Risk Assessment from Heterogeneous Energy Deposition in Tissue. The Problem of Effects from Low Doses of Ionizing Radiation

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= **ABSTRACT** =

Low doses of ionizing radiation from external or internal sources cause heterogeneous distribution of energy deposition events in the exposed biological system. With the cell being the individual element of the tissue system, the fraction of cells hit, the dose received by the hit, and the biological response of the cell to the dose received eventually determine the effect in tissue. The hit cell may experience detriment, such as change in its DNA leading to a malignant transformation, or it may derive benefit in terms of an adaptive response such as a temporary improvement of DNA repair or temporary prevention of effects from intracellular radicals through enhanced radical detoxification. These responses are protective also to toxic substances that are generated during normal metabolism. Within a multicellular system, the probability of detriment must be weighed against the probability of benefit through adaptive responses with protection against various toxic agents including those produced by normal metabolism. Because irradiation can principally induce both, detriment and adaptive responses, one type of affected cells may not be simply summed up at the expense of cells with other types of effects, in assessing risk to tissue. An inventory of various types of effects in the blood forming system of mammals, even with large ranges of uncertainty, uncovers the possibility of benefit to the system from exposure to low doses of low LET radiation. This experimental approach may complement epidemiological data on individuals exposed to low doses of ionizing radiation and may lead to a more rational appraisal of risk.

INTRODUCTION

High doses of ionizing radiations are known to increase the risk of cancer to the exposed individual. Whether cancer is induced in man and mammals from exposure to low doses is unknown; epidemiological studies on exposed human populations are most difficult and many confounding factors need be considered. In order to appreciate potential carcinogenesis also from low doses, experiments are essential as will be discussed in this presentation.

The action of ionizing radiation in the human body has to be considered in a holistic manner. In a region receiving a low dose, radiation is absorbed only by a fraction of cells that constitute organ tissue in that region; energy depositions to individual cells trigger effects primarily at the atomic-molecular level

which is basic within the hierachy of interacting structures in biological systems[16], consisting consecutively of atoms, molecules, cells, specific cell populations and organ tissue.

THE CELL, ELEMENTAL UNIT OF LIFE

The perturbations introduced by ionizing radiation primarily at the basic atomic-molecular level of organization may be transferred to higher levels. This promotion of pertubations depends on the one hand on the initial extent of disorder and on the other hand appears to be inhibited or blocked by mechanisms for protection that operate sequentially at more complex structural planes[11]. Thus, cells have enzymes which defend against potentially toxic agents, such as molecular radicals including oxygen containing free radicals that are produced in the course of normal metabolism and also by ionizing radiation. Other enzymes take care of the repair of molecules, such as DNA, that are damaged. These enzymes are instrumental in substrate synthesis for replacing of damaged or lost structures as long as information for replacement through functional DNA remains available. At the organ-tissue level, mechanisms of defense, repair and replacement operate for example, through the immune system and various biochemical control signals regulating proliferation, differentiation and maturation of cells. For all these responses, the cell is the crucial element of tissue, i.e. the elemental unit of life.

THE RADIATION DOSE TO CELLS

In accord with the practice in radiation protection, the mass of the cell is averaged to have 1 ng, with its cell nucleus of 270 pg, and is proposed to be taken as the gross sensitive volume, GSV, within tissue in assessing the evolution of radiation effects[1,6,7,9,11,12,18]. Although the cell nucleus with its DNA as the most crucial molecule for cellular function is known to be more radiosensitive than the cytoplasm, the multitude of intracellular metabolic interactions is tuned to serve the entire cell. This makes the cell a functional entity to be viewed as an individual composite structure. In the following, the cell is considered to be a spherical volume with an average mass of 1 ng.

With respect to the intercellular matrix, radiation effects are considered to be negligible as long as they do not interfere with cellular function. An example of this would be the attack of toxic substances, such as molecular radicals, on cells from the interstitial spaces. At low doses of ionizing radiation such events are comparatively rare and are here not considered further.

A distinction is made here between cellular and tissue dose. The amount of energy that is deposited per radiation absorption event per GSV is conventionally termed specific energy. Since the GSV is defined here as the cell, this energy is specifically defined as the elemental dose, δ[9]. It has been shown that the number of δ's per unit dose, D, to tissue, the conventionally used indication of absorbed energy, is inversely related to the ioinization density, i.e. the linear energy transfer, LET[2,5,14]. In fact, D is equal to the product of the mean elemental dose, $\bar{\delta}$, the number of such elemental doses, N, and the fraction of cells affected by such doses, F.

$$D = \bar{\delta} \cdot N \cdot F,$$
$$\text{with } 1 \leq N < \infty \text{ and } 0 < F \leq 1 \qquad (1)$$

Below a given level of tissue dose D of a given radiation quality with its corresponding probability distribution of δ's, N remains 1 and F is significantly smaller than 1.

$$D = \bar{\delta} \cdot N \cdot F = \bar{\delta} \cdot F$$
$$\text{for } D \ll \delta \text{ and thus } N = 1 \text{ and } F \ll 1 \qquad (2)$$

Thus it is only F that changes and determines the magnitude of the effect with changing D at the tissue level. For "low dose" in this sense, i.e. for $D \ll \delta$, the fraction of GSVs is much smaller than one, $F \ll 1$. Hence, in the low dose region, most of the GSVs are

not affected by radiation and F is equal to the probability of being hit, a probability which is considerably less than one.

For a better understanding of radiation risk, not only absorbed tissue dose but also the corresponding absorbed dose rate, D is to be considered. Dose rate determines the mean time interval, t, between two consecutive δ's in a given GSV[9].

$$t = \bar{\delta}/D \quad (3)$$

If this mean time interval is larger than the period needed by the hit cell for complete repair and recovery, then the two radiation events can not interact; the risk involved with the second event is independent of the experience of a first event. This is the case e.g., with the background radiation of about 1.5 mGy per year, corresponding to about one δ per GSV per year average.

In situations in which radiation protection is necessary, i.e. with professionally exposed people, the time interval between two δ's may be in the order of hours and thus be comparable to repair and recovery periods. This is then of particular importance because there may be amplification of damage in the cell[15] or possibly a temporary stimulation of the cellular defense system induced by the first radiation absorption event. This may reduce the effect of the second event to near zero as will be shown in this paper.

THE RISK TO CELLS FROM RADIATION

The cell dose concept, as described above, is essential for appraising common difficulties in risk assessment. Risk to tissue is ultimately based on three risks to the cell: 1. The risk of being hit by an elemental dose, δ; 2. the risk of experiencing a given size of δ when hit; 3. the risk of a defined biological effect in response to δ. The first two risks are physical in nature. With a given spectrum of δ's for defined radiation field the probability of a cell being hit, in terms of F, rises linearly with radiation fluence, i.e. with D. The third risk depends on the biological property of the individual cell.

Both the fraction of affected cells, F, and the distribution of δ within the affected cells can be easily measured by a properly scaled microdosimeter[19,20]. The risk of a definded biological effect in a cohort of defined cells in response to being hit by δ is expressed by an appropriate "dose response function", or "hit size effectiveness function", of involved cells[3]. If such dose response tissue and effects observed would be linear. If there would be evidence of variability, then the concept of linearity would need correction[4]. In fact, variability in terms of adaptive response has been observed, as discussed below.

ADAPTIVE RESPONSES BY CELLS TO RADIATION

There is evidence that low doses of ioinizing radiation, i.e. a single δ or few δ per cells, cause in the hit cells, effects that induce biochemical reactions equipping the cell with mechanisms of defense and protection against a repeated experience. Such reactions are here called adaptive responses by which, for example, the detoxification of molecular radicals and repair of damaged DNA are stimulated.

Thus, from about 6 to 60 hours following an acute x-irradiation of human lymphocytes with priming doses of 5 or 10 mGy, the frequency of chromosome aberrations that are induced by a high dose of x-rays of 1, 5 Gy, was significantly reduced compared with nonprimed controls[21,22].

An adaptive response can also be observed regarding the action of molecular radicals. In mouse bone marrow cells, the enzyme thymidine kinase reacts sensitively to changes in intracellular radical concentration[8,10,13]. The enzyme was acutely and temporarily inhibited to a minimum activity of about 60% at about 4 hours with complete recovery about 10 hours after an acute gamma irradiation of

the whole body with less than 10mGy, i.e., in the range of single δ's per cell[8,14]. When a second acute whole body irradiation with the same dose was given four hours after the first, the enzyme activity in the bone marrow cells returned quickly to normal level and remained there as if there had been no radiation exposure at all. Concomitantly, there was at 4 hours a significant increase in the concentration level of free glutathion, a major cellular radical scavenger, in these cells indicative of an improved radical detoxification at that time[13].

This adaptive response has been elucidated further by introducing a disturbance of the radical detoxification by a strong static magnetic field; this was shown in side experiments to momentarily enhance lipid peroxidation at a temperature of 27°C and to depress it at 37°C; the rate of lipid peroxidation is taken to inversely affect the concentration of intracellular radicals. When mice immediately after a first radiation exposure at a controlled body temperature of 27°C, were treated with a static magnetic field of 1.4 T, the radiation effect on the enzyme activity of the bone marrow cells was abolished[10]. The investigation supports the involvement of bilayer lipid membranes and radicals in the observed adaptive response[17]. Concomitant with the disappearance of the enzyme reaction to the irradiation, the adaptive response in terms of resistance to the renewed exposure vanished[12].

Thus, it emerges that there is a radiation induced temporary resistance of the cellular thymidine kinase against potentially detrimental molecular radicals. This is a beneficial effect for the cells following hits by elemental doses. The system of radical detoxification protects cells physiologically against such radicals which are produced during normal metabolism, and it is shown here to be also effective against those generated by ionizing radiation.

THE RISK TO TISSUE FROM THE FRACTION OF CELLS AFFECTED

The cell is the basic element of the tissue system. Transfer of damage from cells to tissue is governed by the tolerance level to which the tissue may experience perturbation, or loss of its cells, without breakdown of tissue structures and functions required for maintaining life. Crucially important for both, acute effects to the organism and the induction of malignant tumors, is the fraction of cells that are hit by δ and respond detrimentally.

In the realm of low dose irradiation, as it is encountered mainly by environmental or occupational exposure or by accident, the number of cells being hit per unit tissue mass is of primary importance. It should perhaps be expressed in terms of a unit, the corresponding reference sample of this quantity[12]. Such a definition would improve clarity of the concept of low dose. The number of cells hit per unit tissue mass is easily measurable and may be applied to predicting effects from measured distributions of δ's in a given radiation field when related response functions of involved cells are known[3]. Mode of exposure, then, would be expressed in terms of numbers of cells hit per unit tissue mass per unit time, with subsequent attention to the distribution of δ's within the population of involved cells. This approach would be somewhat analogous to expressing radionuclide decay per unit time for which the unit is Becquerel and which does not address to the type and quality of radiation that is emitted per decay.

A major conclusion in applying the cell dose concept is the recognition that for low dose irradiation of low ionization density, there are adaptive responses in individual cells with the result that the risk of detriment to individual cells from repeated exposures is generally not additive. Therefore the linear extrapolation of risk to zero dose is not a valid

concept. Moreover, regarding the multitude of cells in tissue, the probability of radiation induced cellular detriment, such as malignant transformation, may be smaller than the probability of respective cell protection through temporary improvement of the radical detoxification system. In this case the net result of low dose irradiation on tissue may be beneficial. This is to be considered because temporary improvement of the radical detoxification system stimulates the cellular defense not only against radiation induced radicals but also against radicals produced by metabolism. Thus the effect of radiation induced improvement of the cellular defense system is amplified beyond radiation effects and the net effect in tissue may be positive.

Indeed, the probability of malignant transformation of a hit blood forming stem cell in man per δ from 100 kV x-rays is exceedingly small and is calculated to range at 10^{-13} for induction of lethal leukemia[12] whereas adaptive responses are easily measured within a relatively small cell population at values of single δ's. Even with the large ranges of uncertainties, first calculations for the case of low doses from radiation of low ionization density in the blood forming cell system favour the benefit. It appears worth while to test this prediction experimentally.

CONCLUSION

The holistic approach to analyzing tissue effects that are initiated primarily at the level of cells, thus leads to new questions in radiation biology, which still need to be answered. Only with the help of the tools of microdosimetry applied at the level of cells, can the sequence of biological responses perpetuating from the atomicmolecular level to the more complex levels of biological organization be integrated into assessing risk to the whole organism. This is important for radiation protection and uncovers the potential for beneficial effects also in terms of a reduced incidence of malignant diseases following low dose irradiation.

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