

Perspectives on the Use of Scopolamine as an Adjunct Treatment to Enhance Survival Following Organophosphorus Nerve Agent Poisoning

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ABSTRACT Scopolamine (SCP) is an anticholinergic drug used clinically for decades to treat motion sickness, as a surgical preanesthetic, and as a smooth muscle antispasmodic. It has also been used experimentally as a pretreatment and/or treatment adjunct to mitigate the toxic sequelae of organophosphorus (OP) nerve agent intoxication. SCP has been reported to increase survival, prevent or terminate seizures, and reduce morbidity from nerve agent intoxication in a number of animal models. The purpose of this study was to evaluate the effect of atropine dose, pyridostigmine bromide (PB) pretreatment, and oxime selection on the efficacy of SCP as an adjunctive treatment to enhance survival following lethal nerve agent exposure in guinea pigs. The results indicate that the use of an effective oxime and/or PB pretreatment was a critical factor in determining the efficacy of SCP. SCP can also reduce the dose of atropine required for survival against lethal nerve agent intoxication.

INTRODUCTION

Standard treatment in the United States for intoxication by organophosphorus (OP) nerve agents (e.g., sarin, V-agents, soman, cyclosarin, and tabun) and OP pesticides is a regimen consisting of the anticholinergic drug atropine and the acetylcholinesterase (AChE) reactivator pralidoxime chloride (2PAM), and in case of severe poisoning resulting in seizures, the anticonvulsant benzodiazepine diazepam. For emergency use by the military in the field, this regimen is administered by i.m. injection at the first onset of symptoms. Atropine antagonizes the effects of excess acetylcholine at peripheral and central nervous system (CNS) muscarinic synapses, 2PAM reactivates inhibited AChE at peripheral nicotinic and muscarinic synapses, and diazepam acts centrally to control seizures and convulsions.¹ In addition, the carbamate AChE inhibitor pyridostigmine bromide (PB) is used orally as a pretreatment to protect a portion of the enzyme from irreversible inhibition by nerve agents that are resistant to reactivation by oximes.²⁻⁴ PB is currently FDA approved only for use against soman.

Scopolamine (SCP) is a well known anticholinergic drug that has been used clinically for decades to treat motion sickness, as a surgical preanesthetic, and as a smooth muscle antispasmodic.⁵ There is a substantial body of published literature on the use of SCP as a component of regimens to protect against and/or treat nerve agent intoxication in experimental animals.⁶⁻¹⁵ In these types of studies, SCP is usually administered as an adjunct to atropine and/or oxime treatment or with a carbamate as a pretreatment. The reports convincingly demonstrate that SCP can improve survival, reduce morbidity, and

terminate seizures in a variety of animal species intoxicated with nerve agents. The beneficial effects of SCP as an adjunct in OP poisoning presumably are linked to its high potency as a CNS muscarinic antagonist and its ability to rapidly enter the brain.¹⁶ These actions enable SCP to more quickly antagonize the action of acetylcholine at central muscarinic receptors.

As an adjunct treatment for nerve agent intoxication, SCP is administered in combination with atropine, an oxime, an anticonvulsant, and carbamate pretreatment. Because of the potential for interaction between SCP and the other components in the regimen, and the differences in the effectiveness of medical countermeasures against the various nerve agents, we undertook this study to examine some of the experimental factors that might affect the capacity of SCP to enhance 24-hr survival rates when used as an adjunct to atropine and oxime treatment of intoxication from OP nerve agents. Specifically, we evaluated the effect of atropine dosage, the presence or absence of PB pretreatment, and the effect of oxime selection on the ability of adjunctive treatment with SCP to enhance survival against sarin, soman, cyclosarin, and Russian V-agent (VR).

METHODS

Animals

Male guinea pigs [Crl HA(Br)] (Charles River, Kingston, NY) weighing 250–400 g were used. Animals were acclimated and observed for evidence of disease for a minimum of 5 days before their use under an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited animal care and use program. Guinea pig ration and tap water were provided ad libitum. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of

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Laboratory Animals (National Research Council, 1996), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

Chemicals and Drugs

Sarin, cyclosarin, soman, and Russian V-agent (VR) were obtained from the Edgewood Chemical Biological Center, Aberdeen Proving Ground, Maryland. A stock solution of each agent was prepared gravimetrically to a nominal concentration of 1 or 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°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 (Atr), pralidoxime chloride (2PAM), and pyridostigmine bromide (PB) were obtained through the Walter Reed Army Institute of Research, Washington, DC. Scopolamine hydrobromide (SCP) was purchased from Sigma Chemical Co., St. Louis, Missouri. MMB4 (1,1'-methylenebis[4-[(hydroxyimino)methyl]-pyridinium]dimethanesulfonate) was synthesized under U.S. government contract. Stock solutions of atropine and 2PAM were prepared in sterile water and stored in the refrigerator. PB, MMB4, and SCP were prepared in sterile water as needed. Atropine and 2PAM or MMB4 were admixed in the same bottle before injection.

Experimental Design

PB (0.026 mg/kg) or saline was injected intramuscularly (i.m.) 30 min before nerve agent challenge. This dose of PB results in 15–30% inhibition of red blood cell (RBC) AChE at 30 min.¹⁷ Animals were challenged subcutaneously (s.c.) between the shoulder blades with $2 \times \text{LD}_{50}$ of sarin (84 $\mu\text{g}/\text{kg}$), cyclosarin (110 $\mu\text{g}/\text{kg}$), soman (56 $\mu\text{g}/\text{kg}$), or VR (22.6 $\mu\text{g}/\text{kg}$). One minute after nerve agent challenge each animal was treated i.m. in a hind limb with 2PAM (25 mg/kg) plus atropine (0.3 or 3 mg/kg). MMB4 (26 mg/kg) was also used in place of 2PAM in experiments against cyclosarin. SCP (0.12 mg/kg) was injected i.m. in the opposite hind limb immediately after atropine and oxime treatment. The doses of the medical coun-

termeasures were selected to approximate human deliverable dosages under current soldier-buddy aid doctrine.¹⁸ The atropine doses are based on body surface area drug scaling formulas recommended by the Food and Drug Administration (FDA).¹⁹ The 0.3-mg/kg and 3-mg/kg doses are the equivalent of about 5 mg and 50 mg human total doses, respectively. The 2PAM dose is based on the mg/kg amount in three Mark 1 autoinjectors. The MMB4 dose is a proposed total human dose in three autoinjectors. The SCP dose is the ED_{50} for terminating soman-induced seizures in guinea pigs.²⁰ Survival was assessed 24 hr after agent challenge. A χ^2 test was used to compare all groups with respect to survival rate, followed by multiple Fisher's exact tests to compare pairs of groups. Fisher's exact test had a Bonferroni adjusted p value to maintain an experimental error rate of $p < 0.05$.²¹

RESULTS

The efficacy of SCP as an adjunct treatment to atropine and 2PAM is depicted in Table I. In the absence of PB pretreatment, SCP enhanced survival in animals intoxicated with sarin, VR, and soman. Against sarin and VR survival increased from 52% (15/29) to 100% (10/10) and 13% (2/16) to 90% (9/10), respectively, in animals treated with SCP in the presence of 0.3 mg/kg atropine plus 2PAM (25 mg/kg). Against soman, SCP enhanced survival from 10% (1/10) to 70% (7/10), but only when the atropine dose was 3.0 mg/kg; SCP did not increase survival in soman-challenged animals when used as adjunct to 0.3 mg/kg atropine and 2PAM. SCP adjunctive treatment also did not enhance survival rates in cyclosarin-challenged animals treated with 2PAM and either dose of atropine. When MMB4 was used in place of 2PAM against cyclosarin, SCP adjunctive treatment in cyclosarin-challenged animals increased the survival rate from 40% (4/10) to 100% (10/10) using an atropine dose of 0.3 mg/kg (Fig. 1).

In the presence of PB pretreatment, SCP increased 24-hr survival in both soman- and cyclosarin-challenged animals treated with atropine and 2PAM. Soman-challenged animals pretreated with PB exhibited increased survival rates with an atropine dose of 0.3 as well as of 3 mg/kg. In cyclosarin-challenged

TABLE I. Efficacy of SCP as an Adjunct Treatment against $2 \times \text{LD}_{50}$ of Nerve Agent Challenge in Guinea Pigs

Pretreatment (mg/kg)	Treatment (mg/kg)	Adjunct (mg/kg)	24-hr Survival Rate			
			Sarin	VR	Cyclosarin	Soman
Saline	Atr (0.3) + 2PAM (25)	—	15/29	2/16 ^b	0/15 ^c	0/10 ^{d,e}
Saline	Atr (3.0) + 2PAM (25)	—	10/10 ^a	1/10 ^b	0/15 ^c	1/10 ^{d,e}
Saline	Atr (0.3) + 2PAM (25)	SCP (0.12)	10/10 ^a	9/10	0/10 ^c	3/10
Saline	Atr (3.0) + 2PAM (25)	SCP (0.12)	—	—	3/10 ^c	7/10
PB (0.026)	Atr (0.3) + 2PAM (25)	—	8/11	1/10 ^b	0/20 ^c	2/10 ^d
PB (0.026)	Atr (3.0) + 2PAM (25)	—	10/10 ^a	2/10 ^b	1/10 ^c	2/10 ^d
PB (0.026)	Atr (0.3) + 2PAM (25)	SCP (0.12)	—	—	2/9 ^c	8/10
PB (0.026)	Atr (3.0) + 2PAM (25)	SCP (0.12)	—	—	10/10	10/10

^a $p < 0.05$ vs. saline/Atr (0.3) + 2PAM against sarin. ^b $p < 0.05$ vs. saline/Atr (0.3) + 2PAM/SCP against VR. ^c $p < 0.05$ vs. PB/Atr (3) + 2PAM/SCP against cyclosarin. ^d $p < 0.05$ vs. PB/Atr (0.3) + 2PAM/SCP and PB/Atr (3) + 2PAM/SCP. ^e $p < 0.05$ vs. PB/Atr (0.3) + 2PAM/SCP, PB/Atr (3) + 2PAM/SCP and saline/Atr (3) + 2PAM/SCP.

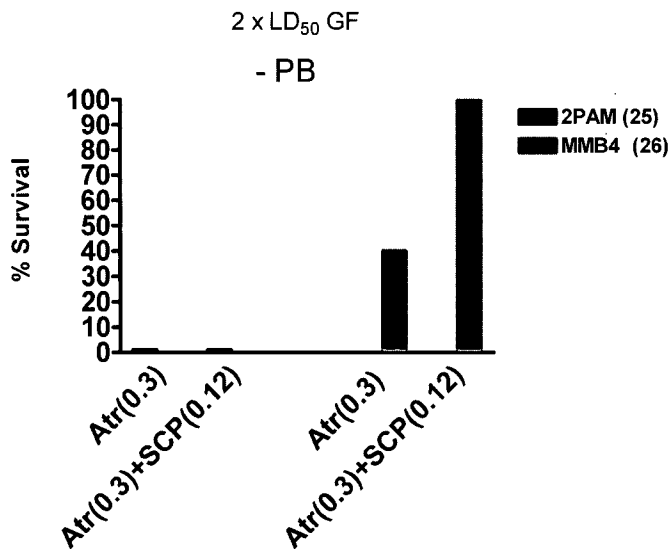


FIGURE 1. Efficacy of SCP (0.12 mg/kg, i.m.) as an adjunct to atropine (0.3 mg/kg, i.m.) and 2PAM (25 mg/kg, i.m.) or MMB4 (26 mg/kg, i.m.) treatment of cyclosarin intoxication in guinea pigs. All treatments were administered i.m. 1 min after s.c. challenge with $2 \times LD_{50}$ of GF. The bars show percentage of animals surviving 24 hr. The * indicates a significant difference between animals treated with SCP and those not treated with SCP.

animals pretreated with PB, increased survival rates were observed with an atropine dose of 3 mg/kg. It is worthwhile to note that in the absence of SCP and/or PB pretreatment, atropine plus 2-PAM treatment afforded significant survival only against sarin.

DISCUSSION

The results of this study show that when used as an adjunct to atropine and oxime treatment, SCP increased survival rates of guinea pigs challenged with $2 \times LD_{50}$ sarin, VR, cyclosarin, or soman. The effectiveness of SCP was dependent on a number of factors: the atropine dose, the presence or absence of PB pretreatment, and the particular nerve agent and oxime utilized in the treatment regimen.

Atropine is the universal treatment for organophosphorus anticholinesterase poisoning. It antagonizes the action of excess acetylcholine at muscarinic receptors in the peripheral nervous system and the CNS that results from inhibition of AChE by nerve agents and blocks many of the toxic sequelae of nerve agent poisoning.⁵ In many published reports evaluating medical countermeasures against nerve agent lethality in experimental rodent models, high doses of atropine were used. It is not uncommon to see experimental reports in rodents where the atropine dose was 10 mg/kg or higher.²²⁻²⁷ These high doses of atropine were used because of differences in the absorption, distribution, metabolism, and excretion of atropine between species and to optimize the effectiveness of the various pretreatment and treatment therapies so as to rank order the most effective regimens. However, high atropine doses may mask the ability to adequately assess differences in the efficacy of the other components in the regimen.²⁸ Therefore, we chose to use doses of atropine that are closer to

human fielded doses for emergency treatment of nerve agent poisoning. Selection of the 0.3- and 3-mg/kg doses of atropine in the present study was made using FDA body surface area dose scaling formulas, and they are equivalent to human doses of approximately 5 mg and 50 mg, respectively. The human emergency dose of atropine used by the military for treatment of nerve agent intoxication is 2–6 mg, i.m., with additional atropine administered in 2-mg increments every 3–5 min until effect. Up to 10–20 mg may be needed in the first few hours following intoxication.^{1,18} For treatment of OP pesticide poisoning, 24–48 mg of atropine may be needed over the first 24 hr in a severely intoxicated individual.²⁹

The dose of SCP used in this study was the ED_{50} for terminating soman-induced seizures in guinea pigs.²⁰ This dose (0.12 mg/kg) also has some human relevance. Based on FDA body surface area dose scaling formulas, the SCP dose used in this study is approximately equivalent to the human ED_{50} (i.e., 20 μ g/kg, i.m.) for lowering the number facility (NF) test scores to 10% of baseline.¹⁶ The NF test is a commonly used test of cognitive function.³⁰

The results of this study also show a clear interaction between SCP and the dose of atropine. When SCP was used as an adjunct to atropine plus 2PAM against sarin intoxication, survival significantly increased from 52% to 100% in the presence of 0.3 mg/kg atropine (Table I). In the absence of SCP, an atropine dose of 3 mg/kg was required to achieve 100% survival against sarin. A similar response was observed in VR-challenged animals. In these animals 0.3 or 3.0 mg/kg atropine plus 2PAM in the absence of SCP provided minimal (10–15%) survival rates against $2 \times LD_{50}$ of VR. The addition of SCP increased the survival rate in VR-challenged animals to 90% at the atropine dose of 0.3 mg/kg. In a previous study, we reported that an atropine dose of 16 mg/kg in combination with 2PAM provided 100% survival against $2 \times LD_{50}$ of VR in guinea pigs.²⁸ These data suggest that SCP adjunctive treatment can reduce the amount of atropine needed or required for effective treatment of nerve agent poisoning. In the case of sarin, SCP adjunctive treatment enabled the use of a dose of atropine that was 10-fold lower than the dose needed to achieve the same survival rate in the absence of SCP, and against VR SCP reduced the amount of atropine needed by 50-fold (i.e., 16 mg/kg to 0.3 mg/kg).²⁸

The ability of SCP to reduce the dose of atropine needed for effective treatment of nerve agent intoxication appeared to be related, in general, to the recovery of AChE activity either through decarbamylation of PB protected enzyme or by use of an effective oxime. The results against cyclosarin demonstrate this principle. In the absence of SCP, we previously reported that an atropine dose of 16 mg/kg in combination with 2PAM treatment provided only about 50–70% survival against $2 \times LD_{50}$ of cyclosarin with or without PB pretreatment in guinea pigs.²⁸ In the current study, SCP did not significantly increase survival in cyclosarin-challenged animals treated with either dose (0.3 or 3.0 mg/kg) of atropine plus 2PAM in the absence of PB pretreatment. When PB pretreatment was utilized, SCP

adjunctive treatment with 3 mg/kg atropine plus 2PAM resulted in 100% survival (Table I). SCP, therefore, in the presence of PB reduced the requirement for atropine by more than 5-fold. PB pretreatment protects a portion of AChE at critical synapses in peripheral tissues, and decarbamylation of this protected enzyme in the first few minutes after intoxication and treatment provides sufficient enzyme activity to sustain survival.^{2,4} A similar atropine dose-sparing response was observed when MMB4 was used in place of 2PAM (Fig. 1). In animals treated with MMB4 plus the 0.3 mg/kg atropine dose, the addition of SCP increased survival from 40% to 100% without the need for PB pretreatment. In the absence of SCP an atropine dose of 16 mg/kg with the same dose of MMB4 resulted in 100% survival against $2 \times LD_{50}$ of cyclosarin.²⁸ Again, SCP adjunctive treatment reduced the requirement for atropine by more than 5-fold. Since MMB4 is the better reactivator of cyclosarin-inhibited AChE than is 2PAM,³¹⁻³³ reactivation of cyclosarin-inhibited AChE in peripheral synapses by MMB4 likely regenerated sufficient enzyme activity to sustain survival.

The ability of SCP to significantly increase the survival rate in soman-challenged animals treated with 3 mg/kg atropine plus 2PAM to 70% in the absence of PB pretreatment (Table I) appears counter to the concept that recovery of AChE is essential, because it is well known that 2PAM cannot reactivate soman-inhibited AChE. We have no explanation for this finding and additional experimentation is needed. However, SCP did show an atropine-sparing effect in the soman-challenged animals that appears related to recovery of AChE activity. A survival rate of 80% was observed in PB-pretreated animals that were treated after soman with SCP and 0.3 mg/kg atropine plus 2PAM; in the absence of PB, SCP increased survival only at the 3 mg/kg atropine dose.

The results suggest that a major factor contributing to the efficacy of SCP as an adjunct treatment to enhance survival, and its ability to reduce the dose of atropine required for effective treatment of nerve agent intoxication, appears to be recovery of peripheral AChE enzyme activity in critical synapses either through use of an effective oxime or decarbamylation of PB-protected enzyme. The rapid or more effective blockade of CNS muscarinic receptors by SCP in itself does not appear to be sufficient. The efficacy of SCP against soman in the absence of an effective oxime in this study appears to be an anomaly since we have not observed this response against other nerve agents that are resistant to oxime reactivation (I. Koplovitz, unpublished data).

In summary, the results suggest that SCP would be a useful adjunct to current emergency, medical countermeasure regimens for nerve agent intoxication. The enhanced survival efficacy provided by SCP in this study is likely a result of its having greater potency than atropine in CNS, and its capacity to enter the CNS more quickly to antagonize the action of excess acetylcholine at critical postsynaptic muscarinic receptors.¹⁶ In addition, some of the benefit provided by SCP adjunctive treatment may also be the result of its capacity to prevent and/or terminate nerve agent-induced seizures in

experimental animal models.^{6,14,15,20} The data also suggest that using SCP as an adjunct treatment could reduce the number of atropine autoinjectors that medics or first responders have to carry. This would reduce the logistical burden of having a sufficient supply of atropine in a mass casualty scenario. Its effectiveness, however, will depend on many factors that are difficult to control in a mass casualty environment. The key determinant of SCP's efficacy in the present studies would appear to be the recovery of peripheral AChE activity either through reactivation by an oxime or through decarbamylation of PB-inhibited AChE. Rapid regeneration of AChE activity following acute OP intoxication should continue to be the major objective of nerve agent treatment regimens.

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