

Radiobiological Research For Improving Tumor Radiotherapy -An Indian Perspective*

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ABSTRACT

Radiation-induced damage to normal tissues within the non- target volume is a major limitation of tumor radiotherapy. Physical methods to obtain superior spatial dose distributions use sophisticated technology and are expensive. Large scale applications of these technologies in a developing country like India; with a large number of cancer patients, poor' instrumental facilities and inadequate infrastructure face several problems. Radiobiological research aiming at developing simple, inexpensive and effective methods to increase the differential response between tumor and normal tissues should be, therefore, strengthened.

Biological end-points are determined not only by the molecular lesions produced due to the absorption of the radiation energy but also by the cellular repair processes, which become operative in response to lesions in the living system. Therefore, enhancement of repair processes in the normal tissues and inhibition of the same in tumors should considerably improve the therapeutic index of radiation treatment. A combination of agents which can **suitably** alter the spectrum of important molecular lesions with modifiers of cellular repair could be an effective strategy. Initial **experiments** using halopyrimidines to increase repairable DNA lesions produced by sparsely ionizing radiations in combination with **2-deoxy-D-glucose** to modulate differentially the repair and fixation processes in the tumor and **normal** tissues have provided promising results. Further research work is warranted since this strategy appears to have great potential for improving tumor radiotherapy.

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*Dedicated to the memory of Brig. S.K. Mazumdar

1. INTRODUCTION

The advent of artificial radiation sources and progress in the medical radiation technology have led to a steady increase in the use of radiations in almost all spheres of health care. Particularly, the present day management of cancer involves radiation-based techniques in a crucial way for the detection, localization as well as curative and palliative therapy of tumors. In principle, radiation should be able to control localized cancer provided sufficiently high absorbed dose can be administered. However, because of inability to selectively irradiate the tumor, normal tissues adjacent to the tumor also receive high radiation dose and are damaged. Thus, the major cause of failure of radiotherapy or treatment related morbidity is radiation damage induced in non-target tissues.

The absorbed radiation dose to the normal tissues can be reduced by using several new technical innovations for improving spatial dose distribution, for example, by conformation therapy employing multiple leaf collimators in conjunction with computerised 3-D treatment planning and image processing systems. Irradiation with heavy charged particles such as pions, protons or helium ions is another possibility which permits very precise dose delivery to the target volume with minimum dose to the surrounding tissues. The accelerators needed for producing high energy particles are, however, extremely expensive, restricting the establishment of such facilities in only a few centres in the economically and technically advanced countries.

In India, the number of new cancer patients needing treatment has been estimated to be of the order of 5×10^5 patients per year. The major types of cancer prevalent in the country, viz. oral, cervical and breast, can be cured by radiotherapy alone, if detected at an early stage. However, the facilities available for radiotherapy in our country are woefully inadequate and non-uniformly distributed. The majority of machines are cobalt-60 teletherapy units (about 100) with a few electron accelerators (8), whereas the projected need is for 500 units. Only very few centres are equipped for computerised treatment planning and imaging. The problems faced in the development of the multidisciplinary speciality of radiation oncology in India have been recently highlighted'. Applications of highly sophisticated, expensive and potentially hazardous technologies in a developing society with inadequate infrastructure, poorly trained personnel and limited resources face special problems. There is a great need, therefore, to improve the management of the radiotherapy departments and to use the existing technology prudently to ensure optimal benefit to the patients.

From a long-term perspective, earnest efforts to develop more effective yet less hazardous, simple and inexpensive techniques are required, if both the rural and urban populations are to be provided with adequate treatment facilities in future. To achieve this objective, basic research is necessary for better understanding the biophysical processes underlying various aspects of radiation oncology. In this presentation, some of the research strategies, which in the author's view, appear to be relevant with reference to the prevalent Indian conditions, will be discussed. However, before undertaking this task, a few general remarks regarding the application

of science and technology for social development are presented in order to provide an appropriate framework for further discussions and action.

2. SCIENCE, TECHNOLOGY AND SOCIAL DEVELOPMENT

The multiple and complex relationships between research in basic sciences, development of technology and its applications for the benefit of the society are shown in Fig. 1. Research in basic sciences systematically attempts to determine truth, thereby leading to generation of new knowledge. Advances in knowledge are necessary for the development of new technology and industry. New technology, if used with responsibility, can be exploited for the benefit of the society.

Translation of advances in knowledge into new technology and industrial development in a reasonably short time span requires cooperation and coordination between a large number of individuals, institutions, organisations and industry. This difficult task can be accomplished only within a flexible and progressive system which encourages easy (with minimum bureaucratic and administrative delay) multiple linkages between research institutes, universities, industry and consumer organisations.

In the absence of any worthwhile development of indigenous technology in the field of radiation medicine in India, we have to depend almost entirely on the transfer of technology from the advanced countries. The successful acceptance and optimal use of any new technology by the society depends, however, upon a number of factors

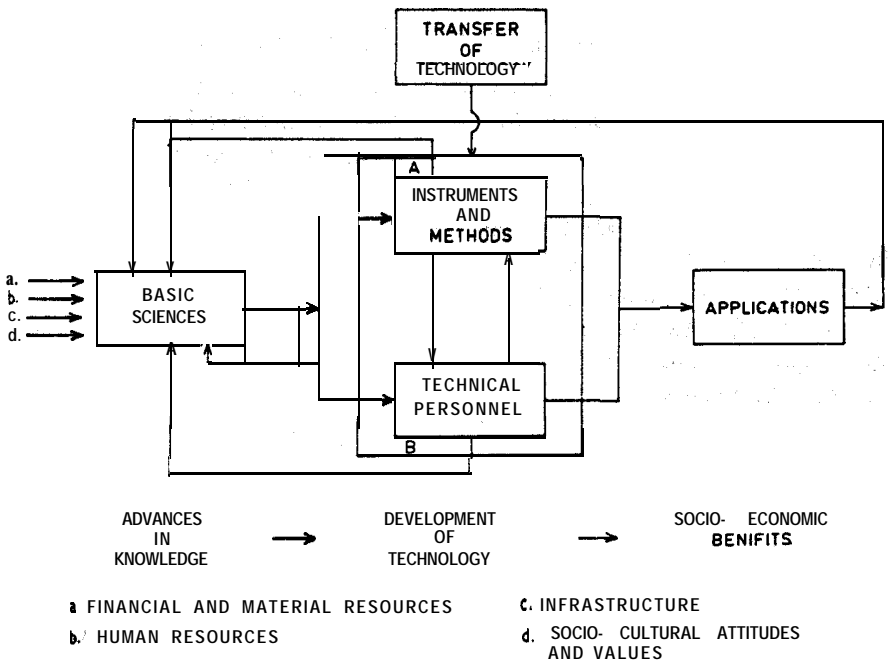


Figure 1. Relationships between science, technology and social development.

including industrial infrastructure, socio-economic and cultural backgrounds besides considerations of technical merits (as compared with the existing technologies) and associated hazards and risks. In order to objectively *evaluate the suitability of the new technology, a total cost-benefit analysis should be carried out. Such an analysis would be helpful in optimizing the use of the technology. Optimization requires that the total cost-benefit ratio, Φ_T , for application in a given situation be minimised. The total cost includes not only the economic costs involved in the application of the technology, but also the cost of the associated hazards and risks.

Adoption of the technology for regular use will be determined by the index of affordability, which may be represented by a critical threshold value Φ_c dependent upon the resources of the society. For a wide acceptance of the technology $\Phi_c \gg 0$.

The calculation of the cost of the hazards, risks and benefits is complex, since it may also depend upon the value systems of the society. Attempts to estimate Φ_T values under different conditions for alternate technological approaches are necessary to objectively compare the alternatives and to help the planners and decision makers.

3. OPTIMIZING USE OF RADIATIONS IN CANCER MANAGEMENT

Within this framework, the present and future prospects of different strategies for optimizing applications of radiation in cancer management in a developing country like India should be examined. It is desirable to develop methods to reduce Φ_c . This could be attempted in a number of ways by (i) reducing the cost of required equipments, (ii) using the existing technology prudently, and (iii) carrying out research to develop better and cheaper technology suited to the Indian environment.

3.1 Reduction in the Economic Cost of the Equipment and Procedures

Cost of most equipment depends upon the industry and market forces. In our country, the field of biomedical instrumentation, has not developed as an industry. Consequently, the users have to rely entirely on imports. The foreign companies quote special prices for India, which are higher, by at least 20-50 per cent than the local price. Furthermore, for subsequent maintenance and servicing, the foreign companies or their Indian agents charge exorbitant rates. In view of the increasing demand of the sophisticated equipment in the field of radiation medicine, it is suggested that adequate financial and human resources be mobilised to encourage inclegenous research, development and manufacture of these equipment suited to the local environment and needs.

3.2 Prudent Use of the Existing Technology

The attitudes and behaviour of health care professionals determine to a great extent the use or misuse of the technology. Through proper education and training, it must be ascertained that the medical practitioners:

- (a) avoid using the technology when not necessary;
- (b) avoid using the more expensive techniques, when the same information and benefit can be obtained through simpler and cheaper techniques;

- (c) use the technique properly and ensure proper quality control to derive the maximum benefit; and
- (d) give due consideration to the potential hazards of the technology and methods employed and take appropriate precautions to **minimise** the hazards.

3.3 Radiobiological Research for Improving Tumor Radiotherapy

The goal of radiobiological research is to understand the effects of radiations on the biological systems and use this knowledge to increase the differential between the radiation responses of the tumor and normal tissues so that a high probability of cure without any significant morbidity at a given radiation dose can be achieved (Fig. 2).

Considerable work to develop radioprotectors for normal tissues and radiosensitizers for the tumor has been going on throughout the world for more than four decades. A large number of physical, chemical and biological agents have been screened (for example, see Uma Devi² and Singh³ for recent reviews). **Inspite** of significant research effort, the success of these radiomodifiers in improving radiotherapy has been rather limited so far. Enormous complexity of the problem, gaps in the basic understanding of the processes determining the radiation responses of the living systems coupled with the large inter- and intra-tumoral heterogeneity displayed by human cancers (even of the same type) could be some of the reasons for the slow progress. A critical review of the literature, however, reveals that a lack of systematic, integrated and holistic approach and greater reliance on **hit** and trial methodology with little emphasis on understanding the basic processes, could also be partially responsible for the sub-optimal results. Recent progress in molecular genetics and development of powerful non-invasive techniques to study structures and functions in the intact organism appear promising to adopt a new approach and to **formulate**

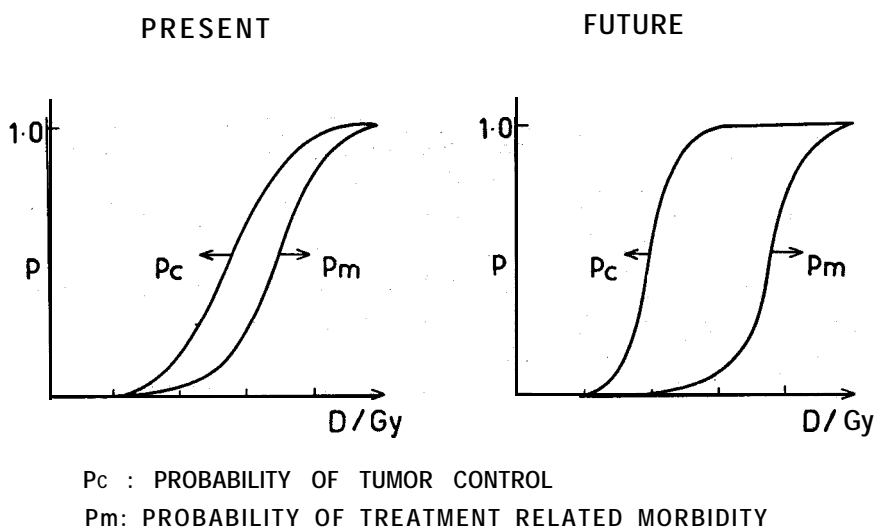


Figure 2. Probabilities of tumor cure (p_c) and morbidity due to damage to normal tissues (p_m) as functions of absorbed radiation dose, D .

a coordinated research programme to achieve the above mentioned desirable yet elusive goal. The rationale of this approach is briefly presented in the following.

3.3.1 Radiation-Induced Biological Damage and Strategies to Improve Tumor Radiotherapy

The biological damage induced by ionizing radiations evolves through a complex network of processes occurring at various levels of organisation and extending over a large time scale (Table 1). For simplicity, we may identify two important stages comprising (i) physico-chemical processes leading to the production of lesions in the biomolecules, and (ii) biological processes representing the responses of the living system to the molecular lesions. Both these phases can be suitably influenced in a number of ways to optimize tumor radiotherapy.

(a) Induction of biomolecular lesions

Absorption of radiation in biological tissues gives rise to excitations and ionizations of atoms and molecules resulting in the formation of highly reactive ion radicals and free radicals which may interact with each other and with other molecules. Some of these reactions may result in the induction of relatively long-lived lesions in important biomolecules. All types of biomolecules including nucleic acids, proteins, lipids and carbohydrates have been shown to be damaged by ionizing radiations. It is now generally agreed that DNA is the most important target for radiation-induced cell death while damage to molecules in biomembranes may also play a role under certain conditions. The most important DNA lesion responsible for cell death is the double strand break (DSB), which is readily repairable in mammalian cells^{4,5}.

The spectrum of molecular lesions induced depends upon the quality of radiation, microenvironment, structures and conformations of the molecules involved.

The quantity and pattern of energy depositions are primarily determined by the radiation quality and the irradiation technique. Passage of radiation through matter results in stochastically distributed energy depositions along the tracks of the ionizing particles. Biological damage, however, is influenced by the density of energy deposition along the particle track. Two small energy depositions, produced by chance in a short distance, may act in a biological macromolecule as one large energy deposition leading to a more severe, h-repairable lesion in the molecule than would be expected by single small energy depositions (repairable lesions). Therefore, densely ionizing radiations (neutrons and heavy ions) are biologically more effective than sparsely ionizing radiations (photons, electrons). Use of heavy charged particles offers many advantages because of their superior depth-dose distribution showing a narrow peak towards the end of their range (Bragg peak) with high relative biological efficiency (RBE) and small lateral scattering. However, production of these particles is prohibitively expensive, as mentioned already in the introduction.

During irradiation with sparsely ionizing radiations, such as fast electrons or gamma rays, indirect action for producing macromolecular lesions may become important. Since 70-80 per cent of biological mass consists of water, reactions with radiolysis products of water, ($*OH$, H , e_{aq} , etc) in particular, free radicals and active

Table 1. Development of radiation effects in a multicellular living organism and strategies for improvement of tumor radiotherapy

Phase	Time scale (s)	Events and processes	Strategies for therapy improvement
1 Physical	10^{-18} - 10^{-15}	Initial distribution of absorbed energy, ionizations and excitations	Optimization of spatial dose distributions
2 Chemical	10^{-15} - 10^{-11} 10^{-11} - 10^{-8} 10^{-3}	Initial radiation products, ions and radicals, interactions controlled by diffusion, secondary products, long-lived lesions in macromolecules	Sensitizers and protectors
3 Biochemical	10^{-3} - 10^4	Enzymatic reactions. repair, misrepair, fixation of lesions	Modifiers of intracellular repair
4 Cellular	10^4 - 10^7	Cell death, and loss recruitment and tissue regeneration	Modifiers of recruitment and tissue regeneration
5 Systemic	10^8 - 10^{10}	Hormonal effects, immune reactions, vascular changes, loss of function, carcinogenesis, aging, death	Rehabilitation

oxygen species contribute significantly to the production of molecular lesions. It has been estimated that 60-65 per cent of cell lethality observed after exposure of eukaryotic cells to sparsely ionizing radiations (low-LET) may be due to lesions induced by indirect effects ($\cdot\text{OH}$ mediated); for densely ionizing radiations (high-LET) this may decrease to about 20 per cent^{6,7}. Thus, the probability of radioprotection by efficient scavengers of hydroxy radicals is **greater** with low-LET radiations than with high-LET radiations.

Radioprotectors

Free radical scavengers and antioxidants have, therefore, been studied extensively as radioprotectors in various model systems. The largest number of compounds acting as free radical scavengers and tested for radioprotection are aminothiols, being chemical analogs of the naturally occurring sulfur-containing amino acid cysteine, which was amongst the earliest substances investigated'. Guanido derivative of cysteine, aminoethylisothiuronium bromide (AET) was shown to reduce the effect

of a radiation dose by a factor of 2.1 in mice', however, it **was found** unsuitable for human use because of high toxicity. The toxicity of AET can be reduced by combining with other thiol compounds^{10,11}. A combination of AET with **5-hydroxytryptophan (5-HT)** appears **to be** promising; it has been shown to give better protection **and is less toxic**".

Synthesis of a phosphorylated aminothiols known as WR-2721 by the Walter Reed Army Institute's research programme was a significant **advance**¹². WR-2721 has been reported to show a dose reduction factor (DRF) of 2.7 in mice when injected intraperitoneally 30 minutes before irradiation. WR-2721 has been studied widely during the last two decades and has been shown to protect different normal tissues to various extents with generally low protection to **tumors**¹³. For this reason, clinical trials on human patients have been initiated **in spite** of some of the side effects observed in animal as well as human studies^{14,15}. Research is in progress to develop less toxic analogs or to combine WR-2721 at **subtoxic** doses with other compounds which may act additively or synergistically. For example, experiments on mice suggest that combination of low doses of WR-2721 with administration of 2-mecaptopropionyl glycine (MPG) could be effective in providing significant protection without serious **toxicity**¹⁶.

Radiosensitizers

A number of studies have also been made to develop radiosensitizers for tumor cells. Amongst a large number of compounds investigated, clinical trials have been carried out using hyperbaric oxygen, electron affinic agents, in particular nitroheterocyclics metronidazole and misonidazole, to sensitize hypoxic cells.

The use of hyperbaric oxygen is very cumbersome. Results of clinical trials with metro- and misonidazoles have shown serious neurotoxicity with little therapeutic **benefit**¹⁷. Attempts are **now being made to develop less toxic analogs**.

Since, biomembranes, besides DNA, could also be important critical targets for radiation-induced biological **damage**^{18,19}, several drugs and chemicals which are known to act specifically on membranes, have been investigated; a few have been shown to modify radiation effects. Phenothiazines, in particular trimeprazine (TMZ) and chlorpromazine (CPZ), have shown **significant** radiosensitization of hypoxic tumor cells and protection of euoxic cells". **Experiments** on animal models have also shown promising results. The mechanisms of action remain to be elucidated. Since these drugs are already being used in clinical practice as tranquilizers, **their pharmacokinetics** are well known and clinical trials can be initiated.

DNA base analogs such as halopyrimidines have been used to increase the radiation sensitivity of proliferating tumor cells. Halogenated pyrimidine analogs like **5-bromo-deoxyuridine (BrdU)** and **2-5 iododeoxyuridine (IdU)** can be incorporated in DNA in place of thymidine in the proliferating **cells**²¹. It has been shown that the incorporation of these analogs into the DNA, increases the radiation-induced DNA strand breaks, chromosome aberrations, micronuclei and cell **death**²²⁻²⁴. The increase in DNA strand breaks is supposed to occur via the production of highly reactive

uracilyl radical due to the interaction of radiation-produced hydrated electron with BrdU²⁵. The uracilyl radical in DNA abstracts a hydrogen atom from the nearby deoxyribose in the same or the opposite strand, resulting in the formation of a single strand DNA break.

Clinical trials using halopyrimidines were initiated in USA and Japan in the early sixties^{26,27} but were abandoned because of problems of drug delivery and severe normal tissue toxicity. For significant radiosensitization, it is essential that sufficient amount of halopyrimidines be incorporated in the tumor cell DNA. This requires the drug to be administered for a long duration, since the cell-cycle times in most human tumors are long. Using continuous intravenous infusions over long periods of time, the clinical trials have been started again by the National Cancer Institute, USA. The initial results do not show any major improvement in case of treatment of glioblastoma patients but the results in a few cases of **unresectable** sarcomas are very encouraging^{28,29}. In these studies also, systemic toxicity due to myelosuppression appear to be the limiting factor. Attempts are being made to increase the incorporations of halopyrimidines in the tumor and minimising in the normal proliferating tissues.

(b) Biological response modifiers

As an adaptive response to attack by cytotoxic agents, the biological systems have developed various repair pathways acting at different organisational levels. These repair pathways attempt to reverse the molecular lesions and 'the tissue injury to restore the original state. Modification of the repair pathways can profoundly influence the biological effects of radiation. Thus, an inhibition of the repair processes in the *tumor can increase the radiation-induced cell-killing and prevent the regrowth of the tumor: Enhancement of the repair processes in the normal tissues; on the other hand, can reduce the radiation-induced morbidity. Studies to understand the regulation and control of repair **pathways** will be helpful in developing differential modifiers of radiation response suitable for improving tumor radiotherapy. This will be discussed in some detail in the remaining part of the present communication.

Intracellular repair

There is considerable experimental evidence to suggest that DNA, being the carrier of genetic information, forms a prominent target for radiation-induced cell death. We may, therefore, classify the molecular damage into DNA damage (A*) and non-DNA lesions (A"). Following the formation of molecular lesions, different cellular responses resulting in various biological consequences (Fig. 3) are possible.

- (a) If the molecular lesions are completely reversed (error-free repair), the damaged cells can return back to the original viable state (A).
- (b) If the DNA lesions are transformed into irreparable forms (fixation) or some errors are introduced during repair (misrepair), alterations and loss of genetic information will result.

If the errors introduced during repair are not detrimental to cell proliferation, mutated cell populations with altered functions (B) may be generated. Somatic cell

mutations could give rise to cancer or lead to organ dysfunction and premature aging. Mutations in the germ cells may cause genetic disorders in the subsequent generations.

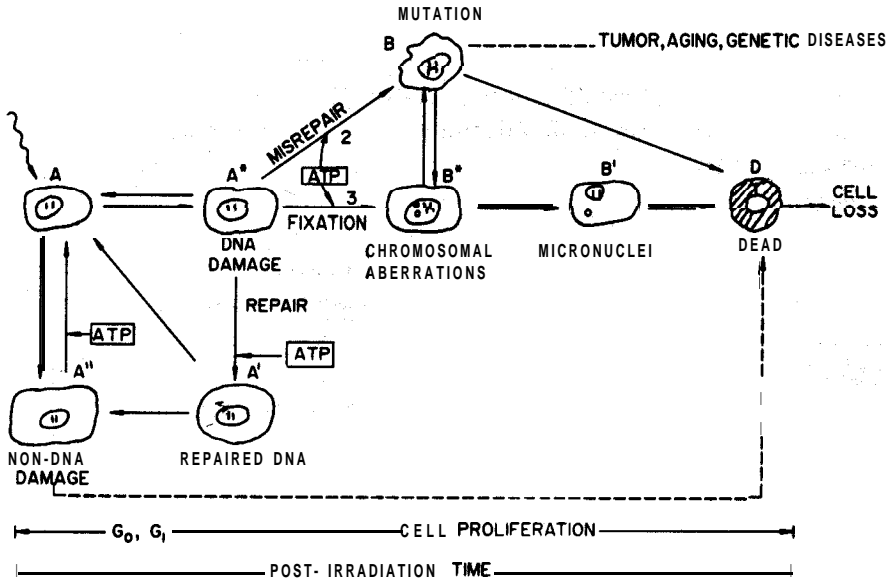


Figure 3. A simple model depicting schematically some of the important cellular responses after radiation-induced molecular lesions.

Processes of misrepair and fixation, associated usually with the processes of cell proliferation, can also lead to chromosome aberrations and formation of micronuclei. The loss of essential genetic information may not be compatible with cell proliferation and survival resulting eventually in cell death (D) and cell loss from the system.

It is obvious from Fig. 3, that the cellular responses to ionizing radiations are essentially determined by competitions between the pathways of (i) error-free repair, (ii) misrepair, and (iii) fixation of radiation-induced DNA lesions. Therefore, suitable modifications in the rates of repair and fixation pathways can alter the cellular radiation responses.

Since DNA double strand breaks are the major potentially lethal lesions induced by ionizing radiation, inhibition of their repair should considerably increase cell death. A number of inhibitors of DNA repair pathways are now available. Specific inhibitors of DNA repair enzymes affect normal and transformed cells in a similar fashion and no differential effects have been demonstrated as yet. Since normal and tumor tissues differ in their cell kinetic organisation and patterns of metabolism, the possibility of influencing rates of repair through modulation of cell proliferation and metabolism has been studied by our group. In particular, the possibility of differential inhibition of DNA repair in neoplastic cells in the presence of a glucose analog, **2-deoxy-D-glucose (2-DG)**, has been demonstrated in a number of model systems³²⁻³⁵.

Our basic studies to investigate the relationship between energy metabolism and repair processes have shown that the rate of error-free DNA repair correlates with the rate of energy production in a sigmoid manner, below a certain **critical threshold**

value of the rate of energy production, DNA repair is completely inhibited. Rates of misrepair and fixation of DNA lesions, which are associated with the processes of cellular proliferation, can also be modified by the energy supply, but quantitatively in a different way than the rates of error-free DNA repair. The differences lead to the existence of cross-over points, which determine the direction of modification of the cellular radiation response under conditions of energy deficiency. Thus, by appropriate modifications of the energy supply, a reduction or an enhancement of radiation injury to the system can be achieved.

2-DG is an inhibitor of glucose transport and glycolysis and therefore, its presence drastically reduces the supply of metabolic energy in the tumor cells which depend to a large extent on the glycolytic pathway for the production of **adenosine-triphosphate (ATP)**, the cellular energy currency. This state of energy deficiency inhibits the rate of DNA repair in tumors. The normal cells, on the other hand, derive most of the energy through the respiratory pathway and therefore, their energy supply is only marginally affected by 2-DG, which is sufficient to reduce the rate of cell proliferation and fixation of lesions, but DNA repair is not reduced significantly under these conditions. Consequently, in the presence of 2-DG in normal proliferating cells, for example bone marrow, the radiation damage is reduced considerably if **2-DG** is administered immediately before or after **irradiation**³⁶.

These results suggest that 2-DG is a unique agent which can increase the radiation-induced damage to tumor cells and reduce the damage to normal cells. Therefore, to investigate the feasibility of using this antimetabolite in humans, clinical trials on brain tumor patients have been **initiated**³⁷.

Intercellular repair and tissue regeneration

Superimposed upon the above mentioned repair pathways, additional mechanisms of repair operate at the tissue level to compensate for the cell loss. A tissue is a complex dynamic system composed of various types of cells in different physiological states. From the point of view of cell kinetics, these cells can be broadly classified into two categories, viz., (i) cycling cells, engaged in proliferative activity (P), and (ii) non-cycling cells (Q and D), as shown in **Fig.4**. The non-cycling cells can be either permanently or temporarily out of **cell cycle** and may be blocked in **G₀**-, **G₁**- or **G₂**-phases of the cell cycle (Q). Cells **which are** permanently non-cycling are the differentiated mature functional cells (F) and **dead cells** (D), which are out of cell cycle (Q) but retain the capability to re-enter the cycle (stem cells) comprise the potential proliferative pool.

Living tissue is a dynamic system; new cells are being constantly produced while some cells are being lost. A non-growing tissue of an adult organism is generally in the steady state-the rate of cell birth and cell loss are constant and equal.

Non-cycling → cycling transitions and their regulations through negative feedback controls provide adaptability to return back to the steady state after perturbations. For example, upon irradiation (Fig. 4(b)), many cells may be killed resulting in an increase in the cell loss. To compensate for the cell loss, the rate of cell proliferation

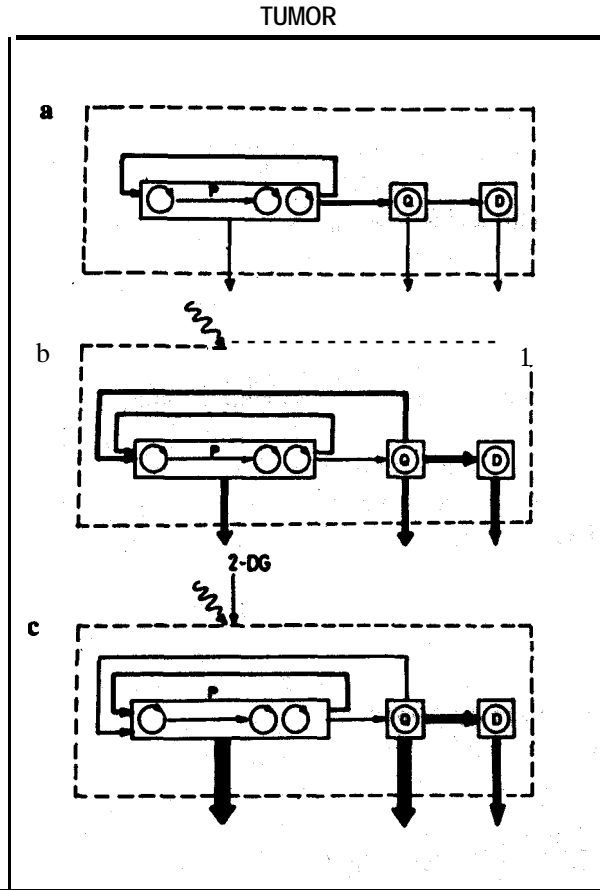


Figure 4. A model showing the effects of radiation and 2-DG on cellular dynamics, (a) undisturbed state, (b) perturbation by irradiation, and (c) effects of 2-DG in combination with radiation.

should increase so that tissue can be repopulated. This can be done in two ways: (i) by reducing the cell cycle time, and (ii) by stimulating the non-cycling cells to re-enter the cell cycle (recruitment). Both these phenomena have been observed to occur in tumors and normal tissues and represent important mechanisms of intercellular or tissue repair.

The tissue regeneration capacity depends upon the number of surviving stem cells and the successful completion of the recruitment process. The recruitment process depends upon complex interactions of various biochemical reactions involved in the signal transduction by small molecules such as cytokinines, growth stimulating factors and receptor mediated mechanisms on the cellular membranes. Recent work has shown that certain immunomodulators such as glucan (β -1,3 polyglucose isolated from the yeast, *Saccharomyces cerevesiae*) and interleukin-1 (IL-1) can significantly enhance the recruitment of stem cells in the bone marrow of whole-body irradiated mice, thereby considerably increasing their survival^{38,39}. These findings are of great interest for protection against radiation and treatment of radiation injuries.

The effects of these modulators on tumors remain yet to be investigated. Our preliminary studies suggest that **2-DG** could differentially inhibit the recruitment process in cell populations exhibiting high rates of **glycolysis**⁴⁰. Thus, the increase in radiation induced tumor regression in mice could be partially explained on this **basis**⁴¹ as shown schematically in **Fig. 4(c)**. Further work to study this possibility needs to be carried out.

The above considerations suggest that it may be possible to develop agents which can stimulate the regeneration of normal tissues after radiation injury and inhibit the repopulation of tumors preventing thereby their regrowth after radiotherapy.

Combination of different strategies

The approaches discussed above are not mutually exclusive and for optimal results combination of different strategies should be attempted. A combination of free radical scavengers and radiosensitizers with biological response modifiers could give additive or synergistic effects. Initial attempts in this direction are quite encouraging. Thus a combination of WR-2721 given before irradiation with soluble glucan administered post-irradiation has shown greater than additive **effect**⁴². Similar results have been reported for the combination of WR-2721 with **IL-1**⁴³.

The radiosensitizing effects of halopyrimidines have been observed to be enhanced in tumor cells in the presence of **2-DG**⁴⁴ and reduced in normal bone marrow cells of **mice**^{45,46}. Therefore, the differential between radiation-induced cell-killing in tumors and normal tissues can be significantly enhanced by combining halopyrimidines and **2-DG** administration with radiation treatment.

A number of new combinations are being studied.

4. FUTURE PROSPECTS

The above discussion shows that the major problems **encountered with** the use of present day radioprotectors and radiosensitizers in clinical radiotherapy concern the toxicity of these agents and inadequate differential effects between the tumor and the normal tissues.

The new approach of combining radioprotectors and radiosensitizers with biological response modifiers appears to be promising. **The** search for an effective and non-toxic combination of radiomodifiers will be greatly facilitated by a better understanding of the processes involved in the biological responses of tumors and normal tissues to radiation induced molecular lesions. Repair, of DNA damage and the recruitment of stem cells in the cell-cycle are of particular interest in this respect. Using techniques of molecular biology, it should be possible to identify the genes (DNA sequences) which code for the various repair enzymes and to study the regulation and control of their expression. The knowledge thus gained will be helpful in developing methods to modulate the contents and activities of repair enzymes appropriately to achieve enhancement of repair in normal and inhibition of repair in tumor cells. Use of **2-DG** to increase the differential effects of cytotoxic agents between tumors and normal tissues is also a step in this direction, though it exploits the differences in the

cellular energy and nucleotide metabolism. Further research work to study the exact mechanisms involved and to carry out clinical trials is warranted.

Non-invasive imaging techniques should be exploited to study and monitor the radiation responses of various tissues in the intact organism. The problems connected with tumor heterogeneity and development of radiation resistance could be better investigated using the non-invasive methods. Improved understanding of tumor biology will be crucial in the development of more effective and simpler therapeutic regimens.

Thus, radiobiological research may eventually achieve the goal depicted in Fig. 2 by developing suitable modifiers of radiation response. If this endeavour is successful, it may be possible to treat local and metastatic malignant disease with whole-body irradiation without any serious toxic effects on normal tissues and without the need for expensive high technology machines. Needless to say that such a development will eminently suit the Indian environment and would go a long way towards the goal of providing health care to all by the year 2000.

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