Protective Effect of Magnesium Pemoline against Lethal Doses of X-Irradiation

Recently a number of investigators have reported that magnesium pemoline (Abbott-30400, Cylert) has the property of enhancing memory and learning[1-4]. During our study of the effect of magnesium pemoline on improving conditioned avoidance response in mice[5], using X-irradiation as an unconditioned stimulus, we also observed that this drug has radioprotective effect on the experimental animals. This interesting observation led us to further experiments studying exclusively the radioprotective effect of magnesium pemoline on mice. The results of this study are reported in this paper.

Methods. CF_1 male mice (20–22 g), 50–60 days old were used throughout the experiments. Each experiment involved 360 mice randomly divided into 3 main groups of 120 each. The animals were housed in standard plastic cages containing 10 in a cage with food and water available at all times. On the first day of the experiment, 1 week after their arrival from the supplier, all the mice were exposed to 50 R of X-irradiation generated by a 400 KV Maxitron with an average dose rate of 80 R/min. On the second day, half of the mice in each group was injected i.p. with 0.6 cm^3 of magnesium pemoline (0.3% solution prepared in 0.3% tragacanth), and the other half injected with bacteriostatic water. All the mice were then immediately exposed to 50 R for the second time. On the third day, 1 day after injection, the first group of mice was exposed to additional 800 R. The second and the third group received this lethal dose on the fifth and the seventh day of the experiment or 3 and 5 days respectively after the injection of water and magnesium pemoline. Mortality in each group was then observed and recorded daily.

Results. During a series of preliminary experiments the mice injected with magnesium pemoline died almost 50% faster than those injected with water. It was soon discovered that Benzalkonium chloride, a preservative agent, was responsible for this effect. In subsequent experiments no benzalkonium chloride was used. The Table shows the post-irradiation survival percentage of 3 groups of mice. In group I, all the water or control mice (total of 60) died on the ninth post-irradiation day while 48% of the experimental or magnesium pemoline mice still survived. The mice in this group were exposed to 800 R 1 day after the injection of water and pemoline. In group II, lethal irradiation took place 3 days after injection and the results showed 35% versus 0% in favor of the experimental mice injected with magnesium pemoline. All the control animals in group III died 8 days after receiving 800 R while more than 50% of the mice injected with magnesium pemoline still survived.

Discussion. It is interesting to note that the survival % of the mice injected with pemoline was highest in group I and lowest in group III. This seems to suggest that the drug was most effective in this experiment when the time between the administration of the drug and the exposure of the animals to lethal amounts of X-rays was the shortest. Since each group was exposed to the final 800 R at different time intervals from the 2 initial 50 R exposures, the total effect of radiation on group I was undoubtedly more damaging than those on group II and III respectively. The survival % in group I, however, was still significantly higher than in the other 2 groups. At the present time we have no explanation for the protective mechanism of magnesium pemoline against radiation. We are in the process of comparing the immediate and the long term radioprotective effects of this drug. In another series of experiments we observed that the drug was still quite effective when the animals were exposed to 850 R (following 2 initial 50 R exposures on the first 2 days) 1 week after drug injection (40% survival for magnesium pemoline versus 10% for water on the seventh day after irradiation)[6,7].

In summary, 2 main effects are quite obvious in this study: (1) magnesium pemoline, known as a memory en-

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Post-irradiation days	Post-irradiation % survival					
	Group I[a]		Group II		Group III	
	H_2O (60 mice)	Magnesium pemoline (60 mice)	H_2O (60 mice)	Magnesium pemoline (60 mice)	H_2O (60 mice)	Magnesium pemoline (60 mice)
1	100	100	100	100	100	100
2	100	100	100	100	100	100
3	100	100	100	100	100	100
4	100	100	100	100	100	100
5	100	100	80	97	90	97
6	84	97	74	94	67	87
7	54	87	47	81	30	71
8	27	64	20	58	0	51
9	0	48	0	35		

[a] Group I, group II, and group III received 800 R 1 day, 3 days, and 5 days after injection respectively.

hancing drug, also possesses radioprotective effect. (2) This effect is significant for both short and long time.

Zusammenfassung. Magnesium-Pemolin, bekannt als gedächtnisstärkendes Arzeimittel, besitzt eine erhebliche Schutzwirkung gegen ionisierende Strahlen. Dieser strahlentherapeutische Effekt betrifft sowohl kurze, als auch langdauernde Wirkungsperioden.

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Age Dependency of the Primary Immune Response in the Hereditary Pituitary Dwarf and Normal Snell-Bagg Mouse

The experiment reported here was undertaken as part of an investigation dealing with the problem of the possible hormonal control of the primary immune response, using as experimental animals the hereditary recessive pituitary dwarf and normal Snell-Bagg mice. Studies on the immunological responsiveness and on the thymus and the peripheral lymphoid tissues of this strain of mice regardless of the age at the time of sacrifice, were previously reported from this laboratory[1-4]. The immunological defects found in dwarf mice are probably related to a congenital underdevelopment of the thymus[2,3]. It is the purpose of this paper to describe the age dependency of immunological reactivity in this particular strain of mice.

Material and methods. Inbred hereditary recessive pituitary dwarf (genetic symbol *dw*) and normal mice of the Snell-Bagg strain were used. Experimental groups were made up of animals of the same age. Since the immunological responses of males and females were similar[5], the sex of the animals is not reported. Four days before sacrifice, the animals were given i.p. 4×10^7 sheep red cells in 0.1 ml of saline, and 24 h before sacrifice an i.p. injection of thymidine-H^3 (Amersham, specific activity 3000 mc/m*M*) at the dose of 0.8 μc/g of body weight. The animals were then killed at different times between 15 and 60 days of age. At the time of sacrifice, body, thymus and spleen were weighed and individual antibody-plaque-forming cells (APFC) from spleen were detected according to the technique of Jerne et al.[6]. Thymus, peripheral and mesenteric lymph nodes and Peyer's patches were fixed in Carnoy's fluid, and histological sections were stained with methyl-green pyronine. Additional sections of peripheral lymphoid tissues were processed for autoradiographic studies, stained with hematoxylin, and the proportion of labelled nuclei was determined by counting 1000 cells randomly chosen in the cortical germinal centres as well as in the paracortical areas. Only cells with 5 or more grains were scored as labelled.

Results. The primary immune response to sheep red blood cells as measured by the capacity to form APFC, and the total number of spleen cells of normal and pituitary dwarf mice of different ages, is reported in the Table, together with the weight values. Normal animals showed a moderate response at 15 days of age; the number of APFC subsequently increased up to the sixtieth day, with a maximum rate of rise between 20 and 30 days of age. Fifteen-day-old pituitary dwarf mice, on the other hand, showed a very poor response. Between 20 and 45 days of age, an only slightly higher, still definitely subnormal plaque-forming capacity was found. Due to the limited life span of pituitary dwarf mice, the experiment was discontinued at this time. A higher total number of spleen cells was constantly found in the normal than in the dwarf mice, in which, on the contrary, a progressive fall was seen.

In histological sections of peripheral lymphoid tissues, the proportion of pyroninophilic cells was greater in normal than in dwarf animals. Starting from 15 to 60 days of age we found a progressively increasing number of pyroninophilic cells, mainly plasmablasts and immature plasma cells in the germinal centres of the normal animals. In these animals sacrificed between 45 and 60 days of age, we found the highest number of these cells with occasionally scattered mature plasma cells. In contrast to this, a constant lack of pyroninophilic cells was found in the germinal centres of the peripheral lymphoid tissues of the pituitary dwarf mice, regardless of the age at time of sacrifice. It was interesting to note that, while in the normal mice we were able to observe a progressive increase of the number of the germinal centres and of the proportion of the pyroninophilic cells, in the dwarf animals a constant pattern of marked lack of pyroninophilic cells as well as of small lymphocytes in the thymus-dependent paracortical areas were the main histological findings throughout the duration of the experiment. In peripheral lymphoid tissues, a constantly high mean % of labelled nuclei was found regardless of the age at the time of sacrifice; in contrast, a constantly lower proportion of labelled nuclei was recorded for these tissues in the dwarf animals (Table).

Histological study of thymus sections from dwarf animals has given results substantially in agreement with our previously reported observations[1,4], showing a progressive early involution with marked loss of lymphocytes and evident fibrosis. In addition, in thymus of dwarf animals sacrificed at 30 and 45 days of age, patterns of cortical inversion resembling those described by Metcalf[7] in preleukemic AKR thymuses, have been noted. This condition is histologically characterized by a variable degree of thickening of the connective capsule and substitution of the medulla with medium and small lymphocytes and scattered reticulum cells.

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