Radiation protection of bone marrow lymphocytes by 2-mercaptopropionylglycine (MPG)

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Summary. The drug, MPG, when administered before irradiation, increases the radioresistance of bone marrow lymphocytes of mice to gamma rays and helps in promoting fast recovery, especially when exposed to sublethal dose.

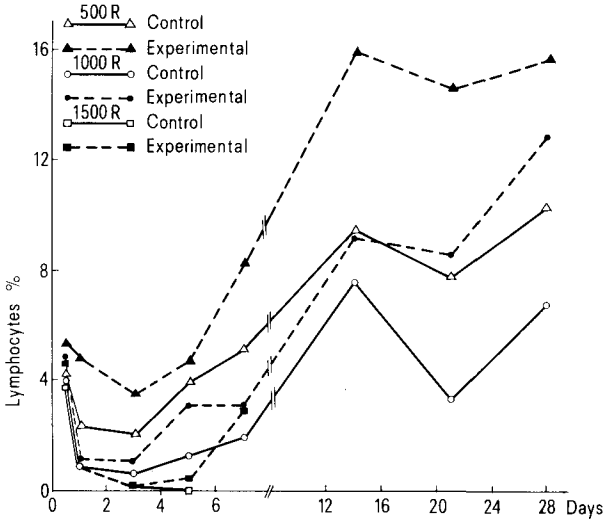
2-Mercaptopropionylglycine (MPG) is an effective radioprotector, having a very low effective dose (20 mg/kg b.wt) and a very high toxic dose (2100 mg/kg b. wt)[1-3]. Recent reports from our laboratory[4-7] provide evidence for its protective action on different mammalian tissues. The present experiment attempts to study the protective effect of MPG on the bone marrow lymphocytes of mice after whole body exposure to gamma radiation.

Material and methods. Male Swiss albino mice of 6-9 weeks, weighing 23-28 g, from an inbred colony were divided into 3 groups. Each group contained an experimental set in which 20 mg/kg b.wt of MPG (received from Santen Pharmaceutical, Osaka, Japan) was injected i.p., and a control set which received an equal volume of distilled water in the same manner. 15-30 min after the injections, the animals of the 3 groups were exposed to 500, 1000 and 1500 R of Co^{60} gamma rays at the dose rate of 24 R/min. The animals were autopsied at 12, 24, 72 h, and 5, 7, 14, 21 and 28 days after exposure. Bone marrow films were prepared by the smear method and stained with Lepahne reagent and Giemsa stain. A total of 500 cells were counted from each slide and the percentage of lymphocytes was determined in relation to the total count.

Observations. Lymphocytes show a drastic reduction in percentage in all the control groups, reaching a minimum value at 72 h. After this, in the 500 R and 1000 R groups, the count increases gradually up to 2 weeks, followed by a second decrease at 3 weeks, the decline being more pronounced in the latter group. Again an increase in the lymphocyte population is observed at 4 weeks, but the value at this interval remains below normal. With 1500 R, the lymphocytes completely disappear by the 5th day (table, figure).

When MPG is given before irradiation, the decline in the lymphocytes does not reach the same level as that of the control. In the 500 R group, the normal count is restored by 2 weeks and is more or less maintained during the next intervals, indicating complete recovery (table, figure). In the 1000 R group, the decline in lymphocytes is higher than in the 500 R group. There is a rise in the value at 2 weeks which is followed by a second drop at 3 weeks, which is not significant. A further increase in lymphocytes is seen at 4 weeks when the value reaches near normal level. In the 1500 R group, there is a continuous decline in lymphocyte percentage till 3 days, followed by an increase at 5 and 7 days (table, figure).

Discussion. The present study shows that lymphocytes are greatly damaged by radiation and the degree of damage increases with dose. MPG increases the percentage of surviving lymphocytes in all the 3 dose groups. With 500 R the MPG treatment affords significant protection, restoring the lymphocyte counts to normal as early as 2 weeks after irradiation. With higher doses, where initial damage is



Graph showing the percentage of bone marrow lymphocytes in mice exposed to different doses of gamma rays.

Percentage of lymphocytes in the mouse bone marrow after whole-body exposure to different doses of gamma radiation. Number of lymphocytes in normal unirradiated mouse bone marrow is 14.80 ± 1.20

Doses	Intervals 12 h	24 h	72 h	5 days	7 days	14 days	21 days	28 days
500 R								
Control	4.20 ± 0.53	2.20 ± 0.28	2.00 ± 0.28	3.93 ± 0.49	5.13 ± 0.57	9.53 ± 0.49	7.80 ± 0.50	10.53 ± 0.51
Experimental	5.33 ± 0.49	4.73 ± 0.68	3.40 ± 0.77	4.73 ± 0.68	8.33 ± 2.03	16.00 ± 1.22	14.68 ± 0.54	15.66 ± 0.40
	NS	NS	NS	NS	$p = 0.05$	$p < 0.05$	$p > 0.01$	$p > 0.01$
1000 R								
Control	4.00 ± 0.58	0.86 ± 0.20	0.60 ± 0.14	1.26 ± 0.00	2.00 ± 0.00	7.60 ± 0.29	3.33 ± 0.20	6.86 ± 0.14
Experimental	4.86 ± 0.82	1.00 ± 0.00	1.00 ± 0.35	3.00 ± 0.70	3.06 ± 0.00	9.33 ± 0.40	8.66 ± 0.41	13.02 ± 0.54
	NS	NS	NS	NS		NS	$p < 0.05$	$p = 0.01$
1500 R								
Control	3.80 ± 0.51	0.86 ± 0.51	0.06 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	No survivors	No survivors	No survivors
Experimental	4.66 ± 0.40	0.86 ± 0.51	0.13 ± 0.00	0.30 ± 0.14	3.00 ± 0.00			
	NS				NS			

± SEM.

more, protection is less. However, the radioresistance of lymphocytes appears to have increased due to the drug treatment, as a higher percentage survives at the early intervals. Hjort[8] also found a 3-fold increase in radioresistance of the lymphocytes with cysteamine; and Crouch and Overman[9] noticed protective effects of AET on peripheral blood in primates.

The protection of the lymphocytes in the present study may be attributed to the following reasons: 1. MPG protects the cells from direct killing, thus reducing the initial depletion; and 2. It protects the chromosomes by restitution, so that when the breaks rejoin, the normal structure is regained, which reduces abnormal divisions and mitotic deaths.

The second possibility serves to explain the failure to notice any significant cell depletion at 21 days in the drug-treated animals. Protection of the stem cell pool is also reflected in the fast recovery and restoration of normal cell count in the sublethally irradiated animals.

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Butanol extracts from myelin fragments. IV. Some interactions between 5-hydroxytryptamine and other neurotransmitters binding

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Summary. To examine the interaction between 5-HT and other neurotransmitters binding to the butanol extracts from myelin, double labelling experiments were done. The binding peaks of C^{14}·ACh and NA were clearly different from that of H^3 · 5-HT. At 5×10^{-7} M, binding of 5-HT, ACh, NA, GABA and DA was 62.7, 2.3, 7.0, 5.8 and 1.9 nmoles/mg protein, respectively. These results suggest that the 5-HT binding components of myelin butanol extracts may have high selectivity and specificity.

From the studies on the 5-HT binding to various membraneous structures of central nervous tissue, Marchbanks[1] and Fiszer and De Robertis[2] have reported the butanol extracts of myelin possessing a 5-HT binding capacity, but detailed biochemical observations were not performed. By Sephadex LH_{20} column chromatography[3] and SDS-urea gel electrophoresis[4], we demonstrated that the butanol extracts from myelin fragments showed binding affinity for C^{14}·5-HT, and these extracts contained basic protein, DM-20 of Agrawal[5] and proteolipid protein.

We also found that acetylcholine (ACh) and dopamine (DA) inhibited the binding of C^{14}·5-HT to these extracts, but noradrenaline (NA) and γ-aminobutyric acid (GABA) had no effect[6]. These results suggested that butanol extracts of myelin might have some interactions with other neurotransmitters as well as 5-HT. Based on these observations, we planned binding experiments of ACh, NA, GABA and DA to the butanol extracts from myelin fragments. In addition, the selectivity of 5-HT binding components is discussed.

Materials and methods. Preparations of myelin fragments and butanol extracts were described previously[3,6]. Briefly, myelin fragments were isolated from the homogenate of rat brain stem (10% in 0.32 M sucrose) by the method of Whittaker et al.[7] and extracted with butanol-water mixtures. The resultant butanol phase was concentrated under N_2 to about one-third of its original volume (TE). An aliquot of TE (64 ml) was treated with water (14%, v/v) to dissolve the insoluble materials, and a 3 ml sample was simultaneously incubated at room temperature for 20 min with H^3·5-HT and 1 of the other neurotransmitters (C^{14}-labelled compound). After incubation, the mixtures were loaded on to a Sephadex LH_{20} column (2×30 cm). Stepwise elution was carried out with solvents of increasing polarity: 100 ml chloroform, 50 ml each of chloroform-methanol (CM) 15:1, 10:1 and 6:1, and then 200 ml of CM 4:1. Protein contents of the TE and collected fractions were assayed using the method of Lees and Paxman[8]. Radioactivity of the collected fractions was counted in a toluene/Triton X-100 emulsion phosphor. When the control experiment was done without protein moiety, the free counts of 5-HT and GABA appeared in the bound areas (11.7 and 21.5%, respectively) and in both cases they were subtracted from the experimental values. H^3·5-HT (27.6 Ci/mmole), C^{14}·NA (52.0 mCi/mmole), C^{14}·GABA (49.4 mCi/mmole) and C^{14}·DA (54.9 mCi/mmole) were obtained from New England Nuclear. C^{14}·ACh (10.2 mCi/mmole) was from Radiochemical Centre.

Results and discussion. In order to examine the interaction between 5-HT and other neurotransmitters binding, myelin butanol extracts were incubated simultaneously with H^3·5-HT and another C^{14}-labelled compound. The bound radioactivity of each of the compounds was determined by Sephadex LH_{20} column chromatography as described else-

Binding of several neurotransmitters to the butanol extracts from myelin fragments

Compound	nmoles/mg protein
5-Hydroxytryptamine	62.7±3.2
Acetylcholine*	2.3±0.3
Noradrenaline	7.0±1.3
γ-Aminobutyric acid	5.8±1.0
Dopamine*	1.9±0.4

Butanol extracts from myelin fragments were incubated for 20 min with 5×10^{-7} M of each of the neurotransmitters. After incubation, mixtures were chromatographed through a Sephadex LH_{20} column as described elsewhere and the bound radioactivity was measured (mean±SEM of 4 experiments). *In both cases, 2 binding peaks are combined.