Radiation protection of pronormoblasts and normoblasts by 2-mercaptopropionylglycine (MPG)

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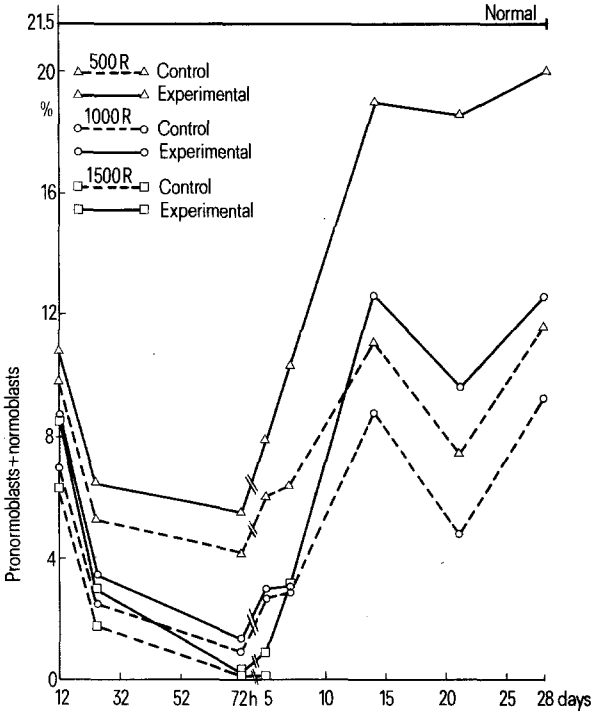
Summary. The pronormoblasts and normoblasts in Swiss albino mice were found to be very sensitive to radiation and their percentage was reduced drastically after exposure to gamma-rays. The degree of damage increased with increase in radiation dose. MPG reduced the initial damage and brought about an early and fast recovery. It is concluded that the drug protects the stem cells and thereby reduces the depletion of the regenerating pool which causes a more efficient and accelerated recovery.

The discovery of a radioprotective effect of MPG (2-mercaptopropionylglycine) at an optimal nontoxic dose of 20 mg/kg b.wt compared with its high toxic dose (2100 mg/kg b.wt)[1] has aroused renewed interest in the field of chemical radioprotection. Radioprotective action of MPG on various tissues of Swiss albino mice after external irradiation has been reported recently from this laboratory[2-5].

Materials and methods. Male Swiss albino mice 6–8 weeks old weighing 23–26 g were selected from an inbred colony. MPG (2-mercaptopropionylglycine, received from Santen Pharmaceutical, Osaka, Japan was dissolved in distilled water so as to give a concentration of 1 mg/ml and pH was adjusted at 6.4 with 0.1 NaOH) was injected i.p. with 20 mg/kg b.wt in the experimental groups, and control animals were given an equal volume of distilled water in the same manner. After 15–30 min of the treatment, the animals were irradiated with 500 R (sublethal), 1000 R (lethal) or 1500 R (supralethal) at the dose rate of 24 R/min. The animals were sacrificed by cervical dislocation at 12,24, and 72 h and 5, 7, 14, 21 and 28 days after irradiation. Bone marrow films were prepared by the smear method and stained with Giemsa stain and Lepahne reagent as a counter stain, for differentiation of pronormoblasts and normoblasts. Differential cell counts were made and a total of 500 cells was counted from each slide. The number of pronormoblasts and normoblasts was considered together and expressed as percentage of the total cell count.

Results. Control. Pronormoblasts and normoblasts show a drastic reduction reaching less than half the normal value at 12 h after irradiation (table, figure). The percentage continues to fall during the following intervals reaching a minimum at 72 h in each dose, the reduction becoming more and more exaggerated as the dose is increased from sublethal to supralethal. After this, in the sublethal (500 R) and lethal (1000 R) doses the count increases gradually upto 2 weeks which is followed by a 2nd decrease at 3 weeks; this 2nd phase of decline is more pronounced in the 1000 R than in the 500 R exposure group. Again an increase in the population of pronormoblasts and normoblasts is observed at 4 weeks but the percentage at this interval does not exceed half of the normal values.

Experimental. The difference in percentage of pronormoblasts and normoblasts is not significant in drug treated and



Graphic representation of pronormoblasts and normoblasts changes in Swiss albino mice exposed to Co60 radiation in presence and absence of MPG.

Percentage of pronormoblasts and normoblasts in the mouse bone marrow after whole-body exposure to different doses of gamma radiation

Intervals doses	12 h	24 h	72 h	5 days	7 days	14 days	21 days	28 days
500 R								
Control	9.8±0.9	5.3±0.8	4.2±0.8	6.1±0.2	6.4±0.8	11.1±0.6	7.5±0.7	11.8±0.4
Experimental	10.8±1.2	6.5±0.4	5.3±0.4	7.9±0.4	10.3±1.0	19.0±1.9	18.5±0.9	20.0±1.4
	NS	NS	NS	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.001$	$p<0.01$
1000 R								
Control	7.0±0.6	2.5±0.7	0.9±0.3	2.7±0.0	2.9±0.0	8.8±0.5	4.8±0.5	9.3±0.5
Experimental	8.7±0.8	3.5±0.2	1.3±0.0	3.0±0.0	3.0±0.5	12.7±0.8	9.7±0.4	12.7±0.5
	NS	NS	NS	NS	NS	$p<0.01$	$p<0.002$	$p<0.01$
1500 R								
Control	6.3±0.8	1.8±0.1	0.1±0.0	0.1±0.0	++	++	++	++
Experimental	8.7±0.9	3.0±0.9	0.2±0.1	0.9±0.1	3.1±0.1	++	++	++
	NS	NS	NS	NS				

±SE of mean. ++No survivors. Percentage of pronormoblasts and normoblasts in normal unirradiated mouse bone marrow is 21.5±1.2.

control animals at each dose till the 3rd day. In the 500 R exposure group the percentage of pronormoblasts and normoblasts increases significantly ($p < 0.05$) from the 5th day on as compared to the control of the same group. At 2 weeks an almost normal value is reached which is more or less maintained during the next intervals. No 2nd phase of decline is noticed at 3 weeks (table, figure). In the 1000 R, there is a rise in pronormoblasts and normoblasts percentage at 2 weeks which is slightly more than half of the normal value. This is followed by a 2nd phase of decline at 3 weeks but the value is significantly higher than that of the control of the same interval ($p < 0.002$). A further decline in percentage is observed at 4 weeks reaching the 2 weeks value of the same dose (table). Supralethally irradiated animals also show an increase in pronormoblast and normoblast percentage on the 5th day. No further observation could be made in this group as there were no survivors.

Discussion. Our findings in the lethally and supralethally irradiated control mice are in conformation with those of Hulse[6] who observed that the pronormoblasts and normoblasts become severely depleted by 1 day and completely disappear by the 3rd day. In our studies the sublethally irradiated animals also show an identical pattern of cell depletion, showing maximum reduction on 3rd day (table). The statistically significant fall in nucleated red cells at 3 weeks and renewed increase at 4 weeks is in complete agreement with the data of Brecher et al.[7] who believe that regeneration of hematopoietic tissues after X-ray injury proceeds in waves. However, the results are different in drug-treated animals. In these animals the initial decrease in percentage is less pronounced as compared with controls which received the same dose. Moreover, the recovery is also earlier and faster in the drug-treated animals especially in the sublethally irradiated group.

In the present observation, more of the pronormoblasts and normoblasts survive in MPG treated animals during the early intervals (upto 3rd day), which indicates protection of these 'blast' cells, thus maintaining a stem cell pool which can actively regenerate the marrow and bring about an early and fast recovery. An early erythropoiesis was reported earlier due to the protection afforded by MEG[8] and AET[9] to the stem cell compartment during the initial radiation damage. The present observation indicates a similar effect by MPG also. However, in the protected animals, the recovery at 4 weeks is less in higher dose (1000 R) as compared to lower dose (500 R). Thus regeneration also appears to be a dose dependent phenomenon; with a higher dose, more of the stem cells are killed and hence the regenerating pool becomes very much reduced in size, which cannot bring about complete recovery within the longest interval studied (28 days).

1 T. Sugahara, Y. Tanaka, H. Nagata, T. Tanaka and E. Kano, Proc. Int. Symp., p.267. Tokyo, Japan 1970.
2 M.R. Saini, B.R. Saharan, H.C. Bhartiya and P. Uma Devi, J. Radiat. Res. *18*, 206 (1977).
3 M.R. Saini, P. Uma Devi and S.S. Yadav, Experientia *34*, 1627 (1978).
4 P. Uma Devi and B.R. Saharan, Experientia *34*, 91 (1978).
5 P. Uma Devi, M.R. Saini, A. Verma and B.R. Saharan, Indian J. exp. Biol. *16*, 86 (1978).
6 H.V. Hulse, Br. J. Haemat. *3*, 348 (1957).
7 G. Brecher, K.M. Endicott, H. Gump and H.P. Brawner, Blood *3*, 1259 (1948).
8 L.H. Smith, Exp. Cell Res. *13*, 627 (1957).
9 P.V. Vittrio, S.B. Dziubato and E.A. Amey, Radiat. Res. *37*, 653 (1969).

Dopamine agonist performance in Planaria after manganese treatment

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Summary. Using Planaria motor performance as model, the authors confirm that Mn^{++} basically inhibits dopaminergic release with transitory hyper-release.

In man, chronic manganese poisoning produces an extrapyramidal syndrome (toxic parkinsonism[1]) with evident dystonic[2,3] and psychiatric symptoms. The metal's interference with the dopaminergic pathways is demonstrated by the considerable drop in striatal dopamine seen in man[4], as well as by the stereotyped behavior and turning produced by intrastriatal administration of manganese in rats[5].

We previously reported[6,7] that when the dopaminergic compartment of *Dugesia gonocephala s.l.* was stimulated, a distinctive motor reaction occurred, i.e. 3-dimensional screw-like movements; this was comparable to mammalian stereotyped behavior. Further research[8] confirmed the validity of this model as an alternative to the conventional experimental models using higher vertebrates.

Within the framework of our investigation of the neurobiological activity of certain metal ions[9-11], it was deemed interesting to test this model in the case of manganese poisoning also.

Materials and methods. Planaria of the species *Dugesia gonocephala s.l.* (Platyhelminthes, Turbellaria, Tricladida) in the agamous scissiparous form, bred by us (Mignone River strain), were placed in solutions of manganese sulfate and chloride (Merck) in distilled water at concentrations increasing from 15.10^{-5} to 180.10^{-4} M (table). The animals were observed in a dimly-lit environment at 18 °C once every 30 min or so for the first 24 h and thereafter about every 12 h for a total of 8 days.

Results. Solutions with a Mn^{++} content ranging from 180.10^{-4} to 18.10^{-4} M immediately or almost immediately induce screw-like movements of the type produced by dopaminergic overstimulation. These movements cease after about 15 min, later recurring spasmodically in the form of repeated 'crises' until the end. In the intercritical phase the animals lie on 1 side, curled up in a 'C'-shape. Slight luminous or mechanical stimulation at this stage leads to the immediate resumption of hyperkinetic behavior for the duration of several minutes. Death ensues in 24-48 h (unlike the situation with sodium salts, which we described earlier[12], the chloride appears to be more toxic than the sulfate, table).