CHAPTER 1

INTRODUCTION
History of radiation therapy as a tumor treatment modality encompasses about ninety years, beginning almost immediately after the discovery of X-rays by Röntgen (1895), of radioactivity by Becquerel (1896) and of radium by the Curies (1898). It was two Swedish physicians, Drs. J.T. Stenbeck and T.A.V. Sjögren in 1898 who claimed the first radiation cure, the removal of a skin tumor from the tip of a patient's nose. Since then radiotherapy has taken its place firmly alongside of surgery as a major curative modality for cancer. The basic aim of radiotherapy is to destroy all cells of a malignant tumor while keeping the damage to the surrounding critical normal tissues to an acceptable level.

But radiotherapy in many cases has not been successful in curing malignancies. This has been attributed to the presence of radioresistant hypoxic cells in tumors (Thomlinson and Gray 1955). The fraction of hypoxic cells in a tumor is not only radioresistant but also forms the foci of regeneration and recurrence, thus constituting a serious barrier in attempts to increase the therapeutic ratio between tumor control and normal tissue damage (Hall 1978, Stratford et al. 1983).

Several methods have been suggested to circumvent the problem of hypoxia which include high-pressure oxygen (HPO), neutron therapy, chemical sensitizers and radiation dose-fractionation. The first clinical trial by Henk et al. (1977) demonstrated that HPO enhanced normal tissue damage in the larynx. However,
it became clear from several controlled trials that the results are not as encouraging as was originally anticipated.

As an alternative approach, neutron therapy has much more to commend in that the neutrons do reach the hypoxic cells in the tumor. But they cannot entirely eliminate the relative radioresistance of these cells and the substantial cost of installing neutron therapy machine limits the use of this technique.

A much less expensive, though equally promising, approach is that provided by chemicals. Hence, several chemicals have been screened as potential radiosensitizers during the last few years. Some of the chemicals which have been contemplated for use as potentiators in cancer radiotherapy include electron-affinic compounds, antitumor antibiotics, membrane-active drugs and platinum complexes.

Adams and Cooke (1969) reported that compounds with strong electron-affinity possess radiosensitizing ability. Appreciable sensitization was observed in cultured Chinese hamster cells irradiated anaerobically in the presence of the electron-affinic sensitizer, paranitroacetophenone (PNAP) (Adams et al. 1971). Encouraging results were obtained with nitrofurans in sensitizing hypoxic cells (Reuvers et al. 1972, Chapman et al. 1972). However, their application in vivo has been limited by their limited water solubility and short biological life (Denekamp 1977). A more soluble version of PNAP, N-dimethylparanitro-2-propiophenone (NDPP) (Adams et al. 1972) was tested in vivo and enhancement ratios upto 1.5
were measured in epidermis made artificially hypoxic, but only at drug doses very close to the lethal range (Denekamp and Michael 1972). Further pharmacological studies have shown that NDPP is metabolized rapidly which would limit its clinical application (Adams et al. 1974).

Several compounds have been shown to enhance the radiation response of hypoxic bacterial and mammalian cells in vitro. Many of these agents, however, show little or no activity in tumor systems irradiated in vivo, either due to toxicity limitations or to poor tumor penetration (Adams et al. 1974, Hall 1978).

One of the most efficient sensitizers is free molecular oxygen. Two chemicals that have been used clinically as oxygen replacements are the nitroimidazole compounds, namely metronidazole and misonidazole. In radiosensitization studies, the importance of the nitroimidazoles arose from the fact that such compounds could specifically sensitize the hypoxic cells but not the oxygenated ones (Asquith et al. 1973, Denekamp et al. 1974). Nitroimidazoles are electron-affinic compounds which can diffuse to distant hypoxic cells because they are not rapidly metabolized by cells (Asquith et al. 1974b, Brown 1975a, Denekamp and Harris 1976). Therefore, these chemicals reach hypoxic zones within the tumor in sufficient concentrations to restore those zones to levels of radioreponsiveness similar to those of well oxygenated tumor and normal tissues. Unlike most anticancer drugs, nitroimidazoles have an equal sensitizing action on cycling and non-cycling cells and on cells at any stage in their cell cycle (Hall and Roizin-Towle 1975, Adams et al. 1976a).
Metronidazole (Metro) [1-(2-hydroxyethyl)-2-methyl-5-nitromidazole] known under the trade name 'Flagyl' and misonidazole (MISO) or Ro-07-0582 [1-(2-hydroxy-3-methoxypropyl)] are unsaturated heterocyclic compounds substituted with the nitro group at positions 5 and 2 respectively. They carry a side chain on the nitrogen, the side chain being an alcohol and ether in MISO and an alcohol in Metro. It is the nitro aromatic nucleus which is essential to the use as a radiosensitizer, whereas the side chain modifies the solubility characteristics of the molecule. The molecular structures of the two nitroimidazoles are as follows:

![Molecular structures of Metro and MISO](image)

Metro has been used for many years clinically in the treatment of Trichomonas. Studies have shown that Flagyl is a general hypoxic cell sensitizer and its radiosensitizing ability has been tested in bean roots, bacteria (Asquith et al. 1973, 1974a), yeast (Krishnan et al. 1978) and mammalian cells in vitro (Foster and Willson 1973, Asquith et al. 1974b). Appreciable radiosensitization has been reported in a wide variety of murine tumors (Rauth 1973, Begg et al. 1974, Stone and Withers 1974, Brown 1975b, Denekamp and Harris 1975).
A distinct advantage of Metro has been reported in cancer patients, where the drug significantly increased the survival rate and improved the cure of cervix cancer and malignancy of corpus uteri (Urtasun et al. 1975, 1976, Karim 1978, Balmukhanov et al. 1987, 1990).

MISO has been reported to sensitize hypoxic Chinese hamster V79-379A cells to irradiation at a concentration of 1 and 10 mM (Adams et al. 1974). Sensitization was only evident for hypoxic cells and was nearly equivalent to the full oxygen enhancement ratio. In vitro studies demonstrated MISO to be an extremely effective sensitizer of hypoxic cells (Asquith et al. 1974b, Moore et al. 1976, Adams et al. 1976b). Significant radiosensitization was also observed in vivo using mouse tumor systems with dose modifying factors of 1.9 - 2.0 (Denekamp and Harris 1975, Stone and Withers 1975a, Adams and Fowler 1976, McNally et al. 1978, Siemann and Kochanski 1981, Hofer et al. 1987).


Earlier reports on nitromidazoles suggested that there was no effect on normal oxygenated cells in vivo (Asquith et al. 1974b, Denekamp et al. 1974, Adams et al. 1976a). However, a small enhancement was reported in mouse normal tissues including skin (Brown 1975a, Stewart et al. 1982), cartilage (Adams and Fowler 1976), Testis (Suzuki et al. 1977) and tail (Hendry 1978a). In addition, clinical
trials in human patients have shown side effects including anorexia, nausea, vomiting, gastrointestinal symptoms and neurotoxicity (Urtasun et al. 1976, Tamulevicius et al. 1981, MRC working party 1984).

Various compounds with antitumor properties have been routinely screened for their radiosensitizing ability in bacterial cells. These include oligomycin, adriamycin, actinomycin-D, mitramycin, bleomycin etc. Antibiotics have undergone extensive testing and are used in the treatment of acute leukemias, sarcomas and a wide variety of carcinomas (Cline and Haskel 1980). Among different antitumor antibiotics, bleomycin (BLM) is suggested as a useful adjuvant to radiochemotherapy and is clinically employed for the treatment of squamous cell carcinomas of the cervix, oesophagus, head and neck, malignant lymphomas and genital organ neoplasms (Carter 1978, Blum et al. 1973, Umezawa 1979). The molecular structure of bleomycin consists of a core which is a pyrimidine chromophore linked to a β-aminoalanine amide side chain and to sugars. It also includes a side chain with amino acids and a carboxylic acid. Bleomycin has the following molecular configuration:
BLM is known to inhibit DNA synthesis and to produce DNA strand breaks (Suzuki et al. 1969, Terasima et al. 1970). It has been suggested that this drug might have a synergistic effect on that of X-radiation (Bleehen 1973), which was demonstrated in bacterial and mammalian cells (Bleehen et al. 1974). The drug was also active against a number of mouse tumors (Umezawa et al. 1967, Jørgensen 1972, Blum et al. 1973).

Clinical data on the antitumor potential of BLM against various types of human cancers were found encouraging (Mathe 1970, Blum et al. 1973, Bedrossian et al. 1973, Parvinen et al. 1985, Maiche 1989). Inspite of its very high antitumor potential, BLM has been reported to induce pyrexia, mucositis, anorexia, nausea, pigmentation of the skin and pulmonary toxicity in patients (Blum et al. 1973, Bedrossian et al. 1973, Maiche 1989). An interaction between BLM and radiation has also been demonstrated in mouse normal tissues such as skin and small intestine (von der Maase 1984 a,b), lung (Catane et al. 1979), lip mucosa (Feng et al. 1986, Vanuytsel et al. 1986) testis and bone-marrow (Uma Devi et al. 1990).

The association between membrane damage and radiosensitization has prompted investigators to test certain therapeutically active drugs known to act on membranes. As a result a new class of radiosensitizers, namely 'membrane-active drugs' has evolved which includes anaesthetics, analgesics and tranquilizers. The advantage with this group of chemicals is their well known toxicities and pharmacokinetics. Procaine hydrochloride, the common local anaesthetic, was the first drug in this series. This drug was selected because of its membrane
specificity and for the presence of a carbonyl group in its structure. This drug and many other membrane-binding agents were reported to sensitize bacterial and mammalian cells to $^{60}\text{Co}$ gamma rays (Shenoy et al. 1975, George and Singh 1984, Maniar and Singh 1985). Other membrane-specific phenothiazines include lignocaine, tetracaine, meprobate, sodium pentobarbitone (Shenoy et al. 1976), triperazine, promethazine, trifluroperazine, proc chloroperazine and chlorpromazine (Singh 1990).

Chlorpromazine (CPZ), locally marketed as 'largactil' is a substituted phenothiazine. It has a 3-ring structure in which two benzene rings are linked by a sulphur and nitrogen atom. It has an aliphatic side chain and a chloride ion on one of the benzene rings.

![Chemical Structure of Chlorpromazine](https://via.placeholder.com/150)

CPZ exhibits radiosensitizing property at comparatively low concentration. In addition, this drug acts on the central nervous system and the brain, locations poorly accessible to many anticancer drugs (Singh 1990). CPZ was found to sensitize rat thymocytes and ascites tumor cells to $^{60}\text{Co}$ gamma rays (Shenoy et al. 1975). It also enhanced the radiation killing of bacterial cells and radiation cure of mouse tumors (Shenoy and Singh 1980, George et al. 1980).

It is now convincingly clear that platinum co-ordination complexes play a significant role in the treatment of human cancers. The biologic
activity of platinum complexes was first noted by Rosenberg et al. (1965) when bacterial cells were grown in a culture containing platinum electrodes. Bacterial cell division was found to be inhibited by platinum products formed in the medium. Further analysis showed that this chemical was Cis-dichlorodiammineplatinum (II). Later it was suggested that an entire class of platinum complexes was capable of causing inhibition of bacterial cell division and that they have an anticancer activity. This class includes dichloroethylenediammine platinum (III), tetrachloroethylenediammine platinum (IV) and tetrachlorodiammine platinum (IV) (Rosenberg et al. 1969).

Cis-dichlorodiammineplatinum (II) C-DDP, Cis-platin or Cis-platinum is an inorganic complex formed by an atom of platinum (II) surrounded by chloride and ammonium ions in the cis-position of horizontal plane.

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\begin{align*}
\text{Cl}^- & \quad \text{NH}_3 \\
\text{Pt}^{2+} & \quad \text{Cl}^- \\
& \quad \text{NH}_3
\end{align*}
\]

Cis-platin given in combination with irradiation has been shown to enhance radiation effects in various experimental tumors both in vitro (Begg et al. 1986, Carde and Laval 1981, Yuhas et al. 1979) and in vivo (Overgaard and Khan 1981, Douple and Richmond 1979a, 1982, Bartelink et al. 1986).

Many clinical trials over the last decade have convincingly demonstrated that Cis-platin is a potential anticancer drug both
individually and in combination with other drugs in the treatment of testicular and ovarian tumors and cancers of bladder, prostate and head and neck (Einhorn and Williams 1979, Creagon et al. 1981, Douple and Richmond 1980). However, despite its usefulness as a potential radiosensitizer, the toxicity associated with the use of Cis-platin has been the cause of great concern. Clinically the drug has been reported to induce mucosal and renal toxicity, profound gastrointestinal symptoms, bone-marrow depression and ototoxicity (Creagan et al. 1981, Haselow et al. 1982, Salem et al. 1984).

In experimental animals also the combination of Cis-platin and radiation resulted in enhanced damage. This was demonstrated in mouse intestine (von der Maase 1984b, Tanabe et al. 1987), rectum (Dewit et al. 1987), Kidney (Moulder et al. 1986) and skin (Bartelink and Kallman 1983).

The goal of cancer therapy is to achieve selective toxicity against tumor cells and to spare normal cells. With few exceptions, antineoplastic agents induce toxicity to many normal tissues (Phillips and Fu 1976, 1978, Muggia et al. 1978, Peckham and Collis 1981) especially, bone—marrow, gastrointestinal and germinal epithelia, hair follicles and lymphoid organs (Mitchell and Schein 1984). Therefore, it is necessary to obtain information regarding the normal tissue response to these agents and their applicability in cancer therapy assessed through preclinical studies in animal models.

Small intestine is the major critical organ in determining the reactions of the patient to radiotherapy of tumor in the abdominal
and pelvic regions. The absorptive surface of the mammalian intestine is greatly enlarged by villi, which project into the intestinal lumen. At the base of the villi are rapidly dividing crypt cells that give rise to cells which move up the villus and become mature and differentiated. The cells on their way towards the tip of the villus secrete enzymes and absorb food and water from the intestinal tract. They are eventually 'pushed off' the tip of the villus by new cells moving up from the crypt (Cheng and Leblond 1974, Coggle 1983). Thus, there is a well defined 'proliferative compartment' comprising of crypts and 'functional compartment' of villi. The intestinal epithelium with its high cell renewal is suitable for assessing the tolerance to treatments with chemical and physical agents.

Ionizing radiation acts on the biological system by altering the molecules and brings immediate changes in all living organisms. Following irradiation, the cells of the organism undergo a series of changes which result in metabolic disturbances leading to cellular damage and, ultimately, death of the organism. Among the numerous problems pertaining to biological effects of ionizing radiation, injury to the gastrointestinal (GI) tract occupies a significant place because the clinical symptoms of radiolesions in this system are quite pronounced. The first few data on gastrointestinal damage in man after irradiation were collected in Hiroshima and Nagasaki after the explosion of atomic bombs. People who suffered bomb exposure showed high fever, leukopenia and nervous, cardiovascular and digestive disorders. Digestive disturbances included anorexia, nausea, vomiting and diarrhoea. Histopathological picture showed edematous mucosa, irregular or sloughed off epithelium, vesicular nuclei and abnormal mitotic figures (Kurtsin 1963).

Death of the mouse from acute intestinal injury following whole-body irradiation has been attributed by Quastler (1956) to the damage to the reproductive capacity of the crypt cells. The resulting radiation syndrome is described as gastrointestinal syndrome. The gross symptomatology of this syndrome is characterized by anorexia, diarrhoea, loss of weight and dehydration, leading to the death of the animal.

The use of single large doses of radiation designed to kill the tumor cells has been found to result in an unacceptable level of damage to the surrounding normal tissues. This problem of acute damage to normal tissues and management of radioresistant hypoxic cells in the tumor has been partly overcome by radiation dose fractionation. Dividing a large acute radiation dose to small fractions allows normal tissues to recover from the radiation injury and at the same time causes reoxygenation of the hypoxic regions (Hall 1985, Cox 1985, Thames and Hendry 1987).

It was Regaud (1920) who, based on his experiments, proposed the clinical application of radiation dose fractionation. He performed experiments in which testes of experimental animals were eradicated.
with X-rays. It was proved to be impossible to sterilize the animals in a single dose without a severe reaction to the skin of the scrotum, whereas if the dose was fractionated over a period of time, sterilization could be achieved with little apparent skin damage. Regaud proposed the hypothesis that the testis with its high rate of cell turnover was a model for the rapidly dividing growing tumor, while the skin represented a dose-limiting normal tissue.

The efficacy of dose fractionation can be understood in terms of the radiobiologic principles which have been described as the four 'R's of radiobiology: repair, reoxygenation, redistribution and repopulation (Withers 1975a). It is proved experimentally that during fractionated irradiation the normal cells repair the sublethal radiation damage and repopulate the degenerated tissue. In tumors, hypoxic cells move from radioresistant to sensitive phases of cell cycle and become oxygenated during interfractional interval (Withers and Mason 1974, Withers 1985, Thames & Hendry 1987, Hall and Cox 1989).

Some reports are available on the changes occurring in rodent small intestine exposed to fractionated irradiation (Withers and Elkind 1969, Hageman et al. 1971, Becciolini et al. 1973, 1984a, Withers et al. 1975, Withers and Mason 1974, Lesher et al. 1975, Maisin et al. 1977, Rao and Fritz-Niggli 1983, Jensen et al. 1986, Dewit and Oussoren 1987). The findings of these investigators demonstrated that the mouse small intestinal epithelium has a large capacity for repair of sublethal radiation injury and is able to tolerate daily clinically relevant doses.
The tolerance to dose fractionation has also been investigated in other normal tissues including hair follicles (Vegesna et al. 1989), spinal cord (van der Kogel 1977), skin (Kal and Gaiser 1977) and mouse tumors (Trott et al. 1977, Kal and Gaiser 1977). There are a few reports on the comparative study of single and fractionated irradiation in patients (Durrant et al. 1977, Nias 1977).

Conventional fractionation radiotherapy is a prolonged course of radiation exposure with daily doses in the range of 2.0 - 2.5 Gy, 5 times per week, usually extending over a period of 4-6 weeks, to a total dose of 60.0-70.0 Gy. In recent years, there has been considerable interest in the use of non-standard fractionation as a way of increasing the therapeutic gain of radiotherapy (Fowler 1984a). Various dose fractionation schemes are devised including hyperfractionation, accelerated fractionation (Multiple daily fractionation, MDF) and hypofractionation (Thames et al. 1983, Withers 1985, Cox 1985).

The clinical advantage of and the normal tissue reactions to altered fractionation schedules has been investigated by several workers and varied response rates have been reported. Many showed encouraging tumor responses and tolerable normal tissue reactions with accelerated fractionation (Angelakis et al. 1973, Saunders et al. 1988, Wendt et al. 1989), while some reported marked early reactions in normal mucosa (Saunders and Dische 1986). However, Kim et al. (1975), Choi et al. (1985), Trott (1987) and Slawson et al. (1988) found no significant difference in the acute reactions and cure rates among patients treated with different fractionation regimens.
However, in altered fractionation schedules, especially in accelerated fractionation, the reduced overall time and interval between fractions may lead to inadequate reoxygenation of hypoxic cells. Further, because of the necessity of limiting the toxicity of the radiosensitizers the best administration schemes appear to be fractionated doses of drugs combined with small doses of irradiation (Sheldon and Fowler 1978, Dische et al. 1979, Parsons et al. 1984). There are several reports which concern the response of normal tissues to fractionated irradiation with or without radiosensitizer (Stewart et al. 1982, Bartelink and Kallman 1983, Hendry and Sutton 1984, Dewit et al. 1986, Tanabe et al. 1987). But there is a comparative paucity of information about the development of small bowel injury following treatment with fractionated doses of radiation and chemotherapeutic drugs.

No sensitizer or combination of sensitizer with radiation schedule will be of any clinical value unless it can be shown that normal body tissues are not sensitized to the same extent as tumors. The present study, therefore, is an attempt to assess the toxic and radiosensitizing effects of different chemotherapeutic drugs and gamma radiation, in acute and fractionated doses, on cellular damage and recovery of one of the critical dose-limiting normal tissues in cancer therapy, namely the small intestine.