XA9745560

IAEA-CN-67/21

BIOLOGICAL EFFECTS OF LOW DOSES OF IONIZING RADIATION: CONFLICT BETWEEN ASSUMPTIONS AND OBSERVATIONS

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Abstract. Recent epidemiological data on cancer incidence among the A-bomb survivors and more importantly experimental studies in cell and molecular radiobiology do not lend unequivocal support to the "linear, no threshold" (LNT) hypothesis; in fact, the discernible evidence that low and high doses of ionizing radiations induce qualitatively different/opposite effects cannot be summarily rejected. A time has come to examine the mechanistic aspects of "radiation hormesis" and "radioadaptive response" seriously rather than proclaiming one's profound disbelief about these phenomena. To put the discussion in a serious scientific mode, we briefly catalogue here reports in the literature on gene expression differentially influenced by low and high doses. These are not explicable in terms of the current radiation paradigm.

1. Introduction. The current radiation paradigm assumes that for genetic effects induced by ionizing radiation, there might not be any "threshold dose", and a "linear, no threshold" relationship between dose and adverse genetic health effect (mutations in offspring, cancer in the exposed survivors) would therefore be expected to increase following exposure to even very low doses. A backward extrapolation from the high to low regions of the dose-effect curve has seemed justifiable. It must, however, be noted that actual experimental as well as epidemiological studies have not provided unequivocal support to the "linear, no threshold" (LNT) hypothesis; instead there has been growing evidence that low dose ionizing radiations induce "hormesis" [1], radioadaptive response [2,3] which are clearly not explicable by the current radiation paradigm. So far as cancer incidence among the A-bomb survivors is concerned, 'hormetic effect' among those exposed to about 8 cGy has also been suggested. A similar trend has also been inferred with regard to the inhabitants of high natural background radiation areas in China [4]. Since epidemiological data are often vitiated by several confounding factors, these are not powerful enough, to constitute the premise for "paradigm shift". There are, however, appropriate data from cellular and molecular radiobiology, to infer that low and high doses elicit *qulitatively* different responses. It is often noted that low and high doses do not simply activate (or inactivate) the expression of genes in quantitative manner, but exert opposite effects on different genes of the genome. The other major observation is that only small conditioning doses induce radioadaptive response, whereas higher doses do not. This again shows that low and high doses induce widely different molecular and cellular events in the cell. We propose to catalogue in this brief review only such major aspects which seem *not* readily explicable on the basis of LNT hypothesis.

2. Induction of gene expression. *a : Proteins* Low and high doses of radiation exert widely varying effects on expression of different genes invloved in synthesis of proteins, control of apoptosis and immunomodulation. Twelve X-ray-induced transcripts differentially expressed, 8 to 230-fold, in X-irradiated versus unirradiated radioresistant human melanoma cells. All transcripts were transiently expressed and induced by lower, but not higher (>600 cGy) doses of radiation. X-ray-inducible genes may function in damaged cells to regulate DNA repair, apoptosis etc. [5]. Such an expression also shows a pleiotropic response. X-irradiation (up to 8

Gy) induced 3 different types of responses in proteins (XIPs, X-ray induced polypeptides) in human malignant melanoma cells. Class I proteins were induced linearly with increasing X-ray doses. The synthesis of class II proteins (a majority of XIPs) increased linearly with low doses, but plateaued at higher doses of 1.5 to 2.5 Gy. In contrast, the expression of class III proteins, decreased with increasing X-ray doses [6]. Mice chronically exposed to 4 cGy γ-rays for 4 weeks showed an increase in the expression of stress proteins (HSP70, HSC70, HSP72) in splenocytes. A higher dose of 10 cGy, however, did not induce these proteins [7]. Low dose irradiation (25 and 50 cGy) stimulates the SH group of membrane proteins and enhances the Na^+K^+-ATPase activity, while doses above 100 cGy significantly decreased enzyme activity, in rat cerebral cortex [8]. (Please see Table 1. for comparative effects)

Table 1. Influence of dose of ionizing radiation on differential gene expression

Parameter	Low dose & effects	High dose & effects	Ref.
1. Expression of X-ray-inducible genes	<6Gy; 12 genes (transcripts) are induced	>6 Gy; no induction of gene expression	5
2. X-ray induced polypeptides -Class I	up to 1.5 Gy; increase	above 1.5 Gy; no change	6
Class II	up to 1 Gy; decrease	above 1 Gy; no change	
3. Expression of stress proteins	4 cGy; induced expression	10 cGy; did not induce expression	7
4. $Na^+K^{(+)}$-ATPase activity in brain	25 & 50 cGy; increased activity	100 cGy; decreased activity	8
5. Thymocyte apoptosis	<20 cGy; reduced below control levels	>50 cGy; increased above controls	9
6. Con-A induced mitogen response	5,10 cGy; increased	>25 cGy; reduced response	13

b: Apoptosis. There was a significant reduction in apoptosis rate, below control levels, with doses below 20 cGy and a dose-dependent increase with those above 50 cGy in thymocytes of whole body irradiated mice and in EL4 cells. A typical J-shaped dose-response curve was observed for X-ray (1-1000 cGy) induced apoptosis in EL4 cells. Only doses above 50 cGy caused an increase in apoptosis and values of 5 dose points were below that of the control and among these 3 were statistically significant from control [9]. Accumulation of p53, the product of tumor suppressor gene involved in the regulation of apoptosis, occurred in the adrenal glands and pancreas of mice exposed to 25 and 50 cGy but not 100 cGy of X-rays [10].

c: Modulation of immune system. 7.5 cGy X-rays enhanced signal transduction in lymphocytes. There was increased mobilization of $[Ca^{2+}]_i$ and activation of protein kinase C in response to ConconavalinA (ConA) and anti CD_3 McAB [11]. In rats exposed to 5 and 10 cGY, ConA-induced mitogen response increased significantly whereas doses above 25 cGy reduced such response [12].

(ii) Adaptive response and the mechanisms involved. Preexposure of cells to a low dose of gamma-ray (1 cGy) caused a decreased susceptibility to gene deletions and rearrangements after a challenging radiation with a high dose [3]. In bone marrow cells of mice exposed to a challenging dose of 1 Gy, adaptive response observed was time-dependent and a lower dose of 2.5 cGy induced a longer-lasting adaptive response as compared to 5 cGy [2]. In human lymphocytes a dose of 1 cGy induced an adpative response, in terms of decrease in the frequency of mironucleated binucleate cells, when exposed to a challenging dose 1 Gy of gamma-rays (Fig. 1) [13].

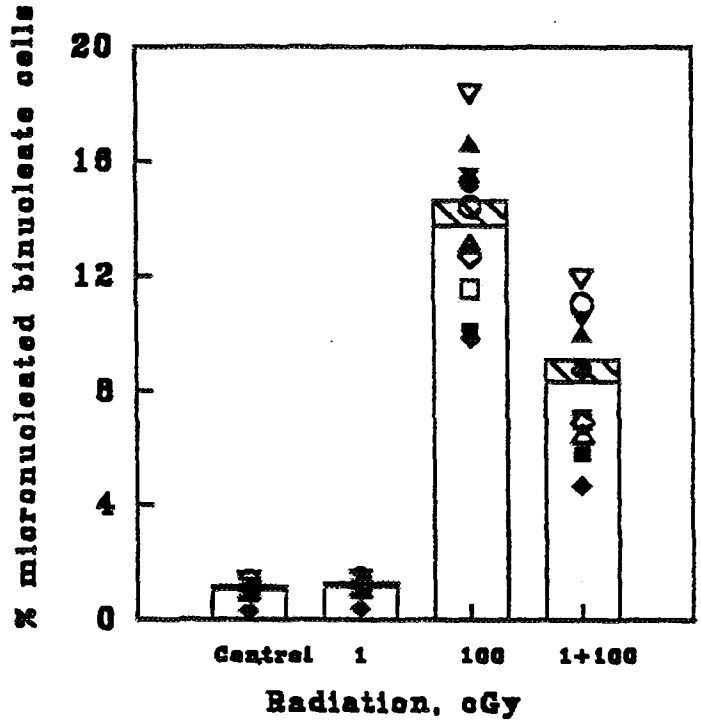

Fig. 1. Effect of pre-exposure to 1 cGy gamma radiation on 100 cGy radiation induced mn-BNCs in human lymphocytes *in vitro* male subjects. Symbols denote different individuals and bars represent pooled mean values [13].

Protein kinase C-mediated signalling pathway is a key step for the transduction of the low-dose-induced signal responsible for adaptive response. Low-dose may trigger changes in the expression of several genes whose products, though most of them are still not identified, would be related to DNA repair and/or control of cell cycle progression [14].

It is likely the the qualitative difference in the responses observed with low and high doses of radiation may be due to the differential response of the signalling pathways. One such pathway deals with reactive oxygen intermediates. They are known to influence apoptosis and membrane functions. It is likely that the quantitative and qualitative differences in the generation of these reactive species may be some of the major factors responsible for the contrasting effects observed with high and low doses. There is a paucity of data in this aspect and further studies are needed to examine the molecular mechanisms involved.

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