LIPOSOME MEDIATED DELIVERY OF 2-MERCAPTOPROPIONYL GLYCINE (MPG) AND CARCINOGENESIS RESPONSE MODULATION

ABSTRACT
The goal of cancer therapy is to eliminate the neoplastic cells without causing any appreciable damage to the normal tissue of the host. Two possible approaches are there in this therapy:

1. Removal of the neoplastic cells/tissues from the host system.
2. Reversal of the process of cellular transformation.

Removal of the neoplastic cells is effected most efficiently by surgical means that has its own obvious fall out. Use of physical, chemical or biological cytotoxic agents are other avenues with their respective limitations. For instance, treatment involving cytotoxic agents is hazardous since they cause serious damage to normal cells. The possibility of reversing the neoplastic cellular transformation may be a better approach but much remains to be done before it can be applicable on population scale. Non-surgically eliminating cancerous growth may be a practical avenue open to us at the moment.

In this non-surgical approach, use of radiations has been one of the best possibilities. The ability of radiations to kill cells by an array of metabolic damages that it causes has been successfully employed to cure cancerous and malignant growth. Radiations, however, act as double edged sword. Since interaction of radiation with matter is random, the radiation interacts with all types of cell - both normal and transformed when a subject is irradiated. Thus, all cells accumulate damages. While the damages transformed cell may not survive and, thereby, cancer may be eliminated, the partially damaged normal cells become a problem. There exists a great probability that these partially damaged normal cell may undergo transformation in due course of time. Several improvisations in the radiotherapeutic protocols, use of different quantities and qualities of radiations and applications of fractionated irradiation schedules have only marginally improved the present clinical efficacy of radiotherapy. It appears that radiotherapy has reached its zenith.

To overcome the limitations of radiotherapy and to improve upon its clinical gains, use of radiomodulatory drugs along with radiation, appropriately named as chemo-radiotherapy, have yielded positive therapeutic advantages in cancer therapy. Two classes of radiomodulatory drugs are in existence in the context of radiotherapy:

1. Radioprotective drugs - This class of drugs essentially should protect normal cells/tissues from the undesirable damaging effects of radiation, thereby, paving way for application of higher doses of radiation for efficient killing of cancerous cells/tissues.

2. Radiosensitizing drugs - The drugs belonging to this class should, in principle, sensitive cancerous cells/tissues so that they are killed even by relatively low doses of
radiations, thereby, reducing the undesirable damages to normal cells.

Chemo-radiotherapy has, indeed, improved the rate of cure of cancer. However, the toxicity of drugs to other tissues, lack of control on the quantity of drug in cancerous tissues, metabolic alteration of drug, non-specific protection/sensitization of tissues, various other side effects have come in way of optimum application of chemo-radiotherapy regimes in clinical practice. In last about two decades, concept of drug delivery system has been tried to overcome the limitations of chemo-radiotherapy regimes to further improve upon it. The concept is based on the fact that certain biologically acceptable carrier may be used to carry the drugs to biological target cells/tissues. Of several possible carriers, liposomes have been found to be promising and convenient.

In the piece of work embodied in this thesis, attempt has been made to test the suitability of liposome as a vehicle for carrying a drug. As it is an exploratory work, 2-mercaptopropionylglycine (MPG), a radioprotective drug, has been used as a model drug. The liposome encapsulated MPG (LEM) is envisaged to be tested for its radioprotective efficacy in normal and transformed system vis-a-vis the free form of MPG. Attempt is also proposed to look into some molecular events triggered by MPG or LEM.

The results embodied in this thesis opens up a possibility of use of liposome as a carrier for radio-modular drugs for use in chemo-radiotherapy. The results very emphatically establish that using liposome carrier may offer significant clinical gains in chemo-radiotherapy. The model drug used in this investigation was 2-mercaptopropionylglycine (MPG), a moderate radioprotective drug that has been shown to be a potential radioprotector both in experimental conditions and in clinical trials. The following are the salient findings emerging from this work:

1. The radioprotection afforded by MPG was enhanced when an equivalent amount of drug was administered through liposome vehicle (chapters 2-5).

2. Of several methods for preparation of liposome to encapsulate MPG, the reverse-phase evaporation method was found to be highly convenient, reproducible and effective (chapter 2). The preparation of liposome by this method involves common and simple laboratory equipments and may be performed at any place.

3. The method offers MPG encapsulation into liposome at a rate of over 50% of the starting concentration of MPG, thus, qualifying to be an efficient method for MPG encapsulation. Five or 10 mM starting concentrations of MPG was found to be optimum for liposome encapsulation (chapter 2).
4. The liposome encapsulated MPG (LEM), as against its free form, afforded significantly higher protection to normal tissues on biological end-points such as, (a) viability of bone marrow and spleen cells and (b) on enzyme acetylcholine esterase (chapter 3).

5. The same was the case in cancer induced or transformed mice. The biological end-points tested for this investigation were cellular glutathione and enzyme γ-glutamyltranspeptidase. On both these limits, the LEM afforded better radioprotection that MPG (chapter 4).

6. It has been shown that MPG was able to influence chromatin organization differently when it was administered in its free form or as LEM (chapter 5).

7. The presence of MPG or LEM was also reflected by the level of cellular poly-ADP-ribosylation, assayed by a new immuno-dot blot assay developed in this investigation (chapter 5).

8. The results point out to the fact that chromatin was poised for better repair in transformed mice when MPG or LEM were administered prior to irradiation (chapter 5).

9. On the parameters of chromatin organization and poly-ADP-ribosylation, MPG and LEM afforded radioprotection was almost similar (chapter 5).

10. Overall, the radioprotection afforded by LEM was higher that free form of MPG (chapters 2-5).

The findings presented in this thesis may have significant impact on the clinical use of radiomodulatory drugs in chemo-radiotherapy as liposome encapsulation could enhance the effectiveness of MPG. A potential use of liposome carrier, however, will be with radiosensitizing drugs. It is known that most of radiosensitizing drugs are highly toxic to normal tissues. Notwithstanding the toxicity problem, use of radiosensitizing drug in chemo-radiotherapy will be more advantageous than that of radioprotective drugs. Liposome as a carrier may be an easy solution to this problem. By liposome encapsulation, the radiosensitizing drug will not be immediately available to other tissues, thus, reducing its toxicity. In addition, it could be visualized that a radiosensitizing drug after liposome encapsulation may be targeted to cancer tissue. Therefore, the cancerous tissue will be sensitized and even a lower dose of radiation may be able to produce enough damage to kill it. Some avenues of specific tissue targeting using immuno-liposome are being investigated.
Even though the work described in this thesis relates to MPG, a radioprotective drug, the findings are of significant clinical value and open up a new line of thinking for improvisation of chemo-radiotherapy.