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Comparative Protection of Hematopoietic and Intestinal System in Mice by Environmetal Hypoxia and Chemical Compounds.

by

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低酸素および化学防護剤によるマウス造血系，腸管系の防護作用の比較

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900R照射を行なつた dd/YF 系マウスの実験で，照射後腸管重量及び，内因性脾コロニー形成に及ぼす放射線防護剤及び低酸素の防護効果を調べた．

防護剤として，SH物質（AET, MEA, cystamine, glutathione）と組織酸素圧降下剤（para-aminopropiophenone, propylene glycol, serotonin）を用いた．それらが組織酸素圧を降下させる，させないにかかわらず，これら防護剤は，脾一つあたりの平均内因性脾コロニー数を増加させた．SH物質は平均内因性脾コロニー数よりも腸管重量によく防護的に作用したが，組織酸素圧降下剤はその両方に同様に防護的に作用した．

It has already been reported (1,2,3) that survival of mammals irradiated in environmental hypoxia indicate protection both the hematopoietic organ and intestinal tract, in contrast to the care of chemical agents by which different effectivenes of protection of these two organs has been demonstrated (4).

The purpose of this study is to compare the protective effect of environmental hypoxia with sulfhydryl compounds, and drugs which decrease oxygen tension in tissues, on the small intestine and hematopoietical system.

Material and methods.

Male mice, strain dd/YF, 12-14 weeks old (22-27 gm) were used. There were housed 5 in a cage and had free access to food (Funabashi farm) and top water. In every group were used 12-15 mice. Experiments were repeated twice and in results pooled data are given.

Irradiation were given with a deep therapy X-ray machine, (200 kV, 25 mA, with a filter of 1.0 mm Cu, and 0.5 mm Al. Mice were housed individually during irradiation in a cylindrical plastic box, 16 cm in diameter and 3 cm in height, containing eight radial compartments. A Radocon Model 575 dosimeter probe was placed in one of the compartment, and the total dose was checked at every exposure. The dose rate was 65 R/min, at a distance of 50 cm from the target, and total dose 900 R.

For estimation of the damage of intestinal tissue, the weight of dry small intestine was determined on the third day after irradiation: Mice were killed by cervical dislocation, small intestine was remooved, washed by saline and dried 7 hrs. at 130°C.

At the same time the wet weight of thymus and spleen was measured.

Protection of hematopoietical tissue was determined by the use of method of endogenous spleen colony (ESC) (5). Ten days after irradiation surviving mice were sacrificed by cervical dislocation and spleen were remooved. After measuring their weight, the spleen were fixed in Bouin's fixative, and the number of nodules was counted two or three days after.

The doses of radioprotective agents were as follow: reduced glutathione 50 mg/kg; MEA (2 mercaptoethylamine HCl) 150 mg/kg; cystamine 2HCl 150 mg/kg; AET (2 aminoethylisothiourea dihydrobromide) 6 mg/mice; PAPP (paraaminopropiophenone in 50% propylene glycol) 60 mg/kg; PG (50% solution of propylene glycol) 0.5 ml/mice; and serotonin 100 mg/kg. All radioprotective drugs were given intraperitoneally 15. min. before the start of irradiation. In the radioprotective drugs used in the present experiment no decrease of oxygen tension in tissues (spleen, muscle) was observed with sulfhydryl compounds such like glutathione, MEA and AET, but the decreased oxygen tension was observed with such drugs as PAPP, PG and serotonin.

For comparison was used the radioprotection by environmental hypoxia resulting by the decrease of the oxygen content of breathed air to 8% oxygen. Mice breathed 8% oxygen 5 min. before and during irradiation.

Results:

Experimental data of the change in the weight of intestine after irradiation are summarized in Fig. 1. Results indicate that the best protective effect on the weight of intestine was obtained with AET and MEA. The intensity of protection with these agents was similar like the effect of environmental hypoxia. The protection by drugs which decreased oxygen tension in tissues such as PG and PAPP was intermediate

Fig. 1. Weight of the dry small intestine in the 3rd day after irradiation 900 R.

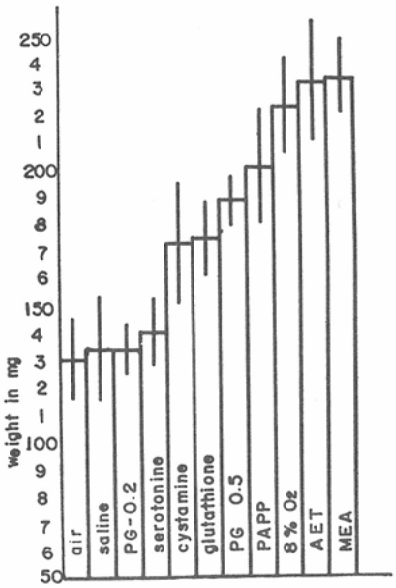


Fig. 2. Endogenous spleen colony count after protection and irradiation.

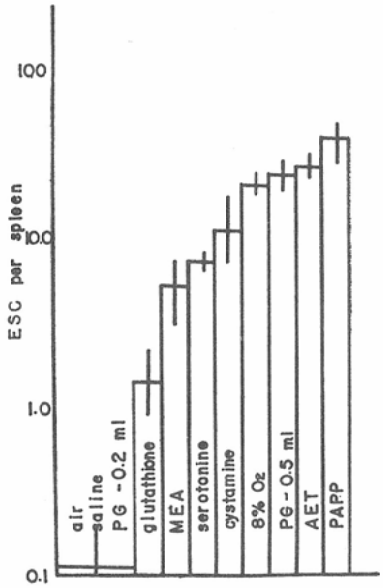


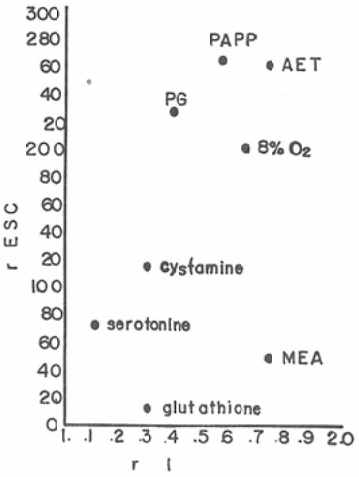
Table 1. Spleen weight after irradiation and protection.

	spleen mg after irradiation	
	in 3 rd day	in 10th day
glutathione	34.5 ± 4.6	50.0 ± 2.6
MEA	39.6 ± 6.0	40.3 ± 2.2
cystamine	34.2 ± 2.0	36.0 ± 2.2
AET	45.0 ± 8.4	58.7 ± 5.4
8% oxygen	41.5 ± 2.5	73.0 ± 12.0
PG	39.5 ± 2.8	50.0 ± 7.0
PAPP	35.0 ± 1.8	75.0 ± 28.0
serotonin	34.0 ± 5.3	39.2 ± 3.4
irrad. only and saline	30.6 ± 6.1	39.2 ± 1.8

amont the used protective agents, while serotonin, which also decreased oxygen tension in tissues did not protected the weight reduction of the intestine. Among sulfhydryl compounds the best protective effect was observed after injection of AET and MEA, while glutathione and cystamine resulted only in slight increase of the weight of intestine as compared with irradiation in air of vehicle.

In Fig. 2 and table 1. are summarized results of the effect of radioprotection on the weight of spleen and endogenous spleen colony count. Fig. 2 clearly indicate that all radioprotective drugs and hypoxia increased the mean count ESC per spleen. The highest increase of the ESC count was observed after PAPP and AET. As compared with ESC count after environmental hypoxia, serotonin again belongs to the group of drugs which heve lower effect on the ESC count than environmental hypoxia. The highest increase of the ESC count was observed, except AET, after injection of drugs which decreased oxygen tension in tissues.

Fig. 3. Relation between intensity of protection of intestine and hematopoietical tissue of mice. vertical line — ratio of increase of ESC after protection (ESC after protection/ ESC after irradiation in air).
horizontal line—ratio of protection of gut (weight of intestine after protection/ weight of intestine after irradiation in air)



Comparison of the intensity of protection by environmental hypoxia (= 1.0) and radioprotective agents on the weight of intestine and ESC count indicate (fig. 3), that drugs which decreased oxygen tension in tissues protect the gut as well as hematopoietical tissue (with exceptione for serotonin), while sulfhydryl compounds are less effective for the protection of hematopoietical tissue than for the gut. Only AET had high protective effect in the gut as well as in hematopoietical tissue.

Discussion.

Environmental hypoxia during irradiation, and radioprotective drugs given before irradiation 900 R, decreased the radiation damage of the intestine and increased the endogenous spleen colony count, as compared with the irradiation in air, or after injection of vehicle only. Endogenous spleen colony count increased in all mice independantly if radioprotective drugs decreased oxygen tension in tissues or not.

The results obtained in this study may suggest that sulfhydryl compounds are better protectors of intestinal system, while environmental hypoxia and drugs which decreased oxygen tension in tissues protected like gut like hematopoietical system. There are two exception: serotonin among drugs which decreased oxygen tension in tissues, and AET among sulfhydryl compounds. Similar results were already reported (4), and was shown, that AET protect small intestine of mice at radiation doses up 2000 R, and marrow regeneration was observed even when doses of 1500 R was used. MEA protected the small intestine of mouse also to a great extent than in bone marrow. But serotonin afforded little protection to the small intestine and bone marrow after 900 R and 1500 R, in contrast to the results obtained by Langendorf et. al (7) who suppose, that serotonin is the best chemical protection known. The reason of the discrepancy remains to be elucidated.

Increase of the mean ESC count after protection by 8% oxygen in environment, or by PG and PAPP in PG are in a good agreement with mortality results reported by us (3,8).

Summary:

In experiments on mice dd/YF, irradiated 900 R was studied protective effect of hypoxia and drugs on the weight of intestine and endogenous spleen colony formation after irradiation. It was observed that all used radioprotective drugs and hypoxia increased the mean ESC per spleen independantly if they decreased oxygen tension in tissues or not.

In sulfhydryl compounds was observed better protection of the weight of intestine than increase of the ESC count, while drugs decreased oxygen tension in tissues protected like intestine like increased ESC count per spleen.

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