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SUBSTITUTED MONOQUATERNARY OXIMES AS REACTIVATORS OF CYCLOSARIN- AND CHLORPYRIFOS-INHIBITED ACETYLCHOLINESTERASE

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This paper describes an *in vitro* study of three potential acetylcholinesterase (AChE; EC 3.1.1.7) reactivators derived from a monoquaternary reactivator pralidoxime. Compounds used were pyridinium-2-aldoxime-4-carbamoyl-N-methyl iodide (TO231), pyridinium-2-aldoxime-4-ethoxycarbonyl-N-methyl iodide (TO237), and pyridinium-2-aldoxime-5-ethoxycarbonyl-N-methyl iodide (TO238). Pralidoxime and obidoxime were used for comparison. Nerve agent cyclosarin and pesticide chlorpyrifos were used as organophosphorus cholinesterase inhibitors. The source of AChE was rat brain homogenate. None of the tested oximes was able to reactivate cyclosarin-inhibited AChE (at 1.0 mmol L^{-1} oxime concentration). In case of chlorpyrifos, TO231 was the most potent AChE reactivator with an 82 % reactivation at 1.0 mmol L^{-1} oxime concentration. This reactivating potency equals that of pralidoxime and obidoxime. TO238 was less effective, and TO237 did not reactivate chlorpyrifos-inhibited AChE at all. None of the tested AChE reactivators, reference compounds included, could be considered universal for both chlorpyrifos- and cyclosarin-inhibited AChE.

KEY WORDS: *brain cholinesterase, cholinesterase inhibition, nerve agents, organophorphorus compounds, pesticides, poisoning, reactivation, treatment*

The toxicity of organophosphorus (OP) compounds - which are used in agriculture as pesticides, in medicine as drugs and in the military as nerve agents - is mostly related to the inhibition of acetylcholinesterase (AChE; EC 3.1.1.7), a very important enzyme which splits the neuromediator acetylcholine (ACh) at the synaptic clefts. Generally, anticholinergics (as functional drugs) and AChE reactivators (as causal drugs) are used as the first aid antidotes in OP poisoning (1-3).

In contrast to anticholinergics that can be administered in case of any nerve agent poisoning, a need has arisen for specific AChE reactivators to counter AChE inhibition. Although there are many potential AChE reactivators, none is able to reactivate all OP inhibitions, regardless of the inhibitor structure (4-6). This is why many laboratories throughout the world are interested in finding a new, broad-spectrum AChE reactivator (7-11). Curretly the most promising AChE reactivator HI-6 has a limited spectrum due to its low efficacy in rectivating tabun- and pesticide-inhibited AChE (12, 13). Pralidoxime, a gold standard among AChE reactivators (14), which is presently used as antidote in the US, is a very poor reactivator of AChE inhibited by a nerve agent (15-17).

This study evaluated monoquaternary oximes TO238, TO231, and TO237, prepared in our department (18). Although these oximes are known for more than eight years, they have never been tested

as AChE reactivators after cyclosarin or chlorpyriphos poisoning. We studied these compounds as *in vitro* reactivators of cyclosarin- and chlorpyrifos-inhibited AChE using our general potentiometric method (19).

MATERIAL AND METHODS

All tested compounds were prepared earlier at the Department of Toxicology of the Faculty of Military Health Sciences (Hradec Kralove; Czech Republic) (18). Their structures are listed in Figure 1. Purities of the tested AChE reactivators were established using TLC (DC-Alufolien Cellulose F; Merck, Germany; mobile phase BuOH-CH_3COOH-H_2O 5 : 1 : 2; detection by solution of Dragendorff reagent). Chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate] was obtained from Riedel-de Haen as analytical standard (99.2 % purity). Nerve agent cyclosarin (GF; O-cyclohexylmethylflourophosphate) was obtained from the Military Facility Brno (97 % purity). All other chemicals used were of reagent grade (Sigma-Aldrich, Czech Republic).

Rat brain homogenate was incubated with the OP compound for 30 min and the AChE reactivator was then added. Ten minutes later the substrate (ACh) was added and the activity measurement started. The released acetic acid from ACh was titrated using sodium hydroxide. The consumption of sodium hydroxide was proportional to the enzyme activity, allowing us to calculate the reactivation potency of the tested AChE reactivators. The whole *in vitro* experiment (including the equation used to calculate the percent of reactivation) was described in detail earlier (19, 20).

RESULTS AND DISCUSSION

Table 1 shows that AChE reactivators TO231 and TO238 had a higher reactivation potency against the pesticide chlorpyrifos than against cyclosarin. Only obidoxime and TO231 achieved a low reactivation potency against cyclosarin (below 10 %). For chlorpyrifos-inhibited AChE, pralidoxime, obidoxime, TO231, and TO238 reached a promising reactivation potency at the concentreation of 1.0 mmol L^{-1}. Only obidoxime showed a promising reactivation potency at the concentration of 10.0 μmol L^{-1} and was thus the most promising oxime in this study.

There is no single AChE reactivator able to serve as a broad-spectrum antidote against both nerve agent and pesticide poisoning. Currently the most promising oxime HI-6 does not sufficiently reactivate tabun- and pesticide-inhibited AChE (12, 13). This is why attempts to synthesize more effective oximes continue (7-11). To find an effective AChE reactivator new compounds are being developed (21, 22). However none of these substances, tested in a number of *in vitro* and *in vivo* studies, can be considered broad-spectrum reactivators (12, 13, 23, 24).

We therefore focused our attention on AChE reactivators that had been known for more than eight years, but had been never tested for their broad-spectrum reactivation potency. Owing to the fact that the only experiments done with these oximes were conducted on mice using a pesticide fosdrine,

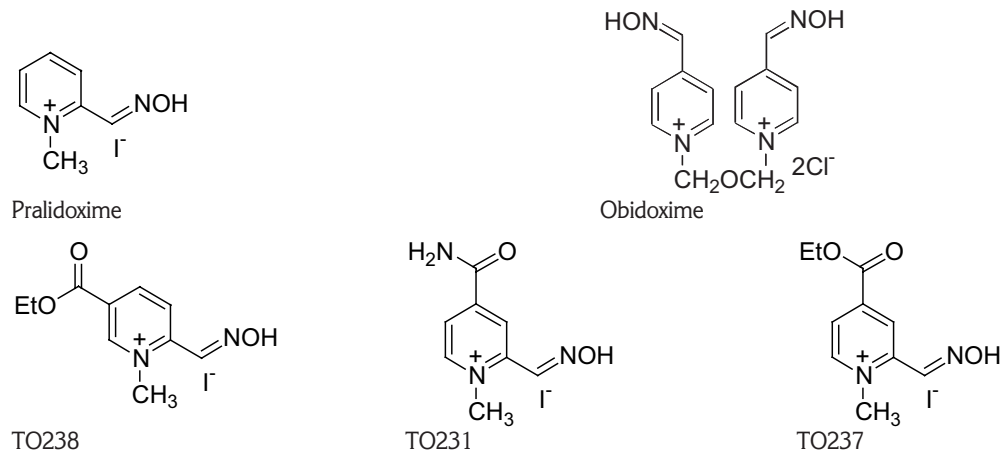

Figure 1 *Structure of tested AChE reactivators*

Table 1 *Reactivation potency of oximes tested (enzyme source: rat brain homogenate; time of inhibition: 30 min; time of reactivation: 10 min; pH 7.6; 25 °C; n=3)*

Oxime	ORGANOPHOSPHORUS COMPOUND			
	Chlorpyrifos		Cyclosarin	
	Reactivation potency / % *	Reactivation potency / % #	Reactivation potency / % *	Reactivation potency / % #
Pralidoxime	9	80	0	0
Obidoxime	43	76	5	2
TO238	0	48	0	0
TO231	6	82	0	3
TO237	0	0	0	0

** oxime concentration = 10.0 μmol L^{-1}*

 # oxime concentration = 1.0 mmol L^{-1}

we decided to test them against the nerve agent cyclosarin and a pesticide group member chlorpyrifos (18). Our results showed that none of the tested oximes was able to reactivate cyclosarin-inhibited AChE, but some oximes reached promising reactivation potency against chlorpyrifos at the concentration of 1 mmol L^{-1}. Our results suggest that there is no point in investigating these reactivators further in order to obtain a broad-spectrum reactivator.

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Sažetak

SUPSTITUIRANI MONOKVATERNI OKSIMI KAO REAKTIVATORI ACETILKOLINESTERAZE INHIBIRANE CIKLOSARINOM I KLORPIRIFOSOM

U ovome *in vitro* istraživanju testiran je reaktivacijski potencijal triju reaktivatora acetilkolinesteraze (AChE; EC 3.1.1.7) dobivenih iz monokvaternog reaktivatora pralidoksima. Testirani su sljedeći spojevi: 4-karbamoil-2-aldoksim-1-metilpiridinijev jodid (TO231), 4-etoksikarbonil-2-aldoksim-1-metilpiridinijev jodid (TO237) te 5-etoksikarbonil-2-aldoksim-1-metilpiridinijev jodid (TO238). Za usporedbu su uzeti pralidoksim i obidoksim. Kao inhibitori kolinesteraze rabljeni su živčani otrov ciklosarin i pesticid klorpirifos. Izvor acetilkolinesteraze bio je homogenat mozga štakora. Nijedan od testiranih oksima nije uspio reaktivirati acetilkolinesterazu inhibiranu ciklosarinom pri koncentraciji oksima od 1.0 mmol L^{-1}. Kod inhibicije klorpirifosom TO231 pokazao se najsnažnijim reaktivatorom acetilkolinesteraze s reaktivacijom od 82 % pri koncentraciji oksima od 1.0 mmol L^{-1}. Ovaj reaktivacijski potencijal jednak je onomu pralidoksima i obidoksima. Slabiju je djelotvornost iskazao TO238, a TO237 nije uspio reaktivirati acetilkolinesterazu inhibiranu klorpirifosom. Stoga nijedan od testiranih spojeva (uključujući i referentne oksime) ne može služiti kao univerzalni reaktivator acetilkolinesteraze inhibirane klorpirifosom i ciklosarinom.

KLJUČNE RIJEČI: *inhibicija kolinesteraza, kolinesteraza mozga, liječenje, organofosforni spojevi, pesticidi, živčani otrovi*

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