CHAPTER V

COMPARISON OF THE TOXIC AND RADIOSENSITIZING EFFECTS OF DRUGS ON THE MOUSE JEJUNUM
A total number of 130 Swiss mice were treated as follows:-

1. The first group of mice received DDW/dextrose (equal volume as used for the drug) prior to an acute dose of 10.0 Gy gamma radiation.
2. The second group received the drug(s) at a dose of 1000 mg/kg (MISO, Metro), 20 mg/kg (BLM, CPZ) or 0.1 mg/kg (c-DDP) b.wt. before irradiation with 10.0 Gy.
3. As controls for cytotoxicity of drugs, the third group of mice was administered with the drug(s) alone, prior to sham-irradiation.
4. The fourth group was treated with a single dose of 15.0 Gy gamma rays without any drug pretreatment.
5. The last group of animals was injected with DDW/dextrose and sham-irradiated to serve as control.

Each of the above groups had 7-10 animals. On day 3 after the treatments all the animals were sacrificed and the jejunum was processed for crypt microcolony assay as mentioned in Chapter III. The jejunal response was evaluated by taking the crypt survival as end point. To compare the effect of the combined treatment with that of radiation alone the dose modifying factor (DMF) was calculated as the ratio between equieffective doses of radiation alone (R) and radiation + drug (R + D),

\[
DMF = \frac{\text{Dose of } R \text{ to produce a given effect}}{\text{Dose of } R + D \text{ to produce the same effect}}
\]
Observations:

Drug toxicity:

All the drugs except Metro showed comparable toxicity in the jejunal epithelium. The pathological changes observed on day 3 after the administration of drug alone included slight oedema in the submucosa and few degenerated crypts with haphazardly arranged large, enucleate and pale cells. Most of the crypts showed an apparent increase in mitotic activity and necrosis. Few abnormal mitoses were also visible. The villi appeared normal except for slight oedema in the lamina propria. There was a significant reduction in the number of surviving crypts in the jejunal mucosa by MISO, BLM, CPZ and c-DDP in comparison with DDW/dextrose injected control. But there was no marked difference in the crypt survival between the different drug-injected groups. Injection of Metro did not produce any significant change in the crypt count from that of the DDW-injected group (Table 2).

Radiation alone:

Whole-body exposure to 10.0 Gy of gamma radiation resulted in extensive damage to the mouse jejunum with outer coats showing oedema. The qualitative changes in the crypts included necrosis, pyknosis, mitotic abnormality, karyolysis, cytoplasmic vacuolation and degranulation, karyorrhexis and distorted arrangement of cells. Many crypts were degenerating and were populated with swollen enucleate cells. The villi were reduced in height and exhibited oedema and hydropic degeneration in the lamina propria (Fig. 5). The damage became more pronounced at 15.0 Gy, the villi showing severe injury. The villi were highly reduced in height and carried very few cells which were of bizarre shape and size. The tips of the villi were
blunt, some of them being ruptured. The crypt and villus showed extensive cell loss. The small intestinal lumen contained mucus and cell debris exuded through the ruptured villi tips (Fig. 6).

Microcolony assay revealed approximately 30% reduction in the number of surviving crypts in the mice receiving 10.0 Gy. Increase in the radiation dose to 15.0 Gy resulted in a further reduction in the surviving crypts to approximately 14% of the sham-irradiated control value (Table 2).

**Combination treatment**:

The histopathological changes in the jejunum of the animals receiving Metro, CPZ and c-DDP were qualitatively similar to those in the irradiation alone group. But MISO and BLM enhanced the radiation injury and the latter was more effective in this than the former. The damage induced by BLM + 10.0 Gy was qualitatively similar to that produced by 15.0 Gy alone (Figs. 7,8).

All the drugs except Metro, when injected before 10.0 Gy of radiation enhanced the radiation damage. Though all the drugs exhibited comparable crypt toxicity in the absence of radiation, when combined with radiation the effect was markedly different. The quantitative evaluation of crypt survival showed a further decrease in the radiation-induced crypt number by BLM, MISO, c-DDP and CPZ. On the other hand, combination of Metro and 10.0 Gy did not bring about any significant change in the radiation response of the jejunum. BLM produced the maximum enhancement of radiation injury giving a DMF of 1.5. The damage produced by combined treatment of BLM and almost 10.0 Gy was quantitatively similar to that produced by 15.0 Gy alone.
MISO pretreatment with 10.0 Gy irradiation also reduced significantly (P < 0.001) the number of crypts, but the effect was less severe than that produced by BLM treatment (Table 2). The effect of the drugs in potentiating the radiation damage decreased in the following order:

\[ \text{BLM} \rightarrow \text{MISO} \rightarrow \text{CPZ} \rightarrow \text{c-DDP} \]

**Discussion:**

It is well established that the cellular depletion of the gut epithelium by irradiation is manifested in the extreme case by complete loss of epithelial cells resulting in the symptoms of severe GI syndrome (Quastler 1956). The present study demonstrates that whole-body exposure to lethal doses of gamma rays resulted in extensive damage to the jejunal mucosa, the damage becoming more severe and extending to the villi at the higher dose. The radiolesions in the jejunum after exposure to 10.0 Gy included reduced crypt cell population, pyknosis, karyolysis, cytoplasmic degranulation, cell death and distorted arrangement of crypt cells. The villi became reduced in height and showed hydropic degeneration in the lamina propria. These observations are in agreement with the earlier reports of cell death with reduction in cell population and decrease in villous size after irradiation with high doses (Quastler 1956, 1963, Uma Devi 1977, Uma Devi and Mathur 1981). Becciolini et al. (1977) reported irregular shape and size of crypt cells and partial disappearance of the villous epithelium exposing the stroma to the lumen in the rat intestine, following exposure to 20.0 Gy of X-rays. Uma Devi et al. (1978) demonstrated degenerative changes in the mouse intestinal crypts which were manifested as a marked destruction of the proliferating epithelial...
lining associated with pyknosis, nuclear and cytoplasmic swelling and lysis of cells in the crypt.

In the present study, after a supralethal dose (15.0 Gy) of gamma irradiation, the villi were severely damaged with highly reduced size and blunt and ruptured tips. Similar changes were also noticed by Quastler (1956), Uma Devi (1977) and Uma Devi et al. (1979) who reported hydropic degeneration and denuded and shrunken villi after exposure to high doses. Reduction in the height of villi and deformity of tips were reported to be due to damage of the proliferative compartment which causes depletion of the functional compartment (Okumara and Matsuzawa 1971). Rao and Fritz-Niggli (1988) also found the villous length in the mouse jejunum to reduce significantly after exposure to 10.0, 11.0 and 12.0 Gy of X-rays. Uma Devi (1977) noticed that post-irradiation changes in the lamina propria consisted of oedema and swollen ground substance. The present findings support the above results and confirm the role of the intestinal mucosa, especially damage to the villi, in producing the GI syndrome.

It was observed that the number of surviving crypts were reduced significantly at 72 h after exposure to 10.0 Gy. This observation supports the earlier findings of Withers and Elkind (1970) and others (Hendry and Potten 1982, Rao and Fritz-Niggli 1988, Ijiri and Potten 1988, Potten et al. 1988) who reported a decrease in the number of crypts in the mouse small intestine 3-4 days after exposure to different doses of radiation. The quantitative results of the present study also agree with those of Rao and Fritz-Niggli (1988) who observed a dose related reduction in the mouse jejunal crypt number following
exposure in the range of 9.0 - 12.0 Gy X-rays. Dewit and Koren (1987) suggested that the reduction in the crypt number due to the loss of the unlimited capacity for cell division of crypt stem cells. Probably, this was because crypt stem cells were unable to proliferate and to form colonies in certain areas of impaired vascularization. Devik (1971) did not find any difference in the relative number of crypts below normal after doses of 7.0, 9.0 and 10.0 Gy. A decrease was observed only after 12.0 Gy.

There are very few published reports on the normal tissue-toxicity of the drugs used in this experiment. The present study shows that all the drugs, except Metro, have toxic side effects. Allan and Smyth (1986) observed that c-DDP alone caused profound damage in the crypt at a dose of 10 mg/kg. The present results demonstrate that toxic effect can be manifested even at a much lower drug dose (0.1 mg/kg), though the severity is reduced. The present findings with MISO support the observations of Bisht (1989) who reported chromosomal toxicity in normal mouse bone-marrow at drug doses of 0.5 mg/g. b. wt. and above. The present results also confirm the findings of Mitelman et al. (1976) that nitroimidazole alone caused chromosomal damage in man. Similarly, Hall et al. (1977) observed that MISO injection reduced the surviving fraction in V79 hamster cells to 0.01. However, contrary to these results, no excess chromosome damage was found in human lymphocytes in vitro after treatment with MISO alone (Prosser and Prisemann 1980).

There are no published reports regarding the CPZ toxicity on normal cells. George et al. (1980) have reported that CPZ alone
at doses of 20, 40 and 100 mg/kg produced a growth delay in mouse fibrosarcoma, the effect increasing with the drug dose, indicating tumor cytotoxicity of the drug.

BLM alone is known to interact with cellular DNA (Ono et al. 1972) and to induce strand breakage in *in vitro* and *in vivo* (Suzuki et al. 1968). Mitosis is reported to be the most sensitive stage to BLM damage (Barranco and Humphrey 1971). In addition, the drug, administered at therapeutic doses, was capable of causing chromosomal damage in human leucocytes (Bornstein et al. 1971). BLM was also demonstrated to exert toxicity by increasing the yield of apoptotic cells in the mouse intestinal crypts at a dose of 20 mg/kg b.wt. (Ijiri and Potten 1987). The present observation on BLM-induced reduction in the crypt survival of mouse jejunum agrees with the above findings.

Except Metro, all the other drugs tested in the study were found to enhance the radiation damage. Radiation and BLM showed a synergistic effect in reducing the crypt survival, while the effect of MISO and radiation was additive. Both CPZ and c-DDP were less effective, showing a sub-additive effect with radiation. A potentiation of intestinal radiation damage by BLM was reported earlier by Phillips et al. (1975) but they found that the effect was mild giving a DEF (dose enhancement factor) of 1.1. This difference in intestinal response from the present results may have resulted from the difference (1) in the drug dose, 20 mg/kg in the present study and 3 mg/kg in Phillips experiment (2) the end point studied, Phillips et al. selected ten microcolonies as the end point and (3) the interval between drug administration and irradiation, 30 minutes in the present study as against 2 h prior to exposure in the study of Phillips et al.
It is possible that the potentiation of radiation damage is dependent on the drug dose and the time of administration before irradiation. Similar enhancement in the radiation damage to the mouse jejunal crypt cells, as seen in the present study, was also noticed by von der Maase (1984b) when BLM was administered 24 h before irradiation, where $D_0$ for radiation was increased by a factor of 1.4. He suggested that the combined effect was probably additive or at least partly additive. According to von der Maase (1984b), BLM probably induces damage to the crypt cells which is fully repaired under normal conditions. However, administration of even small radiation doses within a few hours may interfere with repair mechanisms resulting in irreversible damage to the crypt cells. The synergistic effect observed in the present study may have resulted from the short interval of 30 minutes between the two modalities as against 24 h used by von der Maase. Pharmacokinetic studies of BLM showed that cultured cells rapidly absorbed the drug but the efflux of the drug was relatively slow (Lazo et al. 1989). Thus, irradiation 30 minutes after drug injection might have resulted in both the agents acting simultaneously to produce a synergistic increase in the damage. BLM has also been shown to potentiate the radiation damage to two other proliferative tissues, namely mouse bone-marrow and testes (Uma Devi et al. 1990). According to these authors, this potentiating effect appears to involve an interference with the repair mechanism by the drug. Repair inhibition of radiation damage was also suggested as a possible explanation for synergistic response of cultured cells to BLM and radiation (Bleehen et al. 1974).
Studies have demonstrated unambiguously that BLM cleaves DNA (Umezawa 1979, Twentyman 1984) and produces both single and double strand breaks in DNA (Ajmera et al. 1986). Mitosis has been shown to be the most sensitive to BLM in producing DNA strand breaks while $G_2$ was the most sensitive stage of the interphase. The order of sensitivity of dividing cells in other phases is $G_2 >$ early $S >$ late $S > G_1$ (Barranco and Humphrey 1971, Barranco and Bolton 1977). The high sensitivity of mitotic cells to ionizing radiation is well known. The supra-additive effect of BLM and radiation observed in the present study indicates that radiation and drug may act at the same or different targets. In addition to inducing chromosomal damage on its own (Vig and Lewis 1978), BLM may also interfere with the repair subsequent to exposure.

The present findings on MISO and radiation support the suggestion of Hornsey and Field (1979) that the drug could enhance the radiation damage in normal tissues. A small degree of normal tissue sensitization by MISO has been observed in rodent spinal cord (Yuhas 1979) and hypoxic skin epithelium (Denekamp et al. 1974). Electron-affinic compounds like MISO and Metro have been reported to be cytotoxic preferentially towards hypoxic cells (Sutherland 1974b, Brown 1975b, Foster et al. 1976, Stratford and Adams 1978). But in contrast to hypoxic cell selectivity, some toxicity was also observed under aerobic conditions (Geard and Rutledge-Freeman 1980). The present data support this finding, as indicated by MISO-induced reduction in the mouse jejunal crypt survival. Brown (1977) showed that the drug or its metabolite seems to be toxic both to oxygenated and hypoxic cells. Steel et al. (1979) observed that mice treated with MISO prior to irradiation tended to die at an earlier time than mice not
pretreated with the sensitizer. It was suggested by Arcangeli and Nervi (1980) that MISO cytotoxicity is not highly selective against hypoxic tumor cells. The present observation favours this view. It has been reported that MISO did not sensitize normal mouse bone marrow to radiation at 0.5 mg/g (Uma Devi and Bisht 1987). However, at a higher dose of 1 mg/g, as used in the present study, the drug was shown to enhance the radiation damage to the bone-marrow cells (Bisht and Uma Devi, unpublished data). Thus, the toxic and radiosensitizing effects of MISO seems to be drug dose-dependent. It is also clear from the previous reports and present results that cytotoxicity of MISO is not limited to hypoxic cells.

There is ample evidence that ionizing radiation produces DNA damage. Palcic and Skarsgaard (1978) have shown that DNA is also a target for the cytotoxic effects of MISO and this was subsequently confirmed by Skov et al. (1979) and Olive (1979). Thus, it is proposed that MISO acts, at least in part, by damaging the same cellular target site that is damaged by ionizing radiation. It was suggested that at high concentration the drug damage would suffice to produce outright cell death. At lower drug concentration, the sublethal MISO damage would be superimposed on the radiation damage at the same target site, thereby lowering the radiation dose required to achieve a given level of cell lethality (Hall and Biaglow 1977, Wong et al. 1978, Michaels et al. 1981, Taylor et al. 1987). A reduced capacity to repair potentially lethal radiation damage has been demonstrated in vitro and in vivo with MISO given before or after irradiation of both oxic and hypoxic cells (Guichard et al. 1979, Sakamoto and Aritake (1981).
Earlier report on Metro demonstrated a protective effect on the survival curve of crypt cells in the mouse (Stone and Withers 1974). Although this effect was not observed in the present study, the drug did not enhance the radiation response of the mouse jejunal crypts.

The present observation that c-DDP has a radiation potentiating effect agrees with the earlier findings of other investigators (Burholt et al. 1979, Luk et al. 1979, von der Maase 1984b, Dewit et al. 1985c, Tanabe et al. 1987). Dewit et al. (1985b) reported a DEF of 1.18 for a level of 10 surviving crypt cells per circumference when c-DDP was used at 8 mg/kg prior to exposure to X-rays, concluding thereby that X-rays and c-DDP cause damage to mouse intestinal crypts mainly by independent mechanisms and also by inhibiting sublethal damage repair in these cells. Similarly, Tanabe et al. (1987) reported a high DEF value for mouse intestinal crypts at a drug dose of 12 mg/kg. According to these authors the inhibition of sublethal damage repair may play a significant role in the interaction between c-DDP and X-ray in rapidly proliferating normal cells like those of the intestinal crypts. Similar conclusions were also drawn by Burholt et al. (1979) and Luk et al. (1979) for intestinal crypt cells. The fact that such severe damage could not be found with c-DDP in the present experiment could be explained on the basis that the drug dose in this study was much lower in comparison to the above mentioned studies. The significance of the present results, however, is that c-DDP even at such a low dose (0.1 mg/kg) is capable of producing toxic and radiation sensitizing effects in the mouse jejunum.
There are no published reports on CPZ comparable to the present one. However, a reduction in survival was observed when 15 mg/kg CPZ was injected before LD$_{50}$ whole-body exposure of mice to radiation (G.C. Jagetia, personal communication). Earlier studies demonstrated that administration of the drug before irradiation caused a significant delay in the growth of a murine fibrosarcoma, the combined effect being synergistic (George et al. 1980). It has been shown that ATP is essential for repair of potentially lethal damage in some experimental tumors (Guichard et al. 1977, McNally and Sheldon 1977) and CPZ is known to reduce the cellular ATP content (Grunnel et al. 1955) as well as to inhibit the generation of ATP (Lehrich et al. 1977); hence it would necessarily inhibit cellular recovery. It was also suggested that the inhibition of DNA repair could be brought about by gross disorganization of the cellular membrane at high drug concentration (Maniar et al. 1984). Furthermore, CPZ was found to cause a blockage of cell progression at G$_1$/S and G$_2$+M and to inhibit the repair of X-ray induced potentially lethal damage (Shenoy and Singh 1985b). Significant change in cellular morphology following treatment by CPZ were observed which may influence their radiation response (Schorn et al. 1983). However, the evidence from the literature and present study is insufficient to reach a conclusion on the normal tissue sensitization by CPZ.

In conclusion, though BLM and c-DDP are used as effective anticancer drugs in man and MISO is undergoing clinical trials with a view to be incorporated in cancer treatment in future, the intestinal toxic and radiosensitizing effects of these agents caution against their use with radiation in the treatment of abdominal cancers.
Table 2: Number of surviving crypts/circumference (M ± S.E.) of the jejunum of Swiss mice after different treatments

<table>
<thead>
<tr>
<th>Radiation (Gy)</th>
<th>Drugs</th>
<th>DDW/Dextrose</th>
<th>BLM 20 mg/kg</th>
<th>MISO 1000 mg/kg</th>
<th>METRO 1000 mg/kg</th>
<th>CPZ 20 mg/kg</th>
<th>c-DDP 0.1 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>103.68 ± 1.04</td>
<td>(10)</td>
<td>96.39 ± 0.22</td>
<td>94.77 ± 0.40</td>
<td>101.82 ± 0.40</td>
<td>(10)</td>
<td>95.84 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>(10)</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>(10)</td>
<td>a</td>
</tr>
<tr>
<td>10.0</td>
<td>73.78 ± 0.65</td>
<td>(9)</td>
<td>16.78 ± 0.39</td>
<td>60.33 ± 0.54</td>
<td>73.05 ± 0.71</td>
<td>(10)</td>
<td>71.32 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>(7)</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>(10)</td>
<td>b</td>
</tr>
<tr>
<td>15.0</td>
<td>15.21 ± 0.53</td>
<td>(7)</td>
<td></td>
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<td></td>
<td></td>
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</tbody>
</table>

Numbers in parentheses indicate the number of samples taken.

a: Values are significantly different from that of DDW/Dextrose injected group at P < 0.001

N.S.: Non-significant

b: Compared with 10.0 Gy data