서 발생하였다. 메틸수은은 인체의 장막, 세포막을 투과하여 표적장기(target organ)인 뇌에 도달하고 여기에서 메틸수은은 탈메틸화(demethylation)되어 효소의 SH기와 결합함으로써 독성을 나타내게 된다. FAO/WHO에서 결정하는 총 수은 섭취 허용량은 43 μg day^{-1}, 메틸수은은 20 μg day^{-1}로 되어있다. 수은중독은 일시에 다량이 흡입되어 발생하는 급성중독과 비교적 적은 량이 장기간에 걸쳐 흡입되므로서 발생하는 만성중독으로 구별된다. 그 증상은 유사하며 주로 지각이상, 운동실조, 언어장애, 청력장애, 구심성 시야 협착 등의 Hunter-Russel 증후군으로 불리는 중추신경 증상을 나타낸다. 무기수은염은 장의 작열감, 동통, 구토, 침흘림, 신장기능장해의 원인이 되며 저급알킬수은은 입술 주변 및 팔다리 끝의 감각 이상, 운동의 어려움, 발음 장해, 청력 장해, 시야 협착, 정신장해 등의 신경계장해, 신장, 뇌의 장해를 유발한다. 모체가 메틸수은을 섭취하면 태아에 메틸수은이 이행 축적되기 때문에 태아의 메틸수은은 중독의 위험성은 성인보다 높으며 출생아에게 현저한 중추신경증상을 일으킨다(윤 1984a, b). 세포 수준에서의 유기 수은은 세포막 물질 수송계에서 active transport system을 통해서 유입이 되는 것으로 알려져 있으며, 수은에 의한 신장 독성은 항산화에 관련된 세포(GSH 세포크기와 Gpx 활성)를 변화시켜 산화적 손상을 준다는 것이 MDCK (Madin.Darby canine kidney) cell을 이용한 실험에서 밝혀졌다(Youm *et al.* 2000; Aleo *et al.* 2002).

이온화 방사선 또한 산화적 손상을 일으키는 물리적 요인으로, 특히 DNA에 손상을 일으키는 것으로 알려져 있다(Şener *et al*. 2003). 이온화 방사선에 의한 세포내 DNA 손상의 60～70%는 물의 방사선에 의한 분해로 생성된 OH에 의한 것으로 밝혀졌으며, 과도한 자유라디칼이나 낮은 항산화기능에 의한 산화적 스트레스는 생체분자의 구조적, 기능적 변화를 초래한다. 이러한 변화는 DNA의 치명적인 손상으로 이어질 뿐 아니라 지질과 단백질의 손상과도 관련이 있다(Vijayalaxmi *et al*. 1998).

국내·외 대부분의 실험은 세포 수준에서의 수은 단독 처리에 의한 독성과 그 기작을 알아보거나 이온화 방사선의 단독 처리에 의한 DNA의 손상을 알아보는데 그 초점이 맞춰져 있다. 복합처리에 관한 연구사례로는 사람 림프구에 수은과 이온화 방사선 동시 처리하였을 때 나타나는 DNA 손상 및 수복을 연구한 보고가 있을 뿐이다(Panek *et al*. 2001). 따라서 본 연구에서는 산화적 손상을 일으키는 두 가지 요인, 수은과 이온화 방사선이 수은 처리 전·후에 함께 가해졌을 경우, 그 손상의 상호 작용을 비교하고자 하였다.

재료 및 방법

1. 실험재료 및 처리

본 연구에서 사용된 자궁경부암 세포인 HeLa는 10% FBS (fetal bovine serum)와 1%의 항세균 및 항진균 항생제가 포함된 RPMI-1640 배지에서 37°C의 항온기내에서 배양하였다. 유전 독성 실험을 위해 well 당 1 mL의 배지가 들어 있는 12 well plate에 약 12×10^4 cells $well^{-1}$ 세포 농도로 접종하였고 접종 24시간 후에 수은 및 방사선 처리를 실시하였다. 실험은 수은 및 방사선 단독처리군, 수은 처리 후 방사선 처리군, 방사선 처리 후 수은 처리군으로 나누어 실시하여 수은과 방사선의 단독처리 시 유전독성과 병행 처리 시 유전독성의 시너지효

과를 알아보았다. 수은 처리군은 생리 식염수에 0.001, 0.005, 0.01 μM의 농도로 염화수은(II)을 녹여 5분간 처리하였다. 조사선량은 선량-반응식을 기준으로 단기간 내에 손상이 확인된 선량을 조사하였으며, 방사선 처리군은 기존 보문을 토대로 선량-반응식을 기준으로 단기간 내에 손상이 확인된 선량을 조사하였다(Kim *et al.* 1999). 한국원자력연구소의 ^{60}Co γ-ray를 이용하여 5분간 총 선량이 6.5 Gy가 되게 조절하였으며, 얼음 위에서 행하였다. 수은 처리군은 처리 후에, 방사선 처리군은 조사 전에 trypsin-EDTA를 처리하여 세포를 모아준 후 DPBS (Dulbecco's phosphate buffered saline) 500 μL에 세포를 분산시켜 수은과 방사선으로 인한 유전적 독성 평가를 수행하였다.

2. 수은 처리 시 세포 생존율

세포의 생존율은 MTT (3-[4, 5-dimethylthiazol-2yl]-2, 5-diphenyl-tetrazolium bromide; Sigma) reduction method를 변용하여 측정하였다(Mossman 1983). 이 방법은 세포 내 미토콘드리아의 호흡기능을 측정하는 방법으로 tryphan blue dye-exclusion의 방법보다 정확히 세포의 생존율을 측정할 수 있다고 알려져 있다. 자궁경부암세포를 2×10^4 cell mL^{-1}의 밀도로 6 well-plate에 접종 후 RPMI-1640 배양액에 24시간 배양하였다. 생리식염수로 녹인 수은을 1, 0.1, 0.01 μM의 농도로 2시간 동안 배양액에 처리하였다. 수은 처리 후 배양액을 제거하고 MTT 용액(0.05% in medium)을 30 μL 첨가하여, 배양기 내에서 2시간 동안 반응시키고 반응시킨 MTT용액은 제거하였다. 배양접시 바닥에 형성된 formazan crystals를 DMSO (dimethyl sulfoxide, Sigma) 1 mL에 녹인 후 ELISA reader (Thermo Labsystems)를 사용하여 580 nm의 파장으로 흡광도를 측정하여 세포의 생존율을 계측하였다.

3. 세포 내 유전적 독성 실험

수은과 방사선의 유전적 독성을 알아보기 위하여 comet assay (single cell gel electrophoresis assay)를 실시하였다. 본 연구에서는 P. Grover *et al.*의 방법을 변형하여 실시하였다(Grover *et al.* 2001). 처리 물질이 아닌 시험 과정에서의 DNA 손상을 최소화하기 위해 모든 과정은 어두운 조명 하에서 이루어 졌다. DPBS에 분산시켜 놓은 자궁경부암세포 100 μL와 1% LMP (low melting point agarose) 100 μL를 혼합하여 전날 pre-coating해 놓은 slide glass에 떨어뜨린 후 cover glass를 덮어 전체적으로 퍼지게 하였다. 4°C에서 20분간 굳힌 후 다시 200 μL의 0.5% LMP로 한 층을 덧입혀 4°C에서 굳힌다. 만들어진 slide는 cover glass를 제거한 후, 4°C lysing solution [2.5 M NaCl, 100 mM EDTA, 10 mM Tris (pH 10), 1% sodium sarcosinate, 사용 직전에 1% Triton X-100과 10% DMSO를 혼합]에 담가 1시간 동안 cell lysis를 시킨다. 4°C의 물로 slide에 남은 용액을 제거하고 4°C Electrophoresis buffer (300 mM NaOH and 1 mM, EDTA pH 13)에서 5분간 unwinding 시킨 후 26 V, 300 mA에서 30분간 전기영동을 실시하였다. 전기영동이 끝난 slide는 4°C의 물로 씻어 준 후 400 mM Tris buffer (pH 7.5)에서 15분간 중성화과정을 시행하며 이 과정을 3회 반복하였다. 50 μL EtBr (20 μL mL^{-1})로 염색하여 CCD camera (Hitachi Denshi, Ltd., Japan)가 부착된 형광현미경 (Olympus fluorescence microscope, Japan)에서 200배율로 검경하고, Komet ver. 4.0 image analysis (Kinetic Imaging Ltd, Liverpool, UK)를 이용하여 이미지를 분석하였다. 한 slide 당 50개의 핵을 관찰하여 통계분석 하였다.

4. 통계학적 분석

통계학적 유의적 검정은 일원분산분석 (one-way ANOVA test)을 사용하였고, p값이 0.001보다 작은 경우를 유의하다고 판정하였다. 반복 실험의 결과는 평균±표준편차평균(SEM)으로 나타내었다.

결 과

1. 수은 처리 시 세포의 생존율

세포 생존율에 미치는 수은의 농도를 알아보기 위하여 MTT cell viability assay를 실시하였다. 염화수은(II)을 처리 2시간 경과 후 무처리군의 생존율을 100%로 환산했을 때, 1 μM의 수은 농도에서는 16.9±5.61%, 0.1 μM에서 23.1±17.71, 0.01 μM에서 63.9±57.46%의 생존율을 보였다(Table 1). 이는 모든 농도에서 대조군에 비하여 유의적으로 감소되는 것으로 확인되었다($p<0.001$). 1 μM의 경우 약 87%, 0.1 μM에서 약 73%, 0.01 μM에서 36%의 생존율 감소를 나타낸다. 0.01 μM과 0.1 μM의 염

Table 1. Effects of the Hg on the HeLa cells cultured for 2 hours *in vitro*

Group	Optical density	Cell viability (%)
Control	1.8±0.15	100±8.45
1 μM Hg	0.3±0.10	16.9±5.61
0.1 μM Hg	0.4±0.32	23.1±17.71
0.01 μM Hg	1.2±1.03	63.9±57.46

화수은 농도에서는 약 2배 정도의 급격한 감소율을 보였으며 0.1 μM과 1 μM에서는 완만한 감소율이 나타났다.

2. 수은과 방사선의 세포 내 유전 독성

수은과 방사선의 상호 영향을 알아보기 위해 수은 단독 처리, 방사선 조사 후 수은 처리, 수은처리 후 방사선 조사 시 세포 내 독성 실험을 각각 실시하였다. 육안으로 확인한 결과, 방사선 단독 처리군 보다 수은 단독 처리 시 손상된 DNA의 끌림 현상이 더 많이 관찰되었다. 수은과 방사선을 복합 처리한 실험군에서 DNA 손상이 단독 처리군과 비교하여 확연히 높아지는 것을 확인할 수 있었다(Fig. 1).

수은 단독 처리 시 수은의 처리농도가 높아짐에 따라 손상된 DNA의 끌림 정도가 심화되는 것을 관찰할 수 있었다. 대조군, 0.001, 0.005, 0.01 μM 수은 처리군의 tail extent moment 값은 각각 4.88±1.00, 12.24±1.82, 20.30±1.30, 40.65±2.94로 수은 처리 농도가 높아짐에 따라 유의적으로 증가되며 대조군에 비해 대략 3, 4, 9배의 손상을 가져오는 것으로 나타났다($p<0.001$). Olive tail moment 값은 각각 3.50±0.52, 8.20±2.15, 12.26±0.52, 20.38±1.49로 대조군의 2, 4, 6배 정도의 손상을 나타내었다(Fig. 2).

방사선 조사 후 수은 처리 시 수은 단독 처리나 방사선 단독 처리보다 DNA의 손상 정도가 유의적으로 크게 증가하였다($p<0.001$). 대조군, 방사선 단독 처리군, 방사선 처리 후 0.001, 0.005, 0.01 μM 수은 처리군의 tail extent moment값은 4.88±1.00, 26.90±2.67, 56.50±3.93, 62.03±5.14, 72.72±3.70으로 모든 수은 처리 농도에서 수은 단독 처리의 손상치와 방사선 단독 처리의 손상치를 더한 값보다 20 정도의 손상이 더해졌음을 확인할 수 있었다. Olive tail moment의 경우, 대조군, 방사선 단독 처리군, 방사선 처리 후 0.001, 0.005, 0.01 μM 수은 처리군의 값은 3.50±0.52, 13.16±1.82, 32.69±2.48, 31.56±1.97, 39.44±3.23으로 모든 처리 농도에서 단독 처리 시의 손상치의 합보다 높은 손상치를 보여줬으며 단 0.001과 0.005 μM 수은 처리군에서는 수은의

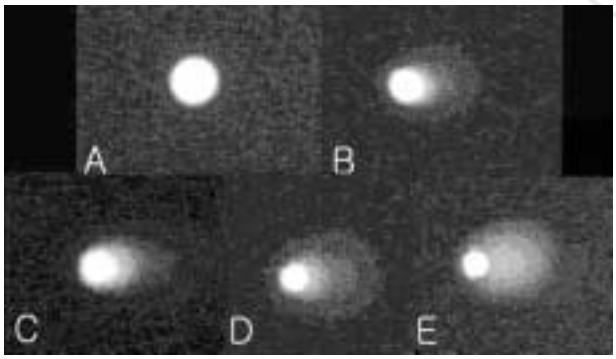

Fig. 1. Comet images of intact and DNA-damaged cells. A: intact cell, B: DNA damaged cell by 0.001 μM Hg, C: DNA damaged cell by irradiation, D: DNA damaged cell by the irradiation after 0.001 μM Hg, E: DNA damaged cell by 0.001 μM Hg after the irradiation.

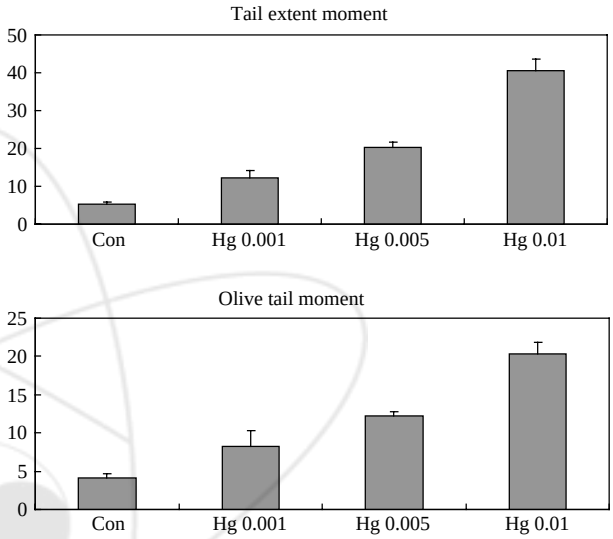

Fig. 2. The *in vitro* genotoxicity assays of only Hg treatment using comet assay of HeLa cell. extent tail moment=tail length×tail% DNA/100, olive tail moment=(tail mean-head mean)×tail% DNA/100; tail extent moment=tail length * tail% DNA/100.

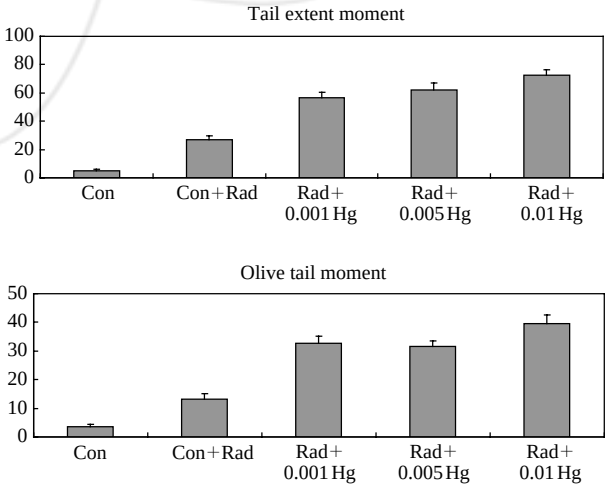

Fig. 3. The *in vitro* genotoxicity assays of Hg treatment after the irradiation using comet assay of HeLa cell. extent tail moment=tail length×tail% DNA/100, olive tail moment=(tail mean-head mean)×tail% DNA/100; tail extent moment=tail length * tail% DNA/100.

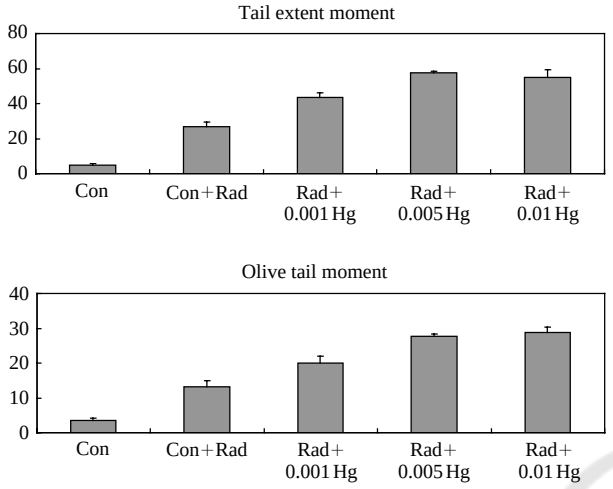

Fig. 4. The *in vitro* genotoxicity assays of the irradiation after Hg treatment using comet assay of HeLa cell. extent tail moment=tail length×tail% DNA/100, olive tail moment =(tail mean-head mean)×tail% DNA/100; tail extent moment=tail length *tail% DNA/100 .

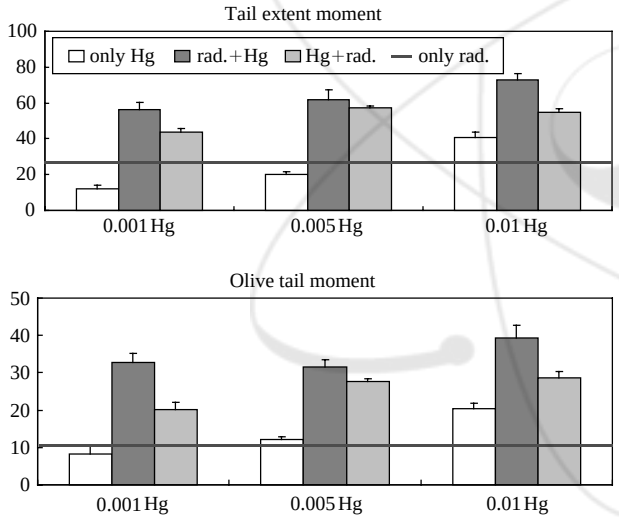

Fig. 5. The *in vitro* genotoxicity assays of the irradiation and Hg treatment using comet assay of HeLa cell. extent tail moment=tail length×tail% DNA/100, olive tail moment =(tail mean-head mean)×tail% DNA/100; tail extent moment=tail length *tail% DNA/100.

농도가 5배 높아졌으나 급격한 차이는 나타나지 않았다 (Fig. 3).

수은 처리 후 방사선 조사 그룹에서는 조사 후 수은 처리군보다 DNA의 손상 정도가 작은 것으로 나타났다. 대조군, 방사선 단독 처리군, 0.001, 0.005, 0.01 μM 수은 처리 후 방사선 조사군의 tail extent moment 값은 4.88±1.00, 26.90±2.67, 43.69±2.60, 57.42±1.19, 54.90±4.46으로 방사선 조사 후 수은 처리군보다 낮은 손상 정도를 보였으며 0.01 μM 수은 농도에서는 0.005 μM 수은 농도와 비슷한 손상 정도를 보였다. 이는 olive tail moment의 값에서도 나타나는데 대조군, 방사선 단독 처리군, 0.001, 0.005, 0.01 μM 수은처리 후 방사선 조사군의 olive tail moment의 값은 3.50±0.52, 13.16±1.82, 20.05±2.01, 27.66±0.67, 28.72±1.69로 0.001 μM과 0.005 μM 사이에서는 약 1.5배 정도의 수치가 증가하였으나 0.005 μM과 0.01 μM 수은 농도 비교 시 손상 정도의 급격한 차이는 관찰되지 않았다(Fig. 4).

고 찰

가장 오래된 중금속 중에 하나인 수은은 그 화학적 형태의 다양성과 산업상의 유용성으로 인하여 대기, 토양 오염을 비롯하여 다방면의 오염을 유발하고 있다. 특히 생화학적 측면에서의 유기 수은은 체내의 대사과정에 참여하는 여러 효소의 활성을 억제함으로서 독성을 유발하는 것으로 알려져 있고 또 수은이 일차적으로 세포막을 구성하는 단백질 중, 특히 수은과 친화력이 높은 sulfhydryl (SH)기를 포함하는 단백질과 결합함으로써 독성을 유발하는 것으로 보고되고 있다(Goyer 1991). 특히 SH기는 세포막의 투과성 및 물질이동에 필수적이기 때문에 이와 관련된 세포독성을 일으킨다. 또한 +2가의 수은이 실험동물의 세포막에서 지질과산화반응을 유발함으로서 세포손상을 초래할 뿐 아니라 손상된 세포로부터 유리된 것으로 추측되는 젖산탈수소효소(lactate dehydrogenase, LDH)의 활성이 증가된다고 보고되고 있는데, 효소의 불활성화나 지질과산화반응의 초기에 O_2, OH, H_2O_2 등이 관여하여, 반응 후 생성된 malondialdehyde와 같은 물질이 강한 세포독성을 나타내는 것으로 보고된 바 있다(Stacey and Klaassen 1980). 이는 결과적으로 DNA 나선구조의 손상, 단백질 thiol 기의 산화 및 항산화 효소의 활성변화 등과 관련된다.

이온화 방사선 또한 산화적 손상을 일으키는 물리적 요인으로, 특히 DNA에 손상을 일으키는 것으로 알려져 있다. 이온화 방사선에 의해 생성된 과도한 자유라디칼이나 낮은 항산화기능에 의한 산화적 스트레스는 생체분자의 구조적, 기능적 변화를 일으키게 되며 그 결과 DNA뿐 아니라 지질과 단백질에도 손상을 초래하는 것으로 알려져 있다(Vijayalaxmi *et al*. 1998).

따라서 본 연구에서는 *in vitro* 내에서 염화수은(II)의 독성과 세포독성을 일으키는 두 가지 요인, 염화수은과 이온화 방사선을 동시처리 시, 그 손상의 상호 작용을

비교하였다. 염화수은(II)의 독성정도를 알아보자고 사람의 자궁암 세포에 농도별로 염화수은(II)을 처리하였다. 세포의 생존율은 3가지 농도(1, 0.1, 0.01 μM) 모두에서 유의하게 감소하였으며 이미 0.1 μM에서 약 73%의 생존율이 감소하는 것으로 나타나 기존 보문의 사람의 간세포에서 25 μM에서 60%의 생존율보다 월등히 낮은 생존율을 나타냈다(Vakharia *et al*. 2001).

염화수은(II)과 방사선의 단독 처리와 복합 처리 시 세포 내의 유전 독성 차이를 알아보기 위하여 comet assay를 실시하였다. Comet assay는 각각의 세포에서 DNA 손상을 직접 가시화하는 전기영동 방법으로 1984년에 Östling과 Johanson에 의해서 처음으로 소개되었다(Östling *et al*. 1984). 전류가 하전을 띠고 있는 DNA를 핵으로부터 잡아당김으로써 완화된 DNA와 깨진 DNA 절편들을 이동시켜 '혜성' 같은 모양을 나타내는데 이 이미지가 DNA 손상정도를 결정하는 척도로 이용된다. 여러 파라메타 중 tail moment 값을 이용하여 DNA의 손상 정도를 비교하였는데, tail moment는 얻어진 혜성모양의 DNA상으로부터 측정한 head, tail 그리고 절단된 DNA 조각의 형광염색 밀도 등을 감안한 값으로 단순 측정된 tail length만을 사용할 경우 실제 DNA 분자 상에 유발된 손상이 지나치게 확대 해석 될 수 있는 단점을 보완할 수 있다고 알려져 있다(Anderson *et al*. 1994). 방사선 전·후 수은 처리를 비교 하였을 경우, 전처리에 비해 후처리의 손상 정도가 더욱 심한 것으로 관찰되었다(Fig. 5). 이러한 결과로 수은 처리 후 방사선을 조사할 경우 수은 단독처리군과 비교하여 손상정도가 심해지기는 했으나 수은과 방사선이 DNA 손상에 각각 다른 독성원으로 작용한 것으로 두 물질간의 상호작용은 일어나지 않은 것으로 사료된다(Fig. 4). 이온화 방사선에 의해 사람세포 DNA에 유발된 손상은 짧은 시간 안에 회복이 진행되는데 손상의 절반이 회복되는데 걸리는 시간은 약 15분 정도로 보고되어 있다(Cebulska-Wasilewska 2003). 그러나 본 실험의 결과에서 볼 수 있듯이 방사선 조사 후 수은을 처리한 경우 DNA 손상 정도가 유의하게 증가됨을 보였다. 이로써 기존 보문(Panek *et al*. 2001)의 수은이 손상된 DNA의 수복 작용을 방해한다는 것을 확인할 수 있었다. 수은은 Fpg protein (the bacterial formamidopyrimidine-DNA glycosylase)에 영향을 미치는 데 이 효소는 손상되어 깨어진 DNA base를 전환하여 DNA를 수복시켜 주는 역할을 하는 것으로 알려져 있다. 이 수복 작용에는 4가지의 시스테인(cystein)과 아연이 결합하여 이루어지는 데 수은은 이 작용에 중요한 역할을 하는 아연을 제거하는 것으로 알려져 있다(Hartwig *et al*. 2002). 따라서 방사선으로 산화적 손상을 입은 DNA가 수복을 하는 과정 중 수은을 처리하여 아연을 제거함으로서 그 손상의 정도가 심해지는 것으로 판단된다.

본 실험 결과들을 통하여 염화수은(II) 처리와 이온화 방사선의 조사에 따른 손상의 차이 및 방사선 전·후 수은 처리 시 그 DNA 손상 정도의 차를 확인 할 수 있었다. 이러한 결과를 토대로 수은 뿐만 아니라 DNA 수복 기작에 영향을 주는 다른 환경오염성 중금속인 Cd (II), Cu (II), Pb (II) 등과 방사선의 상호 작용에 대한 연구도 이루어 질 수 있을 것을 판단된다.

적 요

세포에 미치는 염화수은(II)과 이온화 방사선의 영향과 수은 처리 전·후 방사선 조사 시 그 상호 작용에 관해 알아보고자 본 연구를 수행하였다. 염화수은(II)의 독성정도를 알아보기 위하여 사람의 자궁암 세포에 농도별로 염화수은(II)을 처리하였다. 세포의 생존율은 3가지 농도(1, 0.1, 0.01 μM) 모두에서 유의하게 감소하였으며 이미 0.1 μM에서 약 73%의 생존율이 감소하는 것으로 나타났다. 염화수은(II)과 방사선의 단독처리 시 DNA의 손상 정도에 비해 복합처리 시의 DNA 손상 정도가 2~4배 정도 확연히 높아짐을 볼 수 있었다. 특히 방사선 후 수은 처리군은 DNA 손상의 정도가 다른 처리군에 비하여 높게 나타났는데 이는 이미 기존의 보문에서 밝혀진 바와 같이 수은의 DNA 수복에 관련되어 있는 Fpg protein에 미치는 영향 때문으로 사료된다. 이미 방사선에 의해 산화적 손상을 입은 DNA의 수복 기작을 수은이 방해하여 좀 더 높은 손상을 가져오는 것을 확인할 수 있었다.

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참 고 문 헌

윤임중. 1984a. 수은 중독(상), 대한 산업 보건 협회 산업 보건. 6:7-9.

윤임중. 1984b. 수은 중독(하), 대한 산업 보건 협회 산업 보건. 7:5-7.

Aleo MF, F Morandini, F Bettoni, S Tanganelli, A Vezzola, R

Giuliani, N Steimberg, P Apostoli and G Mazzoleni. 2002. Antioxidant potential and gap junction-mediated intercellular communication as early biological markers of mercuric chloride toxicity in the MDCK cell line. Toxicol. in Vitro 16:457-465.

Anderson D, TW Yu, BJ Philips and P Schmezer. 1994. The effect of various-antioxidants and other modifying agents on oxygenradical-generated DNA damage in human lymphocytes in the comet assay. Mut. Res. 307:261-271.

Cebulska-Wasilewska A. 2003. Response to challenging dose of X-rays as a predictive assay for molecular epidemiology. Mut. Res. 544:289-297.

Hartwig A, M Asmuss, H Blessing, S Hoffmann, G Jahnke, S Khandelwal, A Pelzer and A Bűrkle. 2002. Interference by toxic metal ions with zinc-dependent proteins involved in maintaining genomic stability. Food Chem. Toxicol. 40: 1179-1184.

Kim JK, CJ Lee, KW Song, HH Park and YD Yoon. 1999. Morphological anomaly of primordial follicle in γ-irradiated mice. J Korean Nuc. Soc. 31:385-390.

Mossman T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assay. J. Immunol. Method. 65:55-63.

Goyer RA. 1991. Toxic effects of metal. Casarett Doll's Toxicol. 4:646-651.

Grover P, BS Banu, KD Devi and S Begum. 2001. In vivo genotoxic effects of mercuric chloride in rat peripheral blood leucocytes using comet assay. Toxicology 167:191-197.

Östling O and KJ Johanson. 1984. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells, Biochem. Biophys. Res. Commun. 123: 291-298.

Panek A, JK Kim and A. Cebulska-Wasliewska, 2001. *In vitro* studies of repair efficiency of DNA damage induced by X-rays in lymphocytes exposed to mercury. 12nd Symposium of Polish Radiation Research Society, Sept. 10-12, 2001, Krakow, Poland.

Sauve S, P Brousseau, J Pellerin, Y Morin, L Senecal, P Goudreau and M Fournier. 2002. Phagocytic activity of marine and freshwater bivalves: in vitro exposure of hemocytes to metals (Ag, Cd, Hg and Zn). Aquat. Toxicol. 58:189-200.

Şener G, N Jahovic, OB Tosun, M Atasoy and BÇ Yeen. 2003. Melatonin ameliorates ionizing radiation-induced oxidative organ damage in rat. Life Sci. 74:563-572.

Stacey NH and CD Klaassen. 1980. Comparison of the effects of metals on cellular injury and lipid peroxidation in isolated rat hepatocyte. J. Toxicol. Environ Health. 7:139.

Vakharia DD, N Liu, R Pause, M Fasco, E Bessett, QY Zhang and LS Kaminsky. 2001. Effect of metals on polycyclic aromatic hydrocarbon induction of CYP1A1 and CYP1A2 in human hepatocyte cultures. Toxicol. Appl. Phamacol. 170:93-103.

Vijayalaxmi, RJ Reiter, TS Herman and ML Meltz. 1998. Melatonin reduces gamma radiation-induced primary DNA damage in human blood lymphocytes. Mutat. Res. 397: 203-208.

Youm JH, DH Koh, JY Kim and NS Kim. 2000. Changes on the methylmercury-induced cytotoxicity by control of cell membrane transport system. Korean J. Environ. Health Soc. 26:88-96.

Zalups RK. 2000. Molecular interactions with mercury in the kidney. Pharmacol. Rev. 52:113-144.

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Inhibition of Recovery from Potentially Lethal Damage by Chemicals in Chinese Hamster Cells is Realized through the Production of Irreversible Damage

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Abstract – The inhibition of cell recovery might be proceeded via either the damage of the mechanism of the recovery itself or via the formation of irreversible damage which could not be repaired at all. Both these processes may take place at the same time. Any of these possibilities would result in a decrease in both the rate and extent of cell recovery. To distinguish them, a quantitative approach describing the process of recovery as a decrease in the effective radiation dose was applied to experimental data on the recovery from potentially lethal damage in Chinese hamster cells exposed to X–rays alone or combined with various chemicals (pyruvate, novobiocin, lactate, nalidixic acid, 3–aminobenzamide). For these particular cases, it is concluded that the recovery process itself is not damaged and the inhibition of the recovery is entirely due to the enhanced yield of the irreversibly damaged cells.

Key words : recovery, irreversible damage, chemical, radiation, Chinese hamster cell

INTRODUCTION

It is believed that DNA repair is one of the most important factors in determining cellular sensitivity to ionizing radiation and some other cytotoxic agents (Alper 1979). Many tumours are known to become resistant to ionizing radiation due to the increased efficiency of DNA repair (Burt *et al*. 1991). Therefore, the impairment of the cell ability to recover from radiation damage would be of great relevance in cancer treatment. A lot of chemicals are known to enhance the inactivation effect of ionizing radiation on various cellular systems. It seems generally accepted now that the enhancing effects may be due to both direct drug toxicity and to the enhancement of the cellular radiosensitivity (Streffer and Müller 1984; Hill and Bellamy 1984). It is assumed that drug radiosensitization may be brought about by the inhibition of repair on a cellular level including the recovery from potentially lethal radiation damage. This type of recovery was demonstrated in the cells of various origins and may play a role in the treatment of tumours with ionizing radiation (Weichselbaum 1986). The inhibition of cell recovery (Kumar *et al*. 1985a, b; Little *et al*. 1989; Utsumi *et al*. 1990) and DNA double strand breaks repair (Boothman *et al*. 1989; Yang *et al*. 1995; Takahashi *et al*. 2000) by chemicals is expressed both as a retarded recovery rate and a lesser extent of recovery. It is obvious that these observations may be caused by the following reasons: (i) the damage or inhibition of the recovery process itself, (ii) the increase in the portion of irreversible damage, (iii) both of these reasons. In our recent publications it was shown that the inhibition of the recovery from potentially lethal damage in yeast cells exposed to hyperthermia and ionizing radiation (Petin and Kim 2004) or hyperthermia and UV light (Kim *et al*. 2004) was realized only through the enhanced yield of the irrever-

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sible damage whereas the recovery capacity itself was not damaged or impaired. Although there are considerable interests in combining ionizing radiation with chemicals in order to improve tumor treatment, there are few reports in the literature on a separate estimation of the chemicals influence on the process of recovery itself and the production of irreversible damage. Therefore, it would be of particular interest biophysically to implement such a study. In this paper, a quantitative approach describing the recovery of a cell from potentially lethal damage as a decrease in the effective dose will be used to estimate separately the probability of recovery per unit time and the fraction of irreversible damage. As an example, this approach will be applied to experimental data published by others (Kumar *et al*. 1985a, b) and related with the inhibition of recovery from potentially lethal damage by chemicals (pyruvate, novobiocin, lactate, nalidixic acid and 3–aminobenzamide) in Chinese hamster V79 cells.

MATERIAL AND METHODS

1. Experimental procedures

Experimental data published by others (Kumar *et al*. 1985a, b) have been used for this study. Nevertheless some important points should be mentioned. To determine the time course of the inhibition of recovery from potentially lethal damage, immediately after X–irradiation the stationary phase Chinese hamster cells were incubated with 0, 10, 20 mM of pyruvate, lactate or 3–aminobenzamide, 0, 5, 10, 20 μM of novobiocin or nalidixic acid during 6, 12, and 24 h before they were plated without chemicals to determine their survival by colony–forming ability. Besides, cells were treated with 20 mM of pyruvate and lactate and 20 μM of novobiocin and nalidixic acid starting 1 h after irradiation for 6, 12, and 24 h and then replated for colony formation without chemicals. Chemicals were added following irradiation in order to limit their effect to the recovery period only. For details of protocol see the initially published papers (Kumar *et al*. 1985a, b).

2. Estimation of the recovery parameters

During the recovery process the survival of the irradiated cell increases, which means the decrease in the effectiveness of the initial dose D_1 takes place. Then a certain survival $S(t)$ and the corresponding effective dose $D_{eff}(t)$ can be indicated following the recovery during t hours. In other words, the effective dose is equal to the dose in which the cells should initially be irradiated to attain $S(t)$. It was demonstrated (Korogodin *et al*. 1968; Korogodin 1993) that during the recovery of yeast cells from potentially lethal radiation damage, the decrease in the effective dose could be described by an equation of the form

$$D_{eff}(t) = D_1[K + (1 - K)\, e^{-\beta t}] \tag{1}$$

where D_1 is the initial radiation dose; e is the basis of the natural logarithm, β is the recovery constant that characterizes the probability of recovery from radiation damage per unit time, and K is an irreversible component of the radiation damage expressed as a fraction of the initial irradiation dose by

$$K = K(\infty) = D_{eff}(\infty)/D_1 \tag{2}$$

where $K(\infty)$ and $D_{eff}(\infty)$ are determined for mammalian cells at $t = 24$ h when the recovery curves reach a plateau (conditionally $t = \infty$) and the capability of the cells to recover is saturated or exhausted. The ratio $D_{eff}(\infty)/D_1$ can be considered as an irreversible component of the radiation damage. The cells are believed to be incapable to recover from this part of damage even if they are incubated for a long time at the conditions promoting the recovery. Then the change of this component during the recovery

$$K(t) = D_{eff}(t)/D_1 \tag{3}$$

reflects the relative part of the initial radiation dose or the primary radiation damage, both reparable and irreversible, which has not been repaired during t hours of recovery. Taking into account Eqs. (1) and (2), we have

$$e^{-\beta t} = [D_{eff}(t) - D_{eff}(\infty)]/[D_1 - D_{eff}(\infty)] \tag{4}$$

The right part of this Equation reflects the relative part of the reparable damage that has not been repaired after t hours of recovery. Designating this part through $A(t)$, we can write

$$\beta = -[lnA(t)]/t \tag{5}$$

Thus, by knowing the dependence of the cell survival on a radiation dose and the recovery pattern after exposure to ionizing radiation applied alone or combined with various

chemicals, one can calculate $D_{eff}(t)$ and $D_{eff}(\infty)$. It enables us to calculate $K(t)$ in the dependency of the recovery time t (Eq. 3) and evaluate the irreversible component K (Eq. 2). Having calculated the dependency of $lnA(t)$ on the recovery time t, one can estimate the recovery constant β (Eq. 5). It is worth noting that very similar approach was used before to describe the biological effects after whole body gamma irradiation (Davidson 1957), the recovery of yeast cells irradiated with ionizing radiation (Korogodin *et al*. 1968; Korogodin 1993) or exposed to heat combined with ionizing radiation (Petin and Kim 2004) and UV light (Kim *et al*. 2004). So far its applicability to mammalian cells irradiated alone or combined with chemicals has never been tested.

RESULTS

Fig. 1 shows the survival (curve 1) and recovery (curve 2) curves of the stationary phase cells of Chinese hamster V79 cells irradiated (300 kV X–rays, dose rate being 1.25 Gy min^{-1}) and recovered without chemical treatments. Arrows indicate an example of the effective dose estimation. Both these curves were obtained by averaging six dose–effect and four time–effect curves published by other authors (Kumar *et al*. 1985a, b). Kinetics of the recovery from potentially lethal radiation damage showed (Kumar *et al*. 1985a, b) that the survival increase due to recovery observed in the controls was gradually reduced as the chemical

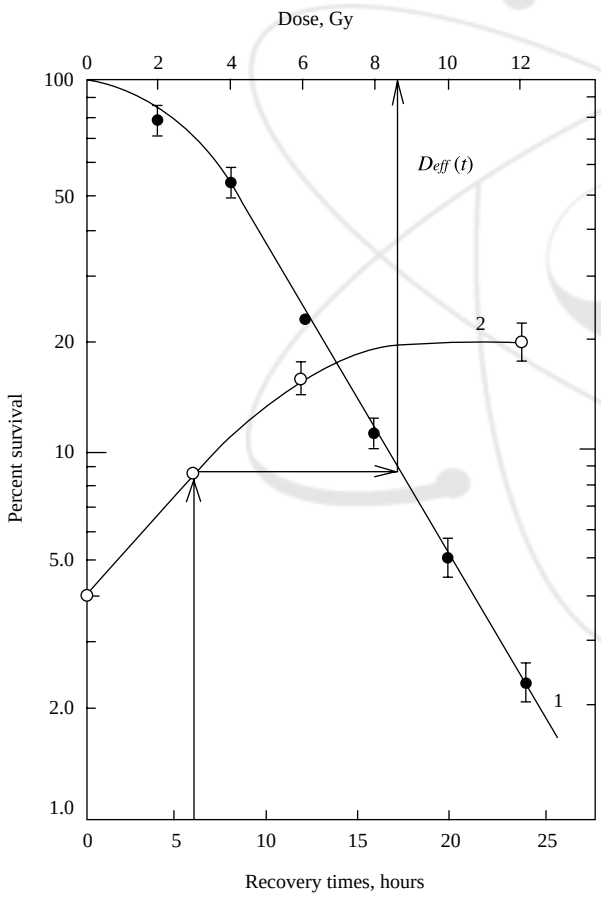

Fig. 1. Survival of Chinese hamster V79 cells in the dependence of X–ray dose (curve 1) and the duration of recovery from potentially lethal damage (curve 2). Cells were irradiated and recovered without chemicals. The results were obtained by averaging of six dose–effect and four time–effect curves published by other authors (Keifer *et al*. 1985a, b). Arrows indicate an example of the initial dose D_1 and the effective dose $D_{eff}(t)$ determination. *Points*, mean; *bars*, SE.

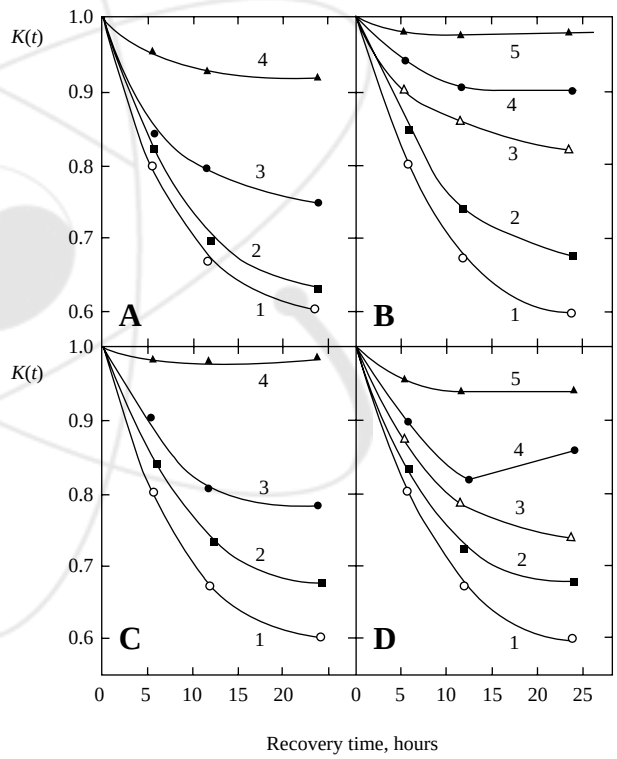

Fig. 2. The dependence of the relative fraction of irreversible damage $K(t) = D_{eff}(t)/D_1$ on the duration of recovery time of Chinese hamster V79 cells recovering after irradiation without chemicals (curves 1) and in the presence of chemical inhibitors of cell recovery. A, pyruvate: 20 mM 1 h after irradiation (curve 2), 10 and 20 mM immediately after irradiation (curves 3 and 4). B, novobiocin: 20 μM 1 h after irradiation (curve 2), 5, 10 and 20 μM immediately after irradiation (curves 3, 4 and 5). C, lactate: 20 mM 1 h after irradiation (curve 2), 10 and 20 mM immediately after irradiation (curves 3 and 4). D, nalidixic acid: 20 μM 1 h after irradiation (curve 2), 5, 10 and 20 μM immediately after irradiation (curves 3, 4 and 5).

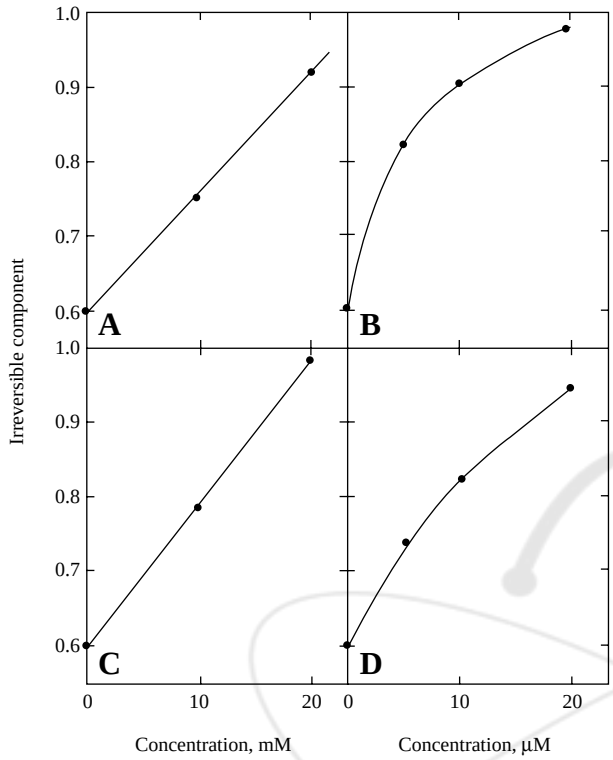

Fig. 3. Irreversible component $K(\infty)$ of radiation damage in Chinese hamster V79 cells recovering from potentially lethal damage in the presence of various concentrations of chemicals: pyruvate (A), novobiocin (B), lactate (C), and nalidixic acid (D).

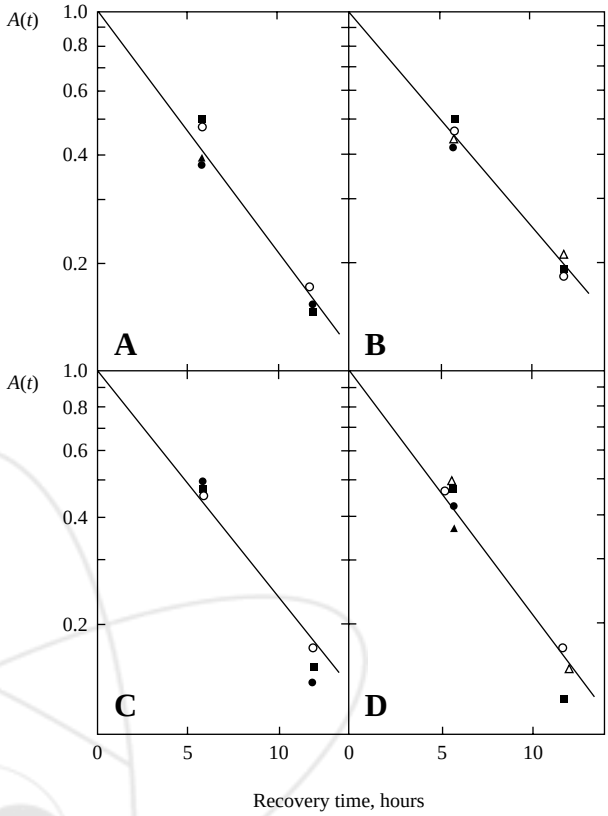

Fig. 4. The dependence of $A(t) = [D_{eff}(t) - D_{eff}(\infty)]/[D_1 - D_{eff}(\infty)]$ on the duration of recovery time of Chinese hamster V79 cells recovering after irradiation without chemicals (open circles) and in the presence of chemical inhibitors of cell recovery. A, pyruvate: 20 mM 1 h after irradiation (closed squares), 10 and 20 mM immediately after irradiation (closed circles and triangles, respectively). B, novobiocin: 20 μM 1h after irradiation (closed squares), 5, 10 and 20 μM immediately after irradiation (open triangles, closed circles and triangles, respectively). C, lactate: 20 mM 1 h after irradiation (closed squares), 10 and 20 mM immediately after irradiation (closed circles and triangles, respectively). D, nalidixic acid: 20 μM 1 h after irradiation (closed squares), 5, 10 and 20 μM immediately after irradiation (open triangles, closed circles and triangles, respectively).

concentration increased, i.e. the inhibition of the recovery was drug concentration dependent. Using these results and the data presented in Fig. 1, we calculated (Eq. 3) the dependency of the relative fraction of the irreversible damage $K(t) = D_{eff}(t)/D_1$ on the duration of the recovery time of the Chinese hamster V79 cells recovering after irradiation without chemicals and in the presence of various chemical inhibitors of the cell recovery. The results are shown in Fig. 2. It can be noted that the untreated cells subjected to post–irradiation recovery showed an appreciable decrease in $K(t)$ whereas this effect became gradually worse as the chemical concentration increased. It appears that the inhibition of the recovery depends on the drug concentration and is almost complete with 20 mM of pyruvate and lactate and 20 μM of novobiocin and nalidixic acid. For instance, the limited values of $K(t)$, i.e. the values of the irreversible component $K = K(\infty)$, are equal to 0.60, 0.75, and 0.92 for the cells recovering from radiation damage without a drug and in the presence of 10 and 20 mM pyruvate, respectively. Qualitatively similar results were obtained for other chemicals. Fig. 3 shows the calculated irreversible component of radiation damage, $K(\infty)$, in Chinese hamster V79 cells recovering from potentially lethal damage with and without various inhibitors of recovery. The obvious increase in the irreversible component with drug concentration should certainly lead to a decrease in the recovery rate because of the decrease in the number of cells capable of recovery.

To clarify whether or not this decrease could explain the observable deceleration of the recovery rate, we estimated

Table 1. Radiobiological parameters of Chinese hamster cells recovery

Chemicals	Conditions of recovery	Irreversible component κ	Recovery constant β, hr^{-1}
Without chemicals	Without chemicals	0.60	0.15
Pyruvate	20 mM 1 h after irradiation	0.63	0.16
	10 mM immediately after irradiation	0.75	0.16
	20 mM immediately after irradiation	0.92	0.16
Novobiocin	20 μM 1 h after irradiation	0.68	0.14
	5 μM immediately after irradiation	0.82	0.14
	10 μM immediately after irradiation	0.90	0.14
	20 μM immediately after irradiation	0.98	–
Lactate	20 mM 1 h after irradiation	0.67	0.14
	10 mM immediately after irradiation	0.78	0.14
	20 mM immediately after irradiation	0.99	–
Nalidixic acid	20 μM 1 h after irradiation	0.68	0.15
	5 μM immediately after irradiation	0.74	0.15
	10 μM immediately after irradiation	0.82	0.15
	20 μM immediately after irradiation	0.94	–
3 – aminobenzamide	20 mM 3 h after irradiation	0.68	0.15
	10 mM 1 h before + after irradiation	0.71	0.15
	20 mM 1 h before + after irradiation	0.95	–

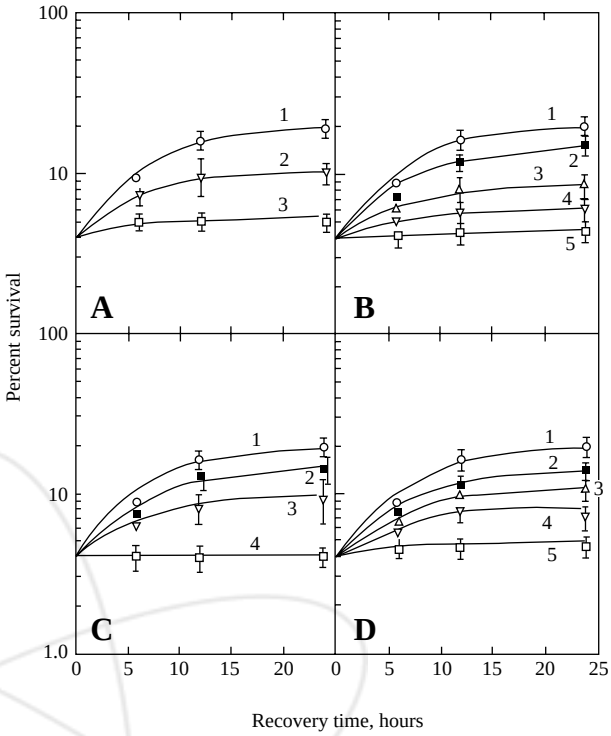

Fig. 5. Survival of Chinese hamster V79 cells in dependence of the duration of recovery from potentially lethal damage recovering after irradiation without chemicals (curves 1) and in the presence of chemical inhibitors of cell recovery. A, pyruvate: 10 and 20 mM immediately after irradiation (curves 2 and 3). B, novobiocin: 20 μM 1 h after irradiation (curve 2), 5, 10 and 20 μM immediately after irradiation (curves 3, 4 and 5). C, lactate: 20 mM 1 h after irradiation (curve 2), 10 and 20 mM immediately after irradiation (curves 3 and 4). D, nalidixic acid: 20 μM 1 h after irradiation (curve 2), 5, 10 and 20 μM immediately after irradiation (curves 3, 4 and 5). Experimental points were taken from (Keifer *et al.*, 1985a). Solid lines were calculated in accordance with Eq. 1 and parameters of the recovery estimated in this study.

the probability of recovery for various conditions of the recovery. The experimental data make it possible to calculate the function $A(t) = [D_{eff}(t) - D_{eff}(\infty)]/[D_1 - D_{eff}(\infty)]$ in the dependency of the recovery time of the Chinese hamster V79 cells recovering after irradiation without chemicals and in the presence of various chemical inhibitors. The results are shown in Fig. 4. One can see that this function declines exponentially with the recovery time independently of whether or not the recovery took place without chemicals or with an increasing concentration of various drugs. By taking Eq. (5) and the results shown in Fig. 4, we calculated the recovery constant β for all the recovery conditions which had been tested. The total set of parameters describing the recovery of the Chinese hamster cells under different postirradiation conditions are summarized in Table 1. This Table also includes the parameters describing the recovery of the Chinese hamster cells in the presence of different concentrations of 3–aminobenzamide estimated on the basis of the experimental data published by others (Kumar *et al.* 1985b) but not presented here in Figures. It can be seen that in all the cases the recovery constant was independent of the recovery conditions (β = 0.15 ±0.01 hour^{-1}) whereas the irreversible component was gradually enhanced as the chemical concentration increased.

To illustrate the correctness of the approach used here,

Fig. 5 exhibits the survival of the Chinese hamster V79 cells in dependency of the duration of recovery from potentially lethal damage recovering after irradiation without chemicals and in the presence of some chemical inhibitors of the cell recovery. Experimental points were taken from (Kumar *et al*. 1985a). Solid lines were calculated in the following way. The effective dose $D_{eff}(t)$ was computed in accordance with Eq. 1 and the parameters of the recovery obtained in this study (Table 1). Then the corresponding survival $S(t)$ was estimated using the example shown in Fig. 1 by the arrow. One can see a close fit of the predicted curves to the experimental data. Thereby the applicability of the mathematical approach to describe the recovery from potentially lethal damage was demonstrated.

DISCUSSION

The aim of this study was to determine whether the inhibition of recovery from potentially lethal damage by chemicals in Chinese hamster cells (Kumar *et al*. 1985a, b) might be causally related to the impairment of the recovery capacity itself or to the production of the irreversible damage, which cannot be repaired. It was shown that the basic effect of the chemicals tested at a concentration sufficient to inhibit recovery from potentially lethal damage appeared to be a reduction in the number of cells capable of recovery owing to the increase in the irreversibly damaged cells. The findings revealed that (i) the irreversible component of radiation damage was gradually enhanced as the chemicals concentration increased and (ii) the probability of recovery was independent of whether the process of recovery happened with or without chemicals sensitizing the radiation effect. It is not excluded that the first inference may be explained by a conversion by the drugs of radiation induced repairable damage so that the enzymes could then not deal with the lesions (Hill and Bellamy 1984; Streffer and Müller 1984). It would seem probable also that chemicals could interfere with the synthesis of the requisite enzymes (Boothman *et al*. 1989; Takahashi *et al*. 2000). The second inference would imply that the same portion of the repairable damage is eliminated for a unit time independently of the recovery conditions investigated. This result strongly suggests that the analysed chemicals don't damage the repair enzymes responsible for recovery. Hence, the observed inhibition of the recovery occurred without the damage of the recovery processing *per se* but due to the decrease in the number of damage which cell is capable of recovery. Similar results have been obtained for diploid yeast cells exposed to hyperthermia and ionizing radiation (Petin and Kim 2004) or hyperthermia and UV light (Kim *et al*. 2004). It follows that some general mechaniosms of radiosensitization or the synergistic effects may underlie the interaction of heat and some chemicals with ionizing radiation. The mechanism should imply the failure of a direct interference with the repair process itself and favor a role of hyperthermia and chemicals analysed in the decreasing rate and the extent of the repair by facilitating either the production of irreversible damage or an early radiation damage fixation before the recovery processes are over or occur.

Detailed analysis of the molecular mechanisms involved is beyond the main aim of this study. However, it can be noted that many mechanisms have been discussed by different authors (Mattern and Painter 1979; Purnell and Whish 1980; Cleaver 1982; Ben–Hur and Elkind 1984; Kumar *et al*. 1985a, b; Utsumi *et al*. 1990). Only as examples, the following possibilities can be mentioned. Because of the critical role of the DNA topoisomerases in the synthesis and the conformation of DNA, and well–known information that ionizing radiation inhibits replicative DNA synthesis, there is a possibility that inhibitors of these enzymes might influence the radiation lethality (Utsumi *et al*. 1990). It can be admitted that a decrease in a quantity of these enzymes wouldn't interfere with the probability of recovery but result in a greater portion of irreversible damage. A similar situation may be realized with insufficient energy metabolism. For instance, novobiocin and nalidixic acid have been shown to inhibit DNA, RNA, and protein synthesis in several mammalian cell lines (Mattern MR and Painter 1979; Kumar *et al*. 1985a; Utsumi *et al*. 1990), their activity may also be expressed through the interference with the function of topoisomerase II in an early stage of DNA repair (Cleaver 1982; Kumar *et al*. 1985a). It was presumed that the inhibition of recovery from potentially lethal damage by lactate and pyruvate may be due to severe metabolic changes such as a decrease in the intracellular ATP concentration (Kumar *et al*. 1985a). These authors postulated that the recovery inhibition might have occurred due to the raising of the lactate and pyruvate levels com-

plicating the repair of DNA damage. This view is strengthened by the data showing that specific inhibitors of poly (ADP–R) synthesis enhance the cell killing and inhibit the DNA strand break rejoining induced by ionizing radiation (Ben–Hur and Elkind 1984). 3–Aminobenzamide has been shown to be a putative specific inhibitor of poly (ADP–R) synthetase (Purnell and Whish 1980). The results obtained by Kumar *et al.* (Kumar *et al.* 1985b) favour a possible role of the chemical in preventing repair by facilitating an early damage fixation before repair can occur, simultaneously reducing the G_2–arrest. All these observations are consistent with the results obtained in this study.

In conclusion, the results of this paper provide opportunity for searching agents, selectively or simultaneously acting on the probability of recovery and the yield of irreversible radiation damage. The recognition that specific inhibitors of recovery may exist, such as an inhibitor of the recovery process itself and that resulting in the increased yield of irreversible damage, would provide both a possibility to analyze the mechanism of a drug and ionizing radiation interaction from this point of view and an expectation that useful regimens in cancer research may be devised to make use of these inhibitors.

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REFERENCES

Alper T. 1979. Cellular Radiobiology. Cambridge University Press.

Ben–Hur E and MM Elkind. 1984. Poly (ADP–ribose) metabolism in X–irradiated Chinese hamster cells: Its relation to repair of potentially lethal damage. Int. J. Radiat Biol. 45: 515–523.

Boothman DA, DK Trask and AB Pardee. 1989. Inhibition of potentially lethal DNA damage repair in human tumor cells by β–lapachone, an activator of topoispomerase I. Cancer Res. 49:605–612.

Burt RK, MC Porier, CJ Jr Link and VA Bohr. 1991. Antineoplastic drug resistance and DNA repair. Ann. Oncol. 2:325–334.

Cleaver JE. 1982. Specificity and completeness of inhibition of DNA repair by novobiocin and aphidicolin. Carcinogenesis 3:1171–1174.

Davidson HO. 1957. Biological Effects of Whole Body Gamma Radiation on Human Beings. Johns Hopkins University Press. Baltimore.

Hill BT and AS Bellamy. 1984. An overview of experimental investigations of interaction between certain antitumor drugs and X–irradiation *in vitro*. Adv. Radiat. Biol. 11: 211–267.

Kim JK, VG Petin and MD Tkhabisimova. 2004. Survival and recovery of yeast cells after simultaneous treatment of UV light radiation and heat. Photochem. Photobiol. 79:349–355.

Korogodin VI, YuG Kapultcevich, MN Myasnik, AF Mosin and VV Gridnev. 1968. Cellular repair processes: survival of irradiated yeast, bacteria, and phages under different postradiation conditions. Adv. Biol. Med. Physics 12:253–274.

Korogodin VI. 1993. The study of post–irradiation recovery of yeast: the 'premolecular period'. Mutation Res. 289:17–26.

Kumar A, J Kiefer, E Schneider and NEA Crompton. 1985a. Inhibition of recovery from potentially lethal damage by chemicals in Chinese hamster V79 A cells. Radiat. Environ. Biophys. 24:89–98.

Kumar A, J Kiefer, E Schneider and NEA Crompton. 1985b. Enhanced cell killing, inhibition of recovery from potentially lethal damage and increased mutation frequency by 3–aminobenzamide in Chinese hamster V79 cells exposed to X–rays. Int. J. Radiat. Biol. 47:103–112.

Little JD, AN Ueno and WK Dahlberg. 1989. Differential response of human and rodent cell lines to chemical inhibition of the repair of potentially lethal damage. Radiat. Environ. Biophys. 28:193–202.

Mattern MR and RB Painter. 1979. Dependence of mammalian DNA replication of DNA supercoiling. II. Effects of novobiocin. Biochim. Biophys. Acta 553:306–312.

Petin VG and JK Kim. 2004. Survival and recovery of yeast cells after combined treatments with ionizing radiation and heat. Radiat. Res. 161:56–63.

Purnell MR and WJD Whish. 1980. Novel inhibitors of poly (ADP–ribose) synthetase. Biochem. J. 185:775–777.

Streffer C and WU Müller. 1984. Radiation risk from combined exposures to ionizing radiation and chemicals. Adv. Radiat. Biol. 11:173–210.

Takahashi S, E Takeda, Y Kubota and R Okayasu. 2000.

Inhibition of repair of radiation–induced DNA double–strand breaks by nickel and arsenite. Radiat. Res. 154:686–691.

Utsumi H, ML Shibuya and MM Elkind. 1990. Novobiocin inhibits the repair of potentially lethal damage but not the repair of sublethal damage. Radiat. Res. 123:55–60.

Weichselbaum RR. 1986. Radioresistant and repair proficient cells may determine radiocurability in human tumors. Int. J. Radiat. Oncol. Biol. Phys. 12:637–639.

Yang L, EB Douple, JA O'Hara and H Wang. 1995. Carboplatin enhances the production and persistence of radiation–induced DNA single–strand breaks. Radiat. Res. 143: 302–308.

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Differential Functions of Caffeine and Ascorbic Acid in γ–Irradiated Male Mice

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Abstract – Radioprotection is of practical importance for the normal tissues of tumor patients subject to radiotherapy, people with planned or accidental exposure to radiation, and the public and radiation workers. Since oxygen enhances radiation–induced biological damage, antioxidants should be related with the function as a radioprotectors. Ascorbic acid or caffeine is an essential component and antioxidant in the diet of humans and a small range of other mammals. The present study investigates functional radioprotection of caffeine and ascorbic acid against gamma radiation in irradiated C57BL/6N mice. Eight–week–old male C57BL/6N mice were irradiated with 6.5 Gy. A caffeine treated group was administered with 80 mg kg^{-1} body weight by intraperitoneal injection, a single treatment 1 hr before irradiation. Ascorbic acid was administered 330 mg L^{-1} in drinking water through all the experimental period. According to time schedules, animals were sacrificed by cervical dislocation. And the samples were collected 2 weeks after whole–body gamma irradiation. The caffeine treated group showed lower decrement of body and organ weights than the other experimental groups. The qualitative analysis of circulating testosterone in serum was performed by means of radioimmunoassay (RIA). The normal level of circulating testosterone was maintained by the treatment of caffeine and ascorbic acid. The change of weight of body and organ and the appearance of seminiferous tubules were improved by an effect of caffeine or ascorbic acid against irradiation. Taken together, caffeine and ascorbic acid protects impairment of spermatogenesis against gamma radiation and may act as a radioprotector.

Key words : caffeine, ascorbic acid, gamma irradiation, male, mice

INTRODUCTION

A requirement of radioprotectors has been rising because of the scattered possibility of exposure to radiation; accidental external or internal exposure, chronic exposure of the lungs to environmental alpha–particles, cosmic radiation exposure during a manned space flight and working over the long haul in an airplane, and therapeutic exposures to radiation. The oxygen effect in radiation biology is well known. Radiation injury occurs through the formation of reactive oxygen species. Since oxygen also enhances radiation–induced biological damage, antioxidants should be radioprotectors. Numerous antioxidants have proven beneficial to radioprotection, among which well known are cysteine, cysteamine, glutathione, WR–compounds, tempace, vitamine A, E, and C, bioactive lipids (dimethylprostaglandin E2, platelet activating factor, and leukotriene C4), and immunomodulators (glucan, synthetic trehalose dicorynomycolate, and interleukin–1) (Goa and Spencer 1995; Nair *et al.* 2001). Ascorbic acid is a well–known antioxidant and an essential component in the diet of humans and a

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small range of other mammals. Ascorbic acid is known to react with free radicals (Singh and Gaby 1991). Because ionizing radiation leads to the production of the activated oxygen species, increased glutathione levels in tissues by the vitamins may contribute to scavenging free radicals generated from irradiation and *in vivo* protection against radiation injury (Tubiana *et al*. 1990). Radioprotective effects of ascorbic acid have been demonstrated in certain cells and animals, which would result from scavenging free radicals (O'Connor *et al*. 1977; Stratford *et al*. 1988). Because the vitamins do not modify tumor growth delay induced by irradiation and concomitantly exhibit radioprotective effects in normal tissues (Okunieff and Suit 1987), these agents have great therapeutic potential as radioprotectants.

Caffeine (1, 3, 7-trimethylxanthine) is one of the most extensively studied ingredients in the food supply. Caffeine exists in diet from a variety of sources-primarily coffee, tea, chocolate, drinks, and both prescription and nonprescription drugs. Recently, caffeine in a cell cycle has shown to override the cell cycle checkpoint in particular a G_2 delay (Powell *et al*. 1995). Caffeine inhibits ATM and ATR in a dose-dependent fashion. The concentration that inhibits ATM by 50% *in vitro* is 1 mM and the 50% inhibitory concentration for ATR is about 3 mM (Sarkaria *et al*. 1999). Studies by George *et al*. (1999) showed that a dose of 80 mg kg^{-1} administered 60 min before a whole-body exposure to 7.5 Gy was optimal for a maximal radioprotection (George *et al*. 1999). Caffeine and its xanthine metabolites had an oxygen radical absorbing capacity comparable with that of ascorbic acid or uric acid (Chul 2000; Azam *et al*. 2003). Moreover, Lu *et al*. (2000) demonstrated that caffeine had an inhibitory effect on UV-induced carcinogenesis. Knowledge of the clear function and side effects of caffeine in the irradiated individuals has been poor. Thus the present studies are to examine the effects of caffeine treatment before whole-body irradiation in male mice. According to the previous investigations, the testis is now recognized as one of the most radiosensitive organs of the body. Interestingly, differential sensitivity of irradiation depends on cell types in the testes (Inano *et al*. 1989). The male mouse gonad contains both radiosensitive (premeiotic germ cells) and radioresistant (somatic) cells. Indeed, irradiation ($\geq$5 Gy) of the male gonad selectively destroys premeiotic (differentiating spermatogonia) germ cells, whereas other cells, i.e., somatic (Leydig and Sertoli cell) cells and postmeiotic (spermatids) germ cells are unaffected (Rodriguez *et al*. 1997). However, it has been reported that fetal irradiation is associated with an increase of hCG binding and testosterone production per Leydig cell, and a decrease in the number of these Leydig cells per testis (Jansz and Pomerantz 1984; Pinon-Lateillade *et al*. 1991). The previous data suggest that somatic cells may be biochemically and physiologically radiosensitive. Therefore, the present study is designed to investigate and compare the radioprotective effects of caffeine and ascorbic acid on spermatogenesis of mice after whole-body gamma irradiation.

MATERIALS AND METHODS

1. Animals and irradiation

The animal was obtained from Daehan Biolink (Chungbuk, Korea). C57BL/6N mice (male, 8-week-old) were used throughout the investigations. All the mice were maintained under the following conditions; temperature (23℃) and lighting (14 hr light : 10 hr dark) and allowed free access to food and water. Irradiated groups were exposed to γ-irradiation using a ^{60}Co source with a total dose of 6.5 Gy, and a dose rate of 12.8 Gy hr^{-1} (Kim *et al*. 1999).

2. Treatments of caffeine and ascorbic acid

The caffeine treated group was administrated with 80 mg kg^{-1} body weight by *i.p* injection, a single treatment, one hour before irradiation (George *et al*. 1999). The ascorbic acidtreated group was administered with 330 mg L^{-1} in drinking water during experiment (Maeda *et al*. 2000). The remaining mice were kept as sham controls. According to the experiment schedules, mice were sacrificed by cervical dislocation 2 weeks after whole-body irradiation. The body weight (BW) and organ weight (OW) were recorded and the indices were calculated with the equation; OW $\times$ 100/BW.

3. Immunohistochemical analysis for Bcl2 and Bax in testis

Testes were fixed overnight at room temperature with

10% neutralized buffered formalin (NBF), dehydrated, and embedded in paraffin. Sections (6 μm) were prepared and mounted on slides. The slides were then hematoxylin and eosin (H-E) stained for morphological observations.

For the immunohistochemical analysis, a rabbit polyclonal IgG antibody against Bcl-2 (N-10) and a mouse monoclonal IgG26 antibody (B-9) from Santa Cruz Biotechnology (Santa Cruz, CA) were used. Sections (5 μm) were mounted onto poly-lysine-coated slides. After rehydration, the slides were washed with distilled water (DW) each followed by the antigen retrieval at 121℃ for 15 min in 10 mM citrate solution (pH 6.0). After the washes with DW, enough drops of 0.05 mL of peroxide blocking solution (0.3% H_2O_2 in methanol) were applied to each section and incubated for 10 min. After washing the slides, enough drops of block solution (1% BSA in phosphate buffer) to minimize the non-specific binding of the antibodies were placed at each section and incubated for 5 min. After blocking, a primary antibody (1 : 400 diluted in phosphate buffered saline) was applied to each section and incubated at 4℃ overnight. Incubation with a secondary antibody and visualization was performed using InnoGenexTM IHC kit (InnoGenex, CA) according to the mansufacturer's instructions. The slides were subsequently counterstained with Mayer's hematoxylin (Sigma).

4. Measurement of testosterone levels by radioimmunoassay (RIA)

The concentration (ng mL^{-1}) of serum testosteone was measured by radioimmunoassay kits (Diagnostic Systems Laboratories, USA) with a sensitivity of 0.08 ng mL^{-1}. The intra- and interassay coefficients of variations were $<3\%$ and $<7\%$, respectively. All experimental groups were assayed in duplicate. The 50 μL of serum or standards was mixed with Testosterone [I-125] reagents. After gentle mixing of the test tube rack by hand, all tubes were incubated in a water bath at 37 ±2℃ for 60 to 70 min. Then, all tubes except the total count tubes were decanted. Radioactivity was measured by a liquid scintillation counter (LS 6500S, Beckman Instruments Int., Fullerton, CA).

5. Statistical analyze

Statistical analysis was performed by Student's *t* test for a simple comparison of the two groups using Sigma Plot® software (Jandel Scientific, Germany). They are expressed as mean ±SEM and $P<0.05$ was considered significant.

RESULTS AND DISCUSSIONS

1. Effect of body and organ weights in irradiated mice

The present studies were designed to investigate the effect of caffeine and ascorbic acid before whole-body irradiation. The dosage and treatment route of caffeine and ascorbic acid used in this experiment referred to the previous reports (George *et al*. 1999; Maeda *et al*. 2000). The rate of increase in the body weight during the experimental period was presented in Figure 1. Two week after whole-body irradiation, the mean body weight of the irradiated control (RC) group showed a significant decrease (−4.08 g, $P<0.02$) compared with those of the control group. The decrease in the body weights of the caffeine-treated irradiated group (AR) was not marked. In the ascorbic acid-treated irradiated (AR) group, the decrease was higher than the RC group. While the unirradiated groups (CT, A and B) showed increases in the body weights, the irradiated groups

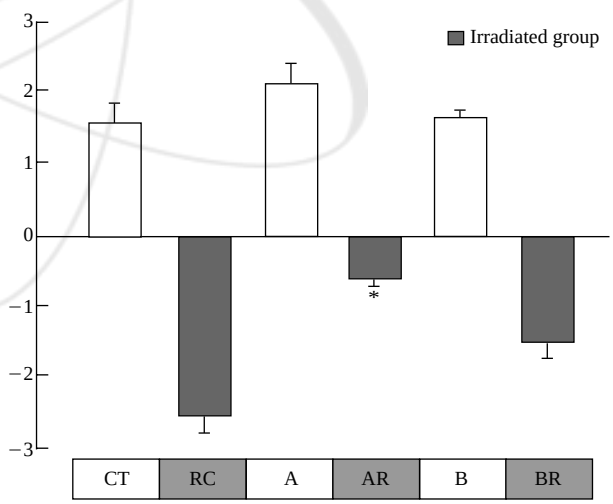

Fig. 1. Increased ratio of the body weights (g) during the experiments. Zero value indicated the mean of the body weights of the experimental groups at the irradiation time. Each bar showed an increase or decrease in body weights 2 weeks after whole-body irradiation. Abbreviations: CT, the control group; RC, the irradiated control group; A, the caffeine-treated group; AR, the caffeine treated before irradiation; B, the ascorbic acid-treated group; and BR, the ascorbic acid-treated before irradiation. *, significantly different from the irradiated control group ($P<0.05$).

(RC, AR and BR) resulted in decreases from the mean body weights at the irradiation point on the whole. It is noteworthy that the treatment of caffeine before whole–body irradiation improved the radiation–induced body weight decreases and that the effect of caffeine was superior to that of ascorbic acid.

To investigate the organ–sensitive defects corresponding to the reduction of the body weights of each group, we were calculated the organ indices of liver, kidney, spleen, and testis. The indices for the experimental groups were shown in Table 1. The index (%) was calculated from the organ weights divided by the body weights as mentioned before. Liver indices in Table 1 did not show difference among the experimental groups. Because kidney indices were affected by gamma irradiation, the value of the RC group was reduced to 87% of those in the control group.

The values of spleen and testis in the RC group decreased significantly by 50% compared with those of the control group ($P < 0.05$). However, the values of the AR group reflected a marked recovery, especially in spleen indices, compared with those of the RC group. While spleen indices of the AR group showed a similar level to the control group, the values of the BR group was not improved into the level of the RC group. Because spleen and testis were the most sensitive to radiation, pretreatments of caffeine and ascorbic acid played important roles in the organ–specific influence on the irradiated groups. In case of testis indices (*ie*, gonad indices), both of the AR and BR group did not differ from the values of the RC group. Though testis indices of the AR and BR group were not better than the RC group, the spleen indices of the AR group indicated that caffeine protected the organ against whole–body irradiation.

Table 1. Calculated organ indices of the experimental groups 2 weeks after whole–body irradiation

	Organ indices after whole–body irradiation			
	Liver	Kidney	Spleen	Testis
CT	3.45 ±0.04 (1.00)	0.62 ±0.01 (1.00)	0.27 ±0.002 (1.00)	0.38 ±0.04 (1.00)
RC	3.83 ±0.01 (1.11)	0.54 ±0.02 (0.87)	0.11 ±0.001* (0.41)	0.20 ±0.02 (0.53)
A	4.22 ±0.01 (1.23)	0.61 ±0.01 (0.98)	0.24 ±0.001 (0.89)	0.34 ±0.02 (0.89)
AR	4.09 ±0.01 (1.18)	0.50 ±0.01* (0.81)	0.28 ±0.001* (1.03)	0.20 ±0.01 (0.53)
B	3.82 ±0.02 (1.10)	0.62 ±0.08 (1.00)	0.20 ±0.002* (0.74)	0.36 ±0.03 (0.95)
BR	3.60 ±0.02 (1.04)	0.58 ±0.02* (0.93)	0.10 ±0.001 (0.37)	0.23 ±0.02* (0.61)

*, significantly different from the value of the irradiated control group. Each value was calculated as described in 'Materials and methods' and represented the mean ±SEM of data from 5 mice/group. A parenthesized value showed a relative potency compared with those of the control group. Abbreviations in this table are the same as in Figure 1.

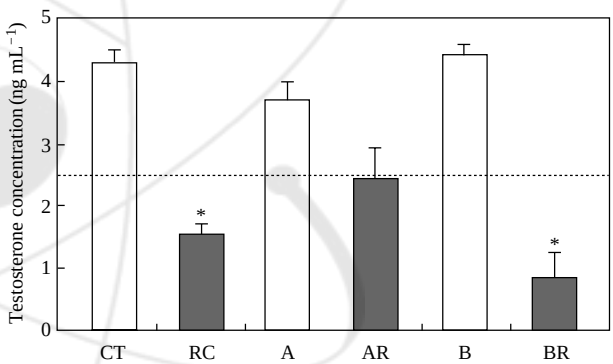

Fig. 2. Changes of the testosterone concentration (ng mL^{-1}) in serum of C57BL/6N mice after whole–body irradiation. Mice were treated with caffeine (80 mg kg^{-1}) in saline by *i.p* injection or ascorbic acid (330 mg kg^{-1}) in drinking water and sacrificed 2 weeks after irradiation. Samples were measured by radioimmunoassay kits with a sensitivity of 0.08 ng mL^{-1}. Striped bars correspond to the irradiated groups. Abbreviations are the same in Figure 1. *, $P < 0.05$.

2. Concentration of the circulating testosterone on irradiated mice

As a functional parameter of radiation–induced testicular defects, we investigated concentration of the circulating testosterone. The level (ng mL^{-1}) of testosterone in serum was determined by a radioimmunoassay with a sensitivity of 0.08 ng mL^{-1} (Fig. 2). In the RC group, the concentration of testosterone was significantly reduced, compared with the value of the CT group ($< 36.2\%$, $P < 0.02$). The caffeine pretreated group showed a similar level to the caffeine only group. However, the level of the AR group did not show a difference from the value of the RC group. We have previously shown that the concentration of circulating testosterone in all the irradiated groups was significantly reduced irrespective of caffeine pretreatment six hours after irradiation (Kim *et al*. 2003). The results obtained in the present study lead to supposition that caffeine pretreatment was associated with protection or recovery of the androgen productive system. As a result of the function of caffeine against γ–ray, the concentration of

testosterone two weeks after irradiation showed a similar level compared with those of the A group. Moreover, ascorbic acids did not play a role like the action of caffeine in the steroidogenic system in the present study.

3. Histological observation

Many studies have been performed by qualitative or quantitative examination to detect the testicular impairments via histological investigations (Russell *et al.* 1990). Qualitative evaluation involves the Leydig cells and their subcellular features, blood vessels, intertubular fluid build–up, cell infiltration, basement membranes, multinucleated giant cells, Sertoli cell morphology, delayed spermiation, missing germ cells, abnormalities of germ cells and germ cell development, absence of the tubular lumen, and disruption of the Sertoli cell barrier. Quantitative evaluation includes testis, fertility, tubular diameter, determination of sperm production, cell ratios, and cell numbers by morphometry.

Irradiation of male reproductive system caused a loss of spermatocytes, missing germ cells, and absence tubular lumen in seminiferous tubules (Muller *et al.* 1999). Results of the morphological assessment using H–E staining 2 weeks after whole–body irradiation showed that, compared with the control mice, the seminiferous tubules of the irradiated group significantly decreased in their diameters and increased a complete atrophy and vacuolation inside the tubules (Fig. 3). While vacuoles of the AR group also remained in quantities, they were not observed inside the tubules of the BR group. However, the BR group showed a reduction in the size of seminiferous tubules and disturbance of the spermatogenesis process. The earlier studies demonstrated that pretreatment with ascorbic acid resulted in a significant reduction of radiation–induced delay in wound healing such as increased collagen contents and fibroblast and vascular densities (Jagetia *et al.* 2003). In the present investigations, ascorbic acid may provide an assistance to regeneration of defects by reducing the vacuole and upkeep the spermatogenesis.

Immunohistochemistry using an antibody raised against Bcl–2 and Bax showed their presence in the seminiferous tubules although the staining did not gave rise to a particularly intense spot. The Bcl–2 family of proteins is a widely recognized group of regulation of apoptosis. This family consists both of pro–(Bax, Bad, Bak, Bik, Bok, Diva, Hrk, and Bid) and anti–apoptotic (Bcl–2, Bcl–xL, Mcl–1, Blc–w, and Bfl–1/A1) proteins that modulate the execution phase of the cell death (Richberg, 2000). Numerous studies have indicated an important role of Bcl–2 family for functional spermatogenesis. Bax–deficient male mice are infertile due to an inappropriate accumulation of premeiotic germ cells (Knudson *et al.* 1995). Transgenic

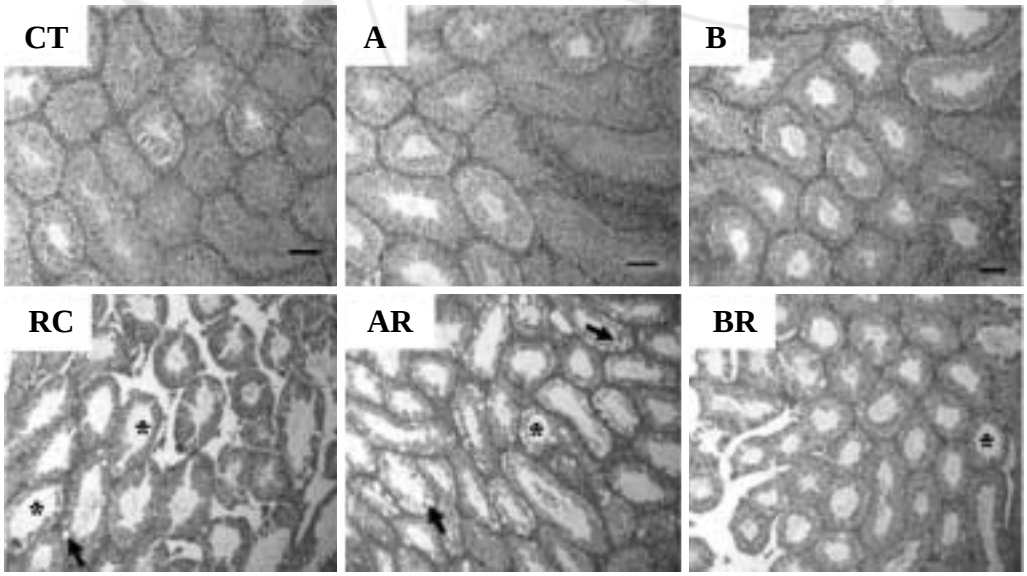

Fig. 3. Photomicrographs of the hematoxylin–eosin staining of the seminiferous tubules from the experimental group two weeks after whole–body irradiation. Abbreviations are the same in Figure 1. An asterisk or arrow indicates a complete atrophy and vacuolation in seminiferous tubules. A scale bar indicates 100 μm.

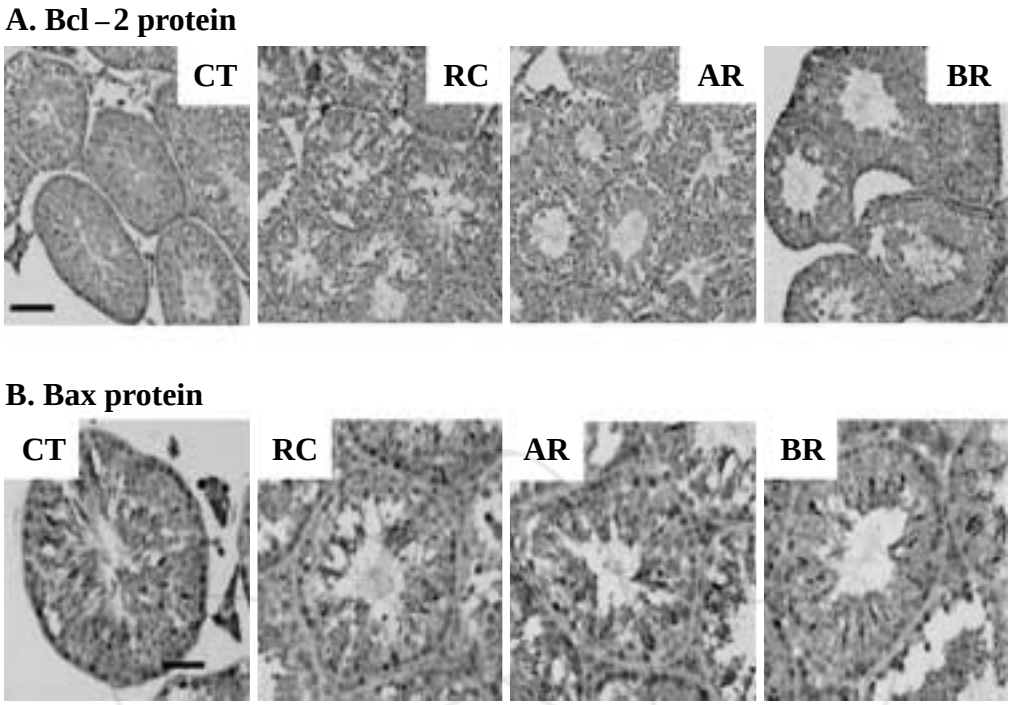

Fig. 4. Bcl-2 (upper panel) and Bax (lower panel) immunohistochemistry in the testis of C57Bl/6N mice two weeks after whole-body gamma irradiation. Abbreviations are the same in Figure 1. A scale bar indicates 50 μm.

mice that overexpress Bcl-2 are infertile and show a disorganization of the cells of the seminiferous tubules (Rodriguez *et al*. 1997).

Two weeks after irradiation, Bax in all the irradiated groups was strongly expressed in the nuclei of the round spermatids and a few primary spermatocytes, but it was also stained in the cytoplasm of the seminiferous tubules and the interstitium (Fig. 4-B). Expression of Bcl-2 was weak in the tubules of all the experimental groups. In case of the CT and BR group, Bcl-2 expression was localized in the cytoplasm of the differentiating spermatids close to the luminal surface (Fig. 4-A). These data were not sufficient to identify the differential expression of regulators among the irradiated groups (RC, AR, and BR group), but further researches for qualitative analysis and isolation upon the cell type in the seminiferous tubules will give the answer to the question whether the treatment of caffeine or ascorbic acid before γ-irradiation affects regulatory proteins which act executively in radiation-induced alteration.

CONCLUSIONS

As radiation-induced defects were concomitant with the oxidative stress, antioxidants should be radioprotectors. Caffeine and ascorbic acid in the present study were essential components in the diets in human and a small range of other mammals. The antioxidative ability of caffeine and ascorbic acid was evaluated in numerous reports, but there was no quantitative approach to their radioprotection role *in vivo*. Therefore, the present study investigated the functional radioprotection of caffeine and ascorbic acid against gamma radiation in irradiated C57BL/6N mice. The caffeine treated mice before irradiation showed higher body weights, spleen indices, and concentrations of the circulating testosterone than those of the other irradiated groups. Ascorbic acid treatment before irradiation exhibited high gonad indices, diameters of the seminiferous tubules, and decrease of vacuoles in cytoplasm of the Sertoli cells. In conclusion, the several defects related with male reproduction caused by γ ray in mice were improved by pretreatment of caffeine or ascorbic acid. Especially, the ascorbic acid treated group was distinguished in structural improvement compared with those of the other irradiated groups and the caffeine treated group showed the excellent steroidogenesis in testis.

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REFERENCES

Azam S, N Hadi, NU Khan and SM Hadi. 2003. Antioxidant and prooxidant properties of caffeine, theobromine and xanthine. Med. Sci. Monit. 9:BR 325–330.

Chul L. 2000. Antioxidant ability of caffeine and its metabolites based on the study of oxygen radical absorbing capacity and inhibition of LDL peroxidation. Clin. Chim. Acta. 295: 141–154.

George KC, SA Hebbar, SP Kale and PC Kesavan. 1999. Caffeine protects mice against whole–body lethal dose of gamma–irradiation. J. Radiol. Prot. 19:171–176.

Goa KL and CM Spencer. 1995. Amifostine. A review of its pharmacodynamoc and pharmacokinetic protperties, and therapeutic potential as a radioprotector and cuytotoxic chemoprotector. Drugs. 50:1001–1031.

Inano H, K Suzuki, H Ishii–Ohba, Y Imada, R Kumagai, S Kurihara and A Sato. 1989. Steroid hormone production in testis, ovary, and adrenal gland of immature rats irradiated in utero with ^{60}Co. Radiat. Res. 117:293–303.

Jagetia GC, GK Rajanikant and SK Rao. 2003. Evaluation of the effect of ascorbic acid treatment on wound healing in mice exposed to different doses of fractionated gamma radiation. Radiat. Res. 159:371–380.

Jansz GF and DK Pomerantz. 1984. Fetal irradiation increases androgen production by dispersed leydig cells of the rat. J. Androl. 5:344–350.

Kim JK, CJ Lee, KW Song, BR Do and YD Yoon. 1999. γ–Radiation accelerates ovarian follicular atresia in immature mice. In Vivo 13:21–24.

Kim JK, JH Kim and YD Yoon. 2003. Evaluation of caffeine as a radioprotector in whold–body irradiated male mice. In Vivo 17:197–200.

Kundson CM, KSK Tung, WG Tourtellotte, GAJ Brown and SJ Korsmeyer. 1995. Bax–deficient mice with lymphoid hyperplasia and male germ cell death. Science 270:96–99.

Lu YP, YP Lou, XH Li, JG Xie, D Brash, MT Huang and AH Conney. 2000. Stimulatory effect of oral administration of green tea or caffeine on ultraviolet light–induced increases in epidermal wild–type p53, p21 (WAF1/CIP1), and apoptotic sunburn cells in SKH–1 mice. Cancer Res. 60:4785–4791.

Maeda N, H Hagihara, Y Nakata, S Hiller, J Wilder and R Reddick. 2000. Aortic wall damage in mice unable to synthesize ascorbic acid. Proc. Natl. Acad. Sci. USA. 97:841–846.

Muller WU, C Streffer, A Woicik and F Niedereichholz. 1999. Radiation–induced malformations after exposure germ cells in various stages of spermatogenesis. Mutat. Res. 425:99–106.

Nair CKK, DK Parida and T Nomura. 2001. Radioprotection in radiotherapy. J. Radiate. Res. 42:21–37.

O'Connor MK, JF Malone, M Moriarity and S Mulgrew. 1977. A radioprotective effect of vitamin C observed in Chinese hamster ovary cells. Br. J. Radiol. 50:587–591.

Okunieff P and HD Suit. 1987. Toxicity, radiation sensitivity modification, and combined drug effects of ascorbic acid with misonidazole in vivo on FSaII murine fibrosarcomas. J. Natl. Cancer Inst. 79:377–381.

Pinon–Lateillade G, MC Viguier–Martinez, AM Touzalin, J Maas and B Jegou. 1991. Effect of an acute exposure of rat testes to gamma rays on germ cells and on Sertoli and Leydig cell functions. Reprod. Nutr. Dev. 31:617–629.

Richburg JH. 2000. The relevance of spontaneous– and chemically–induced alterations in testicular germ cell apoptosis to toxicology. Toxicol. Lett. 112–113:79–86.

Rodriguez I, C Ody, K Araki, I Garcia and P Vassalli. 1997. An early and massive wave of germinal cell apoptosis is required for the development of functional spermatogenesis. EMBO J. 16:2262–2270 .

Russell LD, RA Etllin, APS AHikim and ED Cleff. 1990. Histological and histopathological evaluation of the testis. Cache River Press, Florida.

Singh VN and SK Gaby. 1991. Premalignant lesions: role of antioxidant vitamins and beta–carotene in risk reduction and prevention of malignant transformation. Am. J. Clin. Nutr. 53(1 Suppl):386S.

Stratford MRL, RJ Hodgkiss and RR Watfa. 1988. Studies on the role of antioxidants in radioprotection. Pharmacol. Therap. 39:389–391.

Tubiana M, J Dutreix and A Wambersie. 1990. Introduction to radiobiology, Taylor & Francis, New York.

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제목/부제	이온화 방사선과 화학물질의 복합작용 해석기술 개발
연구책임자 및 부서명	김진규 (방사선전략기술개발부)
연구자 및 부서명	이병헌(방사선공업환경연구부); 신해식(연수생); 김지향(연수생); 우현정(연수생); 김수현(연수생); 박희전(연구생); 오선화(부직대학생) 블라디슬라프 페틴 (해외유치과학자); 안토니나 체불스카-바실레브스카 외 4인 (폴란드핵물리연구소)

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연구위탁기관			계약 번호		

초록(300단어 내외)

이온화 방사선과 화학물질 등과의 복합작용에 의한 생물학적 영향의 평가와 관련 응용기술을 선진화하기 위하여 폴란드핵물리연구소와 공동연구개발을 수행하였다. 이온화 방사선과 수은화합물의 고등동물에 대한 영향평가를 위하여 면역조직화학 검정법, 홀몬 검정법 등의 생체영향 판별 기술을 적용하는 한편 식물에 대한 영향평가를 위해 체세포돌연변이 검정법을 효과적으로 적용함으로써 복합영향 평가의 기술적 수단을 확보하였다. 단세포 실험계인 효모세포 실험을 통하여 다요인 복합작용의 공통적 특성을 검출하고 상승작용에 관한 예측 및 평가 이론모델을 수립하는 한편 이를 라돈과 흡연의 복합영향평가에 활용하였다. 연구결과를 방사선 관련 학술지에 적극 게재함은 물론 국내외 학술대회에 발표하여 연구결과의 학술적 인증을 획득하였다. 기관 간 협력의 경험은 과학기술세계화를 위한 다자간협동연구 등에 적용될 수 있으며 특히, 한국·폴란드 간 과학기술 분야의 호혜적 동반자관계 증진에 기여할 것이다.

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Researcher and Dept.	B.H.Lee(Div. Ind. Env. Res.), H.S. Shin, J.H. Kim, H.J. Woo, (Res. Assistant); Vladislav G. Petin (MRRC, Russia), Antonina Cebulska-Wasilewska *et al.* (INP, Poland)

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Sponsoring Org.				Contract No.	

Abstract(about 300 words)

This research project has been carried out jointly with INP (Poland) to develop technologies for 'Mechanism of Interaction between ionizing radiation and chemicals". Several biological end-points were assessed in experimental organisms such as higher plants, rats, cell lines and yeast cells to establish proper bioassay techniques. The *Tradescantia* somatic cell mutation assay was carried out, and immunohistochemistry and hormone assays were done in Fisher 344 rats and cell lines to analyse the combined effect of ionizing radiation with mercury chloride. Using the common regularities of combined actions of two factors, a theoretical model was established, and applied to the thermoradiation action and synergism between two chemicals, as well. The model approach made it possible to predict the condition under which the maximum synergism could be attained. The research results were published in high standard journals and presented in the scientific conferences to verify KAERI's current technology level. The experience of collaboration can be used as a fundamental tool for multinational collaboration, and make the role of improving relationship between Korea and Poland.

Subject Keywords (about 10 words) Ionizing radiation, chemicals, biological damage, damage repair, synergistic interaction, bioassay, DNA, immunohistochemistry

주 의

1. 이 보고서는 교육과학기술부에서 시행한 특정연구개발사업의 연구보고서입니다.

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