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TITLE Soft X Rays as a Tool to Investigate Radiation-Sensitive Sites in Mammalian Cells

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Soft x rays as a tool to investigate radiation-sensitive sites in mammalian cells

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Abstract

It is now clear that the initial geometrical distribution of primary radiation products in irradiated biological matter is fundamental to the observed end point (cell killing, mutation induction, chromosome aberrations, etc.). In recent years much evidence has accumulated indicating that for all radiations, physical quantities averaged over cellular dimensions (micrometers) are not good predictors of biological effect, and that energy-deposition processes at the nanometer level are critical.

Thus irradiation of cells with soft x rays whose secondary electrons have ranges of the order of nanometers is a unique tool for investigating different models for predicting the biological effects of radiation.

We demonstrate techniques whereby the biological response of the cell and the physical details of the energy deposition processes may be separated or factorized, so that given the response of a cellular system to, say, soft x rays, the response of the cell to any other radiation may be predicted.

The special advantages of soft x rays for eliciting this information and also information concerning the geometry of the radiation sensitive structures within the cell are discussed.

Introduction

It is now well accepted that the inchoate spatial distribution of primary radiation products is fundamental to the biological effectiveness of ionizing radiation--in general, the effect is larger, the more closely spaced the energy depositions. Therefore, very low-energy x rays provide an excellent probe for investigation of different models of radiation action. This is because these x rays interact to produce secondary electrons whose ranges are much smaller than cellular dimensions, and in fact can be of the same order as the DNA diameter. Data on cell survival and cell mutation[1,2] and yields of chromosome aberration[3,4] after irradiation with low-energy x rays have been published.

The most precise characterization of the initial spatial distribution of energy transfers is currently provided by the results of detailed, event-by-event, Monte Carlo simulations of the passage of the radiation of interest through the cell, and thus several groups have developed such computer codes.[5-9] However, very few models[10,11] exist relating such detailed descriptions to the injury directly responsible for the observed end points.

The Generalized Theory of Dual Radiation Action[10] (GTDRA) quantitatively predicts radiation injury yielding observed biological end points, based on the detailed microscopic pattern of energy transfers (depositions) in the sensitive site of a biological object. The GTDRA as originally formulated assumes a uniform field across the sensitive matrix of the cell. However, a particular impediment to the interpretation of the soft x-ray data is the fact that the low-energy x rays are strongly attenuated over dimensions comparable to the cellular size. Thus there are significant variations in dose across the cell (to be distinguished from stochastic variations in energy deposition).

In this paper we first describe the modifications necessary to the GTDRA to take into account the attenuation of the x rays, and we then use this formalism to examine critically the interpretation of the soft x-ray cell-survival results in the framework of the GTDRA. All the analyses in this paper will refer to the published data[1,2,13] on inactivation of V79 Chinese hamster cells.

The GTDRA for uniform fields

The GTDRA has two tenets, which are that

(a) ionizing radiation produces units of elementary injury, termed sublesions, in the sensitive part of the cell at a rate proportional to the energy transferred (deposited) locally, and

(b) these sublesions interact in pairs with a probability, $g(x)$, that is dependent on their separation, x, to produce injury directly responsible for the observed biological end point, these injuries being termed lesions.

If $\tau(x;D)dx$ is the average number of pairs of sublesions whose separation is between x and x + dx, then the second tenet states that the mean number of lesions $\bar{\varepsilon}$ produced by a dose D is

$$\bar{\varepsilon}(D) = \int g(x)\ \tau(x;D)\ dx \quad . \tag{1}$$

$\tau(x;D)$ is comparatively simple to evaluate[12] and $\bar{\varepsilon}$ becomes

$$\bar{\varepsilon}(D) = \frac{1}{2}K^2MD\left(\int_0^\infty g(x)\ \frac{m(x)}{4\pi\rho x^2}\ t(x)dx + D\int_0^\infty g(x)\ m(x)dx\right) \quad , \tag{2}$$

which shows the familiar linear-quadratic dose dependence. In this equation K is a constant relating the energy deposited locally to the number of sublesions produced (see tenet a). $t(x)dx$, termed the proximity function of energy transfers, is the expected energy in a spherical shell of radius x and thickness dx at a randomly selected energy transfer point (here the random selection of transfer points is weighted by the energy deposited at these points). Also in this equation it is necessary to account for the fact that on average only a fraction of the mass, M, of the spherical shell considered above will contribute to sublesion formation, due to the finite size of the sensitive matrix in the cell. This fraction is denoted by $m(x)/4\pi\rho x^2$, where ρ is the cell density.

The physical interpretation of Eq. (2) is the description of lesion formation from the interaction of KDM sublesions produced throughout the sensitive volume with other lesions produced by the same primary particle (first term) and by different, uncorrelated, primary particles (second term).

Modifications for soft x-rays

In Ref. 12 an expression is derived for the average yield of lesions for an attenuated beam of radiation as a function of average absorbed dose, $\bar{D}$, of radiation type "i":

$$\bar{\varepsilon}_i(\bar{D}) = \alpha_i\bar{D} + \kappa(\mu_i)\beta\ \bar{D}^2 \quad , \tag{3}$$

where $\kappa(\mu_i)$ is a function of the attenuation coefficient, μ_i, of the x rays traversing the cell and the geometry of the sensitive matrix; the function is unity for nonattenuating radiations. Eq. (3) differs from the standard linear-quadratic dose-effect prediction of the GTDRA [see Eq. (2)] in that the coefficient of the quadratic term in dose does depend on the radiation used. [In Eq. (2) $g(x)$ and $m(x)$ both depend only on the biological properties of the target.)

We assume that each increment in the number of lesions eliminates a proportionate fraction of cells able to produce colonies, thus, the cell survival is given by

$$S_i(\bar{D}) = \exp[-\bar{\varepsilon}_i(\bar{D})] \quad . \tag{4}$$

Eqs. (3) and (4) in principle allow us to fit the experimental survival data $S_i(D)$ with the parameters α_i and β. We may then use the result (see Ref. 12):

$$\zeta_i = \alpha_i/\beta = \int t_i(x)\ \gamma(x)dx \quad , \tag{5}$$

where $t_i(x)$ is the proximity function of energy transfers for radiation "i" (a function only of the physics of the radiation field, and defined in detail above). $\gamma(x)$ is the probability that two energy transfers, a distance x apart, will produce a lesion; it is a function purely of the biological response of the system. Thus knowing ξ_i from the experimental data and $t_i(x)$ (from calculation, see next section) $\gamma(x)$, the immediate object of this analysis may be unfolded from Eq. (5).

In a more general sense, the confirmation of the GTDRA as a tool for interpreting the soft x-ray data amounts to verifying the following propositions:

(a) There exists a set of parameters $[\alpha_i, \beta]$ that describe the data in a statistically accurate fashion using Eqs. (3-4).

(b) There exists a function $\gamma(x)$ which satisfies Eq. (5).

(c) This function $\gamma(x)$ can be used predictively for data not used in the determination of $\gamma(x)$ (i.e., cell-survival using different radiations), but obtained under otherwise identical conditions.

Each of these points will be examined in detail in the following. Before dealing with the unfolding procedure to obtain $\gamma(x)$, we first discuss the evaluation of $t_i(x)$ and ξ_i.

Evaluation of proximity functions

Proximity functions for all the radiations of interest in this analysis (carbon K x rays, aluminum K x rays, titanium K x rays, 250-kVp x rays, and helium ions of various energies) were calculated using the detailed event-by-event Monte Carlo transport code for water vapor described in Ref. (5). The results of such simulations (the positions and energies of all nonelastic interactions were then analyzed using the procedures described in Ref. (6) to obtain proximity functions. This procedure basically involves calculating the energy-weighted point-pair distance distribution of energy transfers in the field, and binning the product of the two

The x-ray simulations were carried out by transporting the secondary electrons set into motion by photons. In the case of the low-energy x rays the electron energies were 270 eV (photoelectron from carbon K x ray), 955 and 516 eV (photo- and Auger electron from aluminum K x ray, initial electron directions randomly oriented), and 4026 and 516 eV (photo- and Auger electrons from titanium K x rays, initial electron directions randomly oriented). For the 250-kVp x rays, the initial photon spectrum $N(E_\gamma)$ was assumed to be that given by Johns[15] for a half-value layer of 3 mm of copper . For this spectrum a normalized secondary electron energy distribution $N(E_e, E_\gamma)$ was calculated using the code PHOEL2,[16] with each photon undergoing only one interaction. Then the proximity function, t(x), may be calculated[17]:

$$t(x) = \int_0^{E_{e_{max}}} t_{E_e}(x)\ E_e\ N(E_e, E_\gamma)\,dE_e / \bar{E}_e \quad , \qquad (6)$$

where $t_{E_e}(x)$ is the proximity function for an electron of energy E_e and

$$\bar{E}_e = \int_0^{E_{e_{max}}} E_e\ N(E_e, E_\gamma)\,dE_e \quad . \qquad (7)$$

The integrations were performed using 75 electron energies.

The results of the calculations for low-energy and high-energy x rays, and for a representative helium-ion energy are shown in Fig. 1.

Evaluation of ξ_i

As mentioned above, the aim is to fit the experimental survival data to Eqs. (3) and (4) and thus obtain the ratios $\alpha_i/\beta (= \xi_i)$ for the radiations of interest. However, $\kappa(\mu_i)$ is itself a function of the unknown $\gamma(x)$ (see Ref. 12).

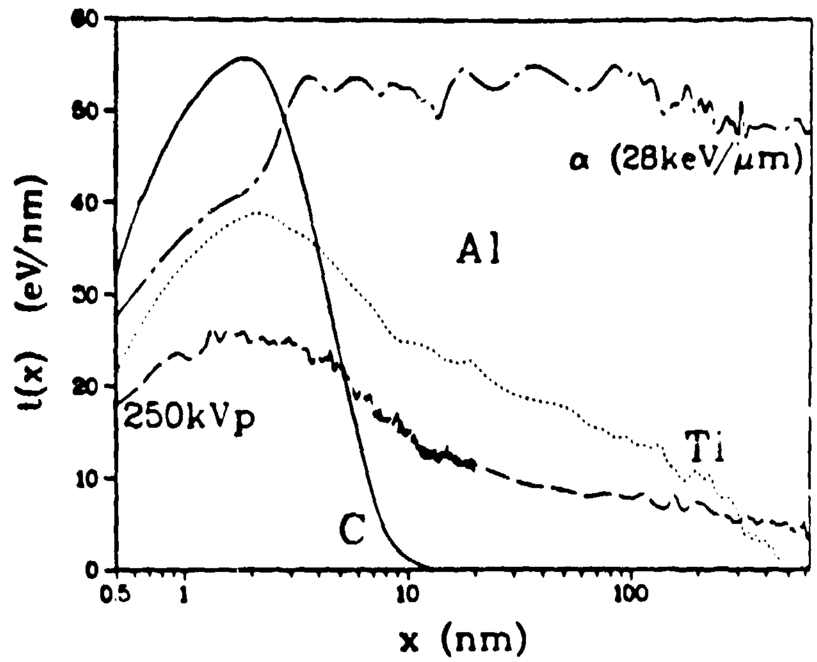

Figure 1. Proximity functions for various radiations.

$$\kappa(\mu_i) = \frac{M}{\bar{M}(\mu_i)} \int_0^\infty \gamma(x) \frac{m(x;\mu_i)}{m(x;o)} 4\pi x^2 \rho \, dx \quad , \tag{8}$$

where $\bar{M}$ and m are described in detail in Ref. 12. Hence, an iterative procedure was carried out (see next section) in which, initially, $m(x;\mu)/m(x;o)$ was replaced by $\delta_{o_i} = m(o;\mu_i)/m(o{\cdot}o)$. Then $\kappa(\mu_i)$ becomes

$$\kappa_o(\mu_i) = \frac{M}{\bar{M}(\mu_i)} \delta_{o_i} \quad . \tag{9}$$

Both $\bar{M}(\mu_i)$ and δ_{o_i} can be calculated (see Ref. 12) given the attenuation coefficients and an assumption concerning the geometry of the sensitive volume. We assumed a hemispherical volume (x ray entering normally through the flat side) with a radius of 7 μm.[18]

The experimental data for the x rays only were fitted to Eqs. (3) and (4) with $\kappa(\mu_i)$ replaced by $\kappa_o(\mu_i)$. A computer program was written to perform the fitting using the maximum likelihood criterion, with β as "global" parameter for all the different x rays, and in a different run, allowing β to vary between radiations. The results are shown in Fig. 2 . Using the standard F test[19] it was not possible to reject the hypothesis of a constant β at the 95% level of confidence. However, when the calculation was repeated putting $\kappa(\mu_i) = 1$ (i.e., not taking into account the effects of attenuation) the hypothesis of a constant β <u>could</u> be rejected.

Iterative procedure

The iterative procedure, initialized in Eq. (9) by replacing $\kappa(\mu_i)$ by $\kappa_o(\mu_i)$, proceeded as follows. Using $\kappa_o(\mu_i)$, values of ζ_i were obtained (see last section) and from Eq. (5), using an unfolding procedure (see next section), an initial value for $\gamma(x)$, $[\gamma_o(x),]$ was obtained. Using this value of $\gamma_o(x)$, an improved value of $\kappa(\mu_i)$ $[\kappa_1(\mu_i)]$ was obtained. The procedure was continued until $\gamma(x)$ converged. In fact, it converged after one iteration $[\gamma_o(x) \approx \gamma_1(x)]$.

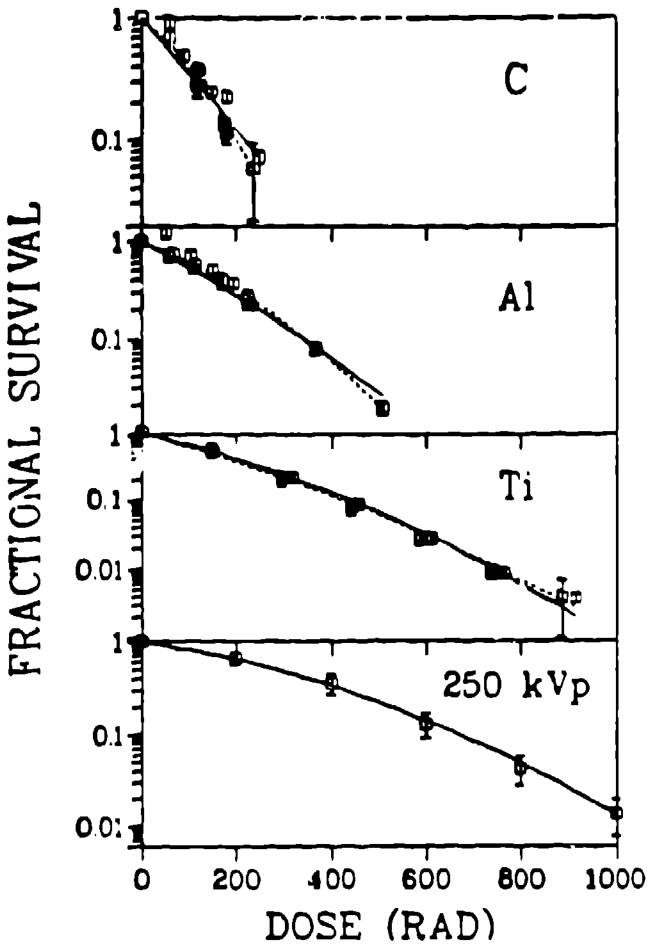

Figure 2. Cell survival after exposure to different x rays. The full lines are from a fit with a "global" β and the broken lines are from a fit in which β was allowed to vary between radiations.

Estimation of $\gamma(x)$

The interaction function $\gamma(x)$ was obtained by solving, using numerical methods, the following equations and constraints.

$$\epsilon_i = \sum_j t_i(x_j)\gamma(x_j)\Delta x_j \tag{10}$$

$$\sum_j x_j^2\, \gamma(x_j)\Delta x_j = (4\pi\rho)^{-1} \tag{11}$$

$$\gamma(x_j) \geq 0 \tag{12}$$

where Eq. (10) is the discrete version of Eq. (5) and Eq. (11) yields the correct normalization of $\gamma(x)$. Eq. (12) simply indicates that $\gamma(x)dx$ is a probability density distribution. The problem was solved using the computer code LSEI,[20] which solves linear equations with linear constraints in a least-squares sense. A suitable grid for the Δx_j was found to have 130 points equally spaced on a logarithmic scale from 10^{-1} nm to 15 μm.

The data used in solving Eqs. (10-12) were restricted to the results from the three soft x-ray sources and the 250-kVp source. The resulting interaction function $\gamma(x)$ is shown in Fig. 3.

Predictive use of $\gamma(x)$

Using $\gamma(x)$ of Fig. 3, which was derived solely from the x-ray data, we may use Eq. (4) and calculated proximity functions to predict survival curves for other radiations incident on the same cell line. As an example, the proximity function of Fig. 1 is used to calculate survival after irradiation with 28-keV/μm helium ions. The resulting prediction together with the experimental survival data[13] is shown in Fig. 4. The agreement is satisfactory.

Discussion

We have shown, firstly, that a set of parameters $\{\alpha, \beta\}$ does exist which describes the experimental data using Eqs. (3-4); secondly, that an interaction function $\gamma(x)$ exists that is consistent with the data, and thirdly, that this function may be used predictively to predict survival after exposure to a very different quality of radiation. Thus the GTDRA is fully consistent with the results of the soft x-ray experiments.

Finally it may be speculated that as the correction factor to the dose-quadratic term is a function of the geometry of the sensitive matrix [see Eq. (3)], a series of experiments with differently attenuated soft x rays would yield information concerning the geometry of the biological structure beyond the function $\gamma(x)$.

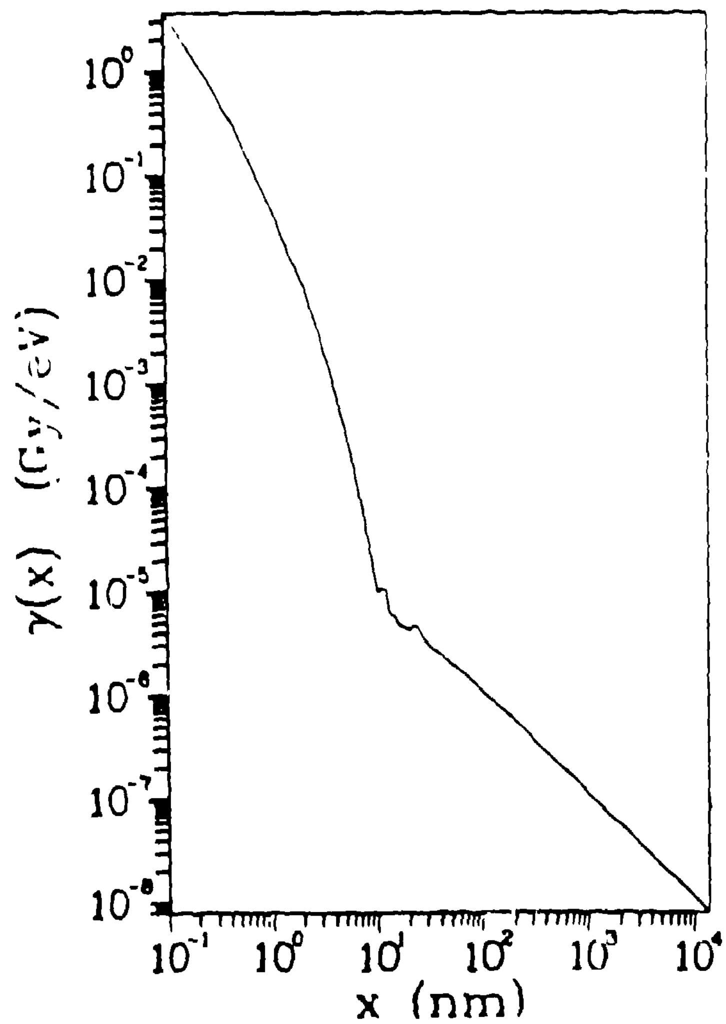

Figure 3. Calculated interaction function $\gamma(x)$.

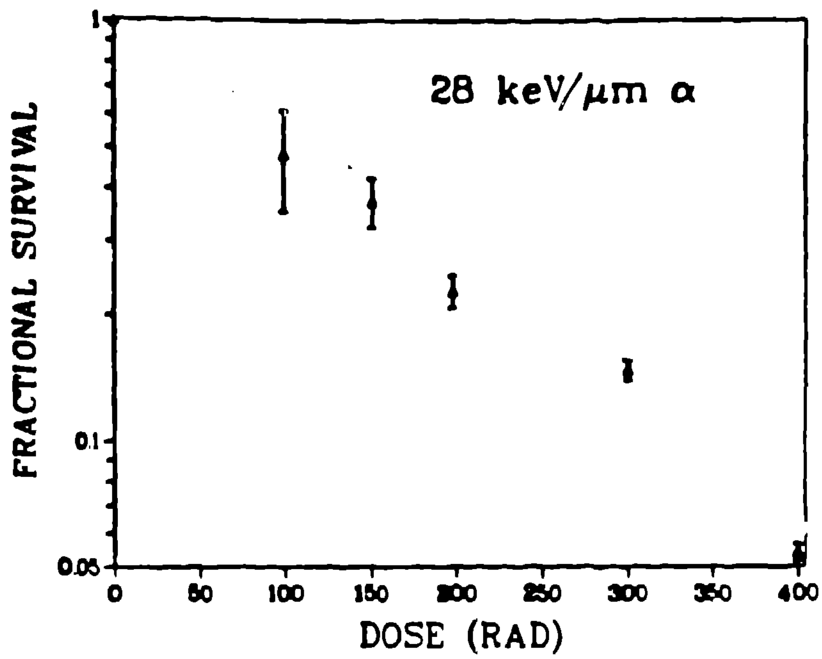

Figure 4. Measured (points) and predicted (curve) survival after exposure to 28-keV/μm alpha particles.

Acknowledgments

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