ORIGINAL ARTICLES

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New synthesized oximes active in nerve agents' hazards

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Abstract: *Object: The aim of the study is to select the most active new imidazolium-quinuclidinum-oxime, from some similar chemical compounds synthesized in our chemistry department, with sufficient efficacy to decrease the acute toxicity of neurotoxic organophosphates known as nerve agents. Method: The experimental study consist in vivo testing the antidotal efficacy of obidoxime and of selected imidazolium oximes synthesized in our chemistry department. Each oxime was included, by equimolar replacing the obidoxime, in an antidotal formula, which also contains atropine. The above mentioned formula containing atropine and obidoxime was used as reference. The protective ratio, defined as the ratio between the lethal median dose of the poisoned and treated study group and the median lethal dose (LD50) of the poisoned and untreated study groups was one of the used parameters in order to select a new active chemical structure in counteracting the neurotoxic organophosphorus compounds acute toxicity. Another studied parameter was the erythrocyte acetylcholinesterase value measured in whole blood 24 hours after exposure. Results: The protective ratio against an organophosphorus compound were the follow: obidoxime chloride: 2; 1,3-dimethyl-2-hydroxyethyl-imidazolyliodide: 1,75;3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl]quinuclidin-dichl-oride: 2,5; 1-methyl-quinuclidin-3-iodide: 1,5. The erythrocyte acetycholinesterase main values were the following: the unpoisoned and untreated study group:3,45 ±0,13mmol/dl; the poisoned and untreated study group: 0,89 ±0,09 mmol/dl; the poisoned and 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl]quinuclidindichloride treated study group:2,89 ±0,11 mmol/dl; the poisoned and obidoxime treated study group: 2,53±0,15 mmol/dl. Conclusions: 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl] quinuclidindichloride synthesized in our chemistry department, has shown a better protective ratio and a more prolonged surviving time than the reference (obidoxime). It has shown the best AChE reactivation of all the synthetized compounds. This compound can be a cheap and good option for replacing obidoxime in the antidotal formula active in nerve agent exposure.*

Keywords: *obidoxime chloride; 1,3-dimethyl-2-hydroxyethyl-imidazolyl-iodide;3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2oxapropyl] quinuclidin dichloride; 1-methyl-quinuclidin-3-on-iodide; organophosphorus compounds; nerve agents*

INTRODUCTION

Strategic expert analyzes have shown that in the next period, there may be international conflicts, amplifying the risk of attempts to use highly toxic chemicals as weapons of mass destruction.[1]

Romania through its geostrategic position, being the member of NATO, can provide a better potential target of such actions.

Neurotoxic organophosphorus compounds (sarin, soman, tabun, most insecticides) are chemical compounds, phosphonic acid esters with central and

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peripheral properties, acetyl-cholinesterase and butyryl-cholinesterase irreversible inhibitors with toxic effects on smooth and striated muscles and central nervous system.[2-4]

They were registered on the list of substances to be destroyed in accordance with international regulations (Convention on the Prohibition of Development, Production and Stockpiling Chimice-Geneva).

Although banned by international conventions, these toxic substances have been used in terrorist attacks against civilians (Tokyo 1995 Halabja in 1987, Anfal-1988). In this context geostrategic and due to soaring use of organophosphorous insecticides, development of medical measures to protect against the lethal toxicity is one of the priorities of research within specialized programs of NATO (NBC – Science for Peace) and European Union (Chemical Program Weapons, Monitoring and Protection, created in the OPCW).

The acute toxicity of organophosphorus compounds known as nerve agents mainly result from their action as irreversible inhibitors of acetylcholinesterase (AChE). They suffer some conformational and chemical changes, resulting a phenomenon known as "aging", whose speed of appearance is directly proportional to the toxicity of compounds.[5,6]

Accumulation of acetylcholine stimulation leads to persistent cholinergic muscarinic receptors that trigger the syndrome whose symptoms include miosis, salivation, bronchial hypersecretion, bradycardia, bronchoconstriction, hypotension and diarrhea.[7]

Another effect of organophosphate anticholin-esterases is the desensitization of nicotinic receptors followed by overstimulation, translated by skeletal muscle twitching and subsequent paralysis.[8] Central nervous system toxic effects include anxiety, restlessness, confusion, ataxia, tremors, convulsions, paralysis, cardiorespiratory effects and coma [9].

The treatment of nerve agents poisoning is based on an antimuscarinic agent (atropine), and an acetyl-cholinesterase reactivator called oxime according to its chemical structure. Atropine blocks the effects of accumulated acetylcholine resulting overstimulation of muscarinic receptors.[9,10]

Acetylcholinesterase reactivators dephosphorylate the acetylcholinesterase – organophosphorus complex, reactivating the enzyme activity.[11] The early appeared seizures were counteracted by benzodiazepines.

Currently available oximes (pralidoxime, obidoxime), have been shown to be less effective against one of the most toxic nerve agents (soman tabun). There is a strong interest in developing new, more potent acetyl cholinesterase reactivators with oxime structure.[12]

The present paper represents an in vivo screening study in selecting more potent chemical compounds, active in counteracting the nerve agents acute toxicity. The paper describes an experimental test of antidotal efficiency of some compounds containing imidazolium, or quinuclidinium rings, which equimolar replace obidoxime chloride in the antidoltal formula.

The results were expressed as the protective ratio representing the ratio between the DL50 of the neurotoxic compound administred to the poisoned and treated rat study group and the DL50 administered to the organoposphate compound of the poisoned and untreated study group.

MATERIAL AND METHODS

Chemicals

- obidoxime chloride (CAS number 111-90-9);
- atropine sulphate (CAS number 5908-99-6) were purchased from Sigma Aldrich;
- diclorvos (PESTANAL CAS number 62-73-7) were purchased from Sigma Aldrich.
- the experimentally tested quinuclidinium-imida-zolium oximes were synthetized in the chemistry department of Medical Military Research Center.

Animals

Male Wistar rats (150-200g) were maintained on rice husk in polypropylene cages. Wistar free access to water and rodent pellet food. The study was

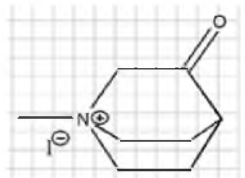
approved by the Ethical Committee on Animal Experimentation.

Acetycholinesterase measurement method: Elisa kit.

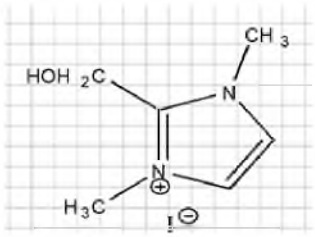
The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of Acetylcholinesterase in human serum, plasma and other biological fluids.

Target Information: Acetylcholinesterase hydrolyzes the neurotransmitter, acetylcholine at neuromuscular junctions and brain cholinergic synapses, and thus terminates signal transmission. It is also found on the red blood cell membranes, where it constitutes the Yt blood group antigen.

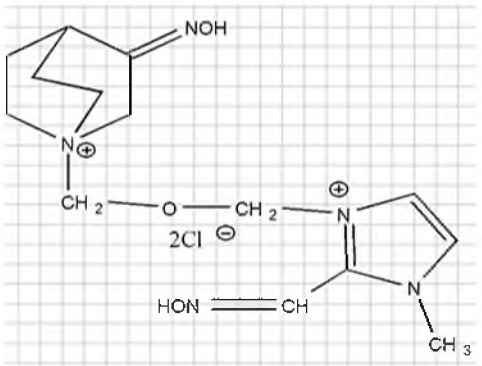
Figure 1: The chemical structure of the in vivo tested compounds as acetycholinesterase reactivators



1methyl-quinuclidine-3-on-iodide



1,3-dimethyl-2-hidroxyethyl-imidazolyl-iodide



3oxim[3(2-hidroxy-iminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride

Treatments

120 Wistar rats were divided into 12 groups, each, including ten rats as follows:

- 1st group: control group unpoisoned, untreated
- 2nd group: poisoned with diclorvos (1,5 DL50) and untreated;
- 3rd group: poisoned with diclorvos (1,5 DL50,) after one minute, were administered the antidotal formula including atropine (2mg) and obidoxime chloride (250 mg);
- 4th group: poisoned with diclorvos (1,5 DL50), after one minute, were administered the antidotal formula including atropine (2 mg) 1methyl-quinuclidine 3-on iodide in an equimolar dose with obidoxime chloride (78,5 mg);
- 5th group: poisoned with diclorvos (1,5DL50,) after one minute were administered the antidotal formula including atropine (2 mg), 1,3- dimethyl-2-hidroxy-ethylimidazolyl iodide in an equimolar dose with obidoxime chloride (61,94 mg/kg);
- 6th group: poisoned with diclorvos (1,5 DL50), after one minute, were administered the antidotal formula including atropine (1,5 mg) and 3oxim[3(2-hidroxy-iminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride (118 mg/kg).

The above mentioned oximes were experimentally tested against 1.5, 1.75, 2, 2.5, DL50.

24 hours after poisoning mortality, protective ratio was registered and erythrocyte achetylcolinesterase activity values were measured.

RESULTS

The aim of the study is to evaluate the most active newly synthesised imidazolquinuclidine-oxime with the great efficacy in reactivating the phosphorylated acetylcholinesterase through some similar chemical compounds synthesized in our chemistry department, able to decrease acute neurotoxic compounds toxicity.

The protective ratio of the obidoxime and experimentally tested formulas correlated with their efficacy as acetylcholinesterase reactivators are represented in the Table 1.

The 3 oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2-oxapropyl] quinuclidine dichloride protective ratio correlated with its acetylcholinesterase reactivator activity is greater than the obidoxime, resulting a

better antidotal efficacy.

Statistical analysis Student's t-test probability associated with statistically significant differences between mean values of erythrocyte acetyl-cholinesterase between the control study group and the poisoned and treated study groups is represented in tables 2-7.

The poisoned and untreated group statistically significantly differs from normal in terms of the acetylcholinesterase inhibition.

Neurotoxic organo-phosphorous compounds cause in the intoxicated group and untreated study group an acetylcholinesterase inhibition of 75.08%, incompatible with survival.

Obidoxime and new synthesized imidazolium-quiniclidinium oximes highlighted new capabilities of acetylcholinesterase reactivation correlated with the protection index.

3-oxime compound [3-(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl]-quinuclidine-dichloride-quinuclidine 3-on iodide revealed a protection index bigger than other obidoxime and other imidazolium compounds studied.

Explanation of this is that the quinuclidine ring, by its position intensifies allosteric oxime group (Table 1).

Tables 2-7 reveals that the intoxicated untreated control group differs statistically significantly from normal and so the intoxicated study groups were treated with different therapeutic formulations.

Table 1: The protective ratio of the antidotal formulas containing the experimentally tested imidazolium oximes as AChE reactivators

Study group	**Acetylcholinesterase reactivator**	**Dose of reactivators** (mg/kg)	**Protective ratio** (DL_{50})	**Acetylcholinesterase reactivation due to oxime** (%)
2	-	-	-	
4	1methyl-quinuclidine 3-on iodide	78.58	1.5	47.82
8	1,3-dimethyl-2-hidroxyethyl-imidazolyl iodide	61.94	1.75	60.28
10	obidoxime chloride	100	2	73.33
12	3 oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride	118.88	2.5	82.31

Table 2: Statistical analysis of the probability P (T test) associated with average values of erythrocyte AchE of unpoisoned and poisoned and untreated study groups

Study group	**The main value of erythrocyte acetylcholinesterase** (mmol/dl)	**P** (T test)	**Observations**
1	3.45 ± 0.13	0.003	$P \leq 0.05$ statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 1: unpoisoned and untreated study group;
Study group 2: paraoxon poisoned and untreated study group

Table 3: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and poisoned and obidoxime treated study groups

Study group	The main value of erythrocyte acetylcholinesterase (mmol/dl)	P (T test)	Observations
10	2.53 ± 0.15	0.007	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 10: paraoxon poisoned and obidoxime treated study group;
Study group 2: paraoxon poisoned and untreated study group.

Table 4: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and poisoned and 1,3-dimethyl-2-hidroxyethylimidazolyl iodide treated study groups

Study group	The main value of erythrocyte acetylcholinesterase (mmol/dl)	P (T test)	Observations
8	2.01 ± 0.07	0.004	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 8: paraoxon poisoned and 1,3-dimethyl-2-hidroxyethylimidazolyl iodide treated study group;
Study group 2: paraoxon poisoned and untreated study group.

Table 5: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and poisoned and 1methyl-quinuclidine 3-on iodide study groups

Study group	The main value of erythrocyte acetylcholinesterase (mmol/dl)	P (T test)	Observations
4	1.65 ± 0.03	0.009	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 4: paraoxon poisoned and iodide treated study group;
Study group 2: paraoxon poisoned and untreated study group

Table 6: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, untreated and 3-oxim[3(2-hidroxyiminomethyl-1- imidazolyl)-2oxapropyl] quinuclidine dichloride- quinuclidine 3-on iodide treated study groups

Study group	The main value of erythrocyte acetylcholinesterase (mmol/dl)	P (T test)	Observations
12	2.84 ± 0.05	0.0002	P ≤ 0.05 statistically significant difference between groups
2	0.86 ± 0.09		

Legend:
Study group 12: paraoxon poisoned and 3-oxim[3(2- hidroxyiminomethyl-1- imidazolyl)-2oxapropyl] quinuclidine dichloride- quinuclidine 3-on iodide treated study group;
Study group 2: paraoxon poisoned study group

Table 7: Statistical analysis of the probability P (T test) associated with average values of erythrocyte acetylcholinesterase of poisoned, obidoxime treated and 3-oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride-quinuclidine 3-on iodide treated study groups

Study group	The main value of erythrocyte acetylcholinesterase (mmol/dl)	P (T test)	Observations
12	2.84 ± 0.05	0.01	P ≤ 0.05 statistically significant difference between groups
10	2.53 ± 0.15		

Legend:
Study group 12: paraoxon poisoned and 3-oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride-quinuclidine 3-on iodide treated study group;
Study group 10: paraoxon poisoned and obidoxime treated study group

Table 8: The correlation between administered dose of acetylcholinesterase reactivator and the pharmacodynamic effect

Study group.	Reactivator dose used in the antidotal formula (mg)	AChE Mmol/ml	Correlation coeficicient
4	61,94	1,65	0,9927
8	78,58	2,01	
10	100	2,53	
12	118,88	2,84	

Legend:
Study group 4: poisoned study group and 1methyl-quinuclidine 3-on iodide treated;
Study group 8: poisoned study group and 1,3-dimethyl-2-hidroxyethyl-imidazolyl iodide treated;
Study group 10: poisoned study group and obidoxime chloride treated;
Study group 12: poisoned study group and 3 oxim[3(2-hidroxyiminomethyl-1-imidazolyl)-2oxapropyl] quinuclidine dichloride treated;

From a medical standpoint, an organophosphoric nerve poisoning that caused inhibition of acetyl-cholinesterase survival limit was applied.

This intoxication was further antagonized with different formulas containing the synthesized imidazoliumquinuclidinium oximes and obidoxime as antidotes.

They showed mean values of acetylcholinesterase significantly different from the poisoned and untreated group. The studied imidasolium quinuclidinium oximes showed average values of acetylcholinesterase statistically significantly different between them, thus emphasizing that the doses used may cause significant variations in therapeutic effect.

Table 8 highlights the so-called "dose finding", the correct correlation between dose and pharmaco-dynamic effect. The correlation coefficient of 0.9927 demonstrates that doses of acetylcholinesterase reactivators used in the antidotal formulas are correct, being those that cause maximum pharmacodynamic effect.

CONCLUSION

• 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2-oxapropyl] quinuclidindichloride synthesized in the Medical Military Research Centre Chemistry Department has shown a better protective ratio and a more prolonged surviving time than obidoxime considered as reference.

• It has shown the best acetylcholinesterase reactivation of all the synthetised compounds and obidoxime

• The very good correlation dose-effect highlights that the correct dose of acetylcholinesterase reactivator was chosen in order to obtain the better pharmacodynamic effect.

• 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2-oxapropyl] quinuclidindichloride can be considered an efficient antidote in neuroparalytic organo-phosphorous hazards.

• 3-oxime-[3-(2-hidroxyimino-methyl-1-imidazolyl-)-2-oxapropyl] quinuclidindichloride can be a cheaper and better option for replacing obidoxime in the antidotal formula active in nerve agent poisoning.

• Thus one can conclude that the result of the experimental study is consistent with the proposed object.

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