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RADIOPROTECTION OF PERIPHERAL BLOOD CELLS WITH A COMBINATION OF HYDROXYTRYPTOPHAN AND A THIOL COMPOUND IN MICE

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Abstract

Radiation induced changes in peripheral blood cells were studied in male Swiss albino mice after 4 Gy and 10 Gy whole body gamma irradiation with and without pretreatment with 2-aminoethylisothiuronium bromide hydrobromide (AET) or with a combination of 5-hydroxy-L-tryptophan (5-HTP) and AET. Combination of 5-HTP with AET or AET alone in a high dose achieved excellent protection against both 4 Gy and 10 Gy whole body irradiation as regards peripheral blood cell counts.

Key words: Radiobiology; mice, chemical radioprotection, blood cells.

Aminoethylisothiuronium bromide hydrobromide (AET), cysteamine, cystamine, serotonin and S-2-(2-aminopropylamine)-ethylphosphorothioate (WR2721) are well known radioprotectors. Due to various phenomena, as toxic reactions, non-protection of some normal tissues (CNS) and protection of tumour tissue (10, 12–16), continued attempts to obtain effective drugs for protection against radiation are needed.

The bone marrow and the lymphocytes suffer extensive damage following whole body irradiation. Damage to bone marrow is known to be the main cause of death in animals following whole body doses of radiation between about 2 and 10 Gy (4).

In our laboratory, significant protection of peripheral erythrocytes by AET (200 mg/kg body weight) has been noted after 7.6 Gy gamma irradiation (9). The objective of the present study was to investigate whether additional radioprotection could be achieved by combination of a suboptimal dose of hydroxytryptophan (5-HTP) with a very small dose of AET.

Material and Methods

AET and 5-HTP were delivered from Sigma Chemicals, USA. Male Swiss albino mice (3–3 1/2 months old and weighing 26 ± 2 g) maintained on standard mice food and water ad libitum were selected from an inbred colony.

The animals were divided into experimental and control groups. In the experimental groups, each consisting of 10 animals, the animals were injected i.p. with distilled water or AET (200 mg/kg) or 5-HTP (100 mg/kg) combined with AET (20 mg/kg). Solutions were prepared in distilled water and the volume injected was 0.5 ml/25 g body weight. The animals in the control groups received an equal volume of distilled water. After 30 minutes, control and pretreated animals were exposed to 4 Gy or 10 Gy whole body gamma radiation, dose rate 4.7–4.5 Gy per minute from a ^{60}Co source in Gamma Cell '220' (Atomic Energy of Canada Ltd). Ten animals in each group were killed at 1, 3, 7, 10, 15 and 30 days after exposure. Blood erythrocytes (RBC) were counted with a hemocytometer. The total number of white blood cells (WBC) per mm^3 of blood was calculated from an average of 4 cell counts obtained from the suspensions. Differential count (DLC) was performed in 200 cells on Jenner-Giemsa stained smears. The method gave the number of surviving cells and not the number of killed cells.

Results and Discussion

After exposure to 4 Gy, the RBC decreased up to 3 days in the irradiated control groups, after which time it

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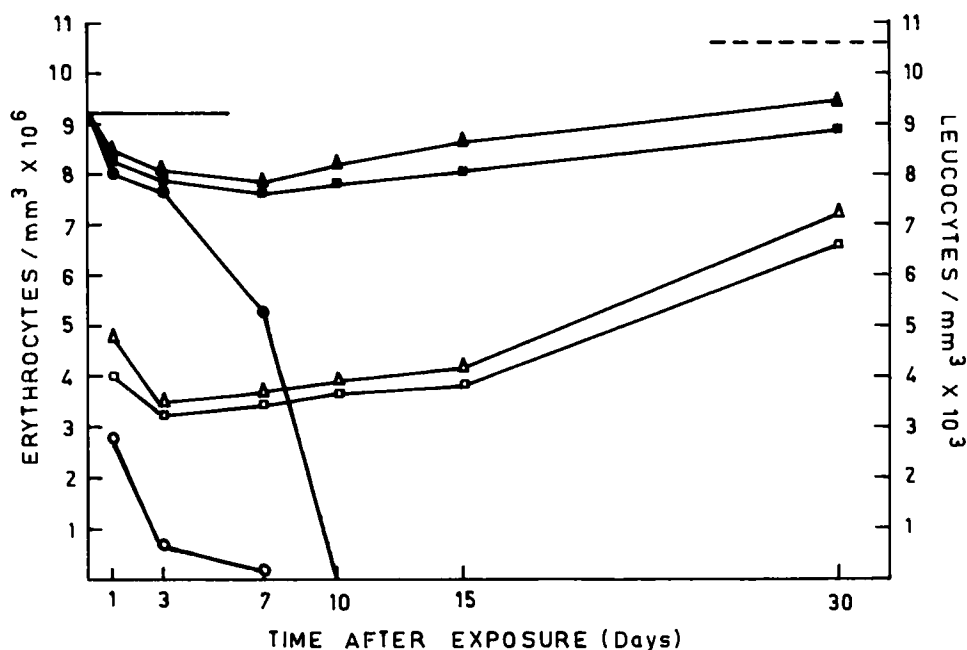


Fig. 1. The erythrocyte (RBC) and leucocyte (WBC) counts in mice after 10 Gy whole body gamma irradiation and the effect of radioprotectors. RBC: Irradiated controls (●—●), 5-HTP (100 mg/kg) and AET (20 mg/kg) (▲—▲), AET (200 mg/kg) (■—■),

normal (—). WBC: Irradiated control (○—○), 5-HTP (100 mg/kg) and AET (20 mg/kg) (△—△), AET (200 mg/kg) (□—□), normal (---).

Table 1

Protective effect of chemical radioprotectors on peripheral erythrocytes and leucocytes against 4 Gy whole body gamma irradiation. Normal RBC ($10^6/\text{mm}^3$) = 9.3 ± 1.8 and WBC ($10^3/\text{mm}^3$) = 11.0 ± 1.7 . Mean \pm SD of 10 animals

Group	Chemical protector		Days after irradiation						
			1	3	7	10	15	30	
1	Distilled water	RBC	8.1 \pm 1.4	7.4 \pm 0.6	7.8 \pm 0.8	8.3 \pm 0.6	8.4 \pm 0.6	8.4 \pm 0.4	
		WBC	4.0 \pm 0.5	2.2 \pm 0.5	5.4 \pm 0.3	5.5 \pm 0.6	6.1 \pm 1.5	8.3 \pm 1.2	
2	AET (200 mg/kg)	RBC	8.7 \pm 1.2	7.2 \pm 0.3	8.5 \pm 1.4	9.3 \pm 1.3	9.2 \pm 1.5	9.3 \pm 1.9	
		WBC	5.4 \pm 0.9	4.8 \pm 0.7	5.7 \pm 1.0	5.8 \pm 1.1	7.0 \pm 0.9	10.6 \pm 1.9 ^b	
3	5-HTP (100 mg/kg)+ AET (20 mg/kg)	RBC	9.0 \pm 1.8	8.5 \pm 1.1	9.0 \pm 1.1 ^c	9.4 \pm 0.7 ^b	9.5 \pm 0.9 ^b	9.6 \pm 1.5	
		WBC	5.6 \pm 0.9	5.1 \pm 1.6	5.8 \pm 0.6	6.1 \pm 0.5 ^c	7.9 \pm 1.0 ^c	11.0 \pm 1.5 ^a	

^a Significant at 0.001 level.

^b Significant at 0.005 level.

^c Significant at 0.05 level.

increased slightly (Table 1). In the groups pretreated with 5-HTP and AET in combination, the decrease in RBC was less marked during the first 3 days and from the 7th day onwards significant recovery was noted (Table 1). AET (200 mg/kg) pretreatment also achieved protection concerning the drop in RBC after 4 Gy gamma irradiation, but did not influence the initial depression up to the 7th day (Table 1). It has been reported that after an exposure to 6 Gy roentgen rays AET (150 mg/kg) does not influence the initial depression of haematopoiesis in bone marrow but favourably affects regeneration (1). It has also been suggested that the decrease in the cell count might be due to

loss through hemorrhage and radiation injury of cell walls or blood vessels (8). In our laboratory, significant protection against erythrocyte cell membrane injury has been noted, as revealed by an Na^+ influx study (11), in lethally irradiated rats, with 5-HTP+AET pretreatment. 5-HTP+AET pretreatment also gave good protection of bone marrow in mice exposed to 6 Gy (6) and 10 Gy gamma irradiation (unpublished observations).

Combination of 5-HTP with AET, or AET (200 mg/kg) alone, produced excellent protection of erythrocytes after 10 Gy gamma radiation up to the 30th day as compared with irradiated controls. In the control group, autopsy

Table 2

Protective effect of chemical radioprotectors on peripheral lymphocytes (%) at different time intervals (days) against 4 Gy and 10 Gy whole body gamma irradiation. Mean \pm SD of 10 animals. Normal lymphocyte count $55 \pm 5\%$

Group	Chemical protector		Days after irradiation					
			1	3	7	10	15	30
1	Distilled water	4 Gy	4 \pm 1	15 \pm 3	17 \pm 3	19 \pm 3	24 \pm 3	51 \pm 7
		10 Gy	3	2	Nil	—	—	—
2	AET (200 mg/kg)	4 Gy	20 \pm 5 ^a	25 \pm 6 ^b	26 \pm 5 ^c	34 \pm 6 ^a	38 \pm 6 ^a	55 \pm 6
		10 Gy	4 \pm 1	14 \pm 3	18 \pm 4	22 \pm 5	28 \pm 5	38 \pm 5
3	5-HTP (100 mg/kg)+ AET (20 mg/kg)	4 Gy	23 \pm 6 ^a	28 \pm 6 ^a	29 \pm 5 ^a	36 \pm 5 ^a	41 \pm 6 ^a	56 \pm 6
		10 Gy	5 \pm 1	16 \pm 3	19 \pm 4	26 \pm 5	31 \pm 5	42 \pm 6

^a Significant at 0.001 level.

^b Significant at 0.005 level.

^c Significant at 0.01 level.

could not be carried out beyond 7 days as all the animals died on the 10th day (Figure).

After exposure to 4 Gy gamma radiation, the WBC decreased sharply showing signs of recovery from the 15th day, and 71 per cent recovery was achieved on the 30th day in the irradiated control group (Table 1). In the 5-HTP + AET pretreated group, protection concerning the drop in the leucocyte count was noted from the 15th day after exposure and complete recovery was observed on the 30th day ($p < 0.001$). AET (200 mg/kg) pretreatment followed a similar trend and highly significant ($p < 0.005$) recovery was noted on the 30th day. The number of circulating lymphocytes, one of the most radiosensitive animal cells, was reduced to 25 per cent of normal in rats after exposure to 1 Gy of roentgen rays (7). All the radioprotectors used in the present study achieved complete protection of lymphocytes after 4 Gy gamma radiation. The combination of 5-HTP with AET, or AET (200 mg/kg) alone, could not give full protection of lymphocytes after exposure to 10 Gy, although the protection caused by these radioprotectors was quite significant on the 30th day as compared with the irradiated control group where studies could not be carried out beyond the 7th day, as all the animals died on the 10th day (Table 2).

The combination of 5-HTP with AET has given excellent protection concerning DNA (2) and survival rate in mice (5) and in rats (3) after an LD 100/30 dose of whole body gamma radiation. This combination has also achieved differential protection of healthy tissues against 80 Gy roentgen irradiation (unpublished observations). The administration of this combination either by the i.p. or the p.o. route, consecutively for 3 days, has not shown any toxic symptoms in rats and mice, to judge from glossiness of the fur and increase in body weight. AET, although an excellent radioprotector, cannot be used due to its toxicity. However, a suboptimal dose of 5-HTP (100 mg/kg) combined with a very low dose of AET (20 mg/kg)

obviously potentiates the radioprotective effect without disturbing toxic effects.

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