Synergism Between Anticholinergic and Oxime Treatments Against Sarin-Induced Ocular Insult in Rats

A. Gore¹, R. Brandeis, I. Egoz, J. Turetz, U. Nili, E. Grauer, and E. Bloch-Shilderman

Department of Pharmacology, Israel Institute for Biological Research, Ness Ziona, 74100, Israel

¹To whom correspondence should be addressed at Department of Pharmacology, Israel Institute for Biological Research, Ness Ziona 74100, Israel. E-mail: Arielg@iibr.gov.il

ABSTRACT

Eye exposure to the extremely toxic organophosphorus sarin results in long-term miosis and visual impairment. As current treatment using atropine or homatropine eye drops may lead to considerable visual side effects, alternative combined treatments of intramuscular (im) oximes (16.8 nmoles/kg, im) with atropine (0.5 mg/kg, im) or with the short acting antimuscarinic tropicamide (0.5%; w/v) eye drops were thus evaluated. The combined treatments efficacy following topical exposure to sarin (1 µg) was assessed by measuring pupil width and light reflex using an infra-red based digital photographic system. Results showed that the combined treatment of various oximes with atropine or with topical tropicamide eye drops rapidly reversed the sarin-induced miosis and presented a long-term improvement of 67–98% (oxime + tropicamide) or 84–109% (oxime + atropine) in pupil widening as early as 10-min following treatment. This recovery was shown to persist for at least 8-h following exposure. All combined treatments facilitated the ability of the iris to contract following sarin insult as tested by a light reflex response. Our findings emphasize the high efficacy of im oxime treatment combined with either atropine im or tropicamide eye drops in counteracting sarin-induced ocular insult. Therefore, in a mass casualty scenario the systemic combined treatment may be sufficient to ameliorate sarin-induced ocular insult. For very mild casualties, who are unlikely to receive im treatment, the combined oxime (im) with topical tropicamide treatment may be sufficient in ameliorating the ocular insult.

Key words: anticholinergic treatments; atropine; HI-6; Long-Evans rats, miosis; MMB-4; organophosphates; oxime; pupillary light reflex; sarin; TMB-4; obidoxime; tropicamide

Abbreviations

ACh, acetylcholine; ChE, cholinesterase; IF, infrared; im, intramuscular; OP, organophosphate; PG, propylene glycol.

The anticholinesterase compound sarin (isopropyl methylphosphonofluoridate) is an extremely toxic organophosphate (OP) nerve agent, which was used as a warfare agent in both terrorist attacks in Japan (Yanagisawa et al., 2006) and in the civil war in Syria (Dolgin, 2013; Rosman et al., 2014). Sarin may be used on the battlefield or against civilians to cause incapacitation or death. Topical OP ocular exposure leads to a local reduction in cholinesterase (ChE) activity in the iris and ciliary muscle (Dabisch et al., 2005; Lund-Karlsen et al., 1976) with subsequent accumulation of acetylcholine (ACh) (Mattié et al., 1984). Following sublethal exposure, hypercholinergic action upon the iris sphincter muscle results in marked miosis with dim
vision, reduction in visual field and difficulty in adapting to both low and high levels of illumination due to desensitization of muscarinic receptors in the iris (Dabisch et al., 2008; Genovese et al., 2008; Lund-Karlsen et al., 1976; Takayanagi et al., 1993). The hypercholinergic action on the ciliary muscle causes an increased tension or even spasm and may lead to blurred vision and myopia accompanied by ocular pain, headaches and nausea (Cannard, 2006; Nohara et al., 1996; Rengstorff, 1985; Smith and Smith, 1980; Yanagisawa et al., 2006). Very mild casualties suffering from sarin-induced ocular insult only, may benefit from topical (Ohbu et al., 1997; Okudera, 2002) rather than systemic anticholinergic treatment (Ohbu et al., 1997; Yanagisawa et al., 2006) since the later may induce systemic side effects (Hurst et al., 2007) with no ocular benefit. Short acting anticholinergic topical eye drops, such as tropicamide, are preferable to the more potent and longer lasting eye drops such as atropine, homatropine or cyclopentolate, which may induce long-term mydriasis and cycloplegia and may even worsen visual acuity and performance (Geoghegan and Tong, 2006; Moylan-Jones and Thomas, 1973; Nozaki and Aikawa, 1995a; Gore et al., 2007; Holstege et al., 1997; Sidell and Borak, 1992). In casualties with additional symptoms such as lacrimation, increased salivation, rhinorrhea, tightness in the chest, sweating, nausea, vomiting, and abdominal cramps (considered mild casualties), systemic treatment of atropine and oximes may have an impact on the ocular symptoms. In 2 cases of sarin-exposed individuals, a combined IM treatment of 2-PAM and atropine alone (Nozaki et al., 1995a; Gore et al., 2012; Holstege et al., 1997; Sidell and Borak, 1992). In casualties with additional symptoms such as lacrimation, increased salivation, rhinorrhea, tightness in the chest, sweating, nausea, vomiting, and abdominal cramps (considered mild casualties), systemic treatment of atropine and oximes is indicated.

In order to minimize repeated treatments or the misuse side effects of mydriasis and cycloplegia (Bartlett et al., 2008), we hypothesized that a combined treatment of oxime and topical tropicamide may be beneficial in counteracting ocular symptoms; as tropicamide will rapidly widen the iris and reduce ciliary spasm while the oxime will reactivate ocular ChE, preventing recurrence of ocular toxic symptoms as has been demonstrated previously using topical combined treatments (Gore et al., 2014). In addition, this combined treatment may enable the reduction of tropicamide dose, reducing the intensity and duration of mydriasis and partial cycloplegia side effects in cases of misuse.

Because the beneficial potential effect of the combined IM oxime with topical (for very mild casualties) or IM (for mild casualties) anticholinergic treatment has not yet been determined, as we aim to examine this concept in this study.

Various IM oxime treatments, which are either presently in use such as HI-6, obidoxime and TMB-4 (Antonijevic and Stojiljkovic, 2007) or in the process of development such as MMB-4 (Lundy et al., 2011) in combination with anticholinergic treatment, were evaluated to determine their beneficial role. The combined treatments used in this work were evaluated for their role in ameliorating the sarin-induced ocular impairment in the rat model by assessing pupil width and iris contraction ability following exposure and treatment.

MATERIALS AND METHODS

Chemicals

Isopropyl methylphosphono-fluoridate (sarin) was synthesized by the department of Organic Chemistry (Israel Institute for Biological Research, IIBR) and its purity of >95% was determined by quantitative NMR. Sarin stock solution of 30 mg/ml in propylene glycol (PG) was prepared and stored at −20°C. Sarin solutions in saline were freshly prepared before each experiment. Tropicamide 0.5% (w/v) was purchased as commercial eye drops Mydramide 0.5% (w/v) (Benzenacacetamid, N-ethyl-s-(hydroxy-methyl)-N-(4-pyridylmethyl)-) from Fischer Pharmaceutical Laboratories, Tel-Aviv, Israel. Saline was purchased as a commercial product from Teva Medical, Ashdod, Israel. HI-6 [1-(2-hydroxyiminomethylpyridinium)-3-(4-karbamoylpyridinium)-2-oxopropan dimethanesulfonate], MMB-4 [bis-1, 1-(4-hydroxyiminomethylpyridinium) methane dichloride], and obidoxime [bis-1, 3-(4-hydroxyiminomethylpyridinium)-2-oxopropane dichloride] were purchased from Chemprotect, Prague, Czech Republic. Trimedoxime [TMB-4; 1, 3-bis-(4-hydroxyiminomethylpyridinium)-propanedichloride], PG and atropine were purchased from Sigma, Rehovot, Israel. Oximes, PG and atropine solutions were prepared in saline before each experiment.

Animals

Male Long-Evans rats (200–300 g) were purchased from Charles River, Boston, Massachusetts. Animals were housed in ambient conditions of 3 animals per cage, temperature-controlled environment at 21 ± 2°C and 40–70% humidity, 12-h light/dark cycle (lights on at 6AM). Rats were provided with Altromin 1324 pellets (lights on at 6AM). Animals were acclimated to the laboratory conditions for at least 1 week following VX (O-ethyl-S-[2-(diisopropylamino)ethyl] methylphosphonothioate) or after sarin exposure (Macisopropylamino)ethyl methylphosphonothioate) and after sarin exposure (Macisopropylamino)ethyl methylphosphonothioate) or after sarin exposure (Macisopropylamino)ethyl methylphosphonothioate) or after sarin exposure (Macisopropylamino)ethyl methylphosphonothioate). Rats were provided with Altromin 1324 pellets (Altromin, Lage, Germany) and tap water ad libitum during the study and were maintained in accordance with the principles enunciated in the Guide for the Care and Use of Laboratory Animals, 8th ed., National Academy Press, Washington DC, 2010. Experimental procedure began at least 1 week following acclimatization.

The experimental protocols were approved by the IIBR committee for animal care and use, and were designed to prevent or minimize any unnecessary pain and stress. Animals were euthanized by carbon dioxide asphyxiation 24h following the experimental procedure. This study is reported in accordance with ARRIVE guidelines for reporting experiments involving animals (Kilkenny et al., 2010; McGrath et al., 2010).

Infrared Photography

Pupil diameter was digitally photographed and measured by an infrared (IR)-based system, adapted from the “ViewPoint Eye Tracker” from humans to small animals. It comprised 3 components: IR spot light, IR video camera, and “Eye Tracker” software (ViewPoint Eye Tracker, PC-60, Arrington Research Inc., 2002). The measurements were based on retina reflection of IR light (Hulet et al., 2006), which unlike white light has no effect on pupil width (Soli et al., 1980) and causes no discomfort and movement of the animal (Dabisch et al., 2007). The optical system supplied a video signal of the pupil in suitable...
magnification and with best contrast so that the pupil was darker than the surrounding. The image was transferred, displayed and analyzed by the “Eye Tracker” software. The pupil was identified in the visual field, designated according to the contrast distinction compared with the surroundings. The pupil width was indicated by a yellow spherical mark and determined in pixel units. Measurements were presented as a ratio of pupil width to the window length of the eye display, which was constant, to correct for variability between measurements due to variations in the distance from the camera to the eye.

**Sarin Exposure, Treatment Protocols, and Ocular Measurements**

The left eye of each rat (N = 12 for each exposure and treatment group) was digitally photographed before (baseline) and following exposure and treatment to determine the degree of pupil constriction produced by topical eye exposure to sarin and the pupil widening following treatment. Pupil width measurements of each rat at all-time points were recorded relative to their own baseline. Images were taken under controlled low-light conditions (2 lux) and only after the animals had been allowed to adjust to darkness for 15 min. Hand restrained animals were topically exposed to 1-μg sarin in the left eye in a fume hood, where animals were held for 10 min after exposure to prevent environmental contamination.

Exposure to 1 eye with 1 μg sarin was carried out based on previous results showing pinpoint pupils and a reduction in visual performance, with no systemic intoxication or change in peripheral blood ChE activity following this exposure (Core et al., 2012). The width of the pupil was measured 15 min after sarin exposure and continued for up to 8 or 48 h.

Fifteen minutes following a single topical exposure to 1-μg sarin or 0.66% (w/v) PG, pupil width measurements were performed (N = 12 for each treatment group). Twenty min after sarin or PG exposure, animals were treated with 1 drop of either tropicamide or saline. Topical saline-treated animals received im treatment of atropine (0.5, 2, 5 mg/kg) or 1 of the oximes TMB-4, HI-6, Obidoxime, MMB-4 at a dose of 16.8 μmol/kg (7.5, 8.03, 6.03, 5.53 mg/kg, respectively) or a combined treatment of 0.5 mg/kg atropine with each 1 of the listed oximes. Topical tropicamide treated animals were additionally treated with im saline injection or with each 1 of the listed oximes as a triple treatment with atropine. All treatments assessed in this study are summarized in Table 1.

Efficacy evaluations of all treatments on pupil diameters were determined 10-min following treatment (30 min after sarin exposure) and at 1, 2, 4, and 8-h following exposure. Volume of all eye drops used in this study was 5 μl/eye and 150 μl/300g animal for the im treatments. All treatments were compared with the saline-treated animals.

**Light reflex.** Alterations in the ability of the iris to contract in response to light, provide a sensitive method to determine the functional state of muscarinic receptors of the papillary sphincter (Dabisch et al., 2007). Thus as described previously (Core et al., 2012), evaluation of pupil response to light (light reflex) in the left eye was measured at 1- and 4-h following exposure. Briefly, the sarin-exposed left eye was illuminated with 350 lux for 2-3 s and pupil diameter was immediately determined. Light reflex is presented as the percent of pupil width constriction change of the prereflex level. The normal physiological range of light reflex change was determined following examination of 36 naïve eyes to be 35-57% with a mean ± SD of 46 ± 11.

### Table 1. Summary of 34 Treatments Evaluated in This Study (N = 12/Group)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarin/Propylene Glycol</td>
<td>Saline</td>
<td>Atropine 0.5 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Atropine 2 mg/kg</td>
<td>Atropine 2 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Atropine 5 mg/kg</td>
<td>Atropine 5 mg/kg</td>
</tr>
<tr>
<td></td>
<td>MMB-4 5.53 mg/kg</td>
<td>TMB-4 7.5 mg/kg</td>
</tr>
<tr>
<td></td>
<td>HI-6 8.03 mg/kg</td>
<td>HI-6 8.03 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Obidoxime 6.03 mg/kg</td>
<td>Obidoxime 6.03 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Atropine 0.5 mg/kg + MMB-4 5.53 mg/kg</td>
<td>Atropine 0.5 mg/kg + MMB-4 5.53 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Atropine 0.5 mg/kg + TMB-4 7.5 mg/kg</td>
<td>Atropine 0.5 mg/kg + TMB-4 7.5 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Atropine 0.5 mg/kg + HI-6 8.03 mg/kg</td>
<td>Atropine 0.5 mg/kg + HI-6 8.03 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Tropicamide</td>
<td>Saline</td>
</tr>
<tr>
<td></td>
<td>MMB-4 5.53 mg/kg</td>
<td>Atropine 0.5 mg/kg + Obidoxime 6.03 mg/kg</td>
</tr>
<tr>
<td></td>
<td>TMB-4 7.5 mg/kg</td>
<td>TMB-4 7.5 mg/kg</td>
</tr>
<tr>
<td></td>
<td>HI-6 8.03 mg/kg</td>
<td>HI-6 8.03 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Obidoxime 6.03 mg/kg</td>
<td>Obidoxime 6.03 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>Atropine 0.5 mg/kg + TMB-4 7.5 mg/kg</td>
</tr>
</tbody>
</table>

**Data Analysis**

Pupil width at different time points following exposure and various treatments was calculated as percent relative to baseline level, which was determined a day before the exposure (an average of 2 measurements). Light reflex was calculated as percent of pupil width change relative to prereflex level and was measured at 1- and 4-h postexposure. Results are presented as mean ± SEM. Results were analyzed separately for each of the measurements by a mixed ANOVA model, with the type of exposure (sarin/PG) and treatment (summarized in Table 1) as between subject factors, and time as within subject factor. Specific comparisons were performed using an analysis of simple main effects for interactions, with a Bonferroni adjustment for multiple comparisons. Values of p < .05 were considered statistically significant for all tests.

### Results

**Effect of im Atropine Treatment on Sarin-Induced Miosis**

In order to determine the impact of im atropine treatment on pupil diameter following sarin exposure, various doses were evaluated (Fig. 1). The group exposed topically to 1 μg sarin and treated with topical and im saline showed pinpoint pupils for up to 4 h (0% of baseline), with partial recovery of approximately 27% of baseline at 8 h. Animals exposed to sarin and treated topically with saline and im atropine showed a dose dependent response. A treatment of 0.5 mg/kg atropine gradually widened pupil width showing a difference of approximately 20% compared with the sarin-exposed nontreated group (p < .05–.001; Fig. 1). Animals treated with higher doses of 2 and 5 mg/kg atropine showed a rapid pupil widening of 60 and 80% of baseline at 30 min and back to normal by 8 h (Fig. 1).

The nonexposed control group treated with 0.5 mg/kg of atropine showed a rapid widening in pupil width to a level of 190% relative to baseline (mydriasis), remaining dilated (140%) 8-h posttreatment (p < .05–.001; Fig. 1). No changes in pupil width were identified in the control group topically exposed to PG and treated with topical saline followed by im saline administration (Fig. 1).
Light Reflex Assessment Following Topical Sarin Exposure and im Atropine Treatments

Light reflex assay was utilized to determine the role of the various treatments used to counteract the deficit in the physiological iris constriction ability following sarin exposure.

A normal light reflex was seen in the control group exposed to PG and treated with topical and im saline at 1- and 4-h following exposure (Fig. 2). No pupil response to light was observed in sarin-induced pinpoint pupils. The control group exposed to PG and treated with im 0.5 mg/kg atropine and all experimental groups exposed to sarin and treated with im atropine presented a light reflex within the normal range at both time points following exposure (Fig. 2).

Effect of im Oxime Treatment on Sarin-Induced Miosis

We next evaluated the impact of various im oxime treatments on pupil diameter following sarin exposure. Nonexposed animals treated im with each 1 of the oximes HI-6, TMB-4, obidoxime, or MMB-4, showed no change in pupil width as compared with the control group (Fig. 3). Animals exposed to sarin and treated im with 1 of the various oximes, presented a gradual pupil widening, which finally reached a level of 57% (MMB-4), 75% (obidoxime), 88% (TMB-4), and 107% (HI-6) relative to baseline, 8-h following exposure.

Light Reflex Assessment Following Topical Sarin Exposure and im Oxime Treatment

Sarin-exposed eyes showed no light reflex response at 1 and 4 h postexposure; however, a recovery in the pupillary contractibility was observed at both time points following im oxime treatment (p < .001; Fig. 4). Control and nonexposed oxime treated animals showed a light reflex ability within the normal range (Fig. 4).

Effect of Combined im Oxime and Atropine Treatment on Sarin-Induced Miosis

Because im atropine or oxime treatments alone showed only a partial pupil dilation recovery, we further tested the benefit of a combined im treatment of both drugs. In the next experiments, we used the 0.5 mg/kg atropine dose due to its poor effect on pupil dilation as atropine had been reported to have had a small if at all, effect in humans following sarin exposure. As was reported previously (Fig. 1) eyes exposed to 1 μg sarin showed pinpoint pupils for up to 4 h, with a slight pupil dilation of approximately 27% at 8 h following exposure (Fig. 5). Sarin-induced pinpoint pupils were significantly dilated at all-time points following the combined treatment of im oximes with 0.5 mg/kg atropine, compared with untreated sarin-exposed eyes (p < .001), showing a significantly higher efficacy in pupil dilation.

FIG. 1. Pupil width alteration following topical sarin exposure and im atropine treatment. Rats were exposed topically to 0.66% PG or 1 μg sarin followed by topical saline treatment and im atropine (0.5, 2, 5 mg/kg) or saline. Pupil width measurement is presented as a percent relative to baseline of the same animal before exposure up to 8 h following exposure. Each point is the mean ± SEM of 12 rats. All differences, between groups, at different time points postexposure are statistically significant (by ANOVA), at a level of *p < .05–.01.

FIG. 2. Effect of atropine treatments on the pupillary light reflex following sarin exposure. Treated eyes with topical saline and im saline or atropine (0.5, 2, 5 mg/kg) were illuminated with 350 lux for 2–3 s at 1- and 4-h following exposure to PG or 1 μg sarin. Each bar is presented as a percentage change relative to the prereflex pupil width, and represents the mean ± SEM of 12 animals. Normal light reflex range is indicated by the dotted horizontal lines. Statistical differences between treatments are statistically significant at both time points postexposure (by ANOVA), at a level of *p < .001 (vs sarin-exposed group treated topically and im with saline at the same time point).
FIG. 3. Evaluation of im oxime treatment on pupil width following topical sarin exposure. Rats were exposed topically to PG or 1-μg sarin and 20-min later were treated topically with saline followed by im oxime treatment of TMB-4, obidoxime, MMB-4, or HI-6. Pupil width was determined at the indicated time points and presented as a percent relative to baseline of the same animal before exposure. Each point is the mean ± SEM of 12 rats. Statistically significant (by ANOVA) differences between groups are indicated by asterisks at a level of *p < .001.

FIG. 4. Effect of im oxime treatments on the pupillary light reflex following sarin exposure. Eyes were illuminated with 350 lux for 2–3 s, 1- and 4-h following topical saline treatment with im oxime treatment or saline (as indicated) and after exposure to PG or 1-μg sarin. Each bar (mean ± SEM) is presented as a percentage change relative to the preriflex pupil width and represents the average performance of 12 animals. Physiological light reflex range is indicated by the dotted horizontal lines. Statistically significant differences (by ANOVA) in pupillary light reflex were observed between sarin-exposed groups treated with topical and im saline at both time points versus other treatments at a level of *p < .001 as indicated.

FIG. 5. Evaluation of the combined im atropine and oxime treatment on pupil width following topical sarin exposure. Rats were exposed topically to PG or 1-μg sarin and 20-min later were given a topical treatment of saline and a combined im treatment of atropine (0.5 mg/kg) with one of the oximes (16.8 μmol/kg) as indicated (TMB-4, obidoxime, MMB-4, or HI-6). Pupil width was determined at the indicated time points and presented as a percent relative to baseline of the same animal before exposure. Each point is the mean ± SEM of 12 rats. Statistical significant differences between groups, at various time points postexposure, are statistically significant (by ANOVA) at a level of *p < .05, **p < .01, ***p < .001 as indicated.
dilation compared with each treatment alone (Fig. 5 vs Fig. 3; $p < .001$).

The various oximes given with atropine presented a significant ($p < .001$) prolonged action in counteracting the sarin-induced pinpoint pupils, with an order efficacy of: HI-6 > TMB-4 > obidoxime = MMB-4 ($p < .001$; Fig. 5). Control, unexposed eyes treated with im oximes with atropine showed rapid pupil dilation (mydriasis) within a few minutes, followed by gradual pupil contraction, approaching baseline at 8 h similar to eyes treated with atropine alone (Fig. 5).

**Light Reflex Assessment Following Topical Sarin Exposure and Combined im Oxime and Atropine Treatment**

Control animals treated with the combined im oxime and atropine showed no change in light reflex response at both time points except for the groups that were treated with HI-6 or obidoxime and atropine, which showed a slight nonsignificant reduction in contraction ability at the first time point examined. Sarin-exposed pupils treated with all combined treatments showed a rapid change in contractility, compared with the nontreated group ($p < .001$), returning to the normal range (Fig. 6) except when using the HI-6 and atropine combination, which showed a slight nonsignificant reduction in normal light response at the first time point (Fig. 6).

**Effect of Combined im Oxime and Topical Tropicamide Treatment on Sarin-Induced Miosis**

We next examined the efficacy of the short-term acting topical tropicamide treatment with im oxime treatment in ameliorating the sarin-induced pupil constriction.

Sarin-induced pinpoint pupils were significantly dilated at all time points following im oxime with 1 drop of 0.5% tropicamide combined treatment, compared with the nontreated sarin-exposed eyes ($p < .001$; Fig. 7). The various oximes given with tropicamide induced a significant synergistic effect (Fig. 3 compare with 7), rapid and prolonged action in counteracting the sarin-induced pinpoint pupils, with an order efficacy of: HI-6 > TMB-4 > obidoxime = MMB-4 ($p < .001$; Fig. 7). Control eyes treated with im oximes with tropicamide showed a sharp pupil widening (mydriasis) within a few minutes, followed by gradual pupil contraction, returning to baseline at 8 h following treatment, similar to those eyes treated with tropicamide alone (Fig. 7).
Light Reflex Assessment Following Topical Sarin Exposure and Combined im Oxime and Topical Tropicamide Treatment

Control animals treated with im TMB-4, MMB-4, and obidoxime with topical tropicamide or tropicamide alone showed a reduction in the contractility of the pupil 1 h following the above treatment, reverted to their normal light response 4-h following treatment (Fig. 8). Sarin-exposed pupils treated with all combined treatments showed a rapid change in contraction ability, compared with the nontreated group ($p < .001$), returning to the normal range at both time points (Fig. 8).

Effect of Combined im Oxime With Atropine and Topical Tropicamide (Triple Treatment) on Sarin-Induced Miosis

We next examined the effect of the topical tropicamide treatment in addition to the standard treatment of im atropine and oxime (triple treatment). Evaluation of the effect of this combination on pupil dilation in a control group simulated misuse, and on a sarin-exposed group simulated over treatment.

A representative oxime, TMB-4 was selected for this experiment. Sarin-induced pinpoint pupils were significantly dilated (mydriasis) at all-time points, following the triple treatment, compared with nontreated sarin-exposed eyes ($p < .05–.001$; Fig. 9), the im oxime and atropine treatment ($p < .001$; Fig. 3) as well as the topical tropicamide treatment ($p < .001$; Fig. 7). Control eyes treated with the triple treatment showed rapid pupil dilation (mydriasis) within a few min, followed by a prolonged mydriasis that returned to baseline only at 24 h (Fig. 9).

DISCUSSION

The objective of this study was to determine the beneficial role of various im oxime treatments combined with im atropine or topical tropicamide treatment in ameliorating the sarin-induced miotic effects following ocular sarin exposure.
This form of ocular insult is likely to be common in mass casualty scenarios, where a high proportion of the victims are exposed to low level sarin vapors.

We show here that an im atropine treatment alone widens pupil width in a dose-dependent manner and ameliorates the light reflex ability following 1 μg sarin exposure. In addition, we show that im oxime (HI-6, TMB-4, obidoxime, and MMB-4) treatment enables gradual pupil dilation and a functional iris constriction ability following topical sarin exposure with an order of efficacy of: HI-6 > TMB-4 > obidoxime > MMB-4.

In contrast to the gradual response in pupil dilation following each treatment alone (im atropine or oxime), the combined administration of 0.5 mg/kg atropine with each of the oximes evaluated induced a rapid synergistic effect with an efficacy order of HI-6 > TMB-4 > obidoxime > MMB-4 and a functional return of light reflex at 1- and 4-h following exposure.

The combined treatment of topical tropicamide with im oximes (HI-6, TMB-4, obidoxime, and MMB-4) showed a similar rapid synergistic effect in pupil dilation enhancing the iris constriction ability at 1 and 4 h, as seen following the im combined treatment. This strong synergistic effect is demonstrated here for the first time and should be considered in the design of appropriate therapeutic doctrines with respect to mitigating the effects of OP ocular poisoning.

A triple treatment of im oxime with atropine and topical tropicamide treatment in control or sarin-exposed group showed a rapid mydriatic effect and restoration of iris contractibility.

Following the sarin terrorist attack experience in Japan, it has been reported that systemic atropine treatment showed no benefit in counteracting the sarin-induced miosis (Ohbu et al., 1997; Yanagisawa et al., 2006). This lack of effect of im atropine treatment on pupil diameter following ChE inhibition was also shown in clinical trials where volunteers were topicaly exposed to physostigmine and treated with im 2 mg atropine (Kay and Morrison, 1988). In an unexposed group, 1 mg of im administered atropine showed a weak pupil widening of only 20% (Mirakhor and Dundee, 1980). In rats, unlike humans, atropine (6 mg/kg, im) has been shown to provide a significant pupil widening following sarin exposure (Dabisch et al., 2005). Even a lower dose of im 0.5 mg/kg atropine provided pupil widening following sarin exposure (Fig. 1). The different efficacy of atropine on pupil widening may be associated with the lower dose used in humans (2 mg/70 kg = approximately 0.03 mg/kg) versus the experiments in rats, which received 0.5 mg/kg, a dose that is approximately 17 times higher. Therefore, the effect of 0.5 mg/kg atropine used in this study may be equivalent to the use of very high doses used effectively in humans (Cannard, 2006) rather than lower doses, which showed no effect on pupil widening following sarin exposure (Yanagisawa et al., 2006). Alternatively, it may be due to differences in the rate of the cholinergic recovery following ocular OP exposure in humans versus rats (Gore et al., 2012; Munro, 1994; Rengstorff, 1985, 1994; Yanagisawa et al., 2006).

The im combination of treatment was not tested following ocular OP insult in humans. However, in 2 single cases a combined treatment appeared to be more beneficial than the treatment with atropine alone. During the Tokyo terrorist attack a few ventilated casualties with respiratory arrest were treated with both intravenous atropine (3–5 mg) and a few grams of 2-PAM, a treatment, which dramatically widened pupil diameter from 1 to 2–3 mm (Ohbu et al., 1997). Another case was of a medical personal with secondary exposure at the University Hospital of Tokyo: Self-treatment of 0.5 mg atropine with 500 mg 2-PAM im showed a marked pupil widening 2 h following treatment compared with other exposed emergency room staff personal treated only with atropine showing a slow pupil widening recovery over 2 days (Nozaki et al., 1995b).

The beneficial effect of combined treatments was clearly demonstrated here in a significant synergistic effect of atropine and the oxime.

This unique synergistic response is likely to be a result of the involvement of 2 different mechanisms both aimed at restoring cholinergic function: The first is the oxime reactivation of the inhibited ChE enzyme, restoring ACh hydrolysis, reduces the hypercholinergic state and indirectly enables muscle relaxation and a gradual pupil widening. The second mechanism is the anticholinergic treatment that blocks the muscarinic response, directly reducing the pupil constriction. The use of a combined treatment, may allow for the reduction in dose and frequency of treatments and elimination of side effects.

We have previously reported that a topical combined treatment may reduce the need of repeated anticholinergic treatments (Gore et al., 2014). As oximes are not approved for ocular use in humans, a combined topical treatment is as yet unavailable. For this reason im oximes was tested and its beneficial role with topical tropicamide was demonstrated.

This combined treatment should be used not only for very mild casualties, but also for continuous treatment following the initial im treatment in mild casualties showing prolonged visual insult with no other systemic symptoms.
Because most casualties following OP exposure will be defined as mild rather than very mild (with ocular insult only) and because military and civilian field medical staff are equipped with auto-injectors containing atropeine and oxime (TA auto-injectors content in Israel: 80 mg TMB-4 with 2 mg atropine for the ages of 10–60 years) the efficacy of this treatment in widening pupil width was also evaluated here. We showed here that this treatment may be beneficial in ameliorating the ocular insult at least at the first stages following intoxication. In the case of TA use, miosis and ocular pain should be monitored before the recommended anticholinergic eye drops treatment is used, as this may cause side effects of mydriasis, as demonstrated by tropicamide (Fig. 9). In addition this may cause side effects of cycloplegia, which cannot be tested in rats and was, therefore, not addressed in this work.

Although this work did not address the effect of the combined therapy on the ciliary muscle, due to the anatomical proximity to the iris and the sharing of the same aqueous humor fluid, it is highly reasonable to expect that OP exposed victims with ciliary spasm will benefit from this treatment, thus reducing ocular pain (Cannard, 2006; Kato and Hamanaka, 1996; Lund-Karlsen et al., 1976) and improving blurred distance vision. Taken together, the data strongly support the use of im oximes combined with im atropeine in mild casualties (as in automatic injectors) or with topical treatment of tropicamide in very mild casualties or casualties with long-term ocular insult. This medical treatment doctrine is expected to shorten the impact of the insult period, reduce the need of repeated anticholinergic treatments and reduce or eliminate possible side effects.

In summary:

1. The use of im oxime with topical ocular or im anticholinergic treatment induces a synergistic beneficial effect in ameliorating the sarin-induced ocular insult.
2. Combined im treatment (as in autoinjectors) may be sufficient in ameliorating ocular insult in “mild” casualties. Thus pupil width should be observed before additional topical anticholinergic treatment is used.
3. Topical ocular tropicamide with im oxime should be considered as the treatment of choice in “very mild” casualties or against sarin-induced “long-term” ocular insult instead of the current treatment of atropeine or homatropine eye drops.

REFERENCES


Mattio, T.G., Richardson, J.S., and Giacobini, E. (1984). Effects of DFP on iridic metabolism and release of acetylcholine and on...


