Influence of topical and oral zinc upon corneal wound healing

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Experimental evidence indicates that the healing rate of avascular penetrating corneal wounds is delayed when compared with vascularized penetrating corneal wounds or with wounds of other vascularized tissues (Gasset and Dohlman, 1968; Howes, Sooy, and Harvey, 1929). This is exemplified clinically by a substantial number of postoperative wound healing complications which may occur after penetrating keratoplasty. The complications resulting from the prolonged period necessary for healing of avascular corneal wounds after penetrating keratoplasty are dehiscence of the wound margins, overriding of host-graft wound edges, anterior iris synechiae, iris prolapse, oedema of the donor corneal button, and even total extrusion of the graft. Each of these complications or any combination of them may be sufficient to compromise the viability of the donor endothelium and lead to graft rejection.

Although it is well known that topically administered corticosteroids have a deleterious effect on the healing of avascular corneal wounds, the majority of postoperative keratoplasty patients receive topical corticosteroids to prevent or ameliorate the postoperative ocular inflammatory response of the anterior segment. However, because of this hazard, topical corticosteroids are given either in sub-optimal doses necessary for achieving suppression of the inflammatory response, or the drug is withheld during the immediate post-transplant period. Therefore, an agent which would accelerate the healing of avascular corneal wounds or nullify the adverse effects of topical corticosteroids on wound healing would be clinically valuable.

In the past many agents have been studied to determine if they are capable of enhancing corneal wound healing, with limited success (Rigg and Rigg, 1959; Thomas, 1955). Recently, systemically administered zinc has been shown to promote wound healing in both animals and man (Pories and Strain, 1966; Pories, Henzel, Rob, and Strain, 1967a, b; Strain, Pories, and Hinshaw, 1960; Savlov, Strain, and Heugin, 1962). The effect of topical antiseptics, including zinc sulphate, upon regeneration of corneal epithelium has been reported (Bellows, 1946); however, the effect of zinc on penetrating corneal wounds has not been studied.

The present study was therefore conducted to evaluate the effect of topically and systemically administered zinc preparations upon the tensile strength of avascular corneal wounds. All experiments were done in a blind fashion unless otherwise noted. Under the experimental conditions employed, statistical analysis of the data shows that the healing of avascular corneal wounds is neither promoted nor retarded by zinc.

Material and methods

ANIMALS

New Zealand albino rabbits weighing 1.8 to 2.3 kg. (4 to 5 lb.) were used in all experiments. There were five groups, with ten rabbits in each group.

CORNEAL WOUNDS

Anaesthesia was achieved with intravenously administered pentobarbital sodium (Diabital) in a dose of 30 mg./kg. body weight. This was supplemented by topically applied proparacaine hydrochloride (Ophthaine). The technique for the linear penetrating central corneal wound was that previously reported (Elliott and Leibowitz, 1966) with certain modifications. After the cornea was marked with two parallel lines, a 9 mm. non-penetrating incision was made in order to allow placement of two black silk (7-0) Stallard type sutures (Fink and Barras, 1956) approximately 5 mm. apart and looped out of the way. The wound was then completed, resulting in a 9 mm. penetrating incision, and the sutures were pulled down and tied. Since Aquavella, Gasset, and Dohlman (1968) observed an effect upon the contralateral control eye from systemic medications, the operative procedure was unilateral in all cases.

Following the surgical procedure, polymyxin B 5,000 units/ml., neomycin sulphate 2.5 mg./ml., gramicidin 0.025 mg./ml. (Neosporin Ophthalmic solution) were dropped liberally on the wound. Thrice daily thereafter 10 per cent. phenylephrine and 1 per cent. cyclopentolate were instilled in all wounded eyes. All animals were observed daily. Eyes in both the experimental and the control group were eliminated from the study if there was evidence of infection, anterior synechia, gross vascularization, or delayed flattening of the anterior chamber.

MEASUREMENT OF TENSILE STRENGTH

The method described by Gasset and Dohlman (1968) was used to determine the corneal wound tensile strength. All tensile strength measurements in control and experimental groups were done on the 11th postoperative day.

DRUG ADMINISTRATION

The drug regimen in each case was started on the day after corneal wounding and continued until the day of the measurement of tensile strength. Five groups of ten animals each were used:

- (1) These received two drops of Zincfrin (zinc sulphate 0.25 per cent., phenylephrine HCl 0.1 per cent., benzalkonium chloride 1/10,000, barbitol 0.26 per cent., barbitol sodium 0.13 per cent.) three times a day. It was estimated that each animal was given 0.4 mg. elemental zinc per day in the operated eye. They were also given approximately 0.03 g. Neosporin Ophthalmic Ointment (polymyxin B 5,000 units/g., zinc bacitracin 400 units/g., neomycin sulphate 5 mg./g.) once daily.
- (2) These were treated as a blind control with Group 1. The only difference was that Group 2 received topical drops containing all the components of Zincfrin except the zinc sulphate (Zincfrin diluent).
- (3) These were given a daily oral dose of 725 mg. zinc sulphate in 1 ml. distilled water, which contained approximately 165 mg. elemental zinc (80 mg./kg.). It has been shown that 300 mg./kg. zinc is toxic to a rabbit, and that 80 mg./kg. causes a significant rise in the plasma zinc level (Benfenstam, 1952). These animals also received the topical drops without zinc sulphate as well as the Neosporin ointment in the same dosage.
- (4) These were treated as a blind control for Group 3, and received the same treatment, except that the oral distilled water contained no added zinc sulphate.

(5) In order to consider the possible effect that the trace amount of zinc (0.015 mg.) in the daily dose of Neosporin ointment might have on healing, this additional control group received Zincfrin diluent and a similar antibiotic ointment, Mycitracin (polymyxin B 5,000 units/g., bacitracin 500 units/g., neomycin sulphate 5 mg./kg.), which contains no zinc.

Results

The tensile strengths of the wounds for the fifty animals in the five groups mentioned above are shown in Table I.

Table I Tensile strength of corneal wounds in g./5 mm. wound on the 11th postoperative day*

Group	1	2	3	4	5
Therapy	Zincfrin (0.4 mg. Zn/day) Neosporin ointment	Zincfrin diluent Neosporin ointment	Oral Zn SO ₄ (165 mg. Zn/day) Zincfrin diluent Neosporin ointment	Oral distilled water Zincfrin diluent Neosporin ointment	Zincfrin diluent Mycitracin ointment (no Zn)
Application	Topical		Oral		Final control
	Zinc	Control	Zinc	Control	
Tensile strength (g./5 mm.)	100	134	76	116	116
	293	313	214	119	155
	291	167	61	132	351
	49	221	72	326	259
	179	63	244	263	188
	138	130	101	—	107
	195	205	225	—	99
	65	102	225	—	164
	216	144	126	—	243
	332	197	51	—	186
	109	233	—	—	—
	247	—	—	—	—
	159	—	—	—	—
	247	—	—	—	—
Mean	187 ± 89	174 ± 70	140 ± 78	191 ± 97	187 ± 79

*All groups were done in blind fashion except Group 5

A formal analysis of these data to test whether there were statistically significant differences among the average tensile strengths for the five groups was carried out using analysis of variance (Table II). This confirms that the mean tensile strengths for the five groups were not significantly different in the statistical sense.

Table II Analysis of variance

Variance	Degrees of freedom	Sums of squares	Mean squares	F
Among treatments	4	17393	4348	< 1 Not significant
Within treatments Among corneae	45	299495	6655	
Total	49	316888		

Comments

The results presented demonstrate that in these dosages, zinc, both topically and systemically, has no significant effect on the normal healing processes of penetrating avascular corneal wounds.

Zinc has been found to be an essential part of several enzymes, including carbonic anhydrase, alkaline phosphatase, and various dehydrogenases. It also functions as cofactor for many other enzymes (Underwood, 1962). In the case of wound healing it has been proposed that zinc acts primarily at the wound site, where it may be incorporated into these enzyme systems (Pories and others, 1967a, b).

There are several factors which must be considered in an analysis of the results. Although high blood levels have been reported after similar oral zinc administration (Berfenstam, 1962), perhaps because of the avascular nature of the cornea, there may not have been an increase in the zinc level at the site of the wound. Furthermore, when evaluating topical application it must be remembered that a corneal wound is covered by epithelial cells within 24 hours (Hilding, 1959). Since the epithelium is known to have a high lipid content, which probably accounts for its preferential permeability to fat-soluble substances, adequate penetration of the epithelium by the ionic topical solution may have been prevented. Use of an additional group of animals treated with subconjunctival zinc salts to circumvent the epithelial barrier was planned; however, this experiment was not performed when we learned that zinc given subconjunctivally causes massive sloughing and necrosis of conjunctival tissues (Gasset, A.R.: oral communication at the Association for Research in Ophthalmology meeting, Tampa, Florida, April 29, 1968).

An additional consideration is the level of zinc which is normally present in the cornea. Although the cornea is not rich in zinc in comparison with other ocular tissues (Bowness, Morton, Shakir, and Stubbs, 1952; Galin, Nano and Hall, 1962), it may nevertheless already contain an optimal amount for wound repair. Thus, supplemental zinc would not be expected to cause a significant change in the wound healing rate.

Summary

Supplemental zinc, given both topically and systemically, failed to accelerate corneal wound healing, as measured by the tensile strength of the wound on the eleventh postoperative day.

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