Inhibition of clastogenic effect of radiation by Liv.52 in the bone marrow of mice

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SUMMARY

The frequency of micronuclei in bone marrow cells of mice treated or not with Liv.52 and then exposed to 4.5 Gy of γ-radiation was evaluated from 6 h to 14 days post irradiation. The frequency of micronuclei increased from 6 h to 24 h post irradiation in both irradiated groups and declined thereafter, the frequency of micronuclei remaining significantly lower in the Liv.52-treated group. These data demonstrate that Liv.52 protects the bone marrow of mice against radiation injury.

Four decades ago Patt *et al*. (1949) for the first time reported that treatment of rats and mice with cysteine before exposure to X-rays resulted in the protection of these animals against radiation-induced sickness and mortality. Since then several other synthetic compounds have been analyzed for their radioprotective ability. However, their practical applicability in human beings has always been limited owing to their high toxicity. Liv.52, a non-toxic herbal preparation composed of *Capparis spinosa, Cichorium intybus, Solanum nigrum, Cassia occidentalis, Terminalia arjuna, Achillea millefolium* and *Tamarix gallica*, is reported to be clinically active in hepatotoxicity and a wide range of hepatic disorders (Mathur, 1957; Sule *et al*., 1968; Deshpande, 1971). This herbal mixture has also been reported to protect mice against radiation sickness, mortality, dermatitis and spleen injury (Saini *et al.,* 1984).

Therefore, the present study was undertaken to assess the radioprotective effect of Liv.52 on the bone marrow of mice exposed to a sublethal dose of γ-radiation by studying the frequency of micro-nuclei.

MATERIALS AND METHODS

Male Swiss albino mice, 6-8 weeks old and weighing 26.9 ± 2.54 g, were selected from an inbred colony maintained under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%) and light (10 and 14 h of light and dark). The animals were given sterile food prepared in the laboratory as per the standard formulation and water *ad libitum*. Throughout the experiments 5-6 animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding.

One group of animals was fed orally with a 5% dextrose solution once a day for 7 days before irradiation and served as the control group, while the other mice received 500 mg/kg b.wt. of Liv.52 powder (supplied by The Himalaya Drug Co., Bombay, India) in 5% dextrose solution in a similar fashion. After 1 hour of administration on day 7 the animals of both groups were exposed to 4.5 Gy of γ-radiation (Gammatron tele cobalt therapy source, Siemens, F.R.G.) in specially designed well-ventilated plastic boxes. The animals were irradiated in groups of 10 at a dose rate of 0.88 Gy/min, at a distance of 60 cm from the source (dosimetry was done by Dr. J.G.R. Solomon, Department of Radiotherapy and Oncology, K.M.C. Manipal, Karnataka, India). Subsequently a few animals were also treated with 5% dextrose and Liv.52 as above but without irradiation for comparison.

Animals of both groups were killed by cervical dislocation 6, 12, 24 h and 2, 3, 7 and 14 days post irradiation. The femora of the animals were dissected out and marrow was flushed out into 0.84% saline and the slides were prepared according to the method of van Beuningen *et al.* (1981). Briefly, the bone marrow cell suspension was centrifuged and kept in hypotonic saline (1:3 physiological saline and distilled water) for 10 minute. After centrifugation cells were resuspended in Carnoy's fixative and dropped over precleaned chilled slides kept in acetone for flame fixation.

The slides were stained in ethidium bromide (Sigma, Cat. No. E-8751) and micronuclei were counted under a fluorescent microscope. 2000 nucleated cells were scored for each point. The results are expressed as the percentage of cells with micronuclei and Student's 't' test was used for statistical evaluation. To test the significance of interaction between the treatments and times a 2-way ANOVA test was carried out according to the following method:

	d.f.	S.S.	M.S.
Between treatments (A)	$q-1$	$\dfrac{1}{c}\dfrac{1}{p}\sum_{1}^{q}y_{i.}^{2}-\dfrac{1}{cpq}y..^{2}$	V_A
Between time (B)	$p-1$	$\dfrac{1}{c}\dfrac{1}{q}\sum_{1}^{p}y_{.j}^{2}-\dfrac{1}{cpq}y..^{2}$	V_B
Interaction (A×B)	$(p-1)\times(q-1)$	* (by subtraction)	V_{Ab}
Between pq cells	$pq-1$	$\dfrac{1}{c}\sum\sum y_{ij}^{2}-\dfrac{1}{cpq}y..^{2}$	V
Residual	$pq(c-1)$	* (by subtraction)	V_e
Total	$pqc-1$	$\sum\sum\sum y_{ijk}^{2}-\dfrac{1}{cpq}y..^{2}$	

RESULTS

The frequency of micronuclei increased beginning at 6 h with a peak value of 4.71% observed 24 h post irradiation in the irradiated group. The frequency then declined abruptly at day 2 and continued to decline throughout the remainder of the post-irradiation period. However, the frequency did not reach normal levels even 14 days post irradiation.

When Liv.52 was given before irradiation the pattern of micronucleus formation remained similar to that in the irradiated control group, but the frequency of micronuclei was much reduced at all post-irradiation time intervals studied. At 24 h and day 2 post irradiation the difference between the 2 groups was highly significant, with the frequency of micronuclei in the Liv.52-treated group some 4 times lower than that in the irradiated group. In the drug-treated group the number of micronuclei declined to almost normal levels 3 days post irradiation (Table 1).

Table 1: The frequency of micronuclei in the bone marrow of mice exposed to 4.5 Gy and Liv.52 + 4.5 Gy of γ-radiation

Post-irradiation time	Frequency of micronuclei ± SEM		$p<$
	4.5 Gy alone	Liv.52 + 4.5 Gy	
6 h	1.10 ± 0.041 (5)	0.47 ± 0.046 (5)	0.001
12 h	1.30 ± 0.047 (5)	0.65 ± 0.020 (4)	0.001
24 h	4.71 ± 0.230 (5)	1.05 ± 0.065 (5)	0.001
2 days	2.36 ± 0.048 (5)	0.58 ± 0.071 (5)	0.001
3 days	0.49 ± 0.095 (5)	0.24 ± 0.053 (5)	0.100
7 days	0.40 ± 0.037 (5)	0.23 ± 0.020 (5)	0.005
14 days	0.36 ± 0.028 (4)	0.26 ± 0.018 (5)	0.025

Normal value: 0.22 ± 0.028 (5)
Liv.52 alone: 0.23 ± 0.020 (5)
Number in parentheses indicates number of animals used.

No significant difference between the frequency of micronuclei in the bone marrow of mice was observed between the normal and Liv.52 (drug alone)-treated animals. A 2-way ANOVA was carried out between the time and treatments. It was found that there was a significant interaction between the time and treatment and also among the 2 treatments themselves (Table 2).

DISCUSSION

The present findings that the frequency of micronuclei increases from 6 h to 24 h after exposure to 4.5 Gy are in accordance with the findings of Heddle (1973) who reported an increased frequency with considerable variability in the number of micronuclei from 12 h to 3 days after exposure of Swiss Webster mice to 2.3 Gy of X-rays. Jensen and Ramel (1978) have also reported an increased number of micronuclei from 6 to 24 h after exposure of mice to 2 Gy of x-rays. Similarly Cole et al. (1981) observed an increased frequency of micronuclei from 6 h to 24 h in the

Table 2: Two-way ANOVA table for the frequency of micronuclei in the bone marrow of mice after exposure to 4.5 Gy γ-radiation

Source of variation	d.f.	S.S.	M.S.		F
Between treatments (A)	1	19.19	19.19	V_A	$\dfrac{V_A}{V_e} = 148.79$ $p < 0.001$
Between times (B)	6	50.72	8.453	V_B	$\dfrac{V_B}{V_e} = 65.54$ $p < 0.001$
Interaction (A × B)	6	18.8814	3.1469	V_{AB}	$\dfrac{V_{AB}}{V_e} = 24.4$ $p < 0.001$
Between 14 cells	13	88.7914	6.83	V	
Residual	54	6.9644	0.12897	V_e	
Total	67	95.7558			

bone marrow and fetal liver of mice exposed to 0.5 Gy of γ-radiation. Thereafter, the frequency of micronuclei starts decreasing from 30 h post irradiation. These results are in agreement with the present findings where a peak value of micronuclei was observed at 24 h, followed by a decline in frequency over the following 14 days post irradiation. Uma Devi and Bisht (1987) have also reported somewhat similar results in the bone marrow of mice exposed to 3.0 Gy of γ-radiation.

Micronuclei start appearing at the end of the first mitotic division after treatment. However, additional micronuclei can be formed in the next few divisions and accumulate for a certain time (Heddle, 1973). The decrease in the frequency of micronuclei after day 2 may be due to the delaying effect of radiation on the progression of the cell cycle as well as a dilution of the micro-nucleated cells with normal cells and the loss of aberrant cells.

The administration of Liv.52 prior to irradiation resulted in a dramatic decrease in the induced frequency of micronuclei at every sample time. At 24 h and day 2-post irradiation, the difference between the 2 groups is approximately 4-fold. This decrease in the frequency of micronuclei induced by Liv.52 clearly demonstrates that this drug is able to protect the cells from the clastogenic effect of radiation.

Liv.52 may combine with free radicals as soon as they are formed in the cells, thus saving the DNA molecule from some of the damage inflicted by radiation. Depletion of intracellular glutathione (GSH) levels has been reported to be one of the causes of radiation-induced damage, while increased levels of intracellular GSH are responsible for the radio-protective action (Revesz *et al.,* 1963). A similar mechanism of action may be attributed to the radio-protective action of Liv.52, which has been reported to restore the intracellular GSH level to normal in rats treated with Liv.52 before exposure to 4.0 Gy of γ-radiation (Sarkar *et al.*, 1989).

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