EFFECTIVITY OF NEW ACETYLCHOLINESTERASE REACTIVATORS IN TREATMENT OF CYCLOSARIN POISONING IN MICE AND RATS

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The present study was performed to assess and compare a therapeutic efficacy of obidoxime, HI-6, BI-6 and HS-6 administered in equimolar doses and combined with atropine in cyclosarin-poisoned mice and rats. It was demonstrated that all the therapeutic regimens tested, were able to decrease the cyclosarin-induced toxicity significantly – at least 1.5 times. Higher therapeutic ratios, almost three times, were achieved in rats in comparison with mice. The highest therapeutic ratio was achieved for therapeutic regimen consisting of HI-6 and atropine in both mice and rats. Obidoxime was the least effective oxime in the treatment of cyclosarin intoxication. The BI-6 oxime was significantly more efficacious than obidoxime (in both mice and rats) and HS-6 (in rats) but its effectiveness did not reach the efficacy of HI-6.

INTRODUCTION

Organophosphorus (OP) compounds such as soman, sarin or cyclosarin are considered potential warfare agents because of their toxicity. The potential for exposure to OP compounds exists on the battlefield (e.g., Iran-Iraq war, Desert Storm), in the civilian sector as a threat by a terrorist group (e.g. Tokyo subway incident), or as an accident as part of current demilitarization efforts. New interest in cyclosarin (GF-agent; \( O-cyclohexyl methylphosphonofluoridate \)) arose shortly after the Gulf War in 1991, when it was recognized that cyclosarin was stockpiled by Iraq1.

The acute toxicity of OP compounds in mammals is generally believed to be due to their irreversible inhibition of the enzyme acetylcholinesterase (AChE, EC 3.1.1.7) and subsequent accumulation of the neurotransmitter acetylcholine (ACh) in synapses of the central and peripheral nervous systems and overstimulation of postsynaptic cholinergic receptors2. Exposure causes a progression of toxic signs, including hypersecretions, fasciculations, tremor, convulsions, coma, respiratory distress, and death.

The current standard antidotal treatment usually includes a muscarinic ACh receptor antagonist to block the overstimulation of cholinergic receptors by ACh, and an oxime to reactivate OP-inhibited AChE3. Pralidoxime, obidoxime and HI-6 are the most widely used representatives of the oximes. These compounds, with quaternary nitrogen that promotes binding in the catalytic site of the AChE, are now a mainstream of treatment for organophosphate exposure. Unfortunately, none from the above mentioned oximes can be regarded as a broad spectrum antidote, because of inability to reactivate AChE inhibited by all nerve agents4. For example, H-oxime HI-6, currently considered as the best known AChE reactivator, is not able to reactivate satisfactorily tabun-inhibited AChE5. Therefore the development of new oximes in an effort to improve the treatment of intoxication with oxime-resistant OP compounds continues. The effective dose of the oxime depends on the nerve agent, the time between poisoning and oxime administration, and other factors6.

The present study was performed to assess and compare a therapeutic efficacy of various oximes administered in equimolar doses and combined with atropine in cyclosarin-poisoned mice and rats.

MATERIALS AND METHODS

Animals

Female mice, weighing 21–27 g and female rats, weighing 190–250 g (Konarovice, Czech Republic) were kept in air-conditioned room with light from 07:00 to 19:00 h and were allowed to free access to standard chow and tap water. The animals were divided into groups of six animals each. Handling of experimental animals was under the supervision of the Ethics Comittee of the Faculty of Military Health Sciences and the Medical Faculty of Charles University (Hradec Králové, Czech Republic).

Chemicals

Cyclosarin of 99.9% purity was obtained from Military Technical Institute in Zemianské Kostolany (Slovak Republic). Its purity was determined by acidimetric titration. The HS-6 oxime (1-(2-hydroxyiminomethylpyridinium)-3-(3-carbamoylpyridinium)-2-oxa-propane dichloride) was a gift of Dr. Stojilkovic, Serbia and Montenegro. The BI-6 oxime (1-(2-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium)-but-2-ene dibromide) and HI-6 oxime
(1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxa-propane dichloride) of at least 98.0 % purity were synthetized earlier in the Department of Toxicology of Faculty of Military Health Sciences in Hradec Králové (Czech Republic). Obidoxime (1,3-bis(4-hydroxyiminomethylpyridinium)-2-oxa-propane dibromide) was purchased from Merck (Germany). All other chemicals and drugs of analytical grade were obtained commercially and used without further purification.

Animal experiments

The oxime therapy combined with atropine was administered intramuscularly (im) 1 min after cyclosarin challenge (im). Dose of each oxime administered for treatment of cyclosarin intoxication was 100 µmol/kg in both mice and rats, atropine dose was 21 mg/kg. The drugs were dissolved in saline and the volume of injection corresponded to 1 % of body weight.

Data analysis

Cyclosarin-induced toxicity was evaluated by the assessment of LD₅₀ values and their 95 % confidence limits within 24 h after administration of cyclosarin at five different doses with six mice per dose. The efficacy of tested treatment was expressed as a therapeutic ratio (LD₅₀ value of cyclosarin in treated animals/LD₅₀ value of cyclosarin in non-treated animals). The statistical differences between LD₅₀ values were considered to be significant when P < 0.05 using t-test.

RESULTS

It was demonstrated that all the therapeutic regimens tested, were able to decrease the cyclosarin-induced toxicity significantly – at least 1.5 times (Table 1). Higher therapeutic ratios, almost three times, were achieved in rats in comparison with mice. The highest therapeutic ratio was achieved for therapeutic regimen consisting of HI-6 and atropine in both mice and rats. Obidoxime was the least effective oxime in the treatment of cyclosarin intoxication. The BI-6 oxime was nearly as effective as HI-6 against cyclosarin but it does not represent a significant improvement of antidotal treatment of cyclosarin poisoning in comparison with HI-6 (at least in mice). On the other hand, obidoxime, that is presently used in COMBOPEN

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animal</th>
<th>Ld50 (µg/kg) ± 95% confidence limits</th>
<th>Therapeutic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mice</td>
<td>279.0 (254.9 - 305.3)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>152.0 (120.0 - 168.0)</td>
<td>-</td>
</tr>
<tr>
<td>Obidoxime + atropine</td>
<td>Mice</td>
<td>432.7 (415.1 - 451.1)</td>
<td>1.55</td>
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<tr>
<td></td>
<td>Rats</td>
<td>735.0 (702.0 - 770.0)</td>
<td>4.8</td>
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<tr>
<td>HI-6 + atropine</td>
<td>Mice</td>
<td>895.5 (769.5 - 1042.2)</td>
<td>3.21*</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>&gt; 2000</td>
<td>&gt; 13.2#</td>
</tr>
<tr>
<td>BI-6 + atropine</td>
<td>Mice</td>
<td>605.6 (559.8 - 655.0)</td>
<td>2.17*</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>&gt; 2000</td>
<td>&gt; 13.2 #</td>
</tr>
<tr>
<td>HI-6 + atropine</td>
<td>Mice</td>
<td>589.1 (557.5 - 622.6)</td>
<td>2.11*</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>848.0 (789.0 - 897.0)</td>
<td>5.6 #</td>
</tr>
</tbody>
</table>

*significantly different from the obidoxime-treated group at the level of P< 0.05
# significantly different from the obidoxime-treated group at the level of P< 0.05

DISCUSSION

When the oximes are administered at the same molar concentration, the acute toxicity of the oximes is not considered, and thus oximes with high acute toxicity might get the intoxication worse. On the other hand, equimolar dosing of oximes is useful when in vitro and in vivo results should be compared. Although cyclosarin is an extremely strong inhibitor of human AChE and butyrylcholinesterase (EC 3.1.1.8) and has obviously an even higher potency than the nerve agent soman, in vivo, cyclosarin is less toxic than soman in several species, including non-human primates, indicating different toxicokinetic behaviour. It was already shown that intoxications with cyclosarin are rather resistant to conventional oxime therapy. Since aging should not be the major cause of its refractory property toward oximes (its half aging time is more than 4 h), the reason of this phenomenon is worthy of investigation in the future. Previous studies with cyclosarin-poisoned animals suggested a poor efficacy of the marketed oximes obidoxime and pralidoxime, whereas the newer oximes HI-6 and HLö 7 proved to be not only very good reactivators of cyclosarin-inhibited AChE in peripheral and central compartments but they were also effective antidotes in the protection of rats against supralethal poisoning with cyclosarin at doses relevant in humans. Accordingly, in this study it was found that therapeutic regimen consisting of HI-6 and atropine showed the highest therapeutic ratio. In addition, the safety factor of HI-6 is considerably greater than the conventional oximes (pralidoxime or obidoxime). The new oxime BI-6 was nearly as effective as HI-6 against cyclosarin but it does not represent a significant improvement of antidotal treatment of cyclosarin poisoning in comparison with HI-6 (at least in mice). On the other hand, obidoxime, that is presently used in COMBOPEN
autoinjector, is not able to protect cyclosarin-poisoned mice and rats such effectively as other oximes tested.

In conclusion, HI-6 still seems to be the most suitable oxime for treatment of cyclosarin poisoning. HI-6 was not only the most effective oxime in cyclosarin poisoning but also its low acute toxicity, in comparison with other oximes, enables to administer relatively high doses for treatment. However, since antidotes in most countries do not use HI-6 in the regimen due to the poor stability of this compound, medical protection against cyclosarin poisoning would remain a problem.

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REFERENCES