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Toxicology 216 (2005) 32–40
TOXICOLOGY
www.elsevier.com/locate/toxicol

Signs of cyclosarin-induced neurotoxicity and its pharmacological treatment with quaternary pyridinium-oximes reactivators

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Received 11 May 2005; received in revised form 15 July 2005; accepted 15 July 2005
Available online 26 August 2005

Abstract

Cyclosarin (GF-agent; *O*-cyclohexylmethylfluorophosphonate) belongs to highly toxic organophosphorus compounds. Potential for exposure to chemical warfare organophosphosphorus nerve agents, such as cyclosarin exists on the battlefield, or in the civilian sector as a threat by a terrorist group, as well as an accident as part of current demilitarization efforts. Cyclosarin was not in a front of scientific interest for long time. The research interest was increased after Operation Desert Shield and Desert Storm with the possibility (later confirmed by the UN special commission) that cyclosarin constituted the Iraqi chemical agent inventory.

In this study, the neurotoxicity of cyclosarin and therapeutic efficacy of three oximes {HI-6(1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxa-propane dichloride), BI-6(2-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium)-but-2-ene dibromide), HS-6(2-hydroxyiminomethylpyridinium)-3-(3-carbamoylpyridinium)-2-oxa-propane dichloride)} as acetylcholinesterase reactivators in combination with atropine was studied in rats. The therapy was administered intramusculary (i.m.) 1 min after i.m. GF-agent challenge (1 LD_{50}). Testing of cyclosarin-induced neurotoxicity progress was carried out using the method of Functional observational battery (FOB). The experimental animals were observed at 24 h and 7 days following cyclosarin administration.

The results were compared to the condition of control rats that received physiological solution instead of cyclosarin and treatment. All tested antidotal compounds induced neuroprotective efficacy, because decrease of neurotoxicity signs was recorded. There were no poisoned experimental group treated with atropine only, because our preliminary study showed no therapeutical effect of atropine alone.

Cyclosarin caused a marked statistically significant change in most of the neurobehavioral parameters (FOB) at 24 h and 7 days after exposure, compared to the saline control group. Survival was 7/10 at 24 h and 5/10 at 7 days. Oxime (BI-6, HS-6 or HI-6) + atropine treatment caused a progressing recovery of the neurobehavioral disturbances caused by cyclosarin at 24 h and 7 days after exposure.

Keywords: Cyclosarin; Functional observational battery; Oximes; Atropine; Rats; Nerve agents; Acetylcholinesterase; Reactivators

1. Introduction

Organophosphates (OP) are used in agriculture and in veterinary practice. Potential for exposure to chemical warfare nerve agents, such as sarin (GB; *O*-isopropylmethylfluorophosphonate), tabun (GA;

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0300-483X/$ – see front matter © 2005 Published by Elsevier Ireland Ltd.
doi:10.1016/j.tox.2005.07.014

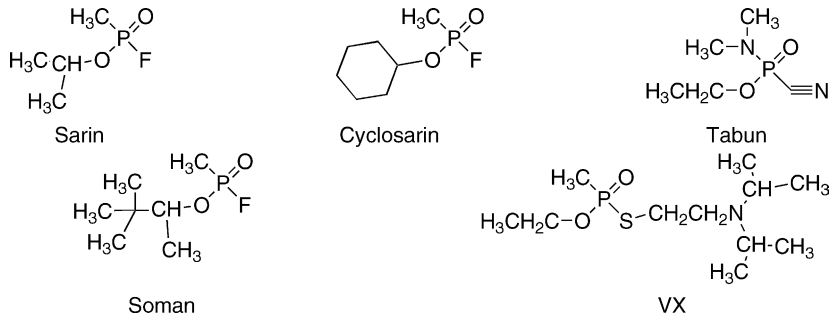

Fig. 1. Chemical structures of currently best-known nerve agents.

O-ethyldimethylamidocyanophosphate), soman (GD; *O*-pinacolylmethylfluorophosphonate), VX (*O*-ethyl-S-(2-diisopropylaminoethyl)-methylthiophosphonate) and cyclosarin (GF; *O*-cyclohexylmethylfluorophosphonate) (Fig. 1) exists on the battlefield (e.g., Iran-Iraq war, Desert Storm), or 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. All the nerve agents share a common mechanism of cholinesterase inhibition and due to this they cause similar symptoms.

OP poison insects and mammals primarily by phosphorylation of the enzyme acetylcholinesterase (AChE, EC 3.1.1.7) at nerve endings. The result is an inibition of the available AChE so that the effector organ becomes overstimulated by the excess acetylcholine (ACh). The enzyme is critical to normal control of nerve impulse transmission from nerve fibers to smooth and skeletal muscle cells, glandular cells, and autonomic ganglia, as well as within the central nervous system (CNS). Some critical proportion of the tissue enzyme mass is inactivated by phosphorylation before symptoms and signs of poisoning become manifest (Reigart and Roberts, 1999).

In animals, generally all cholinesterase inhibitors produce autonomic signs of cholinergic overstimulation (salivation, lacrimation, miosis), hypothermia, mild tremors and mouth-smacking (chewing motions), lowered motor activity, decreased tail-pinch response, and altered neuromuscular function (gait changes and increased foot splay) (Moser, 1995). Whereas the neuropathological lesions associated with OP poisoning are well characterized, there is a considerable lack of information on their behavioral effects, especially after chronic exposure. The importance of neurobehavioral studies lies in the fact that behavior is considered as a functional end product of the various sensory, motor, and integrative processes occurring in the nervous system. This particular aspect, therefore, requires intense investigation, moreover because behavioral changes are now being regarded as a standard indicator of toxicity in humans and animals, chronically exposed to low concentrations of potential neurotoxicants (White and Procter, 1985). Prolonged epileptic seizures in a nerve agent casualty will produce profound, irreversible, brain damage that, in turn, will result in long-term deficits in cognitive function and behavior. Combined prophylaxis (pyridostigmine) and therapy (atropine and AChE reactivators) regimen, however, does not prevent nerve agent-induced seizures in all cases (Filliat et al., 1999). Cyclosarin was not of major interest for many years although research on the treatment of sarin, soman and tabun poisoning was pursued (Kassa and Krejcova, 2002; Kassa et al., 2003; Krejcova et al., 2002; Krejcova and Kassa, 2003). Recently, attention was turned to cyclosarin because of the potential for being misused as a chemical warfare agent. The research interest was increased during Operation Desert Shield and Desert Storm with the possibility (later confirmed by the UN special commission) that cyclosarin constituted an Iraqi chemical agent inventory (Koplovitz et al., 1995; PMC/BAC, 1990).

In the event of poisoning, immediate therapeutic treatment with an anticholinergic drug, such as atropine, which antagonizes the effect of ACh excess at muscarinic receptor sites, and an oxime-reactivator of AChE is used to reactivate any unaged inhibited enzyme. Pralidoxime, obidoxime, methoxime, HI-6 and HLö7 are the most important oximes. However, clinical experience has indicated that mentioned 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, and especially they are not sufficiently effective in the case of cyclosarin (Helden et al., 1996; Krejcova et al., 2005). Therefore, the development of new oximes in an effort to improve the treatment of intoxication with oxime-resistant OP compounds continues. Upon screening in vitro reacti-