[*Current Science* (1995): (69), 7, 601]

Liv.52 Pretreatment Inhibits the Radiation-induced Lipid Peroxidation in Mouse Liver

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ABSTRACT
The effect of Liv.52 on mice liver mitochondrial lipid peroxidation was measured in terms of malanoldehyde (MDA) generation after exposure to 3.0 Gy of gamma radiation at ¼, ½, 1, 2, 4, 8, 24 and 48 h post-irradiation. The lipid peroxidation level increased significantly from ¼ h and reached a peak level at 4 h post-irradiation, and gradually declined thereafter in the irradiated control group. Liv.52 treatment before irradiation inhibited the formation of lipid peroxidation significantly at all the post-irradiation sampling time.

Lipid peroxidation is initiated by reactive oxygen species (ROS) in aerobic living organisms. Superoxide radical (O_2) and hydrogen peroxide (H_2O_2) are generated during metabolism, oxidation and irradiation, and they in turn are converted to hydroxyl free radical (OH). The hydrogen atom in unsaturated fatty acids can be abstracted by the OH radical following the formation of lipid alkyl radical (R+), which initiates the chain reaction of lipid peroxidation in an aerobic condition.

Lipid peroxidation alters the fluidity of biological membranes and then causes cell degradation, affecting the biological defence mechanism. The product of lipid peroxidation, such as malanoldehyde (MDA), damages the enzyme system and DNA[1]. Lipid peroxidation has been used as an end point to study the action of oxidizing and free radical-producing agents as well as to investigate the effects of intracellular radical scavengers (i.e. as an overall measurement of oxidative injury to the cellular membranes). Lipid peroxidation is considered to be an important effect of ionizing radiation on biological membranes[2-4].

Radiation-induced lipid peroxidation has been reported to be caused by superoxide radicals[5]. However, later studies confirmed that the hydroxyl radical is the most active species involved in radiation-induced lipid peroxidation[3,6-8].

Natural products such as herbal medicines have only recently begun to receive some attention as possible modifiers of the radiation response. Liv.52, a non-toxic herbal preparation composed of *Capparis spinosa, Cichorium intybus, Solanum nigrum, Cassia occidentalis, Terminalia arjuna, Achillea millefolium* and *Tamarix gallica*, has been reported to be clinically effective in treating hepatotoxicity and a wide range of hepatic disorders[9-11]. The radioprotective potential of Liv.52 was demonstrated for the first time in mice[12]. Recently, it has been reported that prior administration of Liv.52 protected mouse bone marrow against radiation-induced chromosome damage[13,14]. A significant decrease in lipid peroxidation, followed by an increase in the tocopherol level in Liv.52 fed groups, was observed[15]. Saxena and Garg[16] also reported that the feeding of Liv.52 to rats gave a marked protection against the increase in lipid peroxidation and phospholipid contents of microsomal fraction.

Therefore, it was desired to obtain an insight into the effect of Liv.52 on lipid peroxidation in mouse liver exposed to 3 Gy of ^{60}Co gamma radiation.

Six to eight-week-old male Swiss albino mice weighing 25-30 g were selected from an inbred colony maintained under controlled conditions of temperature (23±2ºC), humidity (50±5%) and light (10 and 14h of light and dark, respectively). The animals were given sterile food prepared as per the standard formulation (wheat 70% Bengal gram 20%, fish meal 5%, yeast powder 4%, sesame oil 0.75% and shark liver oil 0.25%) in the laboratory and water *ad libitum*. Throughout the experiment 5-6 animals were housed in a sterile polypropylene cage containing sterile paddy husk (procured locally) as bedding material. The irradiation was carried out using telecobalt therapy source (Gammatron, siemens, Germany).

A batch of 10 immobilized animals (achieved by inserting cotton plugs in the restrainer) was kept prone in specially designed well-ventilated acrylic box during exposure. The irradiation was carried out at a dose rate of 0.88 Gy/min. The source-to-animal distance was 60-65 cm. The exposure area of 20 x 20 cm was also kept constant. The dosimetry calculation were done by Dr. J.G.R. Solomon, Department of Radiotherapy and Oncology, K.M.C. Manipal, Karnataka.

As the Liv.52 powder is insoluble in water a dose of 500 mg/kg body wt of Liv.52 powder was suspended in 10 ml of 5% dextrose solution containing gum acacia. 0.01 ml/g body weight of Liv.52 or 5% dextrose was administered orally once a day for 7 days using 22-guage oral feeding needles (Popper & Sons Inc., New York, USA).

The animals were divided into two groups. One group of animals was fed with 5% dextrose solution and served as the control group, while the other group received 500 mg/kg body wt of Liv.52 powder in 5% dextrose solution. After 1 hr of administration on day 7, the animals of both groups were exposed to 3 Gy of ^{60}Co gamma radiation. Subsequently, a few animals were also treated with 5% dextrose and Liv.52 but without irradiation, for the sake of comparison. Usually, five animals were used for each time period for each group studied. A total of 90 animals were used for the whole experiment.

The animals from both groups were anaesthetized by administration of overdose of diethyl ether anesthesia and were killed at ¼, ½, 1, 2, 4, 8, 24 and 48h post-irradiation. The livers of the animals were perfused with isotonic saline and removed as weighed. A 10% liver homogenate was prepared in 0.2M Tris-HCl buffer (pH 7.4) using Yamato LSG homogenizer LH-21 (Japan). The lipid peroxidation in liver was estimated by the method Konings and Drijver[17]. Briefly, the homogenate was centrifuged at 50,000 x g (Sorvall, RC 5B, USA) for 10 min at 4ºC and the pellet was discarded. The resultant supernatant was centrifuged again for 60 min at 4ºC to obtain mitochondrial pellet. A homogeneous suspension of mitochondria was prepared in 0.2M Tris-HCl buffer (pH 7.4). To this suspension 150 mM KCl, 0.3mM ascorbic acid and 0.2 M Tris-HCl buffer (pH 7.4) were added, and it was incubated at 37ºC for 1 hr. After incubation, trichoroacetic acid was added followed by the addition of 0.67% TBA. The test tubes were kept in boiling water bath for 15 min. The tubes were removed from the water bath and allowed to cool. The tubes were centrifuged and the resultant supernatant was filtered through Whatman filter paper. The absorbency of the sample was read against the blank at A_{max} 532nm in a UV-VIS recording spectrophotometer (Shimadzu UV-VIS 260, Japan). Mitochondrial protein concentration was determined by the method of Bradford[18]. The data were analysed statistically by Student's 't' test on an IBM/PC.

The lipid peroxidation was expressed in terms of nmol MDA/mg protein. Liv.52 treatment did not alter the level of lipid peroxidation (Table 1).

The exposure of animals to 3.0 Gy ^{60}Co gamma radiation resulted in a significant increase in lipid peroxidation at all the time periods studied compared to the sham-irradiated control. The activity of lipid peroxidation started increasing at ¼ h and reached a peak level by 4 h post-irradiation (Figure 1). This increase was approximately 1.88, 2.4, 2.0, 2.6 and 3 folds higher for ¼, ½, 1, 2 and 4 h post-exposure, respectively compared to the sham-irradiated animals. An abrupt decline in the lipid peroxidation level was observed at 8 h, which was approximately half of the preceding sampling time. However, lipid peroxidation did not attain normal level even by 48 h post-irradiation (Table 1).

Table 1: Effect of Liv.52 on the radiation-induced lipid peroxidation in mouse liver

Time (min/h)	nmol MDA/mg protein (mean ± SEM)	
	3.0 Gy	Liv.52 + 3.0 Gy
¼	3.43 ± 0.22[a]	2.62 ± 0.09[d]
½	4.62 ± 0.20[a]	3.62 ± 0.14[d]
1	3.89 ± 0.13[a]	2.92 ± 0.23[e]
2	5.16 ±0.10[a]	2.89 ± 0.04[a]
4	6.11 ± 0.23[a]	2.93 ± 0.31[e]
8	3.99 ± 0.10[a]	2.92 ± 0.09[c]
24	3.15 ± 0.06[b]	2.86 ± 0.06[f]
48	2.69 ± 0.09[d]	2.13 ± 0.10[d]

Normal control value = 1.95 ± 0.06.
Liv.52 control value = 2.20 ± 0.09.
Normal compared with irradiation group column 1 symbols on right side.
The difference between normal control and Liv.52 control was non-significant.
Liv.52 alone compared with Liv.52 + irradiation group column 2 symbols on left side.
Irradiated groups compared with Liv.52 + irradiated groups
[a] $p<0.0001$, [b] $p<0.0002$, [c] $p<0.0003$, [d] $p<0.001$, [e] $p<0.002$, [f] $p<0.01$.

In the Liv.52 + irradiated group a significant increase in lipid peroxidation compared to sham-irradiated animals was observed only at ½ h post-irradiation. However, Liv.52 administration before irradiation inhibited lipid peroxidation significantly at all the time periods studied. It was 23.61, 21.64, 24.93, 43.99, 52.04, 26.81, 9.21 and 20.81% lower for ¼, ½, 2, 4, 8, 24 and 48 hr post-irradiation, respectively, compared to the concurrent irradiated controls.

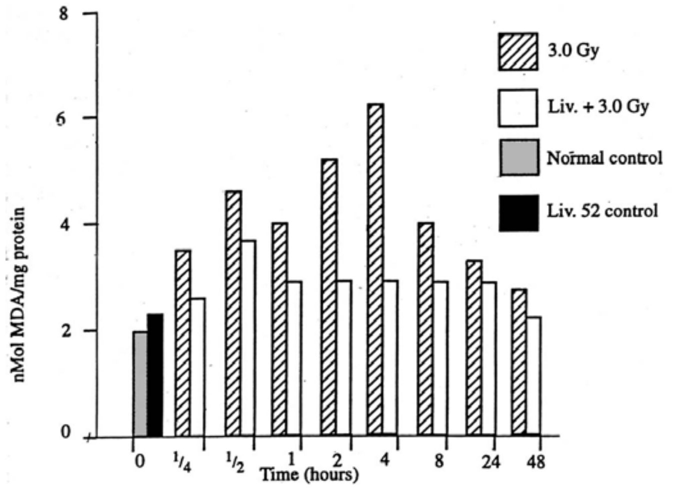

Figure 1: The lipid peroxidation level at different post-irradiation time periods in mouse liver treated or not with Liv.52 before exposure to 3.0 Gy gamma radiations.

Lipid peroxidation induced by radiation is known to be due to the attack of free radicals on the fatty acid component of membrane lipids[7,19,20]. Mitochondrial membranes contain high percentage of polyunsaturated fatty acids and are, therefore, susceptible to free-radical attack[21]. Damage of mitochondrial structures and enhanced lipid peroxidation by ionizing radiation has been reported[22,23]. Lipid peroxidation also results in mitochondrial swelling and disintegration[24].

Irradiation of animals to 3.0 Gy of gamma radiation resulted in a significant increase in lipid peroxidation level at all the time intervals compared to the sham-irradiated group. The lipid peroxidation level started increasing at ¼ h and reached a peak level at 4 h after exposure. This increase was approximately 1.88, 2.4, 2, 2.6 and 3 folds higher for ¼, ½, 1, 2 and 4 h post irradiation respectively, compared to the sham-irradiated group. The glutathione depletion was maximum at 15 and 45 min after exposure to 3.0 Gy of gamma radiation (unpublished data). These findings are in good agreement with the earlier findings, where maximum depletion of GSH at 15-30 min and the highest lipid peroxidation 4h after 3.0 Gy of gamma radiation have been reported[25]. Similarly, a maximum depletion in GSH at 15-30 min and lipid peroxidation as well as liver necrosis at 2-4 hr after administration of allyl alcohol in mice have been observed[26,27]. Lipid peroxidation has been suggested as one the main causes of radiation-induced membrane damage[2,3]. The lipid peroxidation has been found to increase with increase in radiation dose in rat liver microsomes and in mice erythrocytes[28,29].

Several investigators reported that lipid peroxidation in liver homogenate will start as soon as the supply of endogenous GSH is exhausted, and that the addition of GSH promptly stops further peroxidation[30,31]. It has been reported that the addition of GSH markedly inhibits lipid peroxidation in isolated rat liver microsomes[32].

The administration of Liv.52 before irradiation inhibited the lipid peroxidation significantly as compared to the irradiated control. These results are in accordance with the findings of other investigators, who reported a significant decrease in lipid peroxidation, followed by an increase in the tocopherol level in Liv.52-treated group compared to the CCl_4 treated group[15,16]. Similarly, calmodulin antagonists have been found to inhibit the radiation-induced lipid peroxidation[28]. A depletion in lipid peroxidation was observed in mice erythrocytes treated with MPG before irradiation[29]. WR-1065 and GSH were found to be effective inhibitors of mitochondrial lipid peroxidation induced by HDP/Fe/NADH or by ADP/Fe/ascorbate[33].

Saxena *et al.*, reported an increase in the α-tocopherol level in Liv.52 treated rats. In the present study the radiation-induced lipid peroxidation was inhibit significantly by Liv.52 treatment, and this may be due to the elevation of α-tocopherol.

Vitamin E (α-tocopherol) and glutathione are involved in the termination mechanism of lipid peroxidation. Vitamin E donates the hydrogen atom from the chromanol ring hydroxyl group to the lipid radical, generating a tocoperoxy radical. The rate of reaction of vitamin E with lipid hydroperoxy radicals is much faster (-10^4) than the rate of reaction of lipid hydroperoxy radicals with neighbouring polyunsaturated fatty acids, thus preventing further damage. Therefore, vitamin E acts as an effective chain breaker, curtailing progression of the lipid peroxidation. However, vitamin E is very low for serving as a lipid radical scavenger, and its concentration in the membrane would rapidly become vanishingly small to reduce the tocoperoxy radical back to tocopherol. Liv.52 may provide α-tocopherol to reduce the tocoperoxy radicals back to tocopherol, thereby inhibiting the formation of lipid peroxidation.

Therefore, it is reasonable to assume that radioprotective activity of Liv.52 may be due to the inhibition of lipid peroxidation by increasing the level of α-tocopherol and glutathione.

ACKNOWLEGEMENT
We are thankful to Prof. K. Koteshwar Rao, Head, Department of Radiotherapy and oncology, Kasturba Medical College, Manipal for providing the necessary irradiation facilities during the course of this study. Financial assistance in the form of senior fellowship from ICMR, New Delhi to NCG is thankfully acknowledged.

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