ARE WE AT RISK FROM LOW LEVEL RADIATION - DNA REPAIR CAPACITY AS A PROBE OF POTENTIAL DAMAGE AND RECOVERY

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Can the question of risk from low level radiation be answered by an experimental molecular approach? Can we assess accurately biological damage caused by low level radiation? Is there a threshold ? Is there a way to predict future genetic or late somatic expression of damage? What are the processes that occur in a cell and lead, following assaults by radiation or chemicals, towards mutagenicity and possibly carcinogenicity? What is the main reason for differences in radiosensitivity between cells which are otherwise equal?
This last question was answered in the case of bacteria first, when differences in survival from ultraviolet radiation was shown to be dependent on the existence of enzymatic repair systems which are lacking in radiation sensitive mutants (1,2,3). This discovery, soon followed by finding the existence of similar enzymatic repair mechanisms for mammalian cells and for assaults also by ionizing radiations as well as chemicals, opened new prospects for approaching by similar ways the problems of risk from radiation and the raging debate on the question of threshold.

Biological dosimetry and risk estimation may be approached from two different angles: one is to look at damage already inflicted by radiation, and expressed in a biologically important molecule, such as the chromosome, at such low doses that the damage is not apparent in the normal functions of the cell. The other is more directly related to risk evaluation, that is to look for a cellular system which will indicate the potential risk of a radiation assault. Cytological techniques have been developed to enable observing chromosome aberrations in lymphocytes, the most sensitive human cell, and determine in this way the dose of radiation. Aberrations are however the end product of a series of events occuring in the irradiated cell, furthermore they can be easily and accurately seen only following a dose of about 20 rads or more. The biochemical systems responsible for repair of damage to DNA are controlling the ultimate fate of radiation damage, be it specific products (4) or strand breaks (5), and thus are determining both the number of aberrations as well as the radiosensitivity of the cell. Indeed, excision repair and/or postreplication repair function in most normal human cells, and can repair most types of damage to DNA. An impaired activity of any of the enzymes of repair results in lack or reduced capacity of repair. This may result in extreme sensitivity of such cells to radiation, and such a situation has been found to exist in humans carrying genetic auto-immune diseases. Cells from patients with the disease Xeroderma pigmentosum (XP) show extreme sensitivity to UV light, and patients with the disease

Ataxia telangiectasia (AT) show extreme sensitivity to X-rays. These cells may be considered as the human mutants, identical in response to the bacterial mutants which show radiation sensitivity, in comparison to their wild type strains. Impairment in excision repair of UV-irradiated DNA is clearly implicated in XP, and similar impairment is suspected in Ataxia (6,7) and in several other diseases (although the existence of variants with normal DNA excision repair activity sheds doubt whether this is the only factor involved in the appearance of the disease), such as Fanconi's anemia, chronic lymphocytic leukemia and more. An interesting feature of these diseases is characterized in their "chromosome breakage syndrome" and their predisposition to cancer. Several recent reports indicate also an impairment in mitogen-induced transformation of lymphocytes of patients with these diseases. The possibility of a link between chromosome aberrations lymphocyte transformation and DNA repair was recognised by us (8) and led to a decision to develop a biochemical method, based on measurement of DNA repair capability, which would enable predetermination of radiation sensitivity, and a more important feature - an indication of inherent sensitivity which might be expressed ultimately in the future - when the cell or organism might be faced with a situation in which its repair ability will have to function to its full capacity.

EXPERIMENTAL APPROACH AND RESULTS

The aims and steps of these studies have been previously formulated (9) and described (10). We have already shown that induced transformation of human lymphocytes is somewhat impaired following acute radiation dose, in cases where the person from whom the lymphocytes have been drawn was chronically exposed previously to low level beta (tritium) radiation (8). Induced transformation is, however, a black box and not a natural event in the life cycle of the lymphocyte. A clear and exact biochemical reaction was needed and none is better than the study of DNA repair itself. DNA repair may be followed by employing the technique of repair synthesis, whereby the amount of incorporation of labelled thymidine into cellular DNA is measured under conditions which do not permit the normal semiconservative DNA synthesis to occur. The commonly used method of treatment with hydroxyurea results in still too high a background and is subject to increasing criticism as affecting repair. A novel new method was therefore developed in our laboratory in which the cells are treated with trioxalen (trimethylpsoralen, TMP) and near UV light (NUV), bringing about an almost complete cessation of semiconservative DNA synthesis, 99.5 to 99.8% inhibition (Heimer, Kol and Riklis, in preparation). This treatment was previously successfully used by us to study gene expression and the control and regulation of inducible enzymes (11). Now it enabled the accurate measurement of incorporation of labelled thymidine (^{3}H-TdR) into DNA, following assaults by radiation or chemicals, indicating that repair synthesis is occuring.

Details of the experimental procedures are described elsewhere (11, and, Heimer, Kol and Riklis, in preparation; Kol, Heimer and Riklis, in preparation for detailed results). Briefly, it involves irradiation of cells with near UV light for 3-5 minutes in the presence of $5x10^{-6}$M TMP, incubation for 3 hrs, assault by far UV, gamma radiation or a chemical (MMS), incubation with ^{3}H-TdR for 90 min., washing, TCA precipitation and counting in liquid scintillation counter. The method has been found suitable for human fibroblasts, human breast cancer cells, Chinese hamster cells and human lymphocytes. The number of counts incorporated is many thousands above background, indicating repair synthesis following the acute assault. A linear increase of counts is observed up to a certain radiation dose, this being designated as the repair capacity of the cell, given by absolute numbers when the number of counts incorporated while repairing a high dose damage is divided by the counts incorporated following the lowest acute dose or no irradiation-control (Fig.1). The counts were taken up into double stranded DNA, as shown by the S1-nuclease method (13) indicating true repair replication. Thus, the 'repair capacity" is a true measure of the capability of the cells to repair damage.

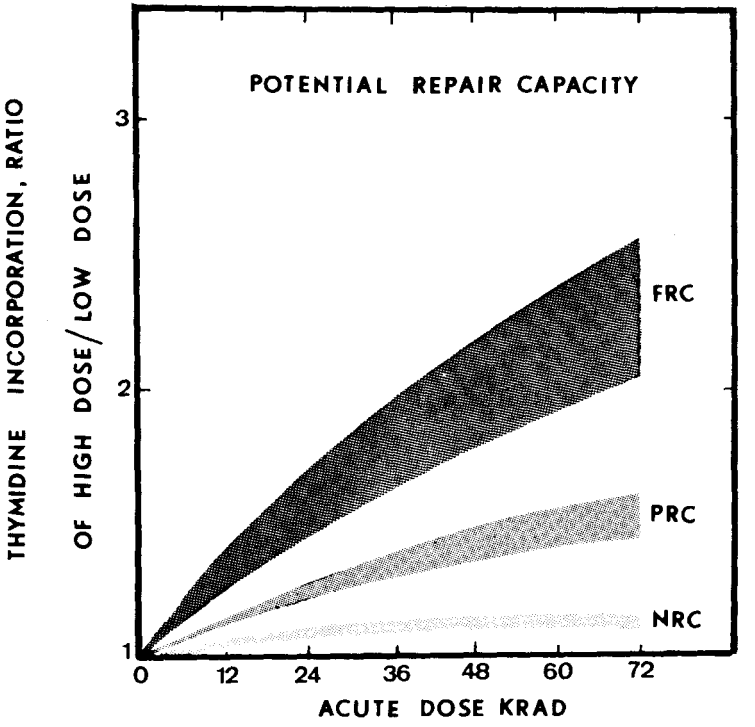

Figure 1. Potential Repair Capacity. Number of counts incorporated per 10^6 cells following each dose is divided by cpm/10^6 of control cells. FRC=full repair capacity; PRC=partial repair capacity; NRC=no repair capacity(12). A hypothetical graph.

A most significant result was obtained in showing that chronically irradiated Chinese hamster cells, with 20 rads per day for 3 months showed the same repair capacity as controls. Their survival was also not affected although their growth rate was slower.

DISCUSSION

Repair synthesis as measured by thymidine incorporation does not indicate the type of repair which has taken place, the exact nature of it is yet to be determined. A more important question to be asked is whether this repair is error-free or error-prone, namely, can we determine whether the cell is fully functional and not mutated. This is clearly a difficult problem, but attempts are being made to answer it by following the performance of an inducible enzyme, Ornithine decarboxylase (14), which if induced only after a radiation assault and if it functions to full capacity, indicates the wholesomeness of the DNA template.

Since the psoralen + NUV method of repair capacity measurement is applicable for lymphocytes, it can easily be used for a world-wide interlaboratory comparative study, in which the range of 'repair capacity' of normal healthy humans will be established, and average figures will be determined for fully repairing cells (FRC), partially repairing cells (PRC) and non-repairing cells (NRC). A threshold for radiation damage will be between the PRC and the NRC regions. Individuals with no repair capacity (NRC), are 'at risk' from any level of radiation above background, while the general public, showing normal repair capacity, may be considered safe from the effects of low level radiations.

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