EFFECT OF CHEMICAL RADIOPROTECTORS ON THE cAMP/cGMP RATIO IN MOUSE BONE MARROW

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The idea that the mechanism of action of chemical radioprotectors is mediated through elevation of the intracellular cAMP level [6] has received much experimental confirmation in recent years. Mitznegg found for the first time a significant rise of the cAMP level in the liver of mice after receiving an injection of cysteamine [7]. It was later shown [1-4] that sulfur-containing radioprotectors and also biogenic amines have the property of raising the cAMP concentration in certain mouse tissues.

Meanwhile, several workers found that an effective radioprotector such as APAETP (S-[N-(3-aminopropyl)-2-aminoethyl]-thiophosphate) had no influence on the cAMP level in the mouse [1] or rat [8] spleen.

In our view the most interesting approach should be to study the ability of radioprotectors to exert an influence on the cyclic nucleotide system of the bone marrow, for involvement of hematopoietic tissue determines the survival rate of animals exposed to the action of ionizing radiation within a certain dose range. No such data can be found in the literature. Accordingly the aim of the present investigation was to determine cAMP and cGMP concentrations in the bone marrow of mice receiving injections of radioprotectors with different chemical structures.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57 black)F_1 mice weighing 20-24 g. In experiments to study postradiation survival of the mice, the animals were irradiated with γ-rays on an "IGUR" apparatus, in the minimal absolute lethal dose of 232.2 mCi/kg (900 R), with dose rate of 212 R/min. The chemical compounds were injected intraperitoneally or subcutaneously in a volume of 0.2 ml of physiological saline before or after irradiation; the pH of the solutions was adjusted to 6-7. Observations on survival continued for 30 days after irradiation. In experiments to determine cyclic nucleotides in the bone marrow of intact and irradiated animals, all the compounds were injected into the mice under the same conditions. At various times after injection of the compounds the mice were killed by cervical dislocation and bone marrow from both fermora was flushed out with 0.5 ml of 0.05M Tris-HCl, pH 7.5, containing 4 mM EDTA, and homogenized in a glass syringe. All the samples were then frozen and thawed quickly 3 times, after which they were boiled on a water bath for 3 min and centrifuged for 20 min at 6000g. The supernatant was used for determination of cAMP and cGMP with the aid of standard kits from "Amersham International" (Great Britain). Radioactivity was counted in dioxan scintillator on a "Delta" counter (The Netherlands). For each experimental point, 5-12 mice were used. The data on survival was subjected to statistical analysis by the chi-square test. The number of animals in each group was not less than 20.

The concentrations of cAMP and cGMP in the bone marrow of intact mice were 6.95 ± 0.48 and 0.83 ± 0.06 pmole/10^7 cells, respectively. The value of the cAMP/cGMP ratio was calculated separately for each animal, and its normal value was 10.8 ± 0.9. All the results are expressed as percentages of the normal or the irradiated control.

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TABLE 1. Radioprotective Properties of Chemical Compounds and Their Effect on the Concentrations of Cyclic Nucleotides and Ratio between Them in Bone Marrow of Intact Mice (M ± m)

Group of compounds	Compounds	Dose, mg/kg	cAMP, % of normal	cGMP, % of normal	cAMP/cGMP, % of normal	Survival rate after 30 days
Sulfur-containing	Cysteamine·HCl	150	169,4±12,7*	77,4±8,1*	173,1±18,6*	60*
	S-[N-(3-aminopropyl)-2-aminoethyl]-thiophosphate	300	113,6±2,7	66,1±6,5*	138,3±12,6*	30*
	S-ethylisothiuronium-diethyl phosphate	100	183,7±13,2	111,1±13,0	165,3±13,6*	42,1*
	Cystine	250	90,5±16,2	87,1±12,1	83,6±18,2	10
	S-butylisothiuronium-butyl phosphate	90	75,9±17,8	104,0±9,5	73,0±14,7	0
	2(N-acetylamino)ethylisothiuronium-diethyl phosphate	300	128,3±17,0	106,0±8,7	120,0±12,2	0
Indolylalkylamines	5-Methoxytryptamine (mexamine)·HCl	75	120,8±4,0*	82,3±5,6*	138,3±13,1*	25*
	5-Chlorotryptamine·HCl	60	121,1±16,3	104,2±18,5	110,3±24,3	5
Arylalkylamines	Isoproterenol·HCl	2,5	176,8±15,9*	75,8±6,5*	188,1±13,9*	54,8*
	Phenylephrine·HCl	15	201,0±27,2*	132,9±10,6*	151,4±14,5*	40*
	Fetanol·HCl (etilefrine)	15	239,6±21,2*	118,2±7,6	202,7±19,2*	55*
	Octopamine adipinate	15	142,6±6,5*	142,0±4,7*	100,2±8,6	10
	Propranolol·HCl	25	88,0±11,8	78,2±10,3	87,2±10,4	10
Other compounds	Nalidixic acid, Na salt	120	143,6±11,5*	102,4±8,9	132,2±9,7*	25*
	Glucosamine	250	149,5±10,6*	108,7±10,3	137,0±10,2*	30*
	Sodium nitrite	120	135,5±10,9*	96,0±8,8	138,2±11,7*	25*
	Benzamide	260	72,4±18,7	160,8±14,3*	59,4±12,6*	0
Control	Physiological saline	0,2 ml	98,3±3,0	94,4±6,5	90,3±5,8	3,2

Legend. Chemical compounds injected in doses equivalent to one-third of LD_{50} 10 min before irradiation or determination of cyclic nucleotides. *$p < 0.05$.

EXPERIMENTAL RESULTS

Parallel with determination of the concentrations of cyclic nucleotides at different times after injection of chemical compounds with different levels of radioprotective effectiveness into the mice the effect of these compounds was studied on the postradiation survival of the mice. It will be clear from Table 1 that compounds ineffective on the radiobiological plane did not cause changes in the cAMP and cGMP levels in the bone marrow cells. Meanwhile, effective radioprotectors of three different chemical classes caused an increase in the cAMP concentration and a decrease in the cGMP concentration, or a combination of these two opposite actions on the concentrations of the two nucleotides. For all compounds with a radioprotective effect an increase in the cAMP/cGMP radio was observed. Thus only radioprotectors (but not inactive substances closely similar to them in their chemical structure) possess the specific property of increasing the cAMP/cGMP ratio in mouse bone marrow.

The time course of changes in value of the cAMP/cGMP ratio in the bone marrow after injection of the radioprotectors is given in Fig. 1 and compared with their radioprotective properties. It will be clear from Fig. 1 that the greatest radioprotective effect of the compounds tested was exhibited when the cAMP/cGMP ratio was at its highest level.

Experiments with injection of the radioprotectors in different doses also clearly demonstrate that the compounds exhibit radioprotective properties only in doses for which the cAMP/cGMP ratio is increased. Compounds in ineffective doses did not affect this ratio (Fig. 2).

In the next series of experiments the radioprotectors cysteamine, mexamine, or isoproterenol were injected into irradiated mice after 2, 15, and 60 min. Control mice received physiological saline. Cyclic nucleotides were determined 10 min after injection, i.e., 12, 25, and 72 min after irradiation. Concentrations of cAMP and cGMP were determined at the same time in the control mice. Irradiation in this dose itself causes no appreciable changes in the cyclic nucleotide concentrations. As will be clear from Fig. 3, only cysteamine when injected 2 min after irradiation caused an increase in the cAMP/cGMP ratio in the bone narrow of the irradiated mice. Irradiation of the animals led to complete loss of the ability of mexamine and isoproterenol to increase the cAMP/cGMP ratio. Simultaneous radiobiological experiments showed that only cysteamine gives a radioprotective effect when injected 2 min after irradiation.

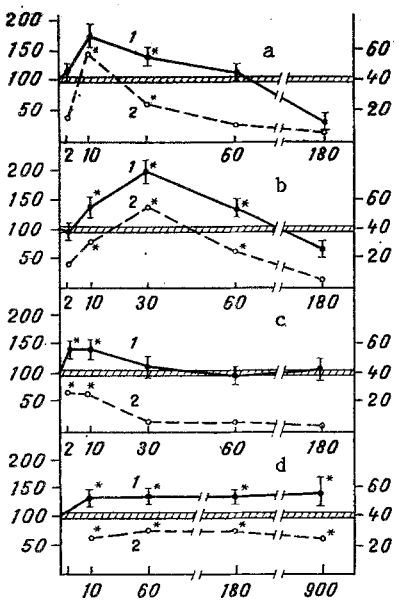

Fig. 1

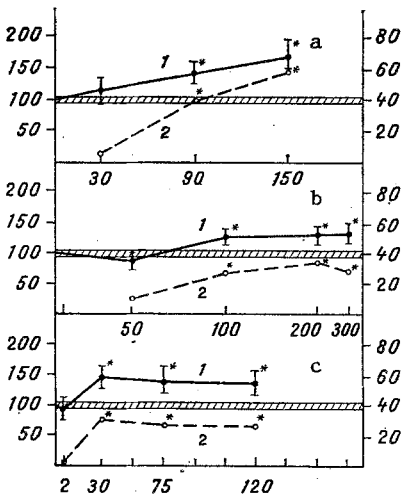

Fig. 2

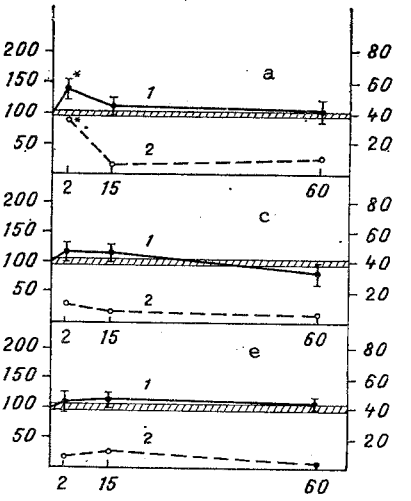

Fig. 3

Fig. 1. cAMP/cGMP ratio in bone marrow of intact mice at different times after injection of radio protectors. Abscissa, time after injection (in min). Here and in Figs. 2 and 3: left ordinate – curve 1: cAMP/cGMP (in % of control); right ordinate – curve 2: 30-day survival of irradiated mice (in %). a) Cysteamine·HCl, 150 mg/kg; b) APAETP, 300 mg/kg; c) mexamine·HCl, 75 mg/kg; d) nalidixic acid, Na salt, 120 mg/kg; *$p < 0.05$.

Fig. 2. cAMP/cGMP ratio in bone marrow of intact mice 10 min after injection of radioprotectors in different doses. Abscissa, dose of compounds (in mg/kg body weight).

Fig. 3. cAMP/cGMP ratio in bone marrow after injection of radioprotectors in irradiated mice. Abscissa, time of injection of compounds after irradiation (in min). e) Isoproterenol·HCl.

All the compounds studied, with marked radioprotective activity, thus led to an increase in the cAMP/cGMP ratio. In some cases this effect was obtained due to an increase in cAMP (isoproterenol, nalidixic acid) or to a decrease in cGMP (APAETP). For cysteamine and mexamine the rise of the cAMP level was accompanied by a fall of the cGMAP. Ineffective compounds did not possess these properties. Comparison of the period of the maximal radioprotective effect of each protector studied with its effect on cyclic nucleotide concentrations in the bone marrow showed that a maximal increase of the cAMP/cGMP ratio was observed in cases when the compound exhibited radioprotective action. Consequently, the radioresistance of the animals was increased during that period of time when the cAMP level was raised or the cGMP level lowered in the bone marrow cells.

Since all the effects of cyclic nucleotides are linked with activation of protein kinases and subsequent phosphorylation of proteins, it can be tentatively suggested that the resistance of the cells to the action of radiation will be highest when cAMP-dependent protein kinases are stimulated and cGMP-dependent protein kinases inhibited. These two categories of protein kinases are known to have opposite effects on many processes (proliferation, nucleic acid synthesis, etc.) [5], which play an important role in the radioresistance of cells.

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