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Radioprotective Effects of Liv.52 and Tissue-Reduced Glutathione (GSH) in Experimental Rats

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SUMMARY

The radioprotective effects of Liv.52 on tissue-reduced glutathione (GSH) levels were studied in rats. Adult female Sprague Dawley rats were exposed to whole body gamma radiation of 4 Gy and 8 Gy. Prior to radiation exposure, Liv.52 was fed, one ml per rat, daily for 15 days. Three days after radiation exposure, reduced glutathione levels in the liver, spleen, kidney and blood were studied.

Liv.52 was beneficial in restoring the spleen weight to body weight ratio in the animals of the 4 Gy group. In the spleen and liver, Liv.52 helped to restore reduced glutathione in sub-lethally exposed rats. Blood-reduced glutathione was found to be normal in both groups of experimental rats who received Liv.52.

The above results exhibit the radio protective effects of Liv.52 in relation to tissue-reduced glutathione in experimental rats exposed to sub-lethal doses of radiation.

INTRODUCTION

Harmful effects of ionizing radiation in mammals are known for a long time and attempts have been made to find out suitable protective agents against the same. But the problem is complex, since the exact mechanism by which radiation induces lethality in mammals is not yet properly understood[1]. Also the lethal effects depend on the dose, specificity, biochemical reactions, oxygen tension, phases of the mitotic cycle etc. Radioprotective agents are defined as compounds that help to diminish the biological effects of ionizing radiation when administered generally before exposure to radiation.

The most important group of chemical protectors so far known is the thiol compounds belonging to the cysteine-cysteamine group. Research on thiol compounds as protectors in mammals started with the pioneering work of Patt *et al*.[2], who discovered that prior injection of cysteine increased the survival rate of lethally irradiated rats and mice. Since then intensive research has been carried out and a large number of compound screened, many of which have been demonstrated to possess protective action[3]. But it has been observed that most of these compounds provided a degree of protection in proportion to the drug administered and many were toxic at the maximum effective dose. Hence these compounds are of little clinical value.

Recently emphasis has been laid on the above topic to explore the possibility of finding some effective, indigenous drug. Indeed, the indigenous remedy Liv.52, which is being used clinically as a detoxicating agent and in the treatment of various hepatic disorders[4-7] has been found to possess radioprotective effects in the peripheral blood of irradiated mice[8].

It has been established that cell membrane constituents, which are concerned with the maintenance of membrane integrity, are affected due to ionising radiation[9]. Reduced glutathione (GSH), which is the most abundant non-protein thiol of mammalian cells[10], helps to preserve the integrity of cell membranes[11]. It has also been reported that cellular glutathione is the key to the oxygen effect in radiation damage[12]. Increase of cellular glutathione is a new hypothesis of the radioprotective mechanism[13]. Hence, it is of great significance to study reduced glutathione (GSH) in the tissue of irradiated animals. The present study reports the protective role of Liv.52 and tissue-reduced glutathione in animals exposed to radiation.

MATERIALS AND METHODS

Adult female Sprague Dawley rats were exposed to whole body gamma radiation of 4 Gy and 8 Gy, at the dose rate of 8 cGy from a Cobalt-60 source (Model G 2000, Atomic Energy of Canada Limited, Ottawa, Canada). Prior to radiation exposure, Liv.52, 1 ml per rat per day, was given for 15 days. Rats of the control group did not receive any radiation and the drug control group of rats received only the drug. All the rats were kept at room temperature. Food and drinking water were supplied *ad libitum*. Experiments were carried out on the third day after radiation exposure, to assess better the role of Liv.52 on the repair and recovery mechanism. Experimental rats along with control ones were anaesthetised with ether, and their blood, spleen, liver and kidney were analysed for reduced glutathione by the colorometric method[14]. The results are shown in Tables 1 to 3.

RESULTS AND DISCUSSION

The figures in Table 1 exhibit appreciable decreases in spleen weight to body weight ratios in both groups of radiation-exposed rats. These are in agreement with other previous findings in rats and mice[15,16]. Animals fed with Liv.52 and exposed to sub lethal doses of radiation exhibited spleen weight to body weight ratios similar to those in the control animals, denoting the beneficial effects of Liv.52. In contrast, rats of the lethal group and fed with Liv.52 did not reveal any beneficial effects. The spleen is normally considered as the most radiosensitive organ[17]. Hence, restoration to normal of spleen weight to body weight ratios confirms the definite beneficial effects of Liv.52 against damage due to radiation.

Table 1: Effect of feeding Liv.52 on the spleen weight to body weight ratio in irradiated rats (Values are Mean ± SE of 6 rats)

Group		Spleen wt./ Body wt. x 10^{-3}
Control		2.966 ± 0.43
Control (Drug)		2.820 ± 0.47
Experimental	4 Gy	1.835 ± 0.32*
	8 Gy	1.85 ± 0.36*
Experimental	4 Gy + Liv.52	3.1 ± 0.4
	8 Gy + Liv.52	1.62 ± 0.3*

Table 2 reveals appreciable decreases in reduced glutathione in the spleens of both groups of irradiated animals. This is in agreement with previous studied in rats[15]. Animals of the 4 Gy group fed Liv.52 did show slight increases in

Table 2: Effect of feeding Liv.52 on the reduced glutathione (GSH) contents in the spleens and livers of irradiated rats (Values are Mean ± SE of 6 rats)

Group		Spleen GSH mg/100 gm wet tissue	Liver GSH mg/100 gm wet tissue
Control		42.4 ± 2.07	165.3 ± 4.6
Control (Drug)		44.8 ± 1.0	159.0 ± 4.0
Experimental	4 Gy	23.8 ± 0.5*	139.1 ± 4.7**
	8 Gy	18.8 ± 1.3*	139.8 ± 5.1**
Experimental	4 Gy + Liv.52	27.4 ± 2.3*	165.5 ± 4.0*
	8 Gy + Liv.52	16.9 ± 2.1*	132.1 ± 4.7**

*p< 0.001 and **p<0.01.

spleen GSH though the values did near the control ones, but some beneficial effects are observed. But rats of the 8 Gy group fed Liv.52 did not exhibit any beneficial effect on spleen GSH.

Liver is the richest source of GSH, which is involved in protecting SH dependent enzymes[18] and radio-resistance[19]. Table 2 denotes significant decreases in liver GSH in both the experimental groups. But animals of the 4 Gy group fed with Liv.52 showed liver GSH values similar to those in the control animals, thus exhibiting the beneficial effects of Liv.52. On the other hand, liver GSH of the 8 Gy group of animals also fed with Liv.52 did not reveal any such effects.

Table 3: Effect of feeding Liv.52 on the reduced glutathione (GSH) contents in the kidneys and blood of irradiated rats
(Values are Mean ± SE of 6 rats)

Group		Kidney GSH mg/100 gm wet tissue	Blood GSH mg/100 ml blood
Control		10.6 ± 0.35	18.7 ± 1.6
Control (Drug)		11.0 ± 0.5	18.1 ± 1.64
Experimental	4 Gy	21.3 ± 3.6*	15.1 ± 0.9**
	8 Gy	19.2 ± 3.0*	16.3 ± 0.6**
Experimental	4 Gy + Liv.52	21.0 ± 2.4*	17.3 ± 0.47
	8 Gy + Liv.52	11.6 ± 1.8	18.6 ± 0.8

*p< 0.001 and **p<0.05.

Table 3 shows that reduced glutathione values in the kidney in radiation-exposed animals were enhanced compared to the control values. Animals of the 4 Gy group fed with Liv.52 did not reveal any protective action. In contrast, beneficial effects were exhibited in the lethally exposed (8 Gy) rats fed with Liv.52. The reason for the above anomalous results is not understood at present and requires further study.

The same table reveals decreases in blood GSH values on the third post-exposure day in both groups of experimental rats, which is in agreement with previous findings[20]. The possible reason could be attributed to increased mobilization of GSH for essential metabolic functions, since much evidence has accumulated on the importance of GSH as an intracellular radioprotector[21]. Irradiated animals of both groups fed with Liv.52 showed blood GSH values similar to control rats, which again points to the radioprotective effects of Liv.52.

It has been stated that GSH may be involved in enzymatic processes of radiation-induced DNA damage[22,24]. The indigenous remedy Liv.52 is being used by clinicians for detoxicating purposes and liver disorders[5-8]. Recently it has been found that Liv.52 is beneficial as a prophylactic remedy against beryllium toxicity[25]. The present study reveals the radioprotective effects of Liv.52 in relation to tissue GSH against sub-lethal does of radiation exposure. The exact mechanism remains to be elucidated.

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