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ANTIRADICAL ACTIVITY AND RADIOPROTECTIVE PROPERTIES OF INHIBITORS OF FREE-RADICAL REACTIONS

Recently, direct experiments have shown the possibility of exchange interaction of phenolic inhibitors with free radicals formed in protein upon irradiation ([1]). These experiments substantiate our hypothesis on the effectiveness of using inhibitors of free-radical reactions as radioprotective compounds (see, for example, ([2])). According to this hypothesis, inhibitors introduced into animals before irradiation, being acceptors of free radicals, reduce the extent of damage caused in the organism by free-radical processes not characteristic of it under normal conditions.

As early as in experiments in 1957, we were able to detect the radioprotective action of 2,4-di-*tert*-butyloxytoluene and butyloxyanisole in experiments on mice irradiated with a lethal dose of radiation ([2]). Subsequently, analogous results were obtained by other authors ([3,4]) using propyl gallate (the normal propyl ester of gallic acid) and other derivatives of gallic acid. In work ([5]), with repeated sublethal irradiation by X-rays, a prolongation of the life of animals protected by certain antioxidant inhibitors was established.

The aim of the present work is to establish a correlation between animal survival and the antiradical activity of inhibitors. The existence of a regular relationship between the radiobiological and chemical-kinetic characteristics could serve as important confirmation of the correctness of the hypothesis concerning the use of low-toxicity inhibitors of free-radical processes in radiobiology. The antiradical activity of an inhibitor A was defined as the product of the relative effectiveness of the inhibitor ε (determined, for example, by the ability of the inhibitor to inhibit a chemical radical-chain reaction ([6])) and its concentration C: $A = \varepsilon C$.

The work was carried out in the concentration range of the preparations in which they do not exert a side toxic effect. Experiments were performed on 1526 mice of both sexes of the Balb line, weighing 18–22 g. Irradiation was car-

ried out on a RUT-200-20-3 apparatus, operating mode 15 ma, Cu filter = 0.5 mm. The irradiation intensity was 40–50 r/min, total dose 650–700 r. Surviving mice were considered to be those that lived 30 days. In control experiments the mortality of mice was 100%. The mean lifetime of control mice was 5–7 days. The preparations were administered intraperitoneally to the animals 15–45 min before irradiation. Water-soluble preparations were administered as solutions in distilled water. The preparations 2,4-di-*tert*-butyloxytoluene and 2,4,6-tri-*tert*-butylphenol, insoluble in water, were administered intraperitoneally in a 10% Tween-80 solution used as a solubilizer. The radioprotective action of sterically hindered phenols, their amino derivatives, polyphenols, and substituted oxypyridines was studied. In their chemical properties these substances are different; however, all of them are acceptors of free radicals and belong to the class of inhibitors of free-radical processes. In experiments on the oxidation of inhibited methyl oleate it was shown that all the tested substances predominantly enter into reaction with free radicals. If these inhibitors react with free radicals formed in the organism upon irradiation, then animal survival should be determined by the antiradical activity of the preparation. In Fig. 1, data are presented graphically on the relationship between survival of animals irradiated with a lethal–

Table 1

Name of preparation	Structural formula	ϵ	No. of mouse in Fig. 1	C, mg/kg	Number of mice in the experiment	Survival, %
Hydrochloride of 4-hydroxy-3,5-di-tert-butylphenylmethylaminomethyl· HCl	Phenol ring: OH; 3,5-di- $C(CH_3)_3$; CH_2ylamine NH_2· HCl	0.85	1	40	45	13.3
Hydrochloride of 4-hydroxy-3,5-di-tert-butylphenylmethylaminomethyl· HCl	Phenol ring: OH; 3,5-di- $C(CH_3)_3$; CH_2ylamine NH_2· HCl	0.85	2	45	30	20
4-Methyl-2,6-di-tert-butylphenol (ionol)	Phenol ring: OH; 2,6-di- $C(CH_3)_3$; CH_3	1	3	30	70	17

Name of preparation	Structural formula	ϵ	No. of mouse in Fig. 1	C, mg/kg	Number of mice in the experiment	Survival, %
4-Methyl-2,6-di-tert-butylphenol (ionol)	Phenol ring: OH; 2,6-di-$C(CH_3)_3$; CH_3	1	4	50	60	20
n-Propyl ester of gallic acid (propyl gallate)	Benzene ring: OH, OH, OH; $COOC_3H_7$	1	5	30	40	17*
n-Propyl ester of gallic acid (propyl gallate)	Benzene ring: OH, OH, OH; $COOC_3H_7$	1	6	50		30**
n-Propyl ester of gallic acid (propyl gallate)	Benzene ring: OH, OH, OH; $COOC_3H_7$	1	7	60	40	42*
3-Hydroxy-2,4,6-trimethylpyridine	Pyridine ring: N; OH; CH_3, CH_3, CH_3	0.3	8	150	30	43.5

Name of preparation	Structural formula	ϵ	No. of mouse in Fig. 1	C, mg/kg	Number of mice in the experiment	Survival, %
3-Hydroxy-2,4,6-trimethylpyridine	Pyridine ring: N; OH; CH_3, CH_3, CH_3	0.3	9	200	150	64.2
Hydrochloride of 3-hydroxy-2-ethyl-6-methylpyridine	Pyridine ring: $N \cdot HCl$; OH; C_2H_5; CH_3	0.25	10	200	110	41.8
Dihydrochloride of 3-hydroxy-2-ethyl-6-methyl-4-dimethylaminomethylpyridine	Pyridine ring: N; OH; C_2H_5; CH_3; $CH_2N(CH_3)_2 \cdot HCl$; HCl	0.3	11	280	280	42.1
2,4,6-Tri-tert-butylphenol	Phenol ring: OH; 2,4,6-tri-$C(CH_3)_3$	0.52	12	100	30	16.6
2,4,6-Tri-tert-butylphenol	Phenol ring: OH; 2,4,6-tri-$C(CH_3)_3$	0.52	13	200	31	40.7
Hydrochloride of 4-hydroxy-3,5-di-tert-amyl-α-methylbenzylamine	Phenol ring: OH; 3,5-di-$C(CH_3)_2C_2H_5$; $CH_3—CH—NH_2 \cdot HCl$	1.65	14	16	30	10.0

Fig. 1. Relationship between the radioprotective and antiradical activity of inhibitors. The numbers at the points correspond to the numbers in Table 1.

Figure 1: Fig. 1. Relationship between the radioprotective and antiradical activity of inhibitors. The numbers at the points correspond to the numbers in Table 1.

Name of preparation	Structural formula	ϵ	No. of mouse in Fig. 1	C, mg/kg	Number of mice in the experiment	Survival, %
Hydrochloride of 4-N,N-di(β-hydroxyethylaminomethyl)-2,6-di-tert-butylphenol	Phenol ring: OH; 2-$C(CH_3)_3$; 6-$C(CH_3)_2$; CH_2—$N((CH_2)_2OH)_2 \cdot HCl$	1.65	15	10	30	0
Control	—				500	0

* Similar results were obtained by A. A. Gorodetskii et al. (3).
** The survival of mice when propyl gallate was administered to them before irradiation at 50 mg/kg was taken by us from work (4).

doses, with protection by the preparations listed in Table 1, and the antiradical activity of these preparations. The established dependence is a simple linear function up to high values of survival of the irradiated mice.

The dependence obtained is statistically reliable. Using the formulas for linear regression (7), the regression coefficients and the variance were determined:

$a = 4.9 \pm 3.1, \quad b = (1.44 \pm 0.13) \cdot 10^2, \quad s = 5.4.$

Fig. 1. Relationship between the radioprotective and antiradical activity of inhibitors. The numbers at the points correspond to the numbers in Table 1.

These experiments confirm the promise of the proposed hypothesis and indicate that free radicals play a major role in the development of radiation injury.

The data on radioprotection by known antiradiation preparations—5-methoxytryptamine and β-mercaptoethylamine—do not fall on the straight line (Fig. 1). Earlier we established a difference in the action of these preparations and of inhibitors in chemical oxidative reactions (8). Apparently, this difference is also retained at the biological level.

As can be seen from Fig. 1, a high value of A can be achieved both by increasing ε and by decreasing the toxicity of the preparation, which makes it possible to

increase the concentration of the administered substance.

However, the ratio of the optimal radiobiological dose to the maximum tolerated dose differs among various inhibitors. For some of them it is close to unity, while for others it is considerably smaller. For example, 2,4-di-tert-butyloxytoluene gives the greatest survival when administered in an amount of 50 mg per 1 kg of body weight; at $C = 100$ mg/kg survival is zero, while the maximum tolerated dose is 400 mg/kg. For 3-oxy-2,4,6-trimethylpyridine the optimal dose is 200 mg/kg, and the maximum tolerated dose is 250 mg/kg.

It is possible that this difference between the optimal and maximum tolerated doses is explained by the fact that inhibitor radicals formed by the reaction $R^{\cdot} + HIn \rightarrow RH + In^{\cdot}$, accumulating in the organism, cease to be harmless to it. In this connection, the low toxicity of a strong inhibitor is a necessary, though insufficient, condition for a high antiradiation effect. Therefore, in radiobiological experiments with inhibitors, in addition to the relative effectiveness ε and the maximum tolerated dose, one should also take into account quantities characterizing the reactivity and toxicity of radicals from the inhibitors. The results obtained make it possible to regard inhibitors as a promising class of radioprotective agents.

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