Summary and Conclusions
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Ionizing radiations such as γ-rays and X-rays are detrimental to life. Since cells consist of about 60 – 80% of water, to great extent the biological effect is mediated through the action of radiation on water. Free radicals generated through the radiolytic dissociation of water molecules, react with various cellular components and alter their structure and functions.

Involvement of radiation as a therapeutic agent in the treatment of pathological conditions particularly cancer was immediately recognized after the discovery of X-rays. However, the hypothesis of Bergonie and Tribondeau (1959) laid the foundation of radiation therapy of cancer. The cancer cells have higher reproductive activity (are less differentiated) and are likely to be more radiosensitive than surrounding normal tissues. Since hypoxia has a dramatic protective effects against ionizing radiation, the presence of hypoxic cells in tumors limits the success of radiation therapy. Relatively higher radiation dose could not be applied to kill tumor cells particularly which are hypoxic in nature, as it would damage the normal tissues intimately associated with the tumor (in the path of treatment beam). Therefore, to protect normal tissues from radiation injury and to potentiate radiation damage of tumors, have been the major goal of the radiation research. A wide range of strategies including the use of radiomodifiers are being devised to achieve this goals. The use of chemical agents either to protect the normal tissues or to enhance the radiosensitivity of tumors opened newer avenues in the radiation therapy of cancer.

Numerous drugs have been tested for their ability to potentiate or protect against radiation effect. Although many chemical agents were shown to be have radiomodifying properties, but were not useful in improving the radiation therapy of cancer even at an experimental level. A survey of literature suggests that without addressing the real problem or understanding the action of modifiers, further work on most of the modifiers was either abandoned or diverted towards the search and testing of the new agents. This kind of endeavour did not bridge the gap between the present state of knowledge of chemical modifiers and radiation therapy of cancer. Therefore, the mechanism of action of radiomodifiers particularly which have protected the normal tissues and potentiated the
tumors simultaneously, needs to be examined. Phenothiazines have shown promising dual effects. Recently their importance and usefulness in improving the radiation therapy has been suggested (Kale 1996). The present study is aimed to understand the biochemical events which make phenothiazines effective radioprotectors in the normal tissues. The role of phenothiazines as radiosensitizers in the tumors is initiated separately.

Although, the effect of phenothiazines as radioprotectors in normal tissues can be attributed to directly acting chemically based mechanisms, like free radical scavenging (Kale and Sitasawad, 1991, Varshney and Kale 1990, Choudhary et. al. 1998), the biological/ biochemical processes are also suggested to play a significant role in the protective action of phenothiazines (Varshney and Kale 1995, 1996, Kale 1996, Chandra and Kale 1998). Available information suggests close links between biological/biochemical processes involving radioprotective action and cytochrome P450 system as well as antioxidant enzymes. Therefore, in the present work, effect of phenothiazines like CPZ, PMZ and TMZ is examined on cytochrome P450 system and antioxidant potential in terms of DTD, GST, SOD and CAT; as well as on lipid peroxidation, XO and LDH which are known to be linked with cellular damage.

To understand the radioresponse of cytochrome P450 system, Swiss albino mice were irradiated with different doses of γ-rays (0 – 9 Gy) at dose-rate of 0.023 Gy/s. The effect on different components of cytochrome P450 system was measured in the liver of mouse. The mode and magnitude of change was found to depend upon dose and time after irradiation. The specific activities of NADPH-cytochrome P450 reductase and NADH-cytochrome b5 reductase; as well as contents of cytochrome P450 and cytochrome b5 were enhanced upto 5 Gy and then decreased progressively.

Dose-rate is one of the important factors which determines the biological consequences of a given absorbed dose. In the present study, the dose-rate effect on the cytochrome P450 system was examined using three dose-rates i.e., 0.437, 0.109, and 0.023 Gy/s. An inverse relationship between change in the activities of different components of cytochrome P450 system and dose-rate was observed.

Influence of γ-rays on the antioxidant potential of animals was also examined in terms of DTD, GST, SOD and catalase in the liver of mouse. The irradiation of mice (0-9 Gy) resulted in the increased activity of DTD, GST and SOD upto 5 Gy; whereas that of
catalase was inhibited in dose dependent manner. The study with dose-rate effect showed inverse relationship with change in the specific activities of antioxidant enzymes.

Lipid peroxidation, XO and LDH are measures of cellular damage. The levels of lipid peroxidation as well as activities of XO and LDH were increased with dose (0 to 9 Gy) in the liver of mouse. In addition, inverse dose-rate effect in their levels was also observed.

Phenothiazines administered intraperitoneally enhanced the specific activity of NADPH-cytochrome P450 in concentration dependent manner in the liver of unirradiated as well as irradiated animals. Phenothiazines showed small but consistent inhibitory effect in case of NADH-cytochrome b5 reductase. However, their levels were significantly higher compared to respective controls. Phenothiazines also enhanced the contents of cytochrome P450 and cytochrome b5 in concentration dependent manner in the liver of irradiated and unirradiated animals.

Since, phenothiazines form complexes with metal ions and Fe²⁺/Fe³⁺ couple influences their radiomodifying ability, the effect of phenothiazines in combination with Fe²⁺/Fe³⁺ ions on cytochrome P450 system was studied at 5 Gy. The combined treatment of phenothiazines and ferrous ions further enhanced the radiation induced activity of different components of cytochrome P450 system. On the other hand, ferric ions in combination with phenothiazines lowered the different components of cytochrome P450 system.

Kinetic studies showed a marked decline in $K_m$, and increase in $V_{max}$ of NADPH-cytochrome P450 reductase with radiation dose. Treatment of CPZ restored the $K_m$ value to a great extent. However, $V_{max}$ was further increased particularly at higher concentration of CPZ.

Cytochrome P450 and antioxidant system seems to be closely linked. As expected, phenothiazines enhanced the specific activities of antioxidant enzymes particularly DTD, GST and SOD. The combined treatment of phenothiazines and ferrous ions showed inhibitory effect on the activities of antioxidant enzymes, whereas, the ferric ions in combination with phenothiazines enhanced the activities of DTD, GST, SOD and CAT.
The radioprotective ability of phenothiazines was tested against radiation-induced peroxidative damage, XO and LDH. The radiation-induced lipid peroxidation, activities of XO and LDH were diminished on the administration of phenothiazines. The radiation damage determined in terms of lipid peroxidation, XO and LDH was further increased with combined treatment of ferrous ions and phenothiazines. Whereas, ferric ions in combination with phenothiazines showed the protective effect against radiation-induced lipid peroxidation and specific activities of XO and LDH.

To study the relevance of the present findings to radiation therapy, the work was initiated to study the survival of animals using 7.0, 8.5 and 10.0 Gy radiation doses, with and without administration of CPZ 60 min prior to irradiation. Radiation caused the lethality in animals. The lethality was reduced on the administartion of CPZ. Interestingly, CPZ enhanced the median survival time of irradiated animals. The early sharp declined death rate was delayed on the administration of CPZ and shifted the curve to the right. Thus CPZ could offer protection against radiation effect. These studies support the idea that phenothiazines could provide radioprotection in vivo system.

In addition, effect of mixed radiation on cytochrome P450 system was examined using very small doses in centigray regions, employing two neutron/gamma ratios (66.7 and 88.5) in liver, spleen and testis of mouse. Mixed radiation enhanced the specific activities of NADPH-cytochrome P450 reductase and NADH-cytochrome b5 reductase in all the tissues. Moreover, the specific activity of DTD was also enhanced on the exposure to n-γ mixed radiation. The neutron/gamma ratios of 66.7 was more effective to induce these changes.

Since, the cytochrome P450 system protects cells against oxidative damage (Morichetti et al. 1989), the radiation-induced activity of cytochrome P450 system in the present study probably suggests the antioxidant potential of animals. The increased specific activities of DTD, GST and SOD support such a possibility. The activated antioxidant and cytochrome P450 systems may attenuate the oxidative stress induced by the active oxygen species generated in radiolytical decomposition of water in the cells. In addition, the enhanced activity of cytochrome P450 system might also help in metabolizing the chemically reactive products generated from the free radical reactions.
The antioxidant function of cytochrome P450 system and antioxidant system seems to be closely linked.

Elevation of cytochrome P450 system along with antioxidant enzymes, and concomitant elevation of lipid peroxidation, LDH as well as XO activities may not be coincidental and rather be closely associated. Considering the antioxidant property, the augmentation of cytochrome P450 system might be regarded as an adaptive response. The radiation-induced activities of different components of cytochrome P450 system were found to decrease with increase in dose-rate. Similar inverse relationship with dose-rate was also found in case of lipid peroxidation, as well as specific activities of DTD, GST, SOD, XO and LDH. These studies support the idea of close relationship between these enzymes and antioxidant property of cytochrome P450.

Phenothiazines are substrate for the cytochrome P450 and hence their interaction might have activated the cytochrome P450 system leading to further enhancement of antioxidant potential of animals. This is also evident from the elevated levels of specific activities of DTD, GST and SOD. Further increase in the activity of cytochrome P450 system on administration phenothiazines, might promote metabolism of the radiolytically formed electrophilic species, and lower the radiation effects. The activation of cytochrome P450 system and simultaneous inhibition of lipid peroxidation and the specific activity of LDH by phenothiazines support this possibility. These observations suggest the involvement of cytochrome P450 system in the radioprotective action of phenothiazines. Since phenothiazines are readily absorbed and accumulated in various organs, similar radioprotective effect is expected to be observed in different tissues of mice. However, the extent of protection would depend upon radiosensitivities of tissues and radiation dose.

The kinetic profile of NADPH-cytochrome P450 reductase suggests that the electron flow in the NADPH-linked electron transport system in the liver of mouse is modulated by radiation and treatment of CPZ, results into change in affinity of enzyme towards substrate; and radioresponse of the cytochrome P450 system.

The phenothiazines in presence of Fe$^{2+}$ ions might have sensitized the radiation effect. Due to increased radiation damage, the cytochrome P450 system might have responded in terms of its enhanced activity. The increased levels of oxidative damage
determined in terms of lipid peroxidation, XO and LDH supported the idea that phenothiazines in presence of Fe$^{2+}$ ions sensitize the radiation effect. The radiation damage potentiated by combined treatment of phenothiazines and Fe$^{2+}$, might have adversely affected the antioxidant system and inhibited the antioxidant enzymes particularly DTD, GST and SOD. Thus, it is quite possible that the combined treatment of phenothiazines and Fe$^{2+}$ has potentiated the radiation effects. In response to radiosensitization the activity of cytochrome P450 system was probably increased.

As mentioned above, the phenothiazines in presence of Fe$^{3+}$ behave as effective radioprotector. Their combined treatment might have provided protection against radiation effects. Therefore, due to lowering of radiation effect, the response of cytochrome P450 system might have also lowered. The enhanced levels of antioxidant enzymes (DTD, GST and SOD) are likely to provide the protection against oxidative damage.

As expected, phenothiazines in presence of Fe$^{3+}$ ions inhibited radiation induced lipid peroxidation, specific activities of XO and LDH. It is quite clear that the presence of iron ions alter the radiomodulating property of phenothiazines. It seems that the redox activity of iron was altered by phenothiazines in such a way that the activity of cytochrome P450 system was enhanced in presence of Fe$^{2+}$ ions and inhibited in presence of Fe$^{3+}$ ions.

The results of present study suggest the possibilities to devise the strategies for differential protection of normal cells based on simultaneous administration of Fe$^{3+}$ and phenothiazines. The administration of phenothiazines-ferric complexes directly perhaps may be more beneficial. Thus the findings of the present study may have significance in understanding the differential radiosensitization of tumor cells and radioprotection of surrounding normal cells by phenothiazines.

Reduced lethality, decreased death rate and enhanced life span of irradiated animals on administration of CPZ were attributed to biochemical/biophysical processes like activation of cytochrome P450 enzymes involved in antioxidant functions. The possible implications of the present survival study are wide ranging and potentially relevant to human beings. Since phenothiazine drugs are in regular clinical use, the
findings of present work may have significance in improving the radiation therapy of cancer.