Treatment of Ocular Tissues Exposed to Nitrogen Mustard: Beneficial Effect of Zinc Desferrioxamine Combined with Steroids

Yair Morad,1,2 Eyal Banin,2,3 Edward Averbukh,5 Eduard Berenshtein,4 Alexey Obolensky,5 and Mordechai Chevion4

PURPOSE. Exposure of the ocular surface to mustard gas chemical warfare leads to a destructive inflammatory reaction. Both steroids and a novel metalocomplex free radical scavenger, zinc desferrioxamine (Zn/DFO)—combined with dexamethasone phosphate (0.1%),—have been shown to be effective separately in reducing ocular damage. The purpose of the present study was to investigate whether the effectiveness of both medications applied simultaneously is superior to the effectiveness of either one applied alone.

METHODS. One eye in each of 52 rabbits was exposed to 2% nitrogen mustard (NM). Topical treatment with eye drops of a metal complex—zinc desferrioxamine (Zn/DFO)—combined with dexamethasone phosphate (0.1%), was compared with the administration of saline or treatment with Zn/DFO or dexamethasone alone. Eight eyes (four animals) that were not exposed to NM served as the control. Examiners masked to the treatment groups assessed the extent of ocular injury and the response to treatment using clinical, histologic, and biochemical criteria.

RESULTS. Treatment with the combination of Zn/DFO and dexamethasone was significantly more effective than was dexamethasone or Zn/DFO alone in reducing NM injury to ocular anterior segment structures. In combination-treated eyes, corneal re-epithelization was faster, corneal neovascularization was less severe, and intraocular pressure was not elevated as severely as in the saline or the Zn/DFO- or dexamethasone-alone groups. In addition, systemic antioxidation status was better conserved in the combination-treated animals.

CONCLUSIONS. The findings suggest that the combination of topically applied Zn/DFO and dexamethasone, by virtue of their additive inhibitory effects on free radical formation and inflammation, should be considered as a basis for the treatment of ocular mustard gas injuries. (Invest Ophthalmol Vis Sci. 2005;46:1640–1646) DOI:10.1167/iovs.04-1165

In a world faced with growing threats of weapons of mass destruction used by countries or small extremist groups, mustard chemical warfare agents are among the most abundant and the easiest to deploy. When mustard agents were first used in World War I, approximately one third of the 1,200,000 soldiers who were exposed required prolonged medical treatment for dermal, gastrointestinal, respiratory, and ocular injuries.1 More recently, mustard gas was used against Kurdish civilians2 and Iranian troops, in the Iran–Iraq conflict, with dire consequences.3

Although airway and gastrointestinal exposure to high concentrations of mustard gas may be lethal, even minimal exposure to this chemical, typical in most casualties, leads to incapacitating ocular chemical burn.4 The standard treatment against mustard chemical injury has not changed for decades. It includes the use of protective gear, ocular irrigation with copious amounts of water,5 and the use of local lubrication, antibiotics, and ocular hypotensive drugs, as required.6 The use of anti-inflammatory agents has not been extensively studied because inflammation was not regarded as a primary cause of the tissue damage inflicted by mustard agents.7 Recently, however, animal studies have promoted the topical use of corticosteroids and nonsteroidal anti-inflammatory agents (such as diclofenac) for the treatment of such injuries.8

In a recent study, our group demonstrated that topical treatment with a novel metalocomplex, zinc desferrioxamine (Zn/DFO), yields marked ocular protection (52%–64%) from nitrogen mustard (NM) injury compared with saline-treated eyes. This included faster healing of corneal epithelial erosions, less scarring and neovascularization, decreased inflammation in the anterior chamber, better maintenance of intraocular pressure (IOP), and less severe changes in the iris and lens.9 No toxic effects of this complex were observed. In an attempt to improve therapeutic options for mustard-induced ocular injury, we sought in the present study to examine whether combining the anti-inflammatory action of topical steroids with the inhibition of reactive oxygen species (ROS) formation by Zn/DFO would yield better protection against nitrogen mustard (NM) ocular injury than would saline or either component alone.

METHODS

Animal Model of Ocular NM Injury

A total of 56 New Zealand Albino rabbits weighing 2.5 to 3.5 kg were used. All animal experiments were conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Animals were anesthetized with ketamine HCl (Ketalar, 50 mg/kg; Pfizer, Sandwich, UK) injected intramuscularly in combination with the relaxing agent xylazine (0.5 mg/kg; VMD, Arendonk, Belgium). Local anesthetic drops (benoxinate HCl 0.4%; Fisher Pharmaceuticals, Tel-Aviv, Israel) were administered. Ocular mustard injury was induced as previously described.10 Briefly, NM (mechlorethamine; Sigma-Aldrich, St. Louis, MO), at a concentration of 2% in saline, was

1Department of Ophthalmology, Assaf Harofeh Medical Center, Zrifin, Israel; and the Departments of 2Ophthalmology and 3Cellular Biochemistry and Human Genetics, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

2Contributed equally to the work and therefore should be considered equivalent authors.

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Corresponding author: Yair Morad, Department of Ophthalmology, Assaf Harofeh Medical Center, Zrifin 73000, Israel; ymorad@013.net.il.
applied to the cornea of one eye of each animal (the experimental eye) for 5 minutes within a trephine. Immediately after application, NM was quickly absorbed from within the trephine with small Weck-Cell sponges (Invotec International, Inc., Jacksonville, FL), followed by washing of the eye with copious amounts of normal saline. Local treatment was immediately initiated according to the protocol described in the next section. In control eyes (four additional animals, eight eyes), saline solution (instead of NM) was applied to the cornea for 5 minutes, also within the trephine. In control experiments, the vehicle (saline) was used as eye drops after exposure to NM. During the experiment and follow-up, intramuscular dipyrone injections (10 mg/kg) were given to animals showing pain or distress. This was necessary mainly during the first 5 to 10 days after exposure.

**Treatment Groups and Experimental Protocol**

After exposure to NM, each animal was treated with either dexamethasone phosphate 0.1% eye drops (TEVA Pharmaceuticals, Tel-Aviv, Israel; group 2); Zn/DFO eye drops (at a concentration of 3.5 mM, prepared as previously described,10 group 4), or a combination of both the complex and the steroid (group 3; Table 1). This concentration of Zn/DFO had been found to be effective in treating NM ocular injuries in our previous study. Dexamethasone phosphate 0.1% had been used to treat corneal alkali burns in previous studies.11-14 Control animals were sham treated with saline, with or without exposure to NM (groups 1 and 5, respectively). In group 5, normal saline was administered in the trephine (no exposure to mustard), and both eyes of each animal served as experimental eyes.

Immediately after exposure to NM, 2 drops of an eye drop solution, as indicated, were administered every hour for 12 hours, in both experimental and control fellow eyes. From the second day onward, until the end of the experiment at 4 weeks after exposure, eye drops were given seven times daily (every 2 hours). Application of the drops was performed in a blind fashion, with caregivers masked to the contents Gentamicin 0.3% (TEVA, Tel-Aviv, Israel); Saline (normal saline); Dexamethasone Sodium Phosphate 0.1% (TEVA Pharmaceuticals, Tel-Aviv, Israel); Zn/DFO = Zinc desferrioxamine; at a concentration of 3.5 mM; ZnCl2 = Zinc Chloride (Aldrich Chemical Company, Inc., Milwaukee, WI); DFO = Desferrioxamine (Desferal; CIBA-GEIGY Limited, Basle, Switzerland). Zn/DFO was prepared by dissolving the chloride salt of the metal in doubly distilled water, maintaining pH at 3, and adding DFO at equivalent concentration, followed by titration to pH 7.4 with sodium bicarbonate. The stock was diluted in saline to a final concentration of 3.5 mM, based on DFO concentration.

Follow-up Parameters

 Experienced examiners, who were masked to the various treatment groups, assessed the magnitude of ocular injury and the response to treatment. Repeated slit-lamp examinations were performed according to a timetable and guidelines. These included scoring of anterior segment injury, measurements of IOP, taking color photographs of the anterior segment, and iris were obtained with a digital camera at 1 to 29 days after injury. Additional observations included extent of eyelid and conjunctiva swelling and injection; anterior chamber reaction; iris atrophy and, when present, hyphema, corneal ectasia, perforation, and the resultant endophthalmitis.

**Slit-lamp examinations.** Slit-lamp examinations were performed at 24 hours after exposure, and subsequently at days 4, 7, 10, 14, 17, 21, 25, and 29. In each examination, the following parameters were recorded:

1. Area of corneal epithelial loss (corneal erosion): The average horizontal and vertical linear dimensions of the epithelial defect as stained by locally applied fluorescein were measured with the adjustable slit lamp beam and the area computed in square millimeters.
2. Degree of corneal opacity: grade 0, clear cornea with details of iris observed clearly; grade 1, mild blurring of iris details; grade 2, moderate opacity with blurred iris crypts; and grade 3, severe corneal opacity, no iris details visible.
3. Degree of iris pigmentation: grade 0, no pigmentation; grade 1, mild; grade 2, moderate; grade 3, severe iris pigmentation.
4. Degree of corneal neovascularization (CNV): grade 0, no pigmentation; grade 1, mild; grade 2, moderate; grade 3, severe iris pigmentation.

Additional observations included extent of eyelid and conjunctiva swelling and injection; anterior chamber reaction; iris atrophy and, when present, hyphema, corneal ectasia, perforation, and the resultant endophthalmitis.

**Intraocular Pressure.** Repeated IOP measurements were performed in all eyes in each group. Baseline IOP was measured before NM (or saline) exposure and remeasured on days 0, 1, 4, 7, 10, and 29, after injury. A hand-held automated tonometer (Tonopen; Mentor, Norwell, MA) was used.

**Color Photographs.** Color photographs of the eye, anterior segment, and iris were obtained with a digital camera at 1 to 29 days after injury (Nikon, Tokyo, Japan).

**Systemic Antioxidant Status.** Ascorbic acid (AA), a naturally occurring antioxidant and free radical scavenger, is normally present in blood. At 1, 7, 14, and 28 days after injury, blood was collected, and systemic oxidative stress was assessed by measurement of OSAA, as described elsewhere.15,16

**Methionine Sulfoxide Reductase Activity.** Methionine is a sulfur-containing amino acid. Its residues are known for their high susceptibility to oxidation. Msr is the enzyme(s), that reduces either the free or the protein-bound methionine sulfoxide back to methionine (Fig. 1). Thus, methionine residues are considered to be the first line of defense of the cells—an antioxidant defense system for proteins. Msr activity (MsrA) can therefore serve as a bona fide indicator for the antioxidant response to stress.

Quantification of Msr activity was performed by incubation the tissue homogenates with dabsyl-methionine sulfoxide (dabsyl-met(O)) for 30 minutes at 57°C, followed by analysis of the reduced product.

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**Table 1. Experimental and Control Study Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment†</th>
<th>Eyes (n)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM (%)*</td>
<td>Dexamethasone</td>
<td>2</td>
<td>Saline</td>
<td>16</td>
<td>2</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone + Zn/DFO</td>
<td>2</td>
<td></td>
<td>12</td>
<td>2</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Zn/DFO</td>
<td></td>
<td></td>
<td>12</td>
<td></td>
<td></td>
<td>Saline</td>
</tr>
</tbody>
</table>

* NM- nitrogen mustard concentration 2%.
† Treatment—the type of treatment drops used in each group; All animals received every day drops contents Gentamicyn 0.3% (TEVA, Tel-Aviv, Israel); Saline = normal saline; Dexamethasone = Dexamethasone Sodium Phosphate 0.1% (TEVA Pharmaceuticals, Tel-Aviv, Israel); Zn/DFO = Zinc desferrioxamine, at a concentration of 3.5 mM; ZnCl2 = Zinc Chloride (Aldrich Chemical Company, Inc., Milwaukee, WI); DFO = Desferrioxamine (Desferal; CIBA-GEIGY Limited, Basle, Switzerland). Zn/DFO was prepared by dissolving the chloride salt of the metal in doubly distilled water, maintaining pH at 3, and adding DFO at equivalent concentration, followed by titration to pH 7.4 with sodium bicarbonate. The stock was diluted in saline to a final concentration of 3.5 mM, based on DFO concentration.
(dabsyl methionine) by HPLC-coupled to spectrophotometric detection at 436 nm.17,18 The assay in a total volume of 100 μL contained 200 μM dabsyl-Met(O) (as a substrate) and the reaction mixture containing 20 mM dithiothreitol (DTT), buffer, and ~100 μg protein. The incubation (reaction) was stopped by adding 100 μL acetonitrile, spinning it down, and discarding the protein fraction. The chromatography was run on a 100-mm 3-μm column (Apex ODS; Jones Chromatography, Lakewood, CO), using a gradient (A to B). A is 19 g sodium acetate (pH 6.0) plus 0.5 mL triethylamine, in one liter of solution; B is acetonitrile (pure). The substrate, dabsyl-Met(O) was prepared according to Moskowitz et al.18

Statistical Evaluation

Gradable parameters were averaged in each treatment group at the various time points. The Mann-Whitney test was used for comparison of parametric data (i.e., erosion area, IOP, OSAA), and the Pearson χ² test was used for comparison of nonparametric data (i.e., degree of corneal opacity or iris pigmentation).

RESULTS

As noted in previous reports10,19 exposure to NM causes severe and long-lasting injury to ocular anterior segment structures. Injury to the conjunctiva and cornea in a saline-administered eye 1 day after exposure to 2% NM is shown in Figure 2. Conjunctival and corneal injury, scarring, and neovascularization evolved over the entire duration of the experiment (4 weeks) in such eyes (Fig. 3A; saline-treated eye 29 days after exposure). In comparison, conjunctival and corneal injury was reduced in eyes treated with dexamethasone alone (Fig. 3B, group 2), Zn/DFO alone (Fig. 3C, group 4), as well as in eyes treated with a combination of both medications (Fig. 3D, group 3). Note especially the marked corneal opacity and neovascularization in addition to the atrophic, chronically dilated pupil in the saline-treated eye versus the relatively clear corneas in the other treatment groups. Some injury in these eyes is still apparent, however, manifested by the pigmentation of the iris caused by hemorrhages that occurred after the exposure. The extent of ocular damage to the various ocular structures in the different treatment groups is further detailed and quantified in Figures 4 to 9.

Corneal Epithelial Defect

One day after exposure to NM, the corneal epithelial defects in the Zn/DFO- and combination-treated groups was reduced, when compared with the saline-administered group (Fig. 4). This difference, however, did not reach statistical significance (P = 0.2 and 0.11, respectively). Steroid-treatment alone delayed corneal re-epithelization, compared with all other groups, including the saline-administered control group. The addition of Zn/DFO to the steroid completely eliminated this delay. Compared with the steroid-treated group, animals treated with Zn/DFO or Zn/DFO plus dexamethasone had significantly smaller epithelial defects at day 1 after exposure (P = 0.04 and 0.01, respectively). This difference was more evident in the

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**FIGURE 1.** Reduction of methionine sulfoxide back to methionine by Msr. MsrA is therefore an indicator of the systemic level of serum antioxidants.

**FIGURE 2.** Injury in a saline-treated eye 1 day after exposure to 2% NM. Note marked injection of conjunctiva and perocular tissues, discharge, corneal clouding, and the large corneal epithelial defect outlined by fluorescein staining.

**FIGURE 3.** Ocular injury 29 days after exposure to 2% NM. Eyes were treated with (A) saline, (B) dexamethasone, (C) Zn/DFO, or (D) Zn/DFO + dexamethasone.

**FIGURE 4.** Average area of corneal epithelial defect 1 and 4 days after exposure to NM.
Zn/DFO+steroid group—the only group in which the epithelial defects completely healed in all animals during the first 4 days after exposure.

**Corneal Opacity**

Figure 5 describes the average corneal opacity score in all four groups, long after exposure. It can be seen that early on, treatment with dexamethasone or a combination of (dexamethasone+Zn/DFO) yielded a more rapid improvement in corneal opacity than did treatment with Zn/DFO alone or with saline. This faster recovery may be attributable to the presence of the steroid component. By day 29 of the study, however, the degree of opacity in animals treated by Zn/DFO alone improved to a level similar to that in the two steroid-treated groups, and all three groups showed significantly less corneal opacity when compared with animals treated with saline (P = 0.025 for dexamethasone; 0.03 for dexamethasone+Zn/DFO, and 0.035 for Zn/DFO alone).

**Corneal Neovascularization**

CNV developed to various degrees in all groups after exposure to NM (Fig. 6). Although treatment with Zn/DFO or dexamethasone alone yielded somewhat lower levels of neovascularization compared with saline treated at day 29, the reduction did not reach statistical significance (P = 0.1 and 0.09, respectively). Combined treatment, however, had a statistically significant protective effect at the last day of follow-up (P = 0.02). Moreover, this was the only group in which the neovascularization did not progress between days 17 and 29 after exposure.

**Intraocular Pressure**

An increase in IOP occurred after exposure to NM, peaking at day 4 in all study groups (Fig. 7). All three experimental treatments reduced this increase when compared with animals administered saline. Combination therapy (dexamethasone+Zn/DFO) was the most effective in reducing IOP (P = 0.005 versus the saline-treated group), significantly more than dexamethasone or Zn/DFO alone (P = 0.03 and 0.05, respectively). In addition, the IOP in the combination treatment group did not significantly differ from the control (non-NM) group as soon as day 4. On day 29, there was no statistically significant difference between the IOP in the various study groups.

**Iris Atrophy and Pigmentation**

Exposure to NM caused iris atrophy and pigmentation together with pupil dilation in all study groups (Fig. 3). This effect was less severe in the three medication treatment groups; however, the difference between these groups and the saline-administered group did not attain statistical significance (data not shown).

**Systemic Antioxidant Status**

OSAA = DHAA/(AA + DHAA) × 100 is a ratio reflecting the systemic antioxidant status of the animal (Fig. 8). On day 1 after exposure to NM, a sharp increase in OSAA in the saline-treated animals was observed. This increase, which reflects the severe oxidative stress accompanying the exposure to NM, was significantly lower in all experimental treatment groups than in the saline-treated group (P < 0.001–0.05). Of these groups, the OSAA in the combination treatment group was the lowest. In fact, contrary to the other treatment groups, the OSAA at day 1 in this group was statistically similar to that of the control (no-NM) group. This difference was still evident on day 29. Compared with the no-NM group, the OSAA ratio was still significantly high in all treatment groups (P < 0.001–0.05) except for the combination treatment group (P = 0.2).

Similarly, NM injury caused a sharp increase in serum MsrA levels in the saline treated group (Fig. 9). This increase was less severe in all treatment groups. On day 28 MsrA levels were still significantly higher than the control no-NM levels in the saline- and dexamethasone-treated groups (P = 0.02, 0.03 respectively). MsrA levels in the Zn/DFO and the combination treatment group had normalized.
Characteristic delayed keratopathy may persist for months, as was demonstrated in animal models.8,9,19,21,22 Long-term ocular damage with characteristic delayed keratopathy may persist for months, as was seen in the Iranian casualties in the Iran-Iraq conflict.21 Another characteristic feature of the exposure to mustard gas is the delayed appearance of the symptoms that unfortunately may lead to a prolonged contact of an unaware victim with this toxic chemical. Usually, after minutes to hours of exposure, symptoms begin with severe eye pain, photophobia, excessive tearing, and blurred vision. Blepharospasm and eyelid swelling may make the assessment of the eye injury difficult and necessitate installation of the topical anesthesia for examination. Physical examination signs are typical of ocular surface destruction and consequences of the anterior segment inflammation. The acute phase of the ocular surface damage may include conjunctival swelling, corneal epithelial defects, vesicles, sloughing of the epithelium, and corneal stromal edema. Later, corneal vascularization and opacity may develop. The early signs of the anterior segment inflammation may include anterior chamber reaction, elevation of the IOP, pupillary constriction, iritis with iris vessel dilatation, and iris necrosis. Scarring and adhesions on a later stage may lead to permanent structural damage and neovascularization.21,23

To date, no specific effective treatment is available to reduce such damage. Current regimens of treatment after ocular exposure to mustard chemicals are nonspecific and do not address the basic pathophysiology underlying the injury. They include use of protective gear, removal of victims from contaminated areas, copious ocular irrigation, systemic analgesics in severe cases, mydriatics to relieve ciliary muscle spasm and prevent formation of iris adhesions, antibiotic drops to prevent secondary bacterial infection, medications to control IOP, and lubricants.23

Recently, the use of steroid compounds has been shown to alleviate clinical signs and decrease the amount of inflammatory cells and mediators in the anterior chamber and cornea in a rabbit model of mustard injury.24 The extent of CNV, a late sequela of mustard injury, was also attenuated.25 The possible explanation for this beneficial effect is based on recent evidence that mustard agents can induce the release of inflammatory mediators, such as TNF-α and IL-1, by direct action on epithelial cells.24 However, steroids were also reported to delay corneal epithelial regeneration in these animal models,24 a known side effect of ocular topical steroid treatment.25

In a recent study, we have demonstrated that topical application of low concentrations of Zn/DFO or Ga/DFO, after corneal exposure to NM, markedly reduces conjunctival, corneal, iris, and anterior chamber injury. In the cornea, faster healing of epithelial erosions, reduced long-term opacification, and lower levels of neovascularization were observed. In the anterior chamber, decreased inflammation and better maintenance of IOP was achieved. Iris pigmentation and atrophy were not as severe, with less posterior adhesions of the iris to the lens. Cataractous changes were also notably milder.10

In the present study, the results suggest that topical combination therapy using both Zn/DFO and dexamethasone is more effective than either component alone in the treatment of NM ocular injury. We hypothesize that this effect is due to the action of each of these two medications on two different arms of the injury response to NM: While Zn/DFO acts to curb formation of ROS, dexamethasone reduces the inflammatory response within the eye that can cause further damage. The ability of steroids to reduce inflammation is well established, including in mustard injury models, as outlined earlier. Support for the action of Zn/DFO on ROS formation is described next. It is assumed that NM injury, at least in part, is mediated by the formation of ROS, in addition to its action as an alkylating agent. Oxidative stress after ocular exposure to mustard has also been observed in other clinical studies26 and in animal models.27,28 The marked depletion of systemic ascorbic acid and the high levels of MrA during the first day in the saline-administered group further support this. The protective effect of Zn/DFO may well be the result of suppressed formation of ROS—an effect supported by both theoretical considerations and previously reported experimental findings.20–26 In the Fenton reaction or in the metal-mediated Haber-Weiss mechanism, the conversion of low reactive species to the highly reactive hydroxyl radicals apparently depends on the availability of trace amounts of redox-active and labile iron or copper ions, which serve as essential catalysts. It is suggested that Zn/DFO exerts its protective effect by intervening in this critical step of hydroxyl radical formation. The two components of this complex, when present at or near the site of injury,
ameliorate the availability and catalytic activity of the redox-active metal ions via “push-pull” mechanisms.\textsuperscript{31} This can be envisioned in the following way: The DFO component of the complex chelates and “pulls” out redox active iron that is responsible for the production of the hydroxyl radicals. At the same time, the relatively inert zinc ion, which is liberated during the exchange of iron within the complex, further acts as a secondary antioxidant by “pushing” out additional iron ions from their catalytic binding sites.\textsuperscript{30,31} The spatial structure of this complex is markedly different from that of desferrioxamine alone, allowing its enhanced infiltrative ability into cells and tissues.\textsuperscript{31}

In addition to its effects in the animal model of mustard injury, a similar protective effect of Zn/DFO was shown in several experimental models in which the formation of free radicals is also presumed to induce tissue damage, including corneal alkali burns\textsuperscript{29} and myocardial\textsuperscript{30} and retinal ischemia-reperfusion insults.\textsuperscript{12,32}

As steroids and Zn/DFO are presumed to inhibit two different mechanisms contributing to tissue injury, an additive protective effect stands to reason. Indeed, in our study, animals treated with a combination of Zn/DFO and steroids had consistently better outcomes in almost all aspects of injury examined. The combination of Zn/DFO + steroid was the only treatment to reduce significantly and stabilize the formation of CNV, a major risk factor for future visual impairment.\textsuperscript{21} Another aspect in which the combination of Zn/DFO + steroid had a clinically significant effect was the induction of rapid corneal re-epithelization compared with treatment with steroids alone. The role of topical steroid treatment soon after chemical corneal injury is controversial. In similarity to our study, others have also reported that steroids cause a delay in re-epithelization.\textsuperscript{12,37} This delay may increase the risk of infection, corneal ulcer formation, and endophthalmitis, and therefore early topical treatment with steroids is currently not advocated in mustard injury.\textsuperscript{26} However, early topical steroid treatment may have a beneficial role in preventing corneal basement membrane damage, which is important for healthy re-epithelization that decreases the risk of future recurrent corneal erosions.\textsuperscript{12} Furthermore, in corneal alkali burn models it was shown that steroid treatment during the first week may be beneficial without increased risk of corneal melting.\textsuperscript{11} We suggest that early treatment with a combination of Zn/DFO and steroids can have the benefits of steroid treatment without the risks of delayed re-epithelization. This may have relevance also for other forms of chemical corneal injury, such as exposure to acid or alkali compounds, in which the timing of steroid treatment is controversial for the same reasons.

In conclusion, we have shown that topical Zn/DFO and steroids manifest a complementary protective effect in treating NM injury. Although ocular tissue damage was not completely prevented, treatment with this combination achieved better results than treatment with either drug alone and was markedly better than treatment with saline. No ocular toxicity was observed. In the current situation, when no specific antidote to mustard injury exists and with the ever-present threat of mustard use in chemical warfare, topical Zn/DFO combined with steroids may be beneficial in treating injuries in the short and intermediate time range. However, the toxic effects of mustard exposure are often prolonged, and the challenge of demonstrating beneficial effects of this combination on long-term outcome remains to be addressed.

References


