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REACTIVATORS OF CHOLINESTERASE ACTIVITY IN METHYLPARATHION EXPOSED RATS

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Abstract. The cholinesterase activity and the biochemical antioxidant parameters in the blood of rats exposed to an organophosphate pesticide, methylparathion, in dose of 1/20 from LD_{50}, and subsequently treated with quaternary ammonium iodides from Mannich bases oximes have been determined. Our research on the reactivator effects of both original synthesized substances named Q_1 and Q_2, compared with toxogonin, showed their reactivator capacity according with cholinesterase activity and the biochemical effects caused by the membrane oxidative stress.

Key words: cholinesterase, methylparathion, toxogonin, Mannich bases oxime, oxidative stress, superoxide dismutase, catalase, glutathione peroxidase

Rezumat. A fost determinată activitatea colinesterazei şi a parametrilor biochimici antioxidanţi, în sângele şobolanilor intoxicaţi cu un pesticid organofosforic, metilparation, în doze de 1/20 din DL_{50} şi trataţi apoi cu ioduri cuaternare de amoniu ale oximelor unor baze Mannich. Cercetările noastre au urmărit efectele reactivatoare ale celor două substanţe originale de sinteză, denumite Q_1 şi Q_2, comparativ cu toxogoninul, demonstrând capacitatea de reactivare a acestora în acord cu activitatea colinesterazică şi cu efectele biochimice cauzate de stress-ul oxidativ membranar.

Cuvinte cheie: colinesteraza, metilparation, toxogonin, oxime ale bazelor Mannich, stress oxidativ, superoxid dismutaza, catalaza, glutation peroxidaza

INTRODUCTION

Oximes, substances generally related with carbonylic group, play an important role in organic synthesis, as well as in modern therapeutics (1). The organophosphate group of pesticides interferes with cholinesterase system. Acetylcholine is the neurotransmitter substance released in the synapses between the axon terminals of the postganglionic fibers and the effector organ cells in the case of parasympathetic system (2). Cholinesterase is found at synapses and neuromuscular junctions, where it destroys acetylcholine after performing its neurotransmitter role. Substances such as: tetraethyl pyrophosphate, diisopropyl fluorophosphates and methylparathion (MP) inhibit or deactivate acetylcholinesterase. The phosphoryl group binds very tightly to the enzyme site at which binding of the acyl group normally occurs, thus preventing attachment of the acetylcholine.

In the case of intoxication with organophosphates, the antidote is a substance with a quaternary ammonium group (NH_4^+), named toxogonin, that the most efficiently passes the blood

brain barrier (3). A series of oximes derived from some Mannich bases were transformed into quaternary ammonium salts at the amine nitrogen atom. Oximes Q_1 and Q_2 were prepared from two series of ketonic Mannich bases, namely1-(2'hydroxy–5'methylphenyl)-3-dimethylamino–1-propanol and 1-(2'hydroxy–4'-morpholineamino)-1-propanol. Quaternization with methyl iodide was attempted in several organic solvents as dioxane, tetrahydrofuran, ethanol (4).

Oxygen free radicals may play a role in the intoxication with organo-phosphates like MP and are neutralized by a series of enzymatic systems (5). Catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) showed significant increase, as the antioxidant defense line against peroxidation damages in organo-phosphates intoxication compared with the control group of animals.

MATERIAL AND METHODS

The experiment was carried out on male Wistar rats (180 - 200 g body weight) from the Biobreeding of the Institute of Virology of Bucharest, Romania. All animals were housed under constant, identical conditions of temperature (18-19 °C) receiving food (a natural diet: milk, bread, oats and carrots) and water ad libitum (6).

The administration route was intraperitoneal injection in a unique dose. The experiment consists in the treatment of five groups of 10 rats each as follows:

Control group:
0.5 ml saline solution (SS) / 100g b.w.

Exposed group:
SS + 0.029 mg MP/Kg/ b.w./day

Standard reactivated group:
SS + 0.029 mg MP / Kg b.w./day + 112 mg toxogonin /Kg b.w.

Reactivated group I:
SS + 0.029 mg MP / Kg b.w./day + 18 mg Q_1 / Kg b.w.

Reactivated group II:
SS + 0.029 mg MP / Kg b.w./day + 9 mg Q_2 / Kg b.w.

The animals from each group were anesthetized with ethyl ether and sacrificed by cutting the *arteria carotis* and the *vena jugularis* after 15 minutes of intoxication with MP or treatment with toxogonin (the standard reactivator) or with both substances Q_1 and Q_2. Blood samples were taken in order to determine the serum cholinesterase activity and biochemical antioxidant parameters in red blood cells: SOD, CAT and GPx.

The serum cholinesterase activity was assessed according to the method described by Ellman (7). This method uses acylthiocholine ester as substrate; thiocholine can be measured by reaction with chromogenic disulfide agents such as dithionitrobenzoate (DTNB). The DTNB reaction produces a yellow color that can be measured at 410 nm.

The following antioxidant parameters CAT, SOD and GPx were investigated in the erythrocytes of rats. CAT was investigated according to the method described by Aebi (8), based on estimation of hydrogen peroxide decomposed during 15 seconds, at room temperature. Enzymatic activity was expressed by K_{15}/g of hemoglobin.

K is the first order rate constant of the enzymatic decomposition of H_2O_2 at 240 nm., the rate being proportional to the concentration of peroxide, during about 1.0 min. at H_2O_2 concentration in the range 0.01– 0.05 M. K_{15} is K at 15 s. time interval (9).

Erythrocyte SOD was investigated according to the method described by Minami and Yoshikawa (10). The method is based on the inhibition by SOD of pirogallol induced nitroblau tetrazolium reduction. The amount of enzyme that causes 50% inhibition of reduction of nitroblautetrazolium by pyrogallol was definited as 1 unit of SOD activity.

Erythrocyte GPx activity was investigated according to the method described by Fukuzawa (11).

The statistical processing of data was made using EPI INFO 6 program and involved means, standard deviations, "t" test and confidence intervals (CI).

RESULTS AND DISCUSSION

In our experiment, cholinesterase activity was significantly decreased for exposed group as compared with the control group (table 1). The decrease may be caused by the irreversibly blocking activity of these compounds on the active site of the enzyme. The catalytic site of acetylcholinesterase, the binding locus for the quaternary nitrogen of acetylcholine, is anionic and involves histidine and serine residues. Some inhibitors, like MP, form stable esters of the esteratic site, resulting in irreversible inhibition. For the standard reactivated group of animals treated with toxogonin, cholinesterase activity was significantly increased, as compared with the exposed group, because the strong cationic group of this compound removes the MP group from the esteratic site and restores the activity of the enzyme. For both reactivated groups, I and II, possessing the quaternary ammonium iodides from Mannich bases oximes (Q_1 and Q_2), the level of cholinesterase activity was significantly increased as compared with the standard reactivated group with toxogonin.

Table 1. The mean values of serum cholinesterase activity (IU/L)

	Control group	Exposed group	Standard reactivated group	Reactivated group I	Reactivated group II
Mean value ± Standard deviation	8439.9 ± 2.8	5377.9± 826.7	6929.5±1054.8	6530.0 ±925.3	6280.0 ±826.4
95%CI	(8438.1 – - 8441.7)	(44890.6 – - 5890.6)	(6275.3 – - 7583.8)	(5956.0 - - 7103.9)	(5767.4 – - 6792.6)
p		≤ 0.05 *	≤ 0.005**	≤ 0.001**	≤ 0.006**

* Compared with the control group **Compared with the exposed group

The new reactivators, Q_1 and Q_2, significantly increase the cholinesterase activity as compared with the MP exposed group of animals.

Erythrocyte CAT, the first line of defense against hydroperoxides, before they can damage membranes and other cell components, was decreased in the intoxication with MP (table 2). The results showed that the CAT was significantly increased after administration of toxogonin and also for new quaternary ammonium iodides derived from Mannich bases oximes Q_1, but not for Q_2.

Tabel 2. The mean values of erythrocyte catalase activity (K_{15}/ g Hb)

	Control group	Exposed group	Standard reactivated group	Reactivated group I	Reactivated group II
Mean value ± Standard deviation	700.0 ±126.8	475.0 ± 142.0	660.3 ±119.5	540.0±97.8	500.0 ±90.5
95% CI	(621.4 -778.6)	(533.9– 426.1)	(586.2 -34.4)	(479.9 - 600.0)	(556.1 – 443.8)
p		< 0.0001*	< 0.0001**	< 0.013**	> 0.05**

* Compared with the control group ** Compared with the exposed group

SOD, an enzyme which destroys the superoxide anion, O_2^-, is significantly decreased for the exposed group as compared with the control group (table 3). By treatment with the reactivator Q_1, the enzyme concentration was significantly increased as compared with the exposed group, but not with the reactivator Q_2.

Table 3. The mean values of erythrocyte SOD activity (U/Ht x 100)

	Control group	Exposed group	Standard reactivated group	Reactivated group I	Reactivated group II
Mean value ± Standard deviation	6.98 ±0.69	5.35±0.52	6.76±0.66	5.95±0.58	5.50±0.54
95% CI	(7.41– 6.54)	(5.03 - 5.67)	(7.16 – 6.35)	(6.31 – 5.59)	(5.16 – 5.83)
p		< 0.05*	< 0.0001**	< 0.001**	> 0.05**

* Compared with the control group ** Compared with the exposed group

GPx provides a second line of defense against hydroperoxides before they damage membranes and other cell components. The activity of GPx was significantly decreased in reactivated group I and II as compared with the exposed group (table 4).

Tabel 4. The mean values of glutathione peroxidase activity (IU/ g Hb)

	Control group	Exposed group	Standard reactivated group	Reactivated group I	Reactivated group II
Mean value ± Standard deviation	3.33±0.59	6.00±1.02	4.05±0.0135	4.63±0.0021	4.20±0.0006
95% CI	(2.96- 3.69)	(5.37-6.63)	(3.96-4.13)	(4.49-4.76)	(4.10-4.29)
p		< 0.0001*	< 0.0001**	< 0.0001**	< 0.0001**

*Compared with the control group ** Compared with the exposed group

CONCLUSIONS

1.The results confirm the important role of the quaternary ammonium iodides based on Mannich bases oximes Q_1 and Q_2 as reactivators of cholinesterase in poisonings with organo-phosphoric compounds.

2.These new reactivators Q_1 and Q_2 increased the antioxidant system but Q_1 appears to be a better reactivator as compared with Q_2 because it increases all the antioxidant systems against hydroperoxides, superoxide anions and hydrogen peroxides before they destroy the cell membranes.

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