SUMMARY AND CONCLUSION

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The glyoxalase system consists of two enzymes: glyoxalase I and glyoxalase II; converts methylglyoxal to D-lactic acid, utilizing reduced glutathione. Although, this system has been known since 1913, lots of interest was generated when Szent-Gyorgi (1967) proposed the electronic theory of cancer and suggested its role as regulator of cell division. Later on it was found to be involved in cell cycle and differentiation.

The glyoxalase system is known to be active throughout embryogenesis, tissue maturation and persists until cell death. It is considered to be biochemical indicator of cell proliferation. It is closely associated with ageing, tumors, diabetic complications and malaria. The glyoxalase system has been conserved. An evolution was accompanied by radiation if not guided. During the development of early life forms, radiaton level were quite high (Crunkite, 1990). The emergence of glyoxalase system in early part of evolution might have experienced irradiation. Radio-sensitivity is directly proportional to the rate of reproductive activity of cell and inversely proportional to degree of differentiation. Glutathione (GSH) which is co-factor of the glyoxalase system, and involved in maintaining the redox status of cell, is one of the determinants of radio-sensitivity. The depletion of GSH leads to increased radio-sensitivity of the cell. Thus radio-sensitivity and glyoxalase system is expected to be closely interlinked. Therefore, radio-modulation of the glyoxalase system at molecular and biochemical levels was examined out using in the liver of mice.

In present work we have examined:

1. Effect of different doses
2. Effect of different dose-rates.
3. Adaptive response
4. Split dose effect

Besides this, we have also evaluated lipid peroxidation and the activities of different antioxidant enzymes like SOD, GST and catalase under similar irradiation conditions.

The findings of present study could be summarized as follows:

1. Animals were irradiated with 3 Gy and mRNA expression was observed with different time interval (0-24h). The expression of glyoxalase I was found to be relatively higher at 6h. The expression results suggest that radiation effect on transcripton persists even in post irradiation period. Since, relatively high mRNA expression was seen at 6h, we have used this time interval for further studies.
2. Glyoxalase I is considered to be biochemical indicator of cell proliferation. When the animals were irradiated with different doses, both the mRNA expression and specific activity of glyoxalase I increased between 0 – 5 Gy and than declined beyond 5 Gy. The enhancement in mRNA and specific activity probably reflects the repair /regeneration in the liver. At higher doses, lowered level of transcription and activity could be due to irreversible damage to liver cells or to the enzyme itself apart from alteration in the induction process.
3. Unlike glyoxalase I, the specific activity of glyoxalase II was progressively decreased. However, its overall activity was above the normal level. The accessibility of radiolytically generated free radicals towards different sites of glyoxalase I and glyoxalase II may not be same, resulting in their differential radio-sensitivity.
4. Peroxidative damage was determined at different doses between 0 – 7 Gy. No significant change in the level of peroxidation could been seen upto 5 Gy. However, beyond 7 Gy the peroxidation was significantly higher compared to unirradiated control.
5. SOD, GST and catalase are important member of antioxidant enzyme system. The specific activities of SOD and GST were increased upto 5 Gy and then declined.
6. The non significant increase in lipid peroxidation between 0 – 5 Gy might be due to the increase in the activities of antioxidant enzymes like SOD and GST. The increased level of peroxidation at 7 Gy could be due to their inhibition caused by radiation.
7. The dose-rate effect at two radiation doses i.e. 3 Gy and 7 Gy was evaluated using different dose-rate i.e. 0.24, 0.06 and 0.015 Gy/sec. The mRNA expression of glyoxalase I was declined with decrease in dose-rate. It may be mentioned that the

lowest dose rate adversely affected the expression which was reduced to 14% and 46% at 3 Gy and 7 Gy respectively. Under similar conditions of irradiation, peroxidative damage was enhanced with decrease in dose rate. These findings support the inverse dose-rate effect on the transcription of glyoxalase I.

8. The dose-rate effect on the activity of glyoxalase I was similar to that of mRNA expression. The response of glyoxalase I and glyoxalase II was also similar. The peroxidation and antioxidant enzymes showed inverse dose-rate effect.
9. Biological effects of radiation are mainly caused by free radicals. At chemical level, the decreased radiation effect at higher dose-rate might be due to their greater recombination. The chances of indirect effect increases when free radicals react with biomolecules without recombination, as it readily happens when dose rate is decreased.
10. The peroxidative effect is known to be transferred from the level of lipids to proteins and nucleic acids. It is possible that, peroxidation might have transferred its effect through its degradation product to nucleic acids and proteins. In event of this possibility the structure and function of glyoxalase I mRNA as well as various regulatory proteins involved in expression of gene including GLY I gene could be altered with greater extend with decrease in dose-rate.
11. Antioxidant enzymes particularly SOD and GST showed inverse dose-rate response which could be due transfer of peroxidative damage or their interaction with free radicals.
12. Not much information is available on biochemical aspects of adaptive response. We have examined the adaptive response using 0.5 Gy conditioning dose and 3 Gy challenging dose. The lowered level of mRNA expression and specific activity of glyoxlase I in the liver of mice irradiated with conditioning dose (0.5 Gy) and then challenging dose (3Gy) was seen as compared to mice irradiated to 3 Gy only. In adaptive response, the conditioning dose is known to protect against the radiation damage induced by subsequently higher doses. The same might have occurred in the present situation and lowered the transcription and specific activity. The reduction of peroxidation supports this possibility.

13. The dose fractionation provides time interval for cells to undergo repair and activate cellular resistant machinery as result cells become less susceptible to damage from the second exposure. A dose 6 Gy was fractionated into two equal fractions (3+3 Gy) and time intervals between two exposures were kept 3, 6 and 12h. With dose fractionation, mRNA expression was lowered. There was increase in the specific activity of glyoxalase I compared to mice irradiated single dose of 6 Gy. The reduced peroxidation indicates lowered level of damage as result of fractionation of radiation. It may be due to repair of the damage during the available time between two exposures. The increased level of antioxidant enzyme activity might have also contributed to lowering the damage. Thus, above findings suggest that the fractionation of dose leads to lesser damage and intern lowers expression of mRNA.
14. It is quite clear that the exposure of animals to different doses resulted increase in transcription as well as specific activity of glyoxalase I upto 5 Gy. The mode of change in transcription and the specific activity of glyoxalase I is similar. However, they differ in magnitude. The concomitant increase in the antioxidant enzymes particularly SOD and GST might not be coincidental, and probably were intimately associated with the change in glyoxalase system. Thus apart from involvement in the repair/regeneration, the present study showed close link between glyoxalase system and antioxidant status of the animals.
15. Interestingly in adaptive response and split dose effect, mRNA expression was lower than the specific activity of glyoxalase I. Its seems the transcription and the activity of glyoxalase I is independently regulated.
16. There has been debate on the role of glyoxalase system in cell proliferation and detoxification. It could be inferred from our findings that its involvement in proliferation and protective action against oxidative stress are not conflicting and probably be intimately interlinked.