ORIGINAL ARTICLE

THERAPEUTIC EFFICACY OF DIFFERENT ANTIDOTAL MIXTURES AGAINST POISONING WITH GF-AGENT IN MICE

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Summary: The toxicity of cyclohexyl methylphosphonofluoridate (GF-agent; cyclosarin) and therapeutic efficacy of four oximes (trimedoxime, methoxime, obidoxime and HI-6) in combination with atropine or benactyzine (BNZ) was studied in mice. The oxime therapy combined with anticholinergic drug was administered intramusculary (i.m.) 1 or 2 min after i.m. GF-agent challenge. All the drugs were applied in dose of 20% of $LD_{50.}$ Obidoxime and trimedoxime that were combined with atropine were less effective than methoxime and HI-6 in combination with BNZ when applied 2 minutes after GF-agent poisoning. When the drugs were administered 1 min after GF-agent challenge already, in case of methoxime, the faster application of therapy resulted in significantly higher protective ratio, while for obidoxime the therapeutic effectivity did not depend significantly on the seasonableness of therapeutic intervention. The present findings show that all four combinations of oxime with anticholinergic drug decrease the GF-agent toxicity more than twofold regardless of the time of treatment administration.

Key words: *GF-agent; Cyclosarin; Methoxime; Obidoxime; Trimedoxime; HI-6; Atropine; Benactyzine; Acute Toxicity; Mice*

Introduction

GF-agent (cyclosarin; cyclohexyl methylphosphonofluoridate) is a highly toxic organophosphorus acetylcholinesterase (AChE, EC 3.1.1.7) inhibitor, known since the end of World War II, which has the potential for being used as a chemical warfare agent. This potential increased during Operation Desert Shield and Desert Storm with the possibility (later confirmed by the UN special commission) that GF-agent constituted of the Iraqi chemical agent inventory (11).

A combined regimen of therapy is now generally considered as the most effective medical approach for the treatment of nerve agent poisoning of military personnel (4). In the event of poisoning, immediate therapeutic treatment with an anticholinergic drug, such as atropine sulfate, antagonizes the effects of excess acetylcholine (ACh) at muscarinic receptor sites, and an oxime, such as pyridinium-2-aldoxime methylchloride (2-PAM), is used for the reactivation of any unaged inhibited enzyme (16).

Clinical experiences have indicated that oximes are not always very efficient reactivators of AChE depending on the type of nerve agent intoxication. Therefore, none of these oximes can be regarded as a broad spectrum antidote, i.e. effective against all nerve agents (5). Two oximes, obidoxime and 2-PAM, that are presently commercially available, are considered to be of insufficient efficacy against certain nerve agents, e.g. soman and GF-agent (19), while in case of sarin poisoning this combination is quite effective. The inhibitory potency of GF-agent against AChE (rabbit brain) is similar to that of soman (6). The in vivo toxicity of GF-agent is similar to that of tabun in mice and rats and to sarin in rabbits and approximately twofold lower than to soman in rats (2). In guinea-pigs the GF-agent is two-threefold less toxic than soman (11). For GF-agent inhibited enzyme, aging should not be the major cause of its refractory property toward the oxime, since its half-time of aging is more than 4 h, a rate comparable with sarin-inhibited enzyme (12).

The purpose of this study was to compare the therapeutic efficacy of various oximes in combination with anticholinergic drug both administered 2 or 1 minute after challenge of GF-agent.

Material and Methods

Animals

Female mice, weighing 21-27 g, from Velaz Prague (Czech Republic) were kept in an air-conditioned room with light from 07:00 to 19:00 h and had free access to standard chow and tap water. The mice were divided into groups of six animals each. Handling of experimental animals was under the supervision of the Ethics Committee of the Purkyne Military Medical Academy and the Medical Faculty of Charles University (Hradec Králové, Czech Republic).

Material

GF-agent (cyclohexyl methylphosphonofluoridate; cyclosarin) of 99.9% purity was obtained from Military Technical Institute in Zemianské Kostolany (Slovak Republic). Its purity was determined by acidimetric titration. The oximes of at least 98.0 % purity were synthetized earlier in the Department of Toxicology of Purkyne Military Medical Academy in Hradec Králové (Czech Republic). All other chemicals and drugs of analytical grade were obtained commercially and used without further purification.

Animal experiment

In our experiment, methoxime (1,1-bis(pyridinium-4-aldoxime)methane dichloride), trimedoxime (1,3-bis(pyridinium-4-aldoxime)propane dichloride), obidoxime (1,3-bis (pyridinium-4-aldoxime)-2-oxapropane dichloride) or HI-6 (1-(pyridinium-2-aldoxime)-3-(pyridinium-4-carbamoyl)-2-oxapropane dichloride) in combination with atropine or benactyzine (BNZ) were used for treatment of GF-poisoned mice. This oxime therapy combined with anticholinergic drug was administered intramusculary (i.m.) 1 or 2 min after i.m. GF-agent challenge. The dose of 20% of LD_{50} for all drugs applied for treatment was chosen in order to evaluate the protective ratio for the maximum applicable therapeutic dose.

Data analysis

GF-agent-induced toxicity was evaluated by the assessment of LD_{50} values and their 95% confidence limits within 1 week after administration of GF-agent at five different doses with six mice per dose (18). The efficacy of tested treatment was expressed as a protective ratio (LD_{50} value of GF-agent in treated mice/ LD_{50} value of GF-agent in non-treated mice). The differences between LD_{50} values were considered to be significant when $P< 0.05$ (13).

Results

The therapeutic efficacy and protective ratio of antidotal treatment administered 2 min following GF-agent challenge are summarized in Table1. Obidoxime and trimedoxime that were combined with atropine were less effective than methoxime and HI-6 in combination with BNZ. Methoxime in combination with BNZ appeared to be the most effective antidotal treatment of GF-agent intoxication in mice from those tested. Therapeutic efficacy of obidoxime and methoxime was further tested when applied already 1 min after GF-agent challenge and the results are presented in Table 2. These oximes were proved in more detail because as medicines they are components of Czech Army antidotal means against nerve agents. In case of methoxime the faster application of therapy resulted in significantly higher protective ratio, while for obidoxime the therapeutic effectivity did not depend significantly on the seasonableness of therapeutic intervention.

Tab. 1: The therapeutic effect of different antidotal mixtures administered 2 min following GF-agent challenge.

Treatment	LD_{50} (μg/kg) with 95% confidence limits	Protective ratio
-	170 (151–190)	-
Trimedoxime, Atropine	465 (374–578)*	2.74
Methoxime, Benactyzine	645 (584–714)*	3.79
Obidoxime, Atropine	369 (291–466)*	2.17
HI-6, Benactyzine	594 (536–658) *	3.49

*significantly different from the untreated group at the level of $P< 0.05$

Tab. 2: The therapeutic effect of different antidotal mixtures administered 1 min following GF-agent challenge.

Treatment	LD_{50} (μg/kg) with 95% confidence limits	Protective ratio
-	170 (151–190)	-
Methoxime, Benactyzine	982 (912–1056)*	5.78
Obidoxime, Atropine	424 (388–464)	2.49

* significantly different from the group treated 2 min following GF-agent challenge at the level of $P< 0.05$

Discussion

The present findings show that all four combinations of oxime with anticholinergic drug decrease the GF-agent toxicity more than 2-fold. In earlier studies it was shown that the reactivating efficacy of methoxime *in vitro* is higher than that of pralidoxime, obidoxime, HI-6 or HLö7 in the case of GF-agent inhibited AChE. The reactivating efficacy of oximes *in vitro* usually corresponds to their therapeutic efficacy *in vivo* (9). In our *in vivo* study the therapeutic regimen consisting of methoxime and BNZ resulted in the highest protective ratio from all the oximes tested in both time schemes of application. *In vivo* the effectiveness of oximes against the toxic effects of nerve agents including GF is usually tested in combination with atropine (1,3). Although, some other anticholinergic drugs (e.g. benactyzine, biperiden) should be more advantageous than atropine for the elimination of toxic effects of nerve agents because of their central antimuscarinic effects. It was confirmed that the anticholinergic drug selection is not as important for the survival as the choice of AChE reactivator (7, 14). However, selection of cholinergic drug in treatment regimen of nerve agent poisoning plays a key role in reduction of epileptiform seizures that are in a strong relationship with acute lethal and neurotoxic effects of nerve agents. Atropine sulfate, the drug used almost universally as an

antidote for anticholinesterase poisoning, is less potent anticholinergic drug than biperiden or trihexyphenidyle for control of nerve agent-induced seizures (15). Although in both therapeutic regimens with the highest protective ratios BNZ as the anticholinergic drug was used, the importance of the choice of anticholinergics needs to be further verified. In our study the oximes were applied in dose of 20% LD_{50}, while usually maximum permissible dose of oxime for treatment of nerve agent poisoning is 10% of LD_{50} (10, 17). Our results show, when compared to data available in literature that the protective ratio in case of obidoxime and atropine is almost 1.5 fold higher when 20% of LD_{50} of oxime was applied (2.49) than for 16% of LD_{50} of obidoxime (1.7) (8). This increase in protective ratio is rather considerable, but another increase of oxime dosing would probably resulted in organism damage by own oxime toxicity.

In conclusion, the presented data indicate that HI-6 and methoxime in combination with BNZ were significantly more effective than obidoxime and trimedoxime in combination with atropine in treatment of GF-agent intoxication.

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