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Radioresponse of Leucocytes in Peripheral Blood of Mice Against Gamma Irradiation and their Protection by Liv.52

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ABSTRACT

Male Swiss albino mice were exposed to 1.20 and 3.60 Gy of whole-body gamma irradiation in the presence (experimental) and absence (control) of a herbomineral formulation of Liv.52. Quantitative variations in the number of total leukocytes count (TLC), lymphocytes and neutrophils were scored in peripheral blood at various autopsy intervals between 0.5 hrs to 28 days. At 1.20 Gy dose, depression in TLC was noticed till day 1, whereas in higher doses until day 5 with a sharpness in first 24 hrs. Prior administration of Liv.52 significantly prevented the deduction in leukocytes count and initiated recovery. Lymphocytes also declined sharply in both radiation doses upto day 1 and after that showed a recovery towards normal count on day 28 in experimental animals at lower dose. The behavior of neutrophils was reciprocal to that of lymphocytes as they showed a rise till day 1 followed by a gradual decline upto day 5 leading to a second peak on day 14 in control as well as experimental groups with both the irradiation doses. Liv.52 helped in restoring normal values of such cells in 1.20 Gy at last autopsy intervals.

Key words: Differential leukocytes count, Mice, Liv.52, Gamma Rays, Radioprotection

INTRODUCTION

Radiation-induced hematological alterations have been extensively studied. Lymphocytes are among the most radiosensitive cells in the living organisms. They are involved in immunological responses and are of immense interest to researchers and clinicians, because of their extreme sensitivity to ionizing radiation[5].

Extensive research has been carried out in recent years to find a suitable chemical radioprotective agent, which can be administered safely before radiation exposure. Several chemical compounds like cystein[14], cysteamin[1], 2-mercaptopropionyl glycine[18] and WR-2721[19] have been known to afford a high degree of protection against radiation in mammals, but most of them were found toxic at their optimum protective dose level. Liv.52 was revealed to be a non-toxic, hepatoprotective as well as radioprotective drug[3,9,12,15].

These findings evoke further thrust to investigate the protective efficacy of this drug against radiation-induced quantitative variations in differential leucocytes count of peripheral blood in mice.

MATERIALS AND METHODS

Young adult male Swiss albino mice of 6-8 weeks age weighing about 20 ± 2 gms were selected from a closely bred colony maintained on standard mice feed (procured from Lipton India Limited, Chandigarh) and water *ad libitum*. The selected mice were divided in two different groups with 42 animals in each. One group of animals was orally given a 5% dextrose solution once a day for 7

days before irradiation to serve as control while the other group received 500 mg/kg body weight of Liv.52 powder (supplied by The Himalaya Drug Co., Bangalore) dissolved in 5% dextrose solution in a similar manner to serve as experimental group. One hour after administration on day 7, the animals of both control and experimental groups were exposed to two different sublethal doses (1.20 Gy and 3.60 Gy) of gamma radiation.

The blood samples of 6 animals were collected from each group at various post-exposure intervals between 0.5 to 28 days from orbital sinus using heparinized micro haematocrit capillaries for quantitative leucocytic variations. The total leucocytes (TLC), lymphocytes and neutrophils counts were performed by adopting routine procedures. Student's 't' test was performed for statistical analysis and results were expressed in mean ± standard error.

RESULTS

The results obtained from the present investigation are depicted in the Table. The leucocytes in general showed an initial decline after irradiation in both the dose level used. The depletion in count was more rapid during first 24 hours, thenceforth it increased slowly till day 28 in both control and experimental groups at 1.20 Gy dose. The normal leucocyte count could not be restored in both the groups even up to the last autopsy interval. However, depression was less marked in drug treated animals and a significant protection was observed at later intervals (Table).

At 3.60 Gy dose, the diminution in number of leucocytes was observed till day 5 and thereafter boosted but remained below normal in both control and experimental groups. The count was significantly higher at later intervals in Liv.52-treated animals.

The variations in lymphocytes number showed a behaviour parallel to total leucocyte count. In 1.20 Gy group, the percentage of lymphocyte declined in both the groups till day 1 after which it increased slightly until day 28 and attained normal value in Liv.52-treated animals only at the last autopsy interval. A significant protection in lymphocytes was noticed at day 5 (Table).

At 3.60 Gy, the lymphocyte count depleted till day 1 but the drop was as high as 50 percent of normal (Table). The percentage of lymphocytes showed an increase but the normal count could not be restored till day 28 in both control and experimental animals. A significant protection in lymphocytes was registered on days 5 and 28 with Liv.52.

The neutrophils exhibited a reciprocal bearing as compared to lymphocytes. The latter showed a sharp decline in first 24 hours followed by a slight increased, but the former demonstrated a steep rise during the first 24 hours post-irradiation and then a gradual decline in both control and experimental groups of animals.

In 1.20 Gy dose, the percentage of neutrophil increased till day 1, thenceforth decreased up to day 5 even reaching below normal. In animals treated with Liv.52 prior to irradiation, the number was restored to normal by the last autopsy interval and a significant difference was observed at day 7 and 14. In 3.60 Gy group, the pattern of neutrophilic variation was similar to the lower dose but not in the Liv.52-treated animals. However, a significant difference in neutrophilic count between the control and the treated groups was noticed on day 28.

Table: Showing changes in leucocyte count in peripheral blood of mice in irradiated (Control) and Liv.52 + irradiated (Treated) mice at different autopsy intervals

Irradiation dose (in Gy)	Type of leucocyte	Mode of Treatment	Post-irradiation time (in days)						
			0.5	1	2	5	7	14	28
1.20	TLC	C	3000 ±63.33	2616 ±43.2	2690 ±70.41	3450 ±82.91	4108 ±59.53	4856 ±53.75	5216 ±53.75
		E	3100 ±47.50	2700 ±47.08	2790 ±53.33	3675 ±92.08	4316 ±67.91 $p<0.05$	5016 ±28.45 $p<0.05$	5416 ±67.91 $p<0.05$
	Lymphocytes	C	54.16 ±2.85	42.30 ±2.21	45.00 ±2.41	49.00 ±2.21	53.33 ±2.06	57.83 ±3.80	62.83 ±2.60
		E	56.00 ±0.89	45.16 ±2.75	48.16 ±3.00	52.00 ±2.41 $p<0.05$	55.00 ±2.26	60.66 ±1.96	66.00 ±2.67
	Neutrophils	C	26.00 ±2.09	36.80 ±2.94	30.00 ±1.41	23.16 ±1.16	25.00 ±1.41	30.16 ±1.32	27.00 ±2.67
		E	25.00 ±0.89	36.00 ±2.67	28.00 ±2.28	24.00 ±1.26	27.00 ±1.26 $p<0.05$	28.00 ±1.41 $p<0.05$	24.10 ±2.75
3.60	TLC	C	2633 ±66.66	2200 ±58.75	2000 ±43.70	1960 ±76.25	2690 ±69.72	3935 ±80.13	4983 ±34.00
		E	2725 ±55.41	2300 ±45.83	2133 ±61.25	2150 ±70.12	2950 ±77.08 $p<0.05$	4208 ±77.50 $p<0.05$	5107 ±42.08 $p<0.05$
	Lymphocytes	C	43.00 ±2.00	33.00 ±0.89	37.16 ±1.32	40.00 ±1.26	41.16 ±1.16	44.80 ±1.32	49.00 ±1.09
		E	44.50 ±1.41	34.00 ±1.00	38.00 ±0.89	42.00 ±1.37 $p<0.05$	43.00 ±1.78	47.00 ±2.28	51.80 ±1.83 $p<0.01$
	Neutrophils	C	31.00 ±2.00	40.16 ±1.72	31.00 ±1.41	22.00 ±2.00	26.00 ±1.41	32.00 ±1.78	30.16 ±1.32
		E	29.8 ±1.50	39.33 ±0.81	32.01 ±1.41	24.00 ±1.41	25.00 ±1.54	30.00 ±1.78	28.00 ±1.89 $p<0.05$

The normal values in sham irradiated mice are: (a) – TLC = 5800 ± 141.42/cumm; (b) - Lymphocyte = 66.0 ± 4%; (c) – Neutrophils = 24.20 ± 1.48%; C = only irradiated (Control); E = Liv.52 + irradiated (Treated)

DISCUSSION

In the present investigation, a drastic reduction in leucocyte count after irradiation is in agreement with the findings of earlier workers[2,5,6]. The leucocyte number showed a drastic decline during the first 24 hours. This initial phase of rapid decrease is due to direct killing of lymphocytes while the slower fall at later intervals in 3.60 Gy is due to the reduced number of new lymphocytes entering the peripheral blood. The peripheral lymphocytes exhibited a maximum depletion at day 1 in the current investigation elucidating an early cell killing effect of radiations on this cell type, which is the most radiosensitive in peripheral blood. This observation is in accordance with the findings of Edmondson[4] and Kumar[10].

The change in neutrophilic count was inverse to that of lymphocytes. It increased during first 24 hours, which can be attributed to “abortive” rise in the neutrophils after irradiation[7,13]. A second

peak of neutrophilic elevation was noted on day 14 after irradiation. Jacobson *et al.*[8], suggested that the first peak can be possibly due to hastening of maturation in bone marrow and for the second peak a mobilization phenomenon in response to radiation-induced tissue injury can be held responsible.

In Liv.52-treated animal groups, the total leucocyte count and lymphocyte percentage were higher than the control group. A similar protection in lymphocyte count has been observed while using cysteine[14] and MPG[11,16] in mice prior to irradiation. Jagetia[9] has demonstrated that Liv.52 provides radio resistance to the bone marrow cells, which possibly accounts for increased number of lymphocytes and hence also for total leucocyte count in drug-treated animals. It is evident that Liv.52 diminishes the direct cell killing against gamma radiation by increasing the cellular glutathione (GSH) level[17] and restores an early recovery of lymphocytes in drug treated animals.

It may also be postulated that Liv.52 may increase the amount of excision repair in cells exposed to gamma rays. Biological factors such as repair capacity or structural alterations in the nucleus may be affected by such substance and could be complimentary or additive to the action of free radicals scavenging for protection from radiation-induced damage.

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