

Effect Of Atropine And Diazepam On The Efficacy Of Oxime Treatment Of Nerve Agent Intoxication¹

I. KOPLOVITZ*, S.M. SCHULZ, R.F. RAILER, M. SIGLER, AND R.B. LEE

U.S. Army Medical Research Institute of Chemical Defense

3100 Ricketts Point Rd

Aberdeen Proving Ground, MD 21010-5400

* Corresponding Author: Attn: MCMR-CDR-P/I. Koplovitz

3100 Ricketts Point Rd.

Aberdeen Proving Ground, MD 21010-5400

USA

Tel: 410-436-5342 | Fax: 410-436-1132 | Email: Irwin.koplovitz@us.army.mil

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ABSTRACT

Standard treatment for anticholinesterase nerve agent intoxication consists of atropine to antagonize acetylcholine at the synaptic receptor, an oxime to reactivate inhibited acetylcholinesterase (AChE), and a benzodiazepine to control seizures. Treatment of oxime resistant nerve agents also requires pretreatment with pyridostigmine bromide to shield a portion of AChE from irreversible inhibition. To determine the influence/role of atropine on the overall therapeutic response, we investigated the effects of selected atropine doses along with other recommended antidotes (pyridostigmine, oxime, diazepam) against 2LD₅₀s of the nerve agents sarin (GB), cyclosarin (GF), Russian V-agent (VR), or tabun (GA) in guinea pigs. Saline or pyridostigmine was administered 30 minutes prior to nerve agent challenge; treatment was administered 1

minute after agent challenge and consisted of atropine (0.3, 3.0, or 16 mg/kg) plus the oxime, 2-PAM chloride (25 mg/kg) or MMB4 dimethanesulfonate (26 mg/kg). Approximately half of the animals were also treated with diazepam (1 mg/kg), injected immediately after atropine and oxime treatment. Survival was assessed at 24 hours. Pyridostigmine pretreatment did not significantly alter survival rates for any treatment combination. Atropine, in combination with 2-PAM or MMB4, gave similar survival responses in GB- or GA- intoxicated animals. Against GB, all three doses of atropine gave high survival rates with either oxime, while against GA only the 16 mg/kg atropine dose afforded high survival rates. Against GF and VR, MMB4 was more effective than 2-PAM in combination with either the 0.3 or 3.0 mg/kg dose of atropine. Diazepam adjunctive therapy significantly increased survival against GF, GA and VR in both MMB4- and 2-PAM-treated animals at these doses of atropine. The findings indicate that the dose of atropine needed for survival depends on the effectiveness of the oxime. MMB4 was more effective than 2-PAM against GF and VR. When the atropine dose was insufficient, diazepam adjunctive treatment significantly increased survival rates against 2LD₅₀s of nerve agent intoxication irrespective of the oxime.

INTRODUCTION

The emergency medical treatment of organophosphorus (OP) nerve agent intoxication in a battlefield scenario consists of self/buddy aid with atropine to block the effect of excess acetylcholine at muscarinic receptors in the brain and peripheral tissues due to inhibition of the enzyme acetylcholinesterase (AChE), an oxime to reactivate the inhibited AChE and restore function at the neuromuscular junction, and in the case of severe poisoning, a benzodiazepine such as diazepam to control convulsions and seizures [Taylor, 2001; Moore et al., 1995]. In addition, for oxime-resistant nerve agents like soman, pyridostigmine bromide (PB) is used as a pretreatment to shield a portion of AChE from irreversible phosphorylation [Dunn and Sidell, 1989; Moore et al., 1995].

Individually, the various components of the regimen are not very effective in preventing lethality from nerve agent intoxication [Dawson, 199]. When used together, however, the components have a marked synergistic effect on survival. There are many factors that determine the overall survival response to medical pretreatment and treatment of nerve agent intoxication, including, but not limited to, the specific agent causing the intoxication, route of agent exposure, time of pretreatment and/or postexposure treatment, dose and pharmacologic activity of the individual drugs and animal species. In this investigation, we evaluated the role of atropine and adjunctive diazepam treatment on the efficacy of the oximes pralidoxime chloride (2-PAM), the oxime currently fielded by the US military, and 1,1'-methylenebis[4-[(hydroxyimino)methyl]-pyridinium] dimethanesulfonate (MMB4), the candidate oxime in development to replace 2-PAM,

against 2LD50s of sarin (GB), cyclosarin (GF), Russian V-agent (VR, O-isobutyl-S-2-(diethylamino)ethylmethyl phosphonothioate) or tabun (GA) in saline- or PB-pretreated guinea pigs.

MATERIALS AND METHODS:

Animals

Male guinea pigs (Crl HA(Br)) (Charles River, Kingston, NY) weighing 250—400 g were used in all studies. 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)-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 the stipulation mandated for a facility accredited by AAALAC.

Chemicals and Drugs

GA (98.5% pure), GB (98.7% pure), GF (96.6% pure) and VR (93.4% pure) were synthesized by the Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground, Maryland. A stock solution of each agent was prepared gravimetrically (weight/volume) 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, 2-PAM-chloride (2-PAM), pyridostigmine bromide (PB), and diazepam were obtained through the Walter Reed Army Institute of Research (WRAIR), Washington, DC. MMB4 dimethanesulfonate was obtained from Southwest Research Institute, San Antonio, TX. Stock solutions of atropine and 2-PAM were prepared in sterile water and stored in the refrigerator. The remaining drugs were prepared fresh daily. Atropine and oximes were admixed in the same bottle for injection.

Experimental Design

Animals were pretreated with saline or PB intramuscularly (i.m.) 30 minutes prior to nerve agent challenge. The dose of PB was 0.026 mg/kg, which results in 20-30% inhibition of acetylcholinesterase (AChE) in erythrocytes (RBC) at the time of agent challenge. Guinea pigs were challenged s.c. with 2LD₅₀s of GA, GB, GF, or VR between the shoulder blades. One minute after challenge each animal was treated i.m. in a hind limb with atropine (0.3, 3.0 or 16 mg/kg), plus either 2-PAM (25 mg/kg) or MMB4 (26 mg/kg). Diazepam (1 mg/kg) was injected i.m. in the opposite hind limb immediately after atropine and oxime treatment in some of the animals. The 2-PAM dose approximated the total dose in three autoinjectors (600 mg per 2-PAM autoinjector) to a 70-kg human. The MMB4 dose approximated the projected total dose in three autoinjectors (600 mg per MMB4 autoinjector) to a 70-kg human. The 0.3 mg/kg atropine dose approximated the total dose in nine autoinjectors (2 mg per atropine autoinjector) given to a 70-kg human [Sidell, 1997]. The 3.0 mg/kg atropine dose is fully antimuscarinic but has minimal anticonvulsant efficacy in nerve agent intoxication [Shih and McDonough, 1999], and the 16 mg/kg atropine dose is an effective dose against soman lethality in guinea pigs [Koplovitz et al., 1995]. The 1.0 mg/kg diazepam dose is approximately the average (effective dose, ED) ED₅₀ for terminating convulsions induced by a number of nerve agents in guinea pigs [Shih et al., 2007]. All pretreatments and treatments were tested in parallel against each agent and were assigned randomly to the animals. Death was the endpoint and those animals that survived could be fully functional to severely impaired. Survival rates were assessed at 24 hour after agent challenge.

Statistical Methods

Response data, survival or death, for all atropine dose, agent, oxime, diazepam and saline/PB groups were analyzed using PROC CATMOD in SAS (version 6.12). PROC CATMOD is a procedure for categorical data modeling and analyzes categorical data similarly to an analysis of variance on continuous response data [SAS Institute, 1989]. PROC CATMOD was used to determine the effects of atropine dose, agent, oxime, diazepam, and PB and their respective two-factor interactions. This analysis was followed by a comparison of the survival proportions for specific treatment groups using a method similar to the multiple comparison Tukey test as described in Zar [1996]. The percent survival for each group was transformed using the arcsine transformation. Differences between the transformed numbers for specific pairs of groups along with estimates of the combined standard error were used to determine whether the pairs of

groups were statistically significant. This was done specifically to compare the percent survival for atropine doses with 2-PAM and MMB4 within each agent and diazepam group (with and without diazepam). Statistical significance was defined as $p \leq 0.05$.

RESULTS

Categorical modeling analysis of the data indicated that, across all agents and treatments, there was no significant difference in the survival rate between saline-pretreated animals (337 of 474 survived [71%]) and PB-pretreated animals (370 of 486 survived [76%]); therefore, the saline and PB pretreatment data were combined when making further comparisons. Table 1 shows the survival rates for the combined group (i.e., saline + PB pretreatment) data for each atropine dose, challenge nerve agent, oxime, and diazepam treatment combination. Categorical modeling analysis of the combined data indicated that there was a significant difference between atropine doses ($p=0.022$), between 2-PAM and MMB4 ($p=0.026$), between treatment with diazepam adjunctive and without diazepam adjunctive ($p \leq 0.01$), and between agents ($p \leq 0.01$).

Figures 1 — 4 show the 24-hour percent survival response for each treatment combination (Panel A) against the 4 nerve agents along with the statistical comparisons between the specified treatment groups (Panel B). In the absence of diazepam adjunctive therapy, all 3 doses of atropine in combination with either 2-PAM or MMB4 provided at least 70% survival of the guinea pigs against 2LD₅₀s of GB (Figure 1). Against 2LD₅₀s of GF (Figure 2), VR (Figure 3) or GA (Figure 4), the 0.3 and 3.0 mg/kg doses of atropine in combination with 2-PAM provided no greater than 20% survival of the guinea pigs (see also Table 1). The 0.3 mg/kg and 3.0 mg/kg doses of atropine in combination with MMB4 resulted in significantly greater survival of the guinea pigs than with 2-PAM against GF (Figure 2) and VR (Figure 3) but not against GA (Figure 4). The 16 mg/kg atropine dose in combination with either oxime was significantly more effective than either the 0.3 or 3.0 mg/kg dose of atropine.

The addition of diazepam significantly improved survival rates for the 0.3 and 3.0 mg/kg doses of atropine in combination with either 2-PAM or MMB4 (Figures 1, 2, 3 and 4). The beneficial effect of diazepam on survival rates was most evident in GF-, VR- and GA-intoxicated guinea pigs.

DISCUSSION

Of the two primary classes of post-exposure nerve agent treatment drugs, atropine and oxime, atropine is usually considered more important because it is universally effective against all nerve agents. Atropine blocks muscarinic receptors in both the

periphery and the brain, reduces secretions and bronchoconstriction, prevents central respiratory failure, and in high doses has an antiseizure action [Shih and McDonough, 1999]. Oximes are generally considered to be an adjunct to atropine, because although they have the critical function of reactivating nerve agent-inhibited AChE and restoring neuromuscular function, their efficacy is nerve agent-dependent (i.e., not broad spectrum), time-constrained because of “aging” of the inhibited enzyme and generally limited to peripheral tissues. Ligtenstein [1991] challenged the primary role for atropine and concluded that atropine should be considered an adjunct to oxime therapy and not vice versa. He investigated the efficacy of atropine and the oxime HI-6 against two very similar S-aminoalkylphosphonothioate AChE inhibitors, one that entered the brain and had a mixed peripheral and central action, and the other that only inhibited AChE in peripheral tissues. Ligtenstein found that atropine (37.5 mg/kg) alone had no beneficial effect on survival against intoxication by the peripherally acting AChE inhibitor and only slight efficacy against the mixed peripheral and central AChE inhibitor. HI-6 (50 mg/kg) alone was more effective than atropine against either inhibitor, but was very effective (i.e., 50-fold shift in the LD₅₀ of the OP) against the peripherally acting OP. The combination of atropine and HI-6 had a large synergistic effect against the mixed acting OP, but very little effect on the peripherally acting compound compared to HI-6 alone. Ligtenstein concluded that reactivation at the neuromuscular junction by oximes is of primary importance and is supplemented by the central action of atropine to improve the function of the respiratory centers.

The results of the present study support Ligtenstein’s view and suggest further that the amount of atropine needed to obtain significant survival rates is dependent on how effective the oxime is in reactivating the nerve agent-inhibited AChE in peripheral tissues. In GB-challenged guinea pigs, 2-PAM and MMB4, which are both efficient reactivators of GB-inhibited AChE [Kassa, 2002], in combination with 0.3 mg/kg atropine resulted in survival rates of 70 and 83%, respectively. The high survival rates of these guinea pigs were most likely due to a significant amount of reactivation of GB-inhibited AChE in peripheral tissues by 2-PAM and MMB4. In the absence of significant reactivation of AChE by either 2-PAM or MMB4, for example, against GA [Kuca et al., 2003; Cabal et al., 2004; Kuca et al., 2006], the 0.3 and 3.0 mg/kg doses of atropine are insufficient to sustain survival of the guinea pigs for 24 hours. In the absence of diazepam, high survival rates after GA exposure were obtained when the animals were treated with 16 mg/kg of atropine.

The importance of oxime reactivation of AChE in peripheral tissues to the survival response is shown further in the GF- and VR-challenged animals. 2-PAM-treated animals, receiving 0.3 or 3.0 mg/kg of atropine, showed little or no survival against 2LD₅₀s of GF or VR, whereas MMB4-treated animals receiving the same doses of atropine resulted in significantly higher survival rates. The difference in survival response

is most likely because MMB4 is a better reactivator of GF- and VR-inhibited AChE than is 2-PAM [Maxwell et al., 1997; Kassa, 2002]. However, the degree of reactivation of GF- and VR-inhibited enzyme in peripheral tissues by MMB4 may not be optimal, since only 40—60% survival was observed at the 0.3 and 3.0 mg/kg doses of atropine. From this we deduce that the poorer the oxime is in reactivating nerve agent-inhibited AChE, the higher the dose of atropine needs to be to ensure survival.

An important finding in this study was that in the absence of adequate doses of atropine and/or sufficient oxime reactivation of peripheral AChE, the presence of diazepam in the treatment regimen enhanced survival. This was observed for both 2-PAM and MMB4 in combination with atropine doses of 0.3 and/or 3.0 mg/kg against GF, VR and GA. This has important operational implications, which suggests that in the field where casualties are not going to be maximally atropinized, administration of diazepam with atropine and oxime therapy may be critical.

The overall survival rates in non-PB- and PB-pretreated guinea pigs were not significantly different for any atropine, oxime and/or diazepam treatment combination, indicating that PB was not needed for survival against 2LD₅₀s of these agents. Furthermore, PB pretreatment did not reduce the atropine dose needed for full survival against 2LD₅₀s of any of the agents. At the 0.3 and 3.0 mg/kg doses of atropine, the 20-30% of peripheral AChE that was protected by PB was not sufficient to enhance survival. Perhaps, a higher dose of PB and more inhibition of peripheral AChE would have increased survival at these doses of atropine. PB pretreatment does enhance survival against oxime-refractory nerve agents like soman when administered with 16 mg/kg of atropine in guinea pigs especially at challenge levels higher than 2LD₅₀s [Koplovitz et al., 1995]. It would appear that the key to PB effectiveness is having a sufficient post-challenge atropine dose. Joiner and Kluwe [1988] found that PB pretreatment afforded little enhancement (i.e., less than a 2-fold increase in the LD₅₀ of soman) of the efficacy of atropine and 2-PAM against soman intoxication in rhesus monkeys when the post-challenge dose of atropine was 0.1 mg/kg. However, when the atropine dose was increased to 0.4 mg/kg, the same doses of PB resulted in a 20- to 40-fold increase in the LD₅₀ of soman. In a subsequent study in rabbits, the same investigators found that PB pretreatment failed to increase the post-challenge treatment efficacy of 2-PAM, MMB4 or HI-6 when the atropine dose was 2.0 mg/kg, but afforded significant enhancement when the atropine dose was increased to 8 mg/kg [Joiner et al., 1989].

Finally, the results suggest that high doses of atropine can mask the responses of other medical countermeasures. For example, the difference in survival efficacy between MMB4 and 2-PAM that was observed against GF and VR with 0.3 or 3.0 mg/kg of atropine was diminished or not observed at an atropine dose of 16 mg/kg. Also, the

survival enhancing action of diazepam in combination with either oxime was masked by the high dose of atropine.

In summary, the dose of atropine was an important factor in protecting guinea pigs from the lethal effects of nerve agent intoxication. However, the presence of a broad spectrum oxime and a benzodiazepine anticonvulsant is at least as important, especially if adequate atropinization cannot be ensured. It would appear that the more efficient the oxime is in reactivating nerve agent-inhibited AChE, the lower the requirement for atropine. Also, in the absence of sufficient atropine, diazepam became a very important adjunct to the oxime for survival of the guinea pigs. The dose of atropine became more important as the ability of the oxime to reactivate AChE became more problematic. PB pretreatment was not required to achieve full survival against a 2LD₅₀ challenge of any of these agents. Indeed, PB could not make up for insufficient amounts of atropine, and its efficacy appeared to be dependent on adequate atropinization and/or the presence of diazepam. PB pretreatment, however, will still be essential for enhancing the efficacy of postexposure medical treatments of oxime-resistant nerve agents at exposure levels greater than 2LD₅₀s. From an operational perspective, the results of the current studies using a standardized pretreatment, nerve agent exposure, and treatment paradigm demonstrate the complexity of dealing with emergency medical management of nerve agent casualties and reveal interesting interactions between the various components in the pretreatment/treatment regimen.

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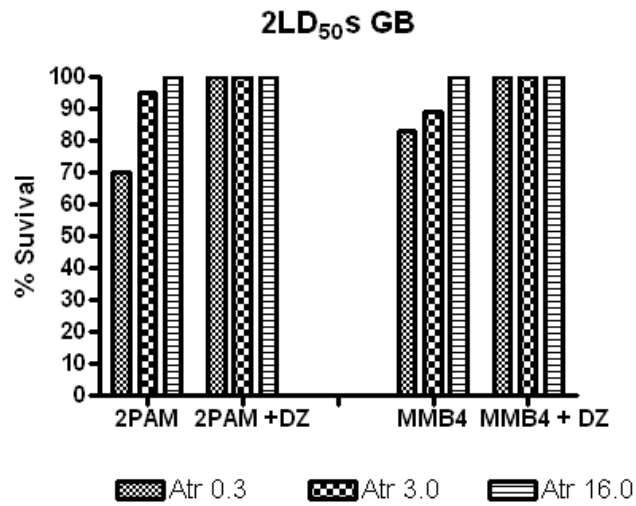
Table 1
Combined Survival Rates from Saline- and PB-pretreated Animals

Treatment ¹ (mg/kg, i.m.)	2LD ₅₀ GB	2LD ₅₀ GF	2LD ₅₀ VR	2LD ₅₀ GA
ATR (0.3) + 2-PAM (25)	14/20	0/20	2/20	1/20
ATR (3) + 2-PAM (25)	19/20	0/20	3/20	4/20
ATR (16) + 2-PAM (25)	22/22	12/20	20/20	20/20
ATR (0.3) + 2-PAM (25) + DZ (1)	18/18	8/20	20/20	10/20
ATR (3) + 2-PAM (25) + DZ (1)	24/24	15/20	19/20	19/20
ATR (16) + 2-PAM (25) + DZ (1)	16/16	17/20	20/20	20/20
ATR (0.3) + MMB4 (26)	15/18	9/20	12/20	0/20
ATR (3) + MMB4 (26)	16/18	8/20	11/20	3/20
ATR (16) + MMB4 (26)	21/21	20/20	20/20	18/20
ATR (0.3) + MMB4 (26) + DZ (1)	22/22	18/20	20/20	13/20
ATR (3) + MMB4 (26) + DZ (1)	21/21	20/20	20/20	18/20
ATR (16) + MMB4 (26) + DZ (1)	20/20	20/20	20/20	19/20

1. Treatment administered 1 minute after sc nerve agent. ATR and oximes were admixed; diazepam was injected separately in the opposite hind limb.

Figure 1

Panel A



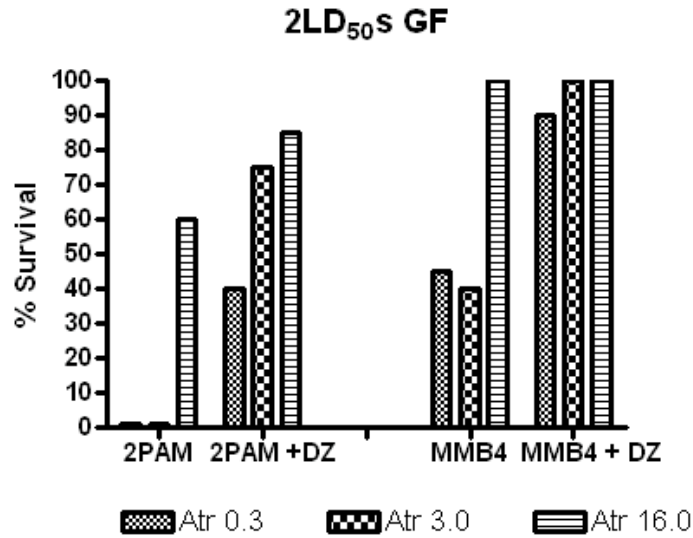
Panel B

GB	ATR (0.3) + 2PAM	ATR (3.0) + 2PAM	ATR (16) + 2PAM	ATR (0.3) + 2PAM + DZ	ATR (3.0) + 2PAM + DZ	ATR (16) + 2PAM + DZ	ATR (0.3) + MMB4	ATR (3.0) + MMB4	ATR (16) + MMB4	ATR (0.3) + MMB4 + DZ	ATR (3.0) + MMB4 + DZ	ATR (16) + MMB4 + DZ
ATR (0.3) + 2PAM		NS	p<0.05	p<0.05	p<0.05	p<0.05	NS	NS	p<0.05			
ATR (3.0) + 2PAM			NS	NS	NS	NS	NS	NS	NS			
ATR (16) + 2PAM				NS	NS	NS	NS	NS	NS			
ATR (0.3) + 2PAM + DZ					NS	NS				NS	NS	NS
ATR (3.0) + 2PAM + DZ						NS				NS	NS	NS
ATR (16) + 2PAM + DZ										NS	NS	NS
ATR (0.3) + MMB4								NS	NS	NS	NS	NS
ATR (3.0) + MMB4									NS	NS	NS	NS
ATR (16) + MMB4										NS	NS	NS
ATR (0.3) + MMB4 + DZ											NS	NS
ATR (3.0) + MMB4 + DZ												NS
ATR (16) + MMB4 + DZ												

Figure 1 Effect of atropine (ATR) dose on the efficacy of 2-PAM and MMB4 with and without diazepam (DZ) adjunctive treatment against 2LD₅₀s of GB. Panel A. Twenty-four-hour survival rates for the various treatment groups. Group numbers (N's) are shown in Table 1. Panel B. Table of statistical comparison between treatments. Numbers in () are mg/kg doses of ATR. NS = not significant (p>0.05)

Figure 2

Panel A



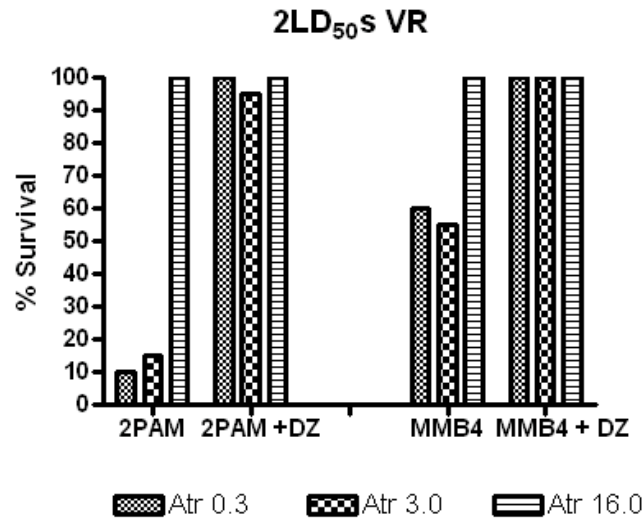
Panel B

GF	ATR (0.3) + 2PAM	ATR (3.0) + 2PAM	ATR (16) + 2PAM	ATR (0.3) + 2PAM + DZ	ATR (3.0) + 2PAM + DZ	ATR (16) + 2PAM + DZ	ATR (0.3) + MMB4	ATR (3.0) + MMB4	ATR (16) + MMB4	ATR (0.3) + MMB4 + DZ	ATR (3.0) + MMB4 + DZ	ATR (16) + MMB4 + DZ
ATR (0.3) + 2PAM		NS	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05			
ATR (3.0) + 2PAM			p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05			
ATR (16) + 2PAM				NS	NS	NS	NS	NS	p<0.05			
ATR (0.3) + 2PAM + DZ					NS	NS				p<0.05	p<0.05	p<0.05
ATR (3.0) + 2PAM + DZ						NS				NS	p<0.05	p<0.05
ATR (16) + 2PAM + DZ										NS	NS	NS
ATR (0.3) + MMB4								NS	p<0.05	p<0.05	p<0.05	p<0.05
ATR (3.0) + MMB4									p<0.05	p<0.05	p<0.05	p<0.05
ATR (16) + MMB4										NS	NS	NS
ATR (0.3) + MMB4 + DZ											NS	NS
ATR (3.0) + MMB4 + DZ												NS
ATR (16) + MMB4 + DZ												

Figure 2 Effect of atropine (ATR) dose on the efficacy of 2-PAM and MMB4 with and without diazepam (DZ) adjunctive treatment against 2LD₅₀s of GF. Panel A. Twenty-four-hour survival rates for the various treatment groups. Group numbers (N's) are shown in Table 1. Panel B. Table of statistical comparison between treatments. Numbers in () are mg/kg doses of ATR. NS = not significant (p>0.05)

Figure 3

Panel A



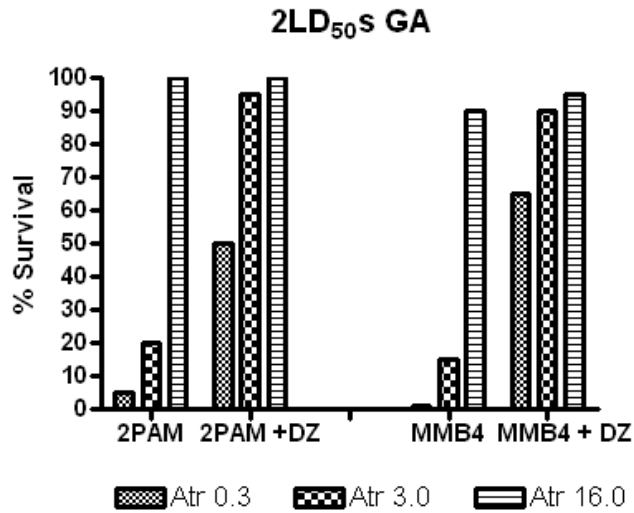
Panel B

VR	ATR (0.3) + 2PAM	ATR (3.0) + 2PAM	ATR (16) + 2PAM	ATR (0.3) + 2PAM + DZ	ATR (3.0) + 2PAM + DZ	ATR (16) + 2PAM + DZ	ATR (0.3) + MMB4	ATR (3.0) + MMB4	ATR (16) + MMB4	ATR (0.3) + MMB4 + DZ	ATR (3.0) + MMB4 + DZ	ATR (16) + MMB4 + DZ
ATR (0.3) + 2PAM		NS	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05			
ATR (3.0) + 2PAM			p<0.05	p<0.05	p<0.05	p<0.05	NS	NS	p<0.05			
ATR (16) + 2PAM				NS	NS	NS	p<0.05	p<0.05	NS			
ATR (0.3) + 2PAM + DZ					NS	NS				NS	NS	NS
ATR (3.0) + 2PAM + DZ						NS				NS	NS	NS
ATR (16) + 2PAM + DZ										NS	NS	NS
ATR (0.3) + MMB4								NS	p<0.05	p<0.05	p<0.05	p<0.05
ATR (3.0) + MMB4									p<0.05	p<0.05	p<0.05	p<0.05
ATR (16) + MMB4										NS	NS	NS
ATR (0.3) + MMB4 + DZ											NS	NS
ATR (3.0) + MMB4 + DZ												NS
ATR (16) + MMB4 + DZ												

Figure 3. Effect of atropine (ATR) dose on the efficacy of 2-PAM and MMB4 with and without diazepam (DZ) adjunctive treatment against 2LD₅₀s of VR. Panel A. Twenty-four-hour survival rates for the various treatment groups. Group numbers (N's) are shown in Table 1. Panel B. Table of statistical comparison between treatments. Numbers in () are mg/kg doses of ATR. NS = not significant (p>0.05)

Figure 4

Panel A



Panel B

GA	ATR (0.3) + 2PAM	ATR (3.0) + 2PAM	ATR (16) + 2PAM	ATR (0.3) + 2PAM + DZ	ATR (3.0) + 2PAM + DZ	ATR (16) + 2PAM + DZ	ATR (0.3) + MMB4	ATR (3.0) + MMB4	ATR (16) + MMB4	ATR (0.3) + MMB4 + DZ	ATR (3.0) + MMB4 + DZ	ATR (16) + MMB4 + DZ
ATR (0.3) + 2PAM		NS	p<0.05	p<0.05	p<0.05	p<0.05	NS	NS	p<0.05			
ATR (3.0) + 2PAM			p<0.05	NS	p<0.05	p<0.05	NS	NS	p<0.05			
ATR (16) + 2PAM				p<0.05	NS	NS	p<0.05	p<0.05	NS			
ATR (0.3) + 2PAM + DZ					p<0.05	p<0.05				NS	NS	p<0.05
ATR (3.0) + 2PAM + DZ						NS				NS	NS	NS
ATR (16) + 2PAM + DZ										p<0.05	NS	NS
ATR (0.3) + MMB4								NS	p<0.05	p<0.05	p<0.05	p<0.05
ATR (3.0) + MMB4									p<0.05	p<0.05	p<0.05	p<0.05
ATR (16) + MMB4										NS	NS	NS
ATR (0.3) + MMB4 + DZ											NS	NS
ATR (3.0) + MMB4 + DZ												NS
ATR (16) + MMB4 + DZ												

Figure 4 Effect of atropine (ATR) dose on the efficacy of 2-PAM and MMB4 with and without diazepam (DZ) adjunctive treatment against 2LD₅₀s of GA. Panel A. Twenty-four-hour survival rates for the various treatment groups. Group numbers (N's) are shown in Table 1. Panel B. Table of statistical comparison between treatments. Numbers in () are mg/kg doses of ATR. NS = not significant (p>0.05)