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Radiation Protection of Mouse Liver by 2-Mercaptopropionylglycine

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The present study is an attempt to investigate the protection imparted by MPG (2-mercaptopropionylglycine) to liver in Swiss albino mice against damages caused by external irradiation. The histopathological changes in the liver of a group of mice exposed to 1000 R of Co-60 gamma rays 15-30 minutes after an intraperitoneal injection of 20 mg/kg body weight of MPG were studied at different post-irradiation intervals. The results were compared with those received from a control group which was irradiated in the absence of the drug. The MPG was found to provide ample protection to the liver leading to an accelerated recovery and restoring the normal structure by 4 weeks after irradiation.

INTRODUCTION

The effect of radiations on the liver is not very clear. The reports of Kolodny,[1] Bollinger and Inglis,[2] indicate that severe parenchymal changes are produced in liver of animals following various doses of radiations. However, other eminent workers failed to observe any visible pathological damage to the hepatic tissue following moderate or large doses of radiations.[3,4]

Dettmer *et al.*[5] have noted that the condition of hepatocytes at the time of irradiation is a determining factor for the subsequent damage. Grad and Stevens[6] and Mehta *et al.*[7] have reported cytoplasmic degranulation, pyknosis, necrosis, and loss of architecture as a result of irradiation.

Since the discovery of chemical radiation protection, a large number of chemicals have been tested to find out their protective action on different biological systems. The radioprotectors which generally impart maximum protection have to be given in very large quantity which is toxic to the organisms. Recently Sugahara and his coworkers,[8] Nagata *et al.*[9] have reported a new synthetic compound 2-mercaptopropionylglycine (MPG), which is an effective radioprotector in a dose, much lower than its toxic level in both mice and man. The present study has been undertaken to observe radiation protection by MPG in liver of mice after whole body irradiation with 1000 R gamma rays.

MATERIAL AND METHODS

Swiss albino mice were obtained from the Cancer Research Institute, Bombay. They were maintained in an air conditioned laboratory and provided with standard mice feed (obtained from Hindustan Lever Ltd., Delhi) and water *ad libitum*. The adult male mice from an inbred colony were used. The drug, 2-mercaptopropionylglycine (MPG) was received from the Santen Pharmaceutical Co. Ltd., Osaka, Japan in the commercial name Thiola. The drug was dissolved in distilled water and pH was adjusted to 6.5 with 0.1 N. NaOH and diluted to give a concentration 1 mg/ml.

42 animals weighing 25 g on an average were divided into two groups of 21 each. The first group of animals was injected intraperitoneally with MPG at a dose of 20 mg/kg body weight, 15 to 30 minutes before irradiation. The second group which served as control received distilled water (volume equal to that injected in the experimental group) in the same method. Animals of both the groups were exposed to a Cobalt-60 source so as to receive 1000 R in 42 minutes. Three animals in each group were sacrified by means of decapitation of neck at 12 hrs, and 1, 2, 5, 7, 14, and 28 days, and small pieces of liver were fixed in Bouin's fluid. The paraffin sections were cut at 5 μ and stained with Harris hematoxylin and eosin for histological studies.

OBSERVATIONS

Control (Animals receiving no drug)

The hepatic cells showed extreme vacuolization of cytoplasm event at the earliest interval studied, i.e., 12 hrs after exposure. The size of the nuclei was reduced due to shrinkage and crenation (Fig. 1). Hyperaemia of blood vessels, lymphocytic infiltration and necrosis were evident. These changes were more exaggerated at 24 hrs and necrosis, pyknosis and karyorrhexis were well marked. The arrangement of hepatic cords got distorted and the nucleus disappeared from several cells (Fig. 2). These changes were more pronounced in the outer region of liver as compared to the central portion. By 48 hrs the cytoplasmic degranulation and vacuolization became more pronounced. The outer region of the tissue showed pyknotic and shrunken nuclei. Severe hyperaemia was also observed (Fig. 3). The nuclear damage was maximum at 5 days after exposure, where most of the cells showed pyknotic nuclei. Nuclear shape was distorted and showed crenation. Hyperaemia and cytoplasmic degranulation continued to be visible; but to a lesser extent. Some cells with giant nuclei were also visible. At 14 days cytoplasmic degranulation pyknosis and crenation of nuclei were seen in a mild form; but lymphocytic infiltration registered an increase.

Although there was an indication of recovery from 2 weeks on, the normal structure was not restored even at 4 weeks. Cytoplasmic degranulation along with shrunken nuclei could be seen even at this interval (Fig. 4).

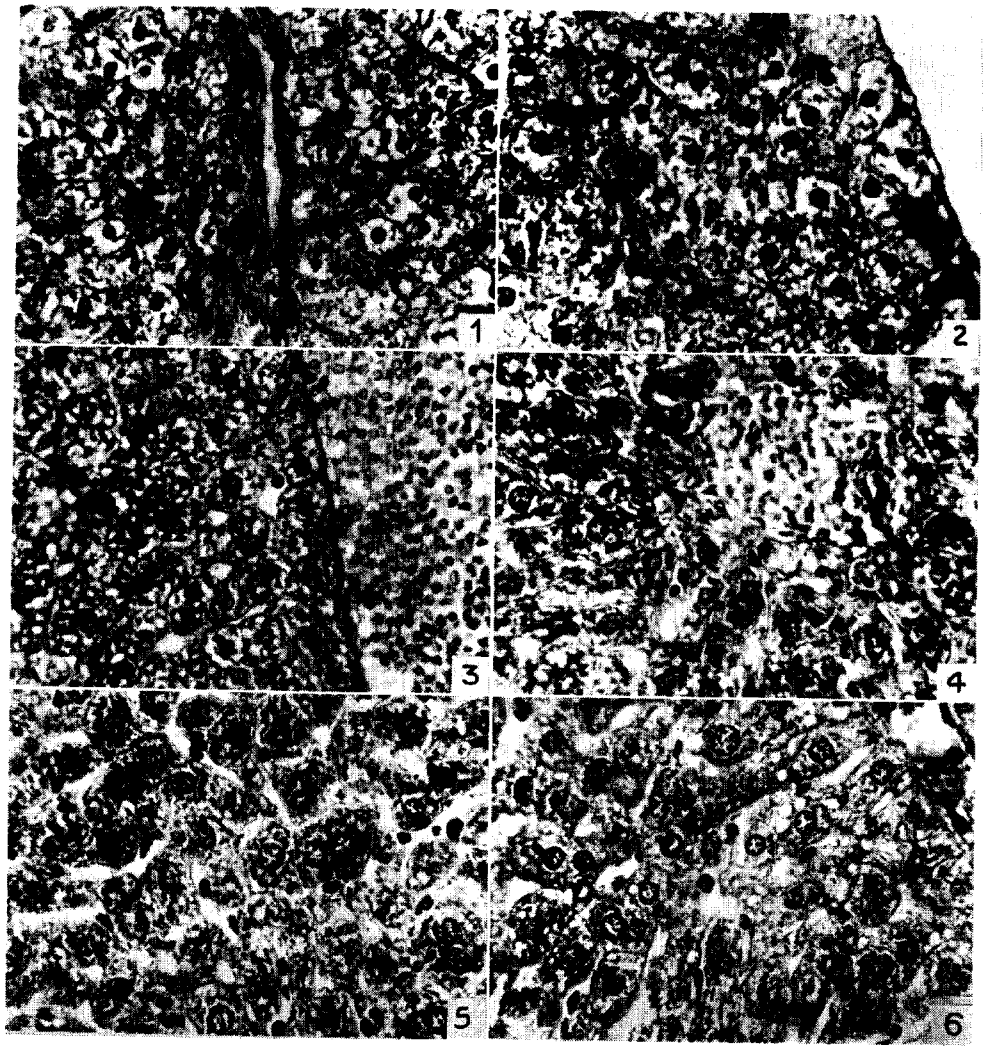

Fig. 1. Control liver 12 hrs. after exposure showing cytoplasmic degranulation, vacuolization and nuclear changes like pyknosis, karyorrhexis and chromatolysis. ×400

Fig. 2. Control liver 24 hrs. after exposure showing relatively more damage in the outer portion. Note the necrotic cells in the outer part. ×400

Fig. 3. Control liver 48 hrs. after exposure. Changes are similar to that of 24 hrs. Note hyperaemia of blood vessels. ×400

Fig. 4. Control liver 4 weeks after exposure showing partial recovery. ×400

Fig. 5. Experimental (MPG injected) liver 48 hrs. after exposures showing recovery of hepatic tissue. ×400

Fig. 6. Experimental 4 weeks after exposure, recovery is almost complete, still some lymphocytes are seen in the hepatic tissue. ×400

Experimental (Animal receiving MPG)

The histological picture at 12 hrs. was very much similar to that of the control at the same interval. But a clear tendency towards recovery was observed by 24

hrs. Neither pyknosis nor crenated nuclei were seen. The nuclei, in fact, showed some increase in size.

By 48 hrs. significant recovery was discernible as almost normal size of the hepatic nuclei could be seen despite lymphocytic infiltration (Fig. 5). There was a progressive recovery with a restoration of the cord-like arrangement of hepatic cells through the next interval studied. The liver revealed a normal structure by 28 days (Fig. 6).

DISCUSSION

The effect of radiations depends upon the condition of hepatocytes at the time of irradiation (Dettmer *et al.*[5]). The present observation showed extreme vacuolization of cytoplasm, reduced size of nuclei, shrinkage and crenation of nuclei at 12 and 24 hrs., after irradiation in the control group. Grad and Stevens,[6] Koletsky,[10] Mignard *et al.*[11] and Mehta *et al.*[7] have reported similar observations.

The outer region (peripheral region) of the liver showed pronounced necrosis, pyknosis, karyorrhexis, haemorrhage, hyperaemia and loss of cells at 24, and 48 hrs after exposure. These findings are in agreement with those of Grad and Stevens,[6] Koletsky,[10] Ogata,[12] Agrawal,[13] Reed *et al.*[14] and Mehta *et al.*[7] There was a definite indication of recovery at four weeks although it remained incomplete.

The damage in hepatic cells after irradiation with gamma rays was not of any specific type and might have been induced by toxaemia brought about by the exposure. In the present experiment the drug (MPG) provided ample protection to the liver. The protective effect was manifested in an early recovery as indicated by the absence of pathological changes like cytoplasmic degranulation, loss of cells, abnormal architecture after 5 days post irradiation and almost normal structure which was restored by two weeks. Thus the result of the present study clearly showed that hepatocytes were radiosensitive and there was an accelerated recovery of the liver in the group receiving MPG before irradiation. It can be concluded from these findings that MPG when given at the dose level of 20 mg/kg body weight, 15 to 30 minutes before exposure to 1000 R gamma rays imparts protection to mouse liver by way of accelerating the process of recovery of the tissue. The mechanism of the drug action in imparting protection against radiation, however, is still unclarified. Further studies, with a view to find out the actual mechanism of action of the drug, is in progress in the authors' laboratory.

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REFERENCES

1. A. Kolodney (1925) Tissue changes after experimental deep roentgen irradiation. *Amer. J. Path.*, 1 : 285-293.
2. A. Bollinger and K. Inglis (1933) Experimental liver disease produced by X-ray irradiation of the exposed organ. *J. Path. Bact.*, **36** : 19-30.
3. G.C. Hall and G.H. Whipple (1919) Roentgen rays intoxication disturbances in metabolism produced by deep massive doses of the hard roentgen rays. *Amer. J. Med. Sci.*, **157** : 453-482.
4. M. Laddage and U. Ramagnoli (1961) Demonstration of some latent lesions in the liver cells. *Minerva Nuclear.*, **5** : 294-295.
5. C.M. Dettmer, S. Kramer, D.H. Driscell and G.E. Ponte (1968) A comparison of the chronic effects of irrodiation upon the normal, damaged and regenerating rat liver. *Radiology*, **91** : 993-997.
6. B. Grad and C.E. Stevens (1950) Histological changes produced by a single large injecjection of radioactive phosphorus (P^{32}) in albino rats and in C3H mice *Cancer Res.*, **10** : 289-296.
7. S. Mehta, L. Mehta and S.P. Mongia (1975) Effect of whole body X-irradiaiion on liver of albino rats. *Int. J. Expt. Biol.*, **13** : 73-75.
8. T. Sugahara, Y. Nagata, H. Nagata, T. Tanaka and E. Kano (1970) Radiation protection by 2-MPG. *Proceedings of international symposium on Thiola*, Santen Pharmaceutical Co., Ltd., Osaka, Japan, pp. 267-272.
9. T. Sugahara (1972) 2-Mercaptopropionylglycine (MPG) and the recent development of chemical radiation protection. *Proceedings of the second international symposium on Thiola*, Santen Pharmaceutical Co., Ltd., Osaka, Japan, pp. 17-22.
10. S. Koletsky and G. Guslafson (1952) Liver damage in rats from radioactive colloidal gold. *Lab. Invest.*, 1 : 312-323.
11. V.A. De. Mignard, P.R. Petek and S. Bernick (1965) Response of the liver to "target" irradiation. *Amer. J. Path.*, **47** : 329-335.
12. K. Ogata, M. Yoshida, T. Kitamuro, G. Akagi, K. Kagawa and F. Fukuda (1963) Hepatic injury following irradiation--a morphological study. *Tokushima J. Exp. Med.*, **9** : 240-251.
13. S.K. Agarwal and R.M.L. Mehrotra (1966) Effects of single dose X-irradiation of liver in albino rats. *Int. J. Path. and Bact.*, **9** : 175-185.
14. G.B. Reed and A.J. Cox Jr. (1966) The human liver after radiation injury. *Amer. J. Path.*, **48** : 596-607.