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Topical Use of Zinc Desferrioxamine for Corneal Alkali Injury in a Rabbit Model

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Purpose. To evaluate the efficacy of topical zinc desferrioxamine in acute corneal alkali injury in rabbits. **Methods.** Twenty rabbits were anesthetized and a standardized alkali burn (1N NaOH) was performed in the center of the cornea (7.5-mm diameter). The animals were randomly divided into two groups and treated (double-masked) with topical zinc desferrioxamine, 220 μ*M*, (group 1) or its vehicle (group 2). Drops were applied 7 times/day for 28 days. Topical gentamicin, 0.3%, was instilled twice a day. Animals were evaluated twice a week. At each examination (using the slit-lamp), the depth of corneal ulcer was graded as follows: 0, no ulcer; 1, tissue loss less than one third of corneal thickness; 2, one third to two thirds tissue loss; 3, more than two thirds tissue loss; 4, descemetocele; or 5, perforation. Ulceration area, vascularization, and epithelial defects also were measured. **Results.** During the study period, the grading of mean corneal ulcerations in group 1 ranged from 0.2 to 1.00, whereas in group 2, it ranged from 1.4 to 2.7. The mean grade and area of ulceration in group 2 were greater than those in group 1 ($p < 0.05$). **Conclusion.** Topical zinc desferrioxamine may be an adjunctive treatment in protecting the cornea against induced alkali injury.

Key Words: Alkali injury—Corneal ulcer—Free radical inhibitors—Zinc desferrioxamine—Rabbit.

Corneal alkali injury is still a challenging disease, having a long-standing course and frequently resulting in severe complications, such as corneal ulceration, perforation, and eye loss (1).

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Different treatment modalities have been tried over the years in an attempt to reduce the incidence of these complications. Therapeutic contact lenses (2), fibronectin (3), and human epidermal growth factor (4,5) were reported to accelerate reepithelialization or stimulate epithelial regeneration. Citrate (6–8) was used to inhibit polymorphonuclear leukocytes activation, and ascorbate (7,8) to increase vitamin C levels in the aqueous humor. In one study, indomethacin reduced the incidence of neovascularization (9). Collagenase inhibitors such as ethylenediaminetetraacetic acid (EDTA) (10), penicillamine (11), tetracycline (12), cysteine, and acetyl cysteine (13) also were used. Recently synthetic inhibitors of matrix metalloproteinases were designed specifically to bind with collagenase and block its collagen-cleaving action (14–17). All these medications showed efficacy on an experimental basis.

Free radicals were shown to play a role in corneal inflammation and alkali burn (8,18–20). They are omnipresent, and protection against them is obviously built into the cornea; those present in the tear film are scavenged before reaching the stroma (21).

After alkali injury, free oxygen radicals gain access to the stroma and exert their destructive effect by their action on membrane lipids, fragmentation of DNA chains, and polymerization and depolymerization of proteins and hyaluronate (19).

Oxygen free radicals include superoxide anion (O_2^-) and hydroxyl radical (·OH). Superoxide anions dismutate to form hydrogen peroxide (H_2O_2), a reaction accelerated by the enzyme superoxide dismutase (SOD):

$$2\,O_2^- + 2\,H^+ \rightarrow H_2O_2 + O_2 \qquad (1)$$

Superoxide also reduces the copper (Cu) and iron (Fe), the redox-active metals, which are necessary for the pro-

duction of the highly reactive and damaging hydroxyl radical:

$$O_2^- + Cu^{+2} \leftarrow Cu^{+1} + O_2 \quad (2)$$
$$O_2^- + Fe^{+3} \leftarrow Fe^{+2} + O_2 \quad (3)$$

Free hydroxyl radical (·OH) is then formed by the Fenton reaction:

$$H_2O_2 + Cu^{+1} \rightarrow Cu^{+2} + \cdot OH + OH^- \quad (4)$$
$$H_2O_2 + Fe^{+2} \rightarrow Fe^{+3} + \cdot OH + OH^- \quad (5)$$

Zinc desferrioxamine (Zn/DFO), a metal-chelator complex, protects the tissue against free radical–induced biologic damage by exchanging with iron or copper to yield ferrioxamine or copper desferrioxamine, respectively. By this "pull mechanism," zinc is liberated and further protected by "pushing out" additional redox-active metal ions from their binding sites (22–26).

In this study, we evaluated the effect of topical administration of Zn/DFO in the corneal alkali-injury model in rabbits' eyes.

METHODS

Alkali Injuries

Rabbits were used in compliance with the ARVO Resolution on the Use of Animals in Research. The animals were housed individually and received food and tap water ad libitum on a 12-h light–dark schedule. Twenty pigmented rabbits weighing 1.7–2.4 kg were anesthetized with intramuscular injection of ketamine, 25 mg/kg, and sodium pentobarbital, 45 mg/kg. A lid speculum was inserted in the right eye, and the cornea was anesthetized with benoxinate 0.2% drops. To create an alkali injury, a 7.5-mm Barron Hessberg radial vacuum trephine (Katena, Denville, NJ, U.S.A.) was attached to the center of the cornea, and the trephine was advanced to contact the stroma. The well was filled with 0.4 ml of 1N sodium hydroxide.

After 60 s of exposure, the sodium hydroxide was removed by insertion of a cellulose microsponge into the well. Then the trephine was removed, and the cornea was irrigated with 10 ml of normal saline. A round 7.5-mm lesion was immediately formed.

Study Design

Ten rabbits were randomly assigned to treatment with 220 m*M* Zn/DFO (MCJ-1), Zn:DFO at ratio 1.0:1.0 in saline, 0.9% (vehicle) (group 1), and 10 rabbits to treatment with saline 0.9% (group 2). Each animal received a drop of the drug or its vehicle from 7 a.m. to 7 p.m. every 2 h during the 28 days of the study, in addition to one drop of gentamicin 0.3% solution twice daily.

Biomicroscopic examination of the cornea, using a slit lamp, was performed twice a week during the study period. The degree of the corneal ulceration was evaluated by ophthalmologists (J.F.,C.S.), who were masked to the treatment regimen. A clinical score was assigned based on the depth of the ulceration, as was described by Pfister et al. (27): grade 0, no ulceration; grade 1, ulceration limited to the anterior third of the cornea; grade 2, ulceration extending to the middle third of the cornea; grade 3, ulceration extending to the posterior third of the cornea; grade 4, descemetocele; and grade 5, corneal perforation.

At each examination, the area of ulceration was calculated by multiplying the largest length of the ulcer and the perpendicular length at the ulcer center; the epithelial defect and the area of vascularization were measured in the same manner.

After the last examination (day 28), the animals were killed.

Statistical Analysis

For each animal, a curve showing the relation between the variables examined and time (days of examination) was plotted, and the area under the curve was calculated (SPSS program for Windows). For each variable, we compared the calculated areas of the treatment and the control groups using the one-tailed Mann–Whitney *U* test.

For the variables showing statistical significance, further calculations using the one-tailed Mann–Whitney *U* test were done to detect the specific examination days where there was a statistically significant difference between the two groups.

The mean values in the text are presented with the standard error of the mean.

RESULTS

One animal in group 1 died on day 10 and was excluded from the statistical calculations. No ocular infections occurred during the study period.

Corneal Ulceration Grades

In group 1, the ulcerative process in the cornea, which began on day 7 (mean score, 0.2 ± 0.1), reached maximal score at day 17 (1.0 ± 0.3) and started to decrease from day 21 (0.8 ± 0.5) to day 28 (0.4 ± 0.2) (Fig. 1).

In group 2, the ulcerative process in the cornea, which began on day 7 (mean score, 1.4 ± 0.5), reached maximal score at day 21 (2.7 ± 0.6) and started to decrease from day 24 (1.9 ± 0.6) to day 28 (1.7 ± 0.7) (Fig. 1).

Grades of ulceration in group 1 were significantly lower as compared with group 2 ($p < 0.02$) during the study. On individual examination days, mean ulceration-

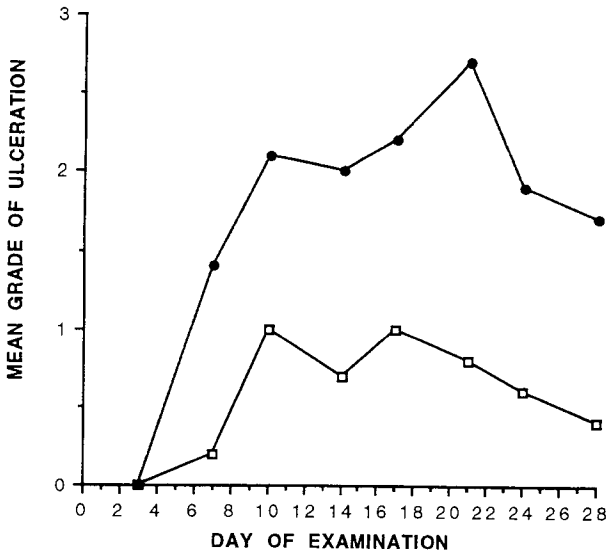

FIG. 1. Plot of mean ulceration grade against time, in rabbits treated with Zn/DFO (group 1, squares) and vehicle (group 2, circles) after corneal alkali burn. Statistical significance was found from days 7–24 using one-tailed Mann–Whitney *U* test.

grade values were significantly lower in group 1 as compared with group 2 from days 7–24 ($p < 0.05$) (Table 1), with borderline significance on day 10.

In group 1, one case had descemetocele (grade 4), and no perforations (grade 5) were recorded, whereas in group 2, two eyes had perforations, and three eyes had descemetoceles (Table 2).

Area of Ulceration

In group 1, the mean area of ulceration during the study period ranged from zero (day 3) to 1.5 ± 0.9 mm^2 (day 17), whereas in group 2, it ranged from zero (day 3) to 5.4 ± 2.4 mm^2 (day 14; Fig. 2).

The area of ulceration in group 1 was significantly smaller than that in group 2 during the study period ($p < 0.05$). On individual examination days, the mean area of ulceration in group 1 was smaller than that in group 2 from days 10–21 ($p < 0.05$; Table 3).

TABLE 2. *Maximal grade of ulceration reached in animals of both groups during the study period*

Grade of maximal ulceration	No. of animals in group 1	No. of animals in group 2
0	1	1
1	5	3
2	1	0
3	1	1
4	1	3
5	0	2
Total no. of animals	9	10

Epithelial Defects and Vascularization

In group 1, the mean area of epithelial defects during the study ranged from 5.0 ± 1.8 mm^2 (day 3) to 11.5 ± 3.2 mm^2 (day 17), and in group 2, it ranged from 3.0 ± 1.3 mm^2 (day 28) to 9.7 ± 4.2 mm^2 (day 21). There was no significant difference in the areas of epithelial defects between the two groups during the study period ($p = 0.23$).

In group 1, the mean area of vascularization during the study ranged from zero (day 3) to 27 ± 7.2 mm^2 (day 28), and in group 2, it ranged from zero (day 3) to 27.2 ± 4.3 mm^2 (day 28). There was no significant difference in the areas of vascularization between the two groups during the study period ($p = 0.34$).

DISCUSSION

Severe alkali injuries of the eye affect the acellular and the cellular parts of the cornea, including epithelium, keratocytes, and endothelium. The acellular tissues show hydrolysis of the glucosaminoglycans and collagenolysis. This devitalization of the cornea stimulates an early and intense inflammatory response and a prolonged stromal repair process (8).

TABLE 1. *Mean grades of ulceration in groups 1 and 2 during the study period*

	Group 1			Group 2			
Day of examination	No. of animals	Mean ulceration grade	SEM	No. of animals	Mean ulceration grade	SEM	*p* Value
03	09	0.0	0.0	10	0.0	0.0	1.000
07	09	0.2	0.1	10	1.4	0.5	0.022
10	09	1.0	0.4	10	2.1	0.5	0.054
14	09	0.7	0.4	10	2.0	0.5	0.027
17	09	1.0	0.3	10	2.2	0.5	0.043
21	09	0.8	0.5	10	2.7	0.6	0.014
24	09	0.6	0.2	10	1.9	0.6	0.048
28	09	0.4	0.2	10	1.7	0.7	0.127

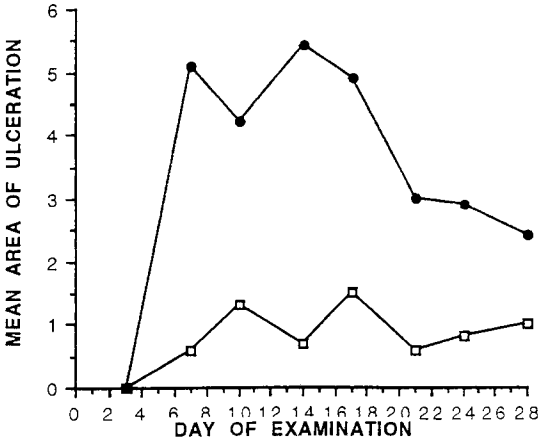

FIG. 2. Plot of mean of ulceration area (mm^2) against time, in rabbits treated with Zn/DFO (group 1, squares) and vehicle (group 2, circles) after corneal alkali burn. Statistical significance was found from days 10–21 using one-tailed Mann–Whitney *U* test.

The products of inflammatory cells are important for the formation of corneal ulceration. Neutrophils dominate the inflammatory response and, when stimulated, undergo a respiratory burst attended by the release of granular enzymes and free oxygen radicals (8).

The cornea, like other respiring tissues, possesses SOD (28). Activity of this enzyme in the cornea is higher than in the lens and similar to that in the retina. Its function is to scavenge superoxide radicals, thereby protecting tissue against damage by free radicals produced from oxygen in its initial stage of reduction (29).

In corneal alkali injury, administration of SOD was shown to have a beneficial effect, suggesting that O_2^- or its derivatives are involved in initiating or promoting ulceration after alkali burns, possibly by producing degradation of collagen and mucopolysaccharides after their direct access to the stroma (19).

Zn/DFO, a metal–chelator complex, was proposed for protection from free radical–induced biologic damage (23). It was suggested that it displaces redox-active metals such as iron and copper from their binding sites, which serve as centers for repeated production of hydroxyl radicals (22,23).

Zn/DFO was shown to protect cats' ischemic retina from severe damage by inhibiting free radical generation during the early reperfusion phase (24). Furthermore, Zn/DFO protected the rats' heart from free radical–induced damage during reperfusion, after regional and global cardiac ischemia, by displacing redox-active metals (25,26). Zn/DFO also inhibits the growth of *Plasmodium falciparum* by scavenging transient pools of intracellular iron (30).

In our study, we used eye drops containing 220 m*M* Zn/DFO, at a Zn/DFO ratio of 1.0:1.0 (MCJ-1) for prevention of free radical–induced corneal damage after alkali burn. Zn/DFO significantly decreased the area and depth of corneal ulceration and prevented perforation in the Zn/DFO-treated corneas. Whereas only one eye had descemetocele in group 1, two perforations and three descemetoceles were discovered in group 2. Zn/DFO most probably decreased the formation of free radicals, resulting in less damage to the cornea.

The process of free radical scavenging by Zn/DFO liberates zinc and may be of significance for the healing after alkali injury.

Zinc is essential for protein (including collagenase) synthesis, and it takes part in the stabilization process of cellular membranes, as well as in reduced macrophage and polymorphonuclear cell motility and phagocytic activity (31). Furthermore, the effect of zinc in wound healing in humans was documented (32,33), but not in the cornea (34,35). On the other hand, zinc is an essential element in the structure of collagenases (14).

Alkali injury–induced damage is multifactorial, and free radicals are only one of many substances released in the tissue during the inflammatory process. It is unlikely that controlling one factor will eliminate the inflammatory cascades and the ulcerative process in the cornea, which may also explain the similar corneal vascularization in the Zn/DFO-treated and untreated eyes, as well as the rate of epithelialization.

TABLE 3. *Mean area of ulceration in groups 1 and 2 during the study period*

	Group 1			Group 2			
Day of examination	No. of animals	Mean area of ulceration	SEM	No. of animals	Mean area of ulceration	SEM	*p* Value
03	09	0.0	0.0	10	0.0	0.0	1.00
07	09	0.6	0.4	10	5.1	3.5	0.066
10	09	1.3	0.7	10	4.2	1.2	0.036
14	09	0.7	0.5	10	5.4	2.4	0.015
17	09	1.5	0.9	10	4.9	1.4	0.014
21	09	0.6	0.4	10	3.0	1.1	0.015
24	09	0.8	0.5	10	2.9	1.1	0.078
28	09	1.0	0.5	10	2.4	1.2	0.219

Values expressed as square millimeters.

We suggest that Zn/DFO may have a role as an adjunctive treatment in alkali injury of the cornea.

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