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EFFECT OF ANTIOXIDANTS ON MEMBRANE-TOXIC EFFECTS OF ANTICHOLINESTERASES

É. P. Zatsepin, N. N. Churaev, T. A. Uspenskaya, G. D. Tirzit, and G. Ya. Dubur

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Some biologically active compounds are known to damage cell membranes [1]. One of the principal mechanisms of the membrane-toxic action of xenobiotics is acceleration of lipid peroxidation (LPO) under their influence. For instance, some anticholinesterases such as malathion, possess a natural pro-oxidant action, whereas other (0,0-dimethyl-2,2-dichlorovinyl phosphate; DDVP) do not possess pro-oxidant activity [3]. For this reason, specific methods of treatment of the corresponding forms of poisoning are frequently insufficiently effective.

The aim of this investigation was to study the possibility of correcting membrane-toxic effects of poisons by means of antioxidants. The organophosphorous insecticides, possessing membrane-stabilizing properties [5], and combining antioxidative activity (AOA) with ability to block Ca^{2+}-channels [2, 6, 8], were used in the experiments.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats (150-180 g) and albino mice. The velocity of LPO was determined by a chemiluminescence method based on slowing of the rise of the "slow flash" of chemiluminescence in supernatant obtained after

Institute of Toxicology, Ministry of Health of the USSR, Leningrad. Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. (Presented by Academician of the Academy of Medical Sciences of the USSR S. N. Golikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 12, pp. 623-624, December, 1990. Original article submitted March 20, 1990.

TABLE 1. Effect of 1,4-DHP Derivatives on LPO in Supernatant ($M \pm m$)

Compound	AOA, M^{-1}
Diludin	$(0{,}84 \pm 0{,}52) \cdot 10^5$
Foridon	$(1{,}93 \pm 0{,}48) \cdot 10^4$
Nifedipine	$(1{,}80 \pm 0{,}87) \cdot 10^3$
Compound I	$(2{,}39 \pm 0{,}88) \cdot 10^2$
Compound II	$(0{,}50 \pm 0{,}47) \cdot 10^2$

TABLE 2. Effect of Diludin on Velocity of LPO and Survival Rate of Animals after Malathion Poisoning ($M \pm m$)

Parameter	Control	Malathion	Malathion+ Tween-20	Diludin, mg/kg	
				45	90
Number of animals dying, %	0	50	93	81	20
MDA concentration	100	138±8	-	99±11*	73±4*
Concentration of DC	0	325±93	-	84±13*	109±20*

Legend. DC) Diene conjugates. MDA concentration expressed in % of control, DC concentration in mmoles/ml homogeneate. $^*p < 0.05$ Compared with poisoning by malathion.

centrifugation of rat brain homogenate (15,000*g*, 15 min) [1]. The velocity of LPO in the rat brain in vivo was estimated from accumulation of LPO products: diene conjugates [4] and malonic dialdehyde (MDA) [1].

The following 1,4-DHP derivatives were used: the disodium salt of 2,6-dimethyl-1,4-dihydropyridine-3,5-*bis*-carbonyloxy acetic acid (compound I), the sodium salt of 2,6,-dimethyl-3,5-dicarbethoxy-1,4-dihydroisonicotinic acid (compound II), 2,6-dimethyl-3,5-dicarbethoxy-1,4-dihydropyridine (diludin), 2,6-dimethyl-3,5-dimethoxycarbonyl-4-(2-nitrophenyl)-1,4-dihydropyridine (nifedipine) and 2,6-dimethyl-3,5-dimethoxycarbonyl-4-(2-difluoromethoxyphenyl)-1,4-dihydropyridine (foridon).

In the experiments of series 1 the effect of 1,4-DHP derivatives possessing AOA on the velocity of LPO and on the survival rate of albino rats after poisoning with malathion in a dose of 1 LD_{50} injected intramuscularly, was studied. The compounds were injected on the appearance of the first signs of poisoning (tremor, salivation): compound I intramuscularly, diludin intraperitoneally. The velocity of LPO and survival rate of the animals were determined 24 h after injection of the poison.

In the experiments of series 2, indices of protection of the albino mice were determined for the 1,4-DEP derivatives in the case of poisoning with malathion (intramuscular injection) and DDVP (intraperitoneal injection). The compounds also were injected intraperitoneally 30 min before injection of the poison for prophylactics purposes.

Atropine and compounds I and II were dissolved in physiological saline, nifedipine, foridon, and diludin in a 10% solution of Tween-20. Equal amounts of solvents were injected into the control animals. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

To assess the role of LPO in the pathogenesis of OPI poisoning, the most active antioxidant, namely diludin, was used (Table 1).

Its effect on the velocity of LPO in the rat brain was studied after poisoning: with malathion which, as was shown previously, possesses a marked pro-oxidant action [3].

The data given in Table 2 show that diludin normalizes the velocity of LPO and reduces the mortality of rats poisoned with malathion.

Unlike malathion, DDVP does not change the velocity of LPO in the brain [3], and a comparative study of the effect of antioxidants on the survival rate of mice poisoned with these two poisons, was accordingly carried out.

TABLE 3. Protective Effect of 1,4-DHP Derivatives and Atropine after Malathion Poisoning ($M \pm m$)

Dose, mg/kg	Atropine	Index of protection		
		nifedipine	foridon	compound 1
0,05	—	1,17±0,09	1,18±0,08	1,01±0,07
0,1	—	1,23±0,05*	1,18±0,07	1,01±0,08
0,5	1,02±0,09	1,24±0,05*	1,30±0,05*	1,02±0,04
1	1,01±0,08	1,09±0,08	1,11±0,09	1,02±0,06
2	1,12±0,07	—	—	1,09±0,07
5	1,13±0,05	—	—	1,08±0,05
10	1,15±0,06	—	—	1,08±0,05

Legend. $^*p < 0.05$.

The specific antidote atropine possessed virtually no protective action against, malathion poisoning, whereas the antioxidants protected the mice from death (Table 3); foridon and nifedipine, which possess high AOA, were more effective than compound I, which is only a weak antioxidant.

In DDVP poisoning, in which there is no effect on LP in the brain, antioxidants and Ca^{2+} channel blockers, in doses reducing the velocity of peroxidation, did not give a protective action and did not potentiate the antidotal effect of the cholinolytics and cholinesterase reactivators; meanwhile, specific antidotes, unlike in malathion poisoning, protected mice with DDVP poisoning from death.

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