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THE TUMORIGENIC ACTION OF BETA, PROTON, ELECTRON AND ALPHA RADIATION IN RAT SKIN

PROGRESS REPORT

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Abstract of Progress Report

The carcinogenic effect of ionizing radiation on rat skin is being studied in an attempt to learn more about radiation carcinogenesis, especially how various parameters of the irradiation, such as dose distribution within the tissue and dose rate, affect the yield of tumors. It was found that when the dose was localized to a small region of skin, the tumor yield was reduced and the magnitude of the reduction indicated that the region of reduced response might extend about 150 μ into the irradiated zone. The proliferative state of the hair follicles at the time of irradiation has relatively little effect on tumor induction although old animals are less susceptible than young or newborn animals. The penetration requirement of at least 0.3 mm for producing tumors suggests that the hair follicle germ cells could be the oncogenic targets. The recovery rate for tumor induction measured by split-dose exposure protocols is 4 hours for electrons. Proton radiation exhibited nearly complete recovery at 24 hours PI. UVL promotion of electron induced tumors did not significantly alter tumor yields. Radioresistance to tumor induction appears to increase with age.

1.0 General Summary

Rat skin has been studied for a number of years as a model of radiation carcinogenesis in a solid tissue. Accessibility of the skin enables the tumors to be detected early so that growth rate, proliferation rate, and onset times can be established accurately and, of course, the superficial location permits an accurate assessment of doses and a localization of the radiation to the tissue of interest. We have been attempting to establish as accurately as possible the nature of the dose response curve, i.e. the rate of tumor occurrence as of function of radiation dose, and the importance to tumor induction of radiologic factors, such as dose rate, fractionation, dose localization, linear energy transfer and of biologic factors, such as the proliferative state of the hair follicles and epidermis at the time of and subsequent to irradiation. The interaction of radiation and other carcinogens, especially ultraviolet light, is under study because of epidemiologic evidence suggesting a potential synergism for induction of scalp tumors.

Radiobiological recovery processes have been studied in tumor response experiments using split doses of radiation separated by various times. The recovery rate for electron induced tumors has been measured, and the oncogenic effects of high LET particles (proton, alpha, argon) are being investigated.

2.0 Progress Achieved in Preceding Year

The general objectives outlined in last year's proposal were achieved. Tumor dose response curves were generated for erythermal and germicidal UVL. Promotion with UVL produced no change in tumor yield in skin initiated with ionizing radiation. An age dependency has been demonstrated for tumor induction in rat skin with older animals exhibiting more resistance to ionizing radiation. Ionizing radiation-chemical interaction studies and high LET fractionation studies are in progress. Epidermal cell mitosis is inhibited in nearly all cells when irradiated in S-phase with 800 rads of electrons. Ionizing radiation was found to increase hair follicle cell proliferation up to 76 weeks post exposure. Damage and repair of rat epidermal cell DNA *in vivo* is being studied.

2.1 The Combined Effect of Ionizing Radiation and Ultraviolet Light on Tumor Induction in Rat Skin

The promoting effect of ultraviolet light (UVL) on ionizing irradiated rat skin is being investigated. UVL and ionizing radiation are both complete carcinogens when administered separately in rat skin. Chronic UVL exposure causes edema, erythema, and scaling with subsequent eschar formation.

Comparison of tumor induction in albino rat skin were made for single doses of erythemal (275-375 um) and germicidal (254 um) UVL; and for repeated doses of erythemal UVL following single doses of electron radiation. Single exposures to erythemal UVL induce tumors of increasing incidence with dose in the range of 0.8×10^7 to 3.2×10^7 ergs/cm^2. The tumor incidence appears constant in the dose range 3.2×10^7 to 25.2×10^7 ergs/cm^2. In this dose range approximately 10 times as much germicidal UVL as erthemal UVL was required for equivalent tumor yields.

Groups of 6 or 12 male albino rats were given 690, 1380, 2060 or 3480 rads of electrons to the dorsal skin at 28 days of age followed by exposure to either 2.1×10^7 or 0.42×10^7 ergs/cm^2 erythemal UVL weekly for 12 or 20 weeks respectively. The electron induced tumors consisted primarily of squamous or basal cell types and were clearly distinguishable, both grossly and histologically, from UVL-induced tumors which were exclusively kerato-sebacous cyctic acanthomas. This marked difference in histology of the tumors may reflect differences in the oncogenic targets of the two radiations. In rats exposed to both UVL and electrons, the incidence and types of tumors indicated that the effects of the two agents were generally additive and independent

except for about an eight week delay in the appearance of electron-induced tumors during the 20 weeks of UVL exposure. For a given total dose, fractionated exposure to erythemal UVL induced fewer tumors than a single exposure, suggesting that recovery or protection occurred during the protracted exposure.

2. 2 The Carcinogenic Interaction of Ionizing Radiation and a Chemical Carcinogen

The carcinogenic potential of ionizing radiation in conjunction with a chemical carcinogen is of practical importance in simulating environmental conditions. Because the tumor response for ionizing radiation is well established in rat skin, the interaction with other carcinogens can be studied with respect to equivalent doses to produce the same response. Temporal additivity can be analyzed by comparing the individual temporal incidence curves for the different carcinogens with the incidence curve obtained with combined treatment.

Arrangements have been made to use the 2.0 MeV Van de Graff accelerator at Union Carbide, Sterling Forest, New York for the initial electron exposures next month (May, 1976). The protocal consists of irradiating the dorsal skin of rats with doses of 750, 1500, 2250 and 3000 rads of electrons followed by weekly applications of 0.002%, 0.01% or 0.05% dimethylbenz(a)anthracene (DMBA) in acetone.

At these doses, the DMBA above should produce low tumor incidences. The objective of the experiment is to determine whether prior electron irradiation alters the susceptibility of the skin to DMBA.

2. 3 Age Dependency in Radiation Carcinogenesis

Rats of various ages were exposed to low voltage x-rays in order to determine the age dependence of the dose response curves for tumor induction. Newborn, 28, 58, 100, 200 and 350 day old rats have been exposed to doses of 500, 1000, 1800, 2250, 3000, 4000 and 5000 rads. An increase in radio resistance with age was noted for the incidence of ulceration at three weeks post irradiation. Tumors were induced with latents periods (time from irradiation to appearance of first tumor) in the range of 15-24 weeks which was not strongly age dependent, with the exception of the 350 day old group which had no tumors at 30 weeks post irradiation. The subsequent tumor appearance rate was approximately constant at each dose. The typical dose response curve (tumors/rat vs. dose) ascends rapidly, reaches a peak incidence, and then descends at higher doses. Although the height of the tumor incidence peak was nearly age-independent, the position of the peak on the dose axis showed a shift to higher doses with increasing age. That is, the older rats became progressively more resistant to the oncogenic effect of the radiation. In terms of the dose at the depth of the follicular bulb, the peak tumor

incidence occurred at 1200-1800 rads in newborns, at 2200 rads in 28 day old rats (previously published data), and at about 2800 rads in the 100 day old rats based on 76, 80, and 28 weeks of observation respectively. The tumor incidence in the 200 day old animals is still too low for analysis at 16 weeks post irradiation.

An increase in radio resistance with age was observed in our previously reported studies on skin tumor in rats irradiated with electrons or protons at 28 and 58 days of age, and supports the general conclusion that several types of radiation become less oncogenic with increasing age.

2. 4 Fractionation with High LET Radiation for Tumor Induction

On the basis of cell lethality data, which exhibits LET dependency, there should be little or no recovery from high LET (about 100 kev/u) radiation for the tumor induction end-point. Investigation of this point for tumor induction in rat skin is possible only by irradiation with high atomic number nuclei such as argon (atomic number 18).

These irradiations are scheduled to take place in June using the Lawrence Berkeley Laboratory heavy-ion linear accelerator (HILAC) and Bevatron in combination (BEVALAC). Preliminary dosimetric considerations and design of a suitable exposure system are underway.

A skin flap technique will be used which involves stretching

a 1 cm. wide flap of dorsal skin vertically above the back of the animal and scanning the beam horizontally through several animals in succession. The experimental protocal designed will determine if linearity exists at high LET for single doses, and also the amount and rate of recovery between split doses.

2. 5 DNA Damage and Repair in Tumor Induction by Ultraviolet Light and Ionizing Radiation

Damage and subsequent repair processes of cellular DNA are believed to be related to the cellular reaction to ultraviolet light and ionizing radiation. Recently these processes have been implicated in the carcinogenic response and several experiments have been undertaken to detect DNA damage and repair following in vivo exposure.

The measurement of UVL induced excision repair DNA synthesis was attempted using the sparse labelling radioautographic technique. This involves injecting (either intradermal or intraperitoneal H^3 thymidine immediately following UVL irradiation and subsequent skin biopsies taken one hour post irradiation. Radioautographs of thin sections from the biopsies will exhibit spare labelling if DNA repair synthesis has occurred because only small segments of newly synthesized DNA will be labelled. The cells in S-phase are heavily labelled and easily distinguishable from sparsely labelled cells. In our experiments using erythemal UVL (275 - 375 nm) in the dose range of

1.58×10^7 to 25.2×10^7 ergs/cm^2 or germicidal (254 nm) UVL in the range of 0.65×10^7 to 26.0×10^7 ergs/cm^2, we found no evidence of sparse labelling above background in non S-phase cells in the dorsal skin of albino rats. This is a carcinogenic dose for both erythemal and germicidal radiations and a dose at which DNA repair synthesis has been demonstrated in hairless mouse epidermis. The erythemal UVL did produce a general decrease in H^3-thymidine uptake in both sparsely and heavily labelled background cells, indicating an inhibition of all cellular DNA synthesis. The erythemal UVL also caused a rapid depopulation of epidermal basal cells to 65% of controls at one hour post-irradiation when biopsies were taken.

The alkaline sucrose gradient technique for detection of single stranded DNA breakage in vitro developed by McGrath and Williams and refined by Elkind and other investigators is being adapted for use with epidermal cells exposed to ionizing radiation in vivo.

2. 6 Hair Follicle Proliferation Following Irradiation

The interest in obtaining mitotic indices of irradiated hair follicles originates from the concept that the hair follicle is a possible tissue of origin for tumor cells. Thus the correlation between tumor yield and mitotic proliferation in hair follicles was examined. Four groups of CD strain rats obtained from Charles River

Laboratories, Brookline, Massachusetts, were exposed to 0.7 MeV electron radiation on their dorsal surfaces at doses of 700, 1150, 1400 and 2300 rads respectively. Animals were observed and appearance of tumors noted. At 68 weeks post irradiation, the animals were injected with vinblastine four hours prior to sacrifice. Tissue sections were made and mitotic figures counted at 1250 x magnification in resting stage hair follicles .08 mm below the epidermis.

The mitotic rate in irradiated hair follicles was significantly higher than that of the controls, although there was no clear relationship between the mitotic rate and either the radiation dose or the subsequent tumor yields. In groups receiving 700, 1150, 1400 or 2300 the rates were $0.68 \pm 0.20\ hr^{-1}$, $0.52 \pm 0.07\ hr^{-1}$, $0.41 \pm 0.07\ hr^{-1}$, and $0.57 \pm 0.09\ hr^{-1}$ respectively, while in the control group the mitotic rate was $0.09 \pm 0.09\ hr^{-1}$. The tumor yields for the various dose groups increased from 0.16 tumors/rat at 700 rads to 3.04 tumors/rat at 1400 rads, and declined to 1.28 tumors/rat at 2300 rads.

The increase in proliferation rate of follicle cells is a striking effect and further studies are being carried out to determine the effects of radiation on proliferation in the overlying epidermis. The possibility that an increased rate of cell turnover

contributes to tumor development has been long recognized for such chemical promoting agents as phorbol myristate acetate (PMA), and it may be that a similar effect is occurring in irradiated hair follicles.

2.7 Chemotherapy of Rat Skin Tumors

The present experiments were undertaken to evaluate two cytotoxic agents, methotrexate and cytosine arabinoside (Ara-C) in preventing the appearance of new rat skin tumors and in causing the regression of pre-existing tumors. Since we have previously noted a high mitotic rate in radiation-induced rat epidermal cell tumors, it was hypothesized that anti-mitotic drugs would be effective in altering the growth rates of these tumors.

Tumor bearing rats from a previous experiment were assigned to treatment groups at 76 weeks post-irradiation. In one group methotrexate was injected in alternate flanks three times weekly at a dose of 1mg/kg, while the same injection protocol was used for Ara-C (3mg/kg) or saline (2.5 ml/kg) in two other groups. The methotrexate treated animals all showed severe weight loss and died after a few injections. As such, the injection protocol with Ara-C was modified so that any animal showing 10% weight loss in two days had its treatment withheld until the weight stabilized. Survival in this group was not different from controls for the 18 week duration of therapy, and the overall weight loss for the group was

less than 10%. All grossly visible lesions were scored at the time of start of the treatments and subsequently at biweekly intervals. Ara-C did not induce tumor regression. Of 27 tumors in the treated group, 3 regressed while 2 of 38 regressed in the control group. Since we have previously observed about 10% false positives in identifying carcinomas from their gross appearance, these "regressions" may not reflect the actual disappearance of carcinomas.

For each of the groups, the number of new tumors appearing during the 18 weeks of treatment may be compared with the number of tumors appearing in the same animals for the 18 weeks prior to treatment. We have previously observed that the rate of appearance of new tumors is approximately constant and, therefore, one expects the number of tumors to be about equal in the two intervals if the chemothereputic agent had no effect. There were 4 new tumors in the Ara-C treated animals, compared with an expected number of 3, and in the saline treated animals, there were 14 tumors compared with 11 expected. We conclude that Ara-C did not reduce the rate of new tumor appearance.

Examination of microscope section showed that many of the tumors in the Ara-C treated animals had evidence of cell death.

Many of the tumor cells were pyknotic, and there was extensive infiltration by lymphocytes.

The antimitotic agent Ara-C was neither able to reduce the new tumor appearance rate, or induce regression in preexisting lesions. The implication of these observations is that tumors of both large (microscopically visible) and small (subvisible) size are able to grow in spite of what appears microscopically to be a large lethal stress. There is no prophylactic value to the antimitotic agent in preventing tumor development under the conditions of this experimental test.

2. 8 Epidermal Cell Survival

Radiation has both toxic and carcinogenic effects on tissue, and it has been suggested that cell lethality at high doses may be responsible for the reduction in tumor yield. The objective of these studies is to evaluate the radiation cell killing in rat skin, for not only single doses, but for split doses of radiation where recovery of both sublethal and suboncogenic injury is likely to be occurring. The assay developed uses the ability of cells to complete the first post-irradiation mitosis as an index of viability.

The experimental procedure involves the pulse labelling of cells with H^3-Thymidine, allowing 24 hours to elapse, and then making whole mount preparations of the epidermis. During the

24 hour interval, the labelled (S-phase) cells will progress through mitosis and appear as adjacent labelled cells (labelled doublets) in the sheet of epidermal cells.

Irradiation increases the number of cells which are incapable of completing mitosis, and thus reduces the fraction of labelled cells appearing as labelled doublets. Variation in cell cycle sensitivity to radiation may be quantitated by varying the timing between the irradiation and ^{3}H-Tdr injection. For example, concurrent irradiation and labelling assays the survival of S-phase cells, while irradiation several days prior to labelling assays the survival of cells that were in G_1 at the time of irradiation.

Preliminary results indicate that S-phase cells irradiated with either electrons or Grenz Rays have a broad shoulder on which little cell killing occurs. 400R reduced the number of labelled doublets only slightly (90 % survival), while doses larger than 800R prevented mitosis in nearly all cells. Results for late G_1 cells (24 hours between irradiation and labelling) indicate the absence of any ^{3}H-Tdr incorporation, which may be the result of DNA synthesis suppression.

Current plans are to more carefully define the shape of the survival function for S-phase cells, and to extend the

interval between irradiation and labelling to longer time periods to overcome the suppression of thymidine incorporation. Plans also include the measurement of split dose recovery for this lethality endpoint, once the survival curves are established.

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