Adaptive Response To Ionising Radiation And Its Role In Influencing Radiation Protection Standards

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Introduction
The linear No-Threshold (LNT) model, the current scientific paradigm for the effect of low level ionising radiation is used for assessing radiation risks and performing cost-benefit analyses in the area of radiation protection. The 1972 BIER report adopted the LNT on practical grounds but noted that “such estimates are fraught with uncertainty”. The 1990 BIER V committee report stated that the derivation of risk estimates for low doses and dose rates through any type of risk model involved assumptions that remained to be validated.

The LNT model is based on the assumption that cancer may result from a single ionising event in a critical cell, that is to say that any radiation dose however small it may be, has a probability of inducing cancer. However, recent experimental evidences prove that protective mechanisms with which mammalian systems are equipped also have a role in averting and annihilating the detrimental effects of environmental mutagens including radiation. DNA repair, antioxidant status, apoptosis are some of these protective mechanisms. Therefore, there is now a need to reconsider the LNT as a predictive model.

Experimental evidences collected over the past two decades point to the observation that low dose of ionising radiation can offer protection to cells against high doses of radiation or in general, a harmful agent. In spite of the fact that the occurrence of radioadaptive response has been to a large extent now accepted by the scientific community and has aroused the attention of policy makers in the field of radiation protection, ambiguities regarding reproducibility, threshold limits and windows within which the phenomenon is known to be operational are some of the factors that baffle decision makers.

Radio-Adaptive Response (RAR)
RAR is referred to the phenomenon by which cells irradiated with very low doses of ionising radiation (few cGy) become less susceptible to the genotoxic effect of a subsequent high challenge dose of radiation or chemicals. Results of experiments with cultured mammalian cells indicate that there exists a narrow window of adapting doses that induce RAR. Doses used to induce AR experimentally are usually in the range 1-2 cGy, however, reports do exist to prove that doses less than 1cGy can also induce the phenomenon. For a review of this phenomenon and its mechanisms refer (1,2,3)

Most experiments on RAR have been conducted by exposing human lymphocytes to adapting and challenging doses in vitro, however, results obtained in such systems may not necessarily correlate well with actual conditions. This is mainly because of the fact that under in vitro conditions the adapting doses are generally administered acutely at a variable dose-rate that usually exceeds 0.2 Gy/min while the rates at which populations living in high natural background radiation and radiation workers are exposed are in the range of a few micro grays per hour.

Studies conducted in vitro have helped ascertain the cellular nature of the phenomenon. However, on comparing the response of cells exposed to adapting doses under in vitro and in vivo conditions, many differences can be observed. Several reports suggest that the induction of RAR might be restricted to specific experimental systems or biological end-points.

Results of experiments conducted in this laboratory both under in vitro and in vivo conditions suggest that the ideal adapting window may lie well below 1cGy. On comparing the extent of UV-induced UDS, significantly higher rates of UDS were observed in the lymphocytes of radiation workers when compared to a corresponding in vitro adapting dose (Fig 1). We postulate that the exposure of human lymphocytes to chronic low doses of radiation in vivo induces an adaptation process that appears to makes cells comparatively more efficient in repairing DNA damage than exposing them to the same amount of radiation in vitro for a shorter time. The observation that the response appears to be pronounced and operative at higher adapting doses in vivo, implies, that cell repopulating events and extra-cellular factors such as hormones, etc. may
Samples exposed in vitro

Fig 1. UV-induced UDS in lymphocytes of radiation workers and blood samples exposed in vitro to low adapting doses of ionising radiation. Control samples were those that received only the challenge UV dose.

Contribute to the full expression of the response. Thus in both cases there is a dose-response, which is, however, limited under in vitro conditions.

Table 1 contains results of some of the many reports on RAR and it can be observed that whenever the adapting dose was delivered in vivo, comparatively much lower doses, and dose-rates (less than 1 cGy) have evoked the response.

Thus, in vivo adaptation process may be different from those observed in vitro indicating a need to perform more experiments with in vivo systems before this phenomenon could influence radiation protection standards.
Table 1.5 Study of adaptive response using various end-points

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Cells used</th>
<th>Adaptive dose</th>
<th>Challenge dose</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Human Lymphocytes (radiation workers)</td>
<td>Elevated natural background radiation (α and γ)</td>
<td>20 J UV</td>
<td>Enhancement in UDS rates</td>
<td>Tuschi et al 1980 (4)</td>
</tr>
<tr>
<td>2</td>
<td>Human Lymphocytes</td>
<td>0.4- 0.98 mGy / 3 month occupational exposure</td>
<td>20 J UV and MMC</td>
<td>Enhancement in UDS rates reduction in SCE</td>
<td>Tuschi 1983 (5)</td>
</tr>
<tr>
<td>3</td>
<td>Mice spleen cells</td>
<td>0 - 0.25 Gy, whole body irradiation</td>
<td>UV</td>
<td>Enhancement in UDS rates</td>
<td>Liu et al 1987 (6)</td>
</tr>
<tr>
<td>4</td>
<td>Chinese hamster V.79 cells</td>
<td>1 - 5 c Gy</td>
<td>1 Gy</td>
<td>Reduction in MN &amp; SCE</td>
<td>Ikushima 1989 (7)</td>
</tr>
<tr>
<td>5</td>
<td>Human Lymphocytes</td>
<td>1c Gy x-rays</td>
<td>150 c Gy x-rays</td>
<td>AR affected by cycloheximide</td>
<td>Youngblom et al 1989 (8)</td>
</tr>
<tr>
<td>6</td>
<td>Mice Splenocytes</td>
<td>5.4 m Gy/ day whole body irradiation</td>
<td>UV</td>
<td>Enhancement in UDS rates</td>
<td>Liu et al 1990 (9)</td>
</tr>
<tr>
<td>7</td>
<td>Mouse Spleen lymphocytes</td>
<td>5 c Gy/ day whole body irradiation</td>
<td>UV and MMC</td>
<td>Enhancement in UDS decrease in SCE</td>
<td>Wojciek &amp; Tuschi 1990 (10)</td>
</tr>
<tr>
<td>8</td>
<td>Mouse bone marrow cells</td>
<td>0.0013 Gy x-rays</td>
<td>1 Gy x-rays</td>
<td>Decrease in CA</td>
<td>Fomenko et al 1991 (11)</td>
</tr>
<tr>
<td>9</td>
<td>Human Lymphocytes</td>
<td>3 c Gy x-rays</td>
<td>1.5 - 3 Gy Gamma rays</td>
<td>Decrease in CA. AR present in cells adapted in G0 cells</td>
<td>Khandogina et al 1991 (12)</td>
</tr>
<tr>
<td>10</td>
<td>Human Lymphocytes</td>
<td>5 c Gy x-rays</td>
<td>2 - 4 Gy x-rays</td>
<td>Decrease in CA</td>
<td>Shadley &amp; Dai 1992 (13)</td>
</tr>
<tr>
<td>11</td>
<td>Human Lymphocytes</td>
<td>1 c Gy x-rays</td>
<td>150 c Gy gamma radiation at 48 hrs</td>
<td>Decrease in CA and MN frequency</td>
<td>Vijayalaxmi et al 1995 (14)</td>
</tr>
<tr>
<td>12</td>
<td>Human Lymphocytes</td>
<td>1-10 mGy at a dose rate of 12 mGy/hr (in vitro adaptation)</td>
<td>30 J UV</td>
<td>Enhancement in UDS rates confirmed by comet assay</td>
<td>Mohankumar et al (1998) (15)</td>
</tr>
<tr>
<td>13</td>
<td>Human Lymphocytes</td>
<td>0.95 – 6.3 mGy / 3 month occupational exposure</td>
<td>30 J UV</td>
<td>Enhancement in UDS rates</td>
<td>Mohankumar et al (2000) (16)</td>
</tr>
</tbody>
</table>

PROBLEMS FACED IN THE APPLICATION OF RAR IN RADIATION PROTECTION

1. Lack of knowledge in the understanding of the mechanism of induction of RAR

The molecular mechanisms underlying RAR is not yet elucidated, this is one reason for ambiguities sited above. Enhanced DNA repair in adapted cells has been repeatedly presented. Several plausible theories and mechanisms have been put forth to explain this phenomenon, among these are apoptosis, enhanced DNA repair, cell repopulating events, scavenging of free radicals by adapting proteins, unknown signal transduction pathways, immunological responses etc. However, results of most studies point out to the theory that the phenomenon is induced by an unknown inducible molecular process triggered by low doses that leads to an enhanced repair of DNA caused by the challenge dose events. The experiments that support this theory are that RAR is known to be abolished by 3-AB, an inhibitor of poly(ADP-ribose) polymerase. Experiments using the emerging single cell gel electrophoresis assay also prove quite convincingly to the fact (17, 16)
2. Equivocal results
The response is known to be modulated by various factors such as pH, dose-rates at which the adapting doses are delivered. While many reports claim a positive response, some others fail to show any and a few even contend a synergistic negative effect.

3. Inter-individual variability
The human population is heterogeneous in its ability to express AR. Differences in the capacity to repair DNA damage may be dependent on factors such as diet, antioxidant status, genetic predisposition etc.

4. Differences in the in vitro and in vivo adapting responses
Reports suggest that the underlying mechanisms of AR observed in vitro differ in some respects from the situation observed after in vivo exposures as for instance in populations living in areas with high natural background radiation levels and in persons occupationally exposed to ionising radiation. Moreover many reports on the phenomena contend that the response is evoked only in cycling cells. If this is true then RAR has no real implications in radiation protection since most of the adult cells are in G0 state.

PROBABLE CAUSES FOR THE DIFFERENCES IN RESPONSE UNDER IN VITRO AND IN VIVO CONDITIONS
Reports indicate that DNA repair can be modulated by the functioning of the thymus in response to radiation exposure as for instance T cell differentiation (18). Liu (19) observed significant increase in the percentage of CD4- and CD8- cells in the thymus following whole body irradiation of mice. Tusche et al (20) also observed an increase in the ratio of CD4 and CD8 cells in lymphocytes of radiation workers compared to an unexposed population. These and other studies (21) indicate that the adaptation process is likely to be more due to cell renewal mechanisms rather than selective cell deletion. In this study, since the increase in UDS indicate an increase in DNA repair, it is possible that the in radiation workers there could have occurred a shift in the lymphocyte population in favour of a cell type with greater DNA repair capacity.

In addition to the thymic involvement, hormonal changes observed as the lowering of serum corticosterone and the increase of serum testosterone (19), indicate the involvement of the endocrinological system in the complete manifestation of the phenomenon of adaptive response. Thus the secretory products produced in response to the adapting dose delivered in vivo may be enhancing the DNA repair process or offer protection against the challenge dose. Further, some studies indicate that cells in vivo are more radio resistant than in vitro (22,23). All these findings suggest that cell repopulating events and other extra cellular factors are capable of influencing adaptive response causing the response in vivo to be much more pronounced than in vitro.

RELATIONSHIP BETWEEN RAR AND RADIATION HORMESIS (RH)

The RAR phenomenon is now generally accepted to be closely linked with radiation hormesis (RH), a term used to highlight the beneficial effects of low dose radiation. Thus RH may be considered as a special case of RAR. The principal differences between these two phenomena lies in the detection end points and the presence or absence of the challenge dose.

A comparison of in vivo, in vitro effects also imply a comparison of RAR and RH describes the stimulatory effects following single or chronic exposures to low doses of ionising radiation, whereas RAR involves the effect of the second insult the challenge dose. However the demarcation between RH and RAR is in the assessment of the end-points studied. While cytogenetic parameters like CA, DNA repair markers and mutation frequencies are used to detect RAR, survival and cancer incidence are the preferred ones to detect RH

SHOULD THE KNOWLEDGE OF RAR AFFECT CURRENT RISK ASSESSMENT METHODS FOR RADIATION PROTECTION?

Although it may be not too early to accept the beneficial or null biological effects of low doses of ionising radiation, it is certainly so to set standards and threshold doses for purposes of cancer risk estimates and radiation protection. The potential associated with AR will be considered for application only when the underlying mechanisms of this phenomenon are fully understood. The parameters that need to be assessed in this regard are, genes that are activated by the adapting dose, signal transduction pathways that determine and activate
genes associated with this phenomenon, characterisation and function of the induced proteins as well as other inducible responses to DNA damage such as apoptosis, cell cycle checkpoints etc. Only when the functions of these parameters are fully deciphered will it be possible to have a clear idea of the potentials of RAR. Although most researchers agree to the above statement completion of this venture at present appears to be a massive task with no significant progress made. Nevertheless, the most important parameter that needs to be analysed is in our opinion is the dose-rate factor and the dose ranges at which RAR is evoked under in vivo conditions. This is obviously because of the fact that if RAR has to affect and determine RP standards then it has to tested on exposed populations. Also, RAR can influence radiation protection only if it can correlate well with radiation hormesis, since the ultimate deciding factor and endpoint in risk assessment and radiation protection is the delay or onset of detrimental late tissue effects such as carcinogenesis.

Long-term systematic studies on occupationally exposed personnel and epidemiological surveys of areas with high natural background radiation are therefore required before meaningful conclusions could be drawn and RAR could influence radiation protection standards.

References

5. H. Tuschl, R. Kovac, H. Altman. UDS and SCE in lymphocytes of persons occupationally exposed to low levels of ionizing radiation, Health Physics, 45 1-7 (1983).