SUMMARY AND CONCLUSIONS
The radioresistance of hypoxic cells in the tumor is one of the major obstacles in the successful cure of malignancies (Hofer et al. 1981). One method aimed at overcoming this problem is the use of chemical agents which increase the radiation sensitivity of hypoxic cells. However, the use of chemicals along with radiation in the treatment of abdominal tumors is limited by the high sensitivity of the actively proliferative normal tissues. Radiation-dose fractionation is a clinically accepted treatment modality to minimize the damage to normal tissues surrounding the tumor area.

The small intestine is one of the most rapidly proliferating tissues of the mammalian body and, as such, represents one of the limiting tissues in terms of radiotherapy and chemotherapy exposures, as extensive GI damage will be life-threatening. In contrast to a considerable amount of data on the mouse intestinal response to irradiation, there is a dearth of information about its response to altered treatment modalities such as combination treatment of chemicals and radiation and nonconventional radiation-dose fractionation. The present investigation is an attempt to study the mouse jejunal response to combination of chemotherapeutic drugs and radiation, in single and fractionated doses.

An experiment was carried out to determine the LD\textsubscript{50/30} and LD\textsubscript{100/7} after whole-body irradiation of adult BALB/c mice with different doses (7.5, 8.5, 10.0, 12.0, 15.0 Gy) of gamma radiation. After acute exposure the animals were observed daily for radiation sickness and mortality. Deaths occurring due to GI syndrome (within 3-7 d) and haemopoietic syndrome (within 12-30 d) were recorded and percent survival was plotted against radiation dose. From the
Preliminary screening of five chemotherapeutic agents (MISO, Metro, BLM, CPZ, c-DDP) was done using the microcolony survival assay (Withers and Elkind 1970) to select the two most effective drugs for further study. In this study, different groups of adult Swiss mice were exposed to an acute dose of 10.0 Gy with or without an injection of one of the drugs, MISO, Metro (1000 mg/kg), BLM, CPZ (20 mg/kg) and c-DDP (0.1 mg/kg), 30 min before irradiation. Separate groups of animals received only drug(s) and served as drug-controls. A group of animals was exposed to an acute dose of 15.0 Gy and another group was sham-irradiated for comparison. Histological observation on day 3 after irradiation with both the doses (10.0 and 15.0 Gy) indicated extensive damage to the jejunal mucosa, the damage becoming very severe at the higher dose. Qualitative changes included cell death, karyolysis, crypt degeneration and shortening of villi. After 15.0 Gy, there were very few crypts and the villi had completely collapsed. Quantitative analysis indicated significant ($P < 0.001$) reduction in the crypt number after both the doses of radiation.

All the drugs, except Metro, exerted comparable toxicity in the jejunum as indicated by the reduction in crypt survival. Combination of drugs with radiation enhanced significantly the radiation damage in the jejunal mucosa, the combined effect being supra-additive for BLM + 10.0 Gy, additive for MISO + 10.0 Gy and sub-additive for CPZ + 10.0 Gy and c-DDP + 10.0 Gy. Metro had no effect on the irradiated jejunum.
Based on these results, the two most effective drugs, viz. MISO and BLM, were selected for detailed study with fractionated radiation-dose schedules.

In the radiation-dose fractionation study, different groups of BALB/c mice received radiation in acute dose of 15.0 Gy and different fractionated schedules of the same total dose with or without fractionated MISO pretreatment (accelerated: 2.5 Gy x 2 x 3; rapid fractionation: 5.0 Gy x 1 x 3; hypofractionation: 7.5 Gy x 2).

In experiments with BLM combined with radiation, the animals could not tolerate a total dose of 30 mg/kg given in 3 fractions (10 mg x 1 x 3) in combination with radiation (5.0 Gy x 1 x 3). Hence the doses of the drug and radiation were reduced to 10 mg/kg x 1 x 2 and 5.0 Gy x 1 x 2 respectively. In the accelerated fractionation BLM was administered only once a day 30' before the first fraction of 2.5 Gy. Jejunal response to different treatments was evaluated by assessing the changes in the crypt survival and crypt and villous cell population on day 3 after the last exposure and compared with the sham-treated controls.

The results revealed that fractionation of an acute dose (15.0 Gy) into 7.5 Gy x 2 schedule resulted in considerable sparing of the jejunal mucosa and indicated repair and regeneration during interfractional intervals, as evidenced by an increase in the crypt survival, crypt cell number, mitotic activity and decreased cell death and villous injury. Fractionation to still smaller doses of 5.0 Gy and 2.5 Gy for 3 consecutive days resulted in further sparing and recovery.
of the jejunal mucosa. Accelerated fractionation and rapid fractionation produced an almost identical effect.

MISO alone, in either fractions exerted slight toxicity, in the form of an increased mitoses in the jejunal crypts. Combination of fractionated doses of MISO did not alter the irradiated jejunal response at the cellular level, though a definite effect on the mitotic cells of MISO treated animals was observed. There was a significant increase in the number of mitoses along with a high number of abnormal mitoses. Changing the dose schedule from three fractions to six fractions did not result in any noticeable change in the jejunal response. BLM in both the fractionated schedules significantly enhanced the radiation damage in the crypt and villus after both the fractionated schedules. The crypt survival and crypt cell number decreased significantly and villous size was drastically reduced with a decline in the cell number and severe damage to the tips.

The effect of MISO in single dose (1000 mg/kg) on sublethally irradiated (1.5, 3.0, 5.0 Gy) mouse jejunum was studied at different post-treatment intervals from 3 h to 28 d. Three groups of adult BALB/c mice were exposed to the above sublethal doses of gamma-rays with or without prior administration of MISO. Jejunal reaction to radiation alone and radiation with drug pretreatment was studied by assessing the crypt survival and cellular alterations in the crypt and villus. Results revealed that all the three doses of radiation produced qualitative and quantitative changes as early as 3 h after exposure. The qualitative changes included pyknoses, necroses, mitotic
suppression, cytoplasmic degranulation and vacuolation. Quantitatively there was reduction in the crypt cells and mitoses and increase in the cell death. Radiation-induced changes increased progressively, reaching a maximum on day 1 after all the doses. However, the degree of damage was dose-dependent, the damage being more severe and appearing earlier after higher doses. Recovery followed at 72 h, the rate of which was also a function of the radiation dose, being complete within a week after the low dose (1.5 Gy) and delayed following higher doses (3.0 and 5.0 Gy).

The damage in the crypt was followed by villous injury, which was manifested as hydropic degeneration in the lamina propria and reduction in the height and cell population. As in the case of crypts, the villous damage was more severe and appeared earlier following exposure to higher doses. Recovery followed the maximum damage and was complete within a week in the low dose group (1.5 Gy) and the normal villous structure was observed after 2 weeks in 3.0 Gy and 3 weeks in 5.0 Gy groups.

Administration of MISO before radiation resulted in lesions which were similar to those produced after radiation alone, both qualitatively and quantitatively. The only difference between the radiation control and MISO + radiation group was in the number of mitotic cells, which was significantly lower in the latter group. Following MISO + 5.0 Gy, there was a total absence of mitoses at 3 h. However, mitotic cells appeared at 6 h and increased gradually with time at all the doses. The rate of repair in MISO pretreated animals was slower and prolonged
at the higher exposures when compared to that in the radiation control groups. The changes occurring in the villi of MISO + radiation group also followed the same pattern as that in the radiation control animals. As in the case of the crypts, the rate of recovery in the former was delayed when compared to that in the latter.

Based on the above experiments the following conclusions can be drawn:

1. Death of animals within 3-10 days after exposure to lethal doses in the range of 10.0 - 15.0 Gy is predominantly due to GI damage. The secondary influences which contribute to GI death may include water and electrolyte imbalance, nutritional impairment and deficiency in the defense mechanisms due to damage to the bone-marrow.

2. The small intestine is an acutely radiosensitive organ which is extensively damaged by whole-body irradiation with acute lethal doses (10.0 and 15.0 Gy). However, when the same total doses of radiation are delivered in smaller fractions, the damage in the mucosa is efficiently repaired and the lost tissue is rapidly regenerated during the interfractional intervals. Among different fractionation regimes, accelerated and rapid fractionation schedules were better tolerated than hypofractionation. This confirms the tolerance and remarkable repair capacity of mouse jejunum to dose fractionation. This could be important from the clinical point of view.

3. Of the five chemotherapeutic drugs (MISO, Metro, BLM, CPZ and c-DDP) tested, MISO and BLM are both effective as potentiators of damage to mouse jejunum by acute lethal exposure to γ-radiation but BLM is the most effective in reducing crypt survival.
4. Though MISO is toxic and enhanced the damage when combined with a lethal dose (10.0 Gy) of radiation, the drug does not seem to alter the jejunal response to sublethal exposures (1.5, 3.0 and 5.0 Gy). Also, when given in multiple doses, MISO has no detectable effect on the jejunal architecture following exposure to fractionated γ-rays. However, a reduction in the mitotic activity is obvious in both the cases. On the other hand, BLM significantly enhances the radiation damage in the jejunum, even at fractionated doses, indicating a cumulative toxicity which is not altered by further fractionation of drug. These observations should be taken into consideration while planning the treatment of intraabdominal malignancies with combined modality of drug(s) and radiation.

Though the present findings have characterized the drug—radiation interactions in normal intestine only, similar interaction in other proliferative tissues may also be expected. Assuming that the situation in human is not completely dissimilar to that observed in mice, it is warranted that the possible enhanced normal tissue reactions are taken into account while planning cancer treatment schedules using combined modalities.