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Efficacy of the Tertiary Oxime Monoisonitrosoacetone (MINA) Against Lethal Sarin Intoxication in the Guinea Pig

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14. ABSTRACT

A major limitation of currently available oximes is their inability to readily cross the blood brain barrier and reactivate acetylcholinesterase (AChE) that has been inhibited by nerve agents. MINA is a tertiary oxime that readily enters the brain and was reported more than 50 years ago to be effective against lethal nerve agent intoxication. The purpose of this preliminary study was to re-evaluate the efficacy of MINA as a treatment for lethal sarin (GB) intoxication in guinea pigs. Male animals were challenged subcutaneously (s.c.) with 2 LD_{50}s of GB and treated 1 min later with MINA, 2PAM, atropine, or diazepam alone or in various combinations. A total of 14 different treatment groups were evaluated, 7 with MINA and 7 without MINA. Survival was assessed 24 hr after GB challenge. All 69 (100%) animals treated with MINA survived, whereas only 26 of 70 (37%) animals without MINA survived. The results clearly demonstrate the effectiveness of MINA and the potential benefit of using oximes that are readily able to enter the brain.

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Abstract

A major limitation of currently available oximes is their inability to readily cross the blood brain barrier and reactivate acetylcholinesterase (AChE) that has been inhibited by nerve agents. MINA is a tertiary oxime that readily enters the brain and was reported more than 50 years ago to be effective against lethal nerve agent intoxication. The purpose of this preliminary study was to re-evaluate the efficacy of MINA as a treatment for lethal sarin (GB) intoxication in guinea pigs. Male animals were challenged subcutaneously (s.c.) with 2 LD_{50}s of GB and treated 1 min later with MINA, 2PAM, atropine, or diazepam alone or in various combinations. A total of 14 different treatment groups were evaluated, 7 with MINA and 7 without MINA. Survival was assessed 24 hr after GB challenge. All 69 (100%) animals treated with MINA survived, whereas only 26 of 70 (37%) animals without MINA survived. The results clearly demonstrate the effectiveness of MINA and the potential benefit of using oximes that are readily able to enter the brain.

Introduction

Currently available oximes are quaternary monopyridinium or bispyridinium salts that do not readily cross the blood brain barrier (BBB). The permanent positive charge on the pyridinium nitrogen(s) restricts the ability of these drugs to cross biological membranes and particularly the BBB. As a result, these drugs are relatively ineffective in reactivating acetylcholinesterase (AChE) inhibited by organophosphorus (OP) anticholinesterase agents in the brain. This is a major limitation of current oxime therapy for OP nerve agent intoxication.

Before the development of the quaternary pyridinium oximes (e.g., 2PAM) in the late 1950s, many of the early reactivators were lipid soluble, tertiary oximes that were able to cross the BBB. These early oximes fell by the wayside because the quaternary pyridinium oximes were several orders of magnitude better reactivators of phosphorylated AChE (Hobbiger, 1963). One of the most widely studied early oximes was monoisonitrosoacetone (MINA) (Figure 1). This oxime was shown to protect rats against the lethal effects of GB when used alone or in combination with atropine (Askew, 1956, 1957). Cohen and Wiersinga (1960) demonstrated that MINA was able to enter the brain and reactivate GB-inhibited AChE.

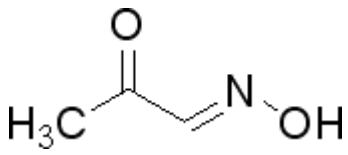

Figure 1. Chemical Structure of MINA.

The importance of central oxime reactivation in the overall treatment of nerve agent intoxication is unclear. Given the dramatic effect that an oxime can have in peripheral tissues, an oxime that readily crosses the BBB could increase survival and reduce seizures and neuropathology that accompany nerve agent intoxication. The purpose of this study was to re-assess the efficacy of the tertiary oxime MINA as treatment for GB intoxication in guinea pigs. The goal was to determine that MINA could significantly improve survival when used as a post-challenge treatment for lethal GB intoxication.

Materials and Methods

Animals

Male Hartley (CrlHA(BR)) guinea pigs (Charles River Laboratory, Kingston, NY) weighing 250-400 gm were used. Animals were quarantined and observed for evidence of disease for a minimum of five days prior to their use under an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International-accredited animal care and use program. Guinea pig ration and tap water were provided *ad libitum*. All animal procedures described in this report were performed in accordance with the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animal Resources, National Research Council, in accordance with Department of Defense regulations.

Chemicals and Drugs

GB (isopropylmethylphosphonofluoridate) was obtained from the Edgewood Chemical Biological Center, Aberdeen Proving Ground, Maryland. A stock solution of GB was prepared gravimetrically to a nominal concentration of 2 mg/ml in saline. The actual concentration was verified by gas chromatography with flame ionization detection. Stock solutions were stored in 1-, 3- or 5-ml aliquots at -70^{o}C until needed. Dilutions were prepared in saline on the day of use from an aliquot of thawed stock solution and maintained on ice. Atropine sulfate, 2PAM-chloride (2PAM) and diazepam were obtained through the Walter Reed Army Institute of Research, Washington, D.C. MINA was purchased from Sigma Chemical Co., St. Louis, MO. Stock solutions of atropine and 2PAM were prepared in sterile water and stored in the refrigerator. MINA was dissolved in sterile water on the day of use. Diazepam was dissolved in a vehicle consisting of 40% propylene glycol, 10% ethanol, 2.5% benzyl alcohol, 48.5% water on the day of use. Atropine and 2PAM when administered in combination were admixed in the same bottle. Diazepam and MINA were each given as separate injections.

Experimental Design

Each guinea pig was challenged with 2 LD_{50}s GB subcutaneously (s.c.) between the shoulder blades. One minute after challenge each animal was treated intramuscularly (i.m.) in a hind limb with 2PAM (25 mg/kg), atropine (0.3 mg/kg), diazepam (1 mg/kg), MINA (60 mg/kg) or various combinations of the 4 drugs (see Table 1). The 2PAM dose approximates the total dose in 3 autoinjectors (600 mg per 2PAM autoinjector) given to a 70-kg human. The atropine dose (0.3 mg/kg) approximates the dose in 9 autoinjectors (2 mg per atropine autoinjector) given to a 70-kg human. The dose of diazepam is greater than the ED_{50} for terminating seizures induced by GB in guinea pigs pretreated with pyridostigmine and treated with 0.1 mg/kg (diazepam ED_{50} 0.47 mg/kg) or 2 mg/kg (diazepam ED_{50} 0.79 mg/kg) atropine plus 2PAM (Shih et al., 2006). The MINA dose is approximately 4 times the dose of 2PAM Cl on a µmol/kg basis. A total of 14 treatment groups with 9 or 10 animals per group were tested. Survival was assessed 24 hr after agent challenge. Fisher's exact test (1-tailed) was used to compare survival rates with significance at $p<0.05$ (Winer, 1971).

Results and Discussion

The survival rates for all 14 treatment groups are summarized in Table 1. All 69 (100%) animals treated with MINA or MINA plus one or more of the other treatment drugs survived. In contrast, only 26 of 70 (37%) animals without MINA survived. The only treatment without MINA that resulted in 100% survival was the group treated with a combination of atropine plus 2PAM plus diazepam.

The results of this study clearly demonstrate that MINA was a very effective treatment against 2 LD_{50}s of GB intoxication in guinea pigs. MINA alone afforded 100% survival. MINA was significantly ($p<0.05$) more effective than atropine, diazepam, or 2PAM alone, and atropine plus 2PAM or atropine plus diazepam. These data suggest that oxime reactivation of AChE in the brain inhibited by nerve agents was responsible for the efficacy of MINA against GB. The fact that 100% of the animals treated with MINA alone survived points to the potential importance of oxime reactivation of nerve agent-inhibited AChE in the CNS.

The efficacy of MINA against GB was most likely due to reactivation of AChE in peripheral tissues and the brain (Askew, 1957; Cohen and Wiersinga, 1960). Preliminary in-house data have shown that MINA reactivates AChE in the brain inhibited by GB in the guinea pig (Shih, personal communication). The results for MINA would have been more dramatic had 60% of the animals treated with 2PAM alone not survived. This outcome was unexpected. It is unclear why 60% of the animals survived in this group, while only 30% survived in the group treated with atropine plus 2PAM. Additional animals will have to be exposed to GB and treated with 2PAM to determine whether this response was aberrant or real.

Further comments are warranted about some of the drug doses used in this study. We used a relatively low dose of atropine for several reasons. First, it enabled the use of a reasonable challenge level of GB. With standard doses ($\geq$16 mg/kg) of atropine in combination with oxime treatment, guinea pigs can survive challenge levels of GB greater than 20 LD_{50}s (Dawson, 1994). This latter level of GB challenge is not practical experimentally, nor is it thought to be achievable on the battlefield. Second, the doctrinal human self-buddy-aid dose of 6 mg atropine or 3 autoinjectors is approximately 0.1 mg/kg to a 70-kg human. Although the 0.3 mg/kg atropine dose used in this study is higher than the doctrinal dose, it is well within the range of the amount of atropine needed in one day to treat a severely intoxicated individual (Sidell et al., 1997; Eyer, 2003; Rotenberg and Newmark, 2003). If dose-scaling formulas are taken into consideration to extrapolate the atropine from guinea pigs to human based on body surface area, the 0.1 mg/kg human dose in 3 autoinjectors would equate to 0.5 mg/kg in the guinea pig (Food and Drug Administration, 2002). Finally, it has been our experience that the standard dose (16 mg/kg) of atropine in rodents can mask differences between the efficacy of oximes against nerve agent intoxication and would have prevented us from discriminating between 2PAM and MINA (unpublished data).

The dose of MINA was selected from preliminary data showing that 60 mg/kg reactivated about 50% of the brain AChE inhibited by GB in guinea pigs (Shih, personal communication). In the same experiments, 2PAM (25 mg/kg) did not reactivate brain AChE. While the dose of MINA was 5 times greater that of 2PAM on a molar basis, it was also about 5-fold less efficient than 2PAM *in vitro* in reactivating human RBCs inhibited with GB (Hobbiger, 1963).

The results also reinforce the importance of the oxime in the treatment regimen for GB poisoning. In the absence of MINA or 2PAM, neither atropine nor diazepam treatment individually, or in combination, afforded any protection against $2LD_{50}$s of sarin. Reactivation of AChE in peripheral tissues and the brain should be the most important goal of emergency medical treatment of nerve agent poisoning.

In summary, this preliminary study demonstrates the potential usefulness of administering an oxime that can readily cross the BBB to reactivate AChE that has been inhibited by a nerve agent. Additional work is needed with MINA and other centrally penetrating oximes to evaluate the role of CNS oxime reactivation on enhancing survival, preventing or terminating nerve agent-induced seizures, and reducing or eliminating neuropathology and incapacitation against a broad spectrum of nerve agents.

Table 1

Survival Rates of Guinea Pigs Challenged with $2LD_{50}$s of GB

Treatment (mg/kg)	**#Survived/#Tested**
Atropine (0.3)	0/10*
Diazepam (1)	0/10*
Atropine (0.3) + Diazepam (1)	0/10*
2PAM (25)	6/10*
Atropine (0.3) + 2PAM (25)	3/10*
Diazepam (1) + 2PAM (25)	7/10
Atropine (0.3)+2PAM (25) +Diazepam (1)	10/10
MINA (60)	10/10
Atropine (0.3) + MINA (60)	10/10
2PAM (25) + MINA (60)	10/10
MINA (60) +Diazepam(1)	10/10
Atropine (0.3)+ MINA(60) +Diazepam (1)	10/10
2PAM (25) + MINA(60) + Diazepam (1)	10/10
Atropine (0.3)+MINA(60)+2PAM (25)	9/9

* $p < 0.05$ compared to treatments with MINA

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