Corneal wound healing

I. Inhibition of stromal healing by three dexamethasone derivatives

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Dexamethasone alcohol, dexamethasone-21-acetate, and dexamethasone-21-phosphate inhibit corneal wound healing in rabbits. Wound healing was studied at 3 day intervals by the anterior chamber pressure necessary for wound rupture. No inhibition of healing was noted after only three days of treatment for any steroid; when present, it was observed on day 6 and continued for at least 15 days. Dexamethasone alcohol inhibited wound healing in treated eyes at concentrations as low as 0.001 per cent, dexamethasone-21-phosphate was inhibitory at 0.01 per cent, and dexamethasone-21-acetate was inhibitory at 0.1 per cent following six days of treatment with two drops of the respective concentration five times per day. Dexamethasone alcohol was absorbed systemically to the extent that wound healing was inhibited in the vehicle-treated contralateral eye with treatments of 0.1 and 0.01 per cent concentrations. Similarly, dexamethasone-21-phosphate at the 0.1 per cent treatment level lessened wound strength in the contralateral eye. No contralateral effect for dexamethasone-21-acetate was observed at the highest treatment level of 0.1 per cent. Indomethacin and phenylbutazone did not affect corneal wound healing in the treated or contralateral eyes as measured by this technique.

Key words: corneal wound healing, dexamethasone, phenylbutazone, indomethacin.

Numerous reports describe the effects of corticosteroids on corneal wound healing.1–8 In reviewing these reports, one is hampered because (1) of conflicting evidence of steroid inhibition of corneal wound healing, (2) of the variety of routes of steroid administration, and (3) of incomplete data on the steroid derivative used. There has been little concern with systemic absorption and the effect on the contralateral eye and there are no observations on the intrinsic activity, solubility, transport (active and/or passive), and local metabolism of corticosteroids. Therefore, little is known about what role steroids play in corneal wound healing.

Corneal wounds heal by the following sequence of events: (1) spreading of the epithelium to cover the wound, (2) ac-
celerate mitosis of epithelial cells to plug the wound (day 1), (3) conversion of keratocytes to fibroblasts on day 3, (4) migration of fibroblasts to the wound site, and (5) synthesis of sulfated mucopolysaccharides between fibroblasts which then combine with protein secretions of the fibroblast to form collagen. It has been suggested that inhibition of corneal wound healing following steroid treatment is a result of the failure of keratoblasts to proliferate.

The purpose of this study was to determine the effect of dexamethasone alcohol, dexamethasone-21-phosphate, and dexamethasone-21-acetate on corneal wound healing. The inhibition of corneal wound healing of the treated and contralateral eyes was determined following topical treatment with graded concentrations of the three dexamethasone derivatives.

Methods and materials

New Zealand albino rabbits of both sexes weighing between 1.4 and 1.8 kilograms were used. Animals were maintained on 6 oz. of rabbit chow (Purina) per day and water ad libitum.

**Corneal wound.** The rabbits were anesthetized with sodium pentobarbital (30 mg. per kilogram) by intravenous injection, topical application of 0.1 ml. (two drops) of 0.5 per cent tetracaine hydrochloride, and retrobulbar injection of 0.5 ml. of 1 per cent hexylcaine hydrochloride. A limbal incision was made with a cataract knife and enlarged with scissors to 6 mm. at 3 o’clock in the left eye and at 9 o’clock in the right eye. The wound was closed with two silk sutures (6-0).

**Treatment of wound.** Treatment of the right eye with a test formulation and of the left eye with vehicle, by five topical applications of 0.1 ml. (two drops every two hours) per day, was started immediately. Treatment was continued until wound strength was determined.

**Wound rupture.** On days 3, 6, 9, 12, or 15, animals were anesthetized with sodium pentobarbital (30 mg. per kilogram) and 0.1 ml. of 0.5 per cent tetracaine hydrochloride. The silk sutures were carefully removed and the animal’s head was secured in a stereotaxic holder (No. 1240, David Kopf Instruments, Tujunga, Calif.). A Busher Automatic Injector (Becton, Dickinson and Company, Rutherford, N. J.) was adapted to the electrode carrier of the stereotaxic holder with a ring clamp. A 1 cc. insulin syringe was fitted into the injector and a 3 way stopcock with a 1 inch, 27-gauge, regular point needle attached. Adjustment of the electrode carrier brought the needle to within 6 mm. of a point on the dorsal surface of the eye so that the needle entered 1 mm. from the limbus. Upon triggering the release lever, the needle was propelled forward and entered the eye a distance of 4 mm. The stopcock was adapted to a sensitive air regulator (No. HFS-501, Southwest Air Equipment Co., Fort Worth, Texas) and pressure gauge, 0 to 30 pounds per square inch (p.s.i.) in 1 pound graduations. The regulator was connected to an oxygen tank and kept under a constant pressure of 40 p.s.i. Oxygen flow was directed to the anterior chamber at an initial pressure of 2.5 p.s.i., then raised to 5 p.s.i. after five seconds. Increments of 1 p.s.i., at five-second intervals, were made until wound rupture. Rupture was detected by flooding the eye with water prior to rupture and noting the escaping oxygen in the water. If wound rupture occurred during an increase from 1 p.s.i. to the next, it was recorded as the 0.5 p.s.i. between; e.g., 6.5 p.s.i.

**Test formulations.** Stock formulations of 0.1 per cent dexamethasone alcohol (in suspension), 0.11 per cent dexamethasone-21-acetate (in suspension), and 0.126 per cent dexamethasone-21-phosphate (in solution) were prepared in 0.5 per cent hydroxypropyl methylcellulose. These concentrations provided an equal molar concentration of dexamethasone. Log<sub>10</sub> dilutions of each derivative were made using the hydroxypropyl methylcellulose vehicle. Phenylbutazone (1.0 per cent) and 0.5 per cent indomethacin (active in immunouveitis according to Baldwin and Borgmann, personal communication) in 0.5 per cent hydroxypropyl were also tested.

**Statistics.** Results are expressed as means and standard deviations. Significance of the difference between mean values was determined by an analysis of variance and Student’s t test with a P value of 0.05. All comparisons were made to the vehicle-treated group in which both eyes were treated with the vehicle only for the same number of days.

**Results**

An almost linear relationship of wound tensile strength versus time (days) was observed (Fig. 1) in control eyes. Tensile strength appeared to plateau slightly by days 12 and 15. Normal untreated wounds showed a similar healing relationship with time. Anterior synechiae, when and if
formed, did not alter the healing pattern for these 6 mm. limbal wounds.

Dexamethasone alcohol (0.1 per cent) completely inhibited the increase in corneal wound strength from days 3 to 6 (Fig. 2). After six days, the rate of increased tensile strength paralleled that of the controls. A decrease was noted in the tensile strength of the contralateral wound, although this inhibition was not as great as that observed for the treated eye. Dexamethasone-21-acetate (0.11 per cent) demonstrated significant (P < 0.05) inhibition of corneal wound healing on days 6, 9, and 15 (Fig. 3). Corneal wound healing was not inhibited in the contralateral eye with dexamethasone-21-acetate. Dexamethasone-21-phosphate (0.126 per cent) significantly (P < 0.05) inhibited corneal wound healing on days 6, 9, 12, and 15 (Fig. 4), with the difference between treated and control wounds remain-
Fig. 4. Effect of 0.126 per cent dexamethasone-21-PO₄ on corneal wound tensile strength of treated and contralateral eyes (4 eyes per point) from day 3 to day 15. Vehicle (control) = 10 eyes per point. Pooled standard deviation = 1.2 p.s.i.

Fig. 5. Effect of log₁₀ concentrations of dexamethasone alcohol on corneal wound tensile strength of treated and contralateral eyes (12 eyes per point) on day 6. Vehicle (control) = 12 eyes.

Dose response of log₁₀ concentrations of the dexamethasone derivatives was determined after six days of treatment. Dexamethasone alcohol was significantly (P < 0.05) inhibitory in the treated eye at 0.1, 0.01, and 0.001 per cent relative to vehicle-treated eyes (Fig. 5) and in the vehicle-treated contralateral eye at 0.1 and 0.01 per cent when the results on day 6 were compared statistically. The only concentration of dexamethasone-21-acetate that significantly (P < 0.05) inhibited wound healing at day 6 for treated eyes was 0.11 per cent and no concentration of dexamethasone-21-acetate tested inhibited corneal wound healing in the contralateral eye (Fig. 6). Significant (P < 0.05) inhibition of corneal wound healing in the treated eye was observed at 0.126 and 0.0126 per cent and in the contralateral eye at 0.126 per cent (Fig. 7).

Indomethacin (0.5 per cent) and phenylbutazone (1.0 per cent), both nonsteroid anti-inflammatories, did not decrease corneal tensile strength as measured by this technique.

Discussion

Inhibition of corneal wound healing was exhibited by dexamethasone alcohol, phosphate, and acetate; however, the degree of inhibition for equal molar concentrations was quite different. This demonstrates, with respect to the wound-healing mechanism, that the activities of these three closely related molecules, different at the C-21 position (Fig. 8), are different.

This is the first report to compare different derivatives of a corticosteroid, under comparable conditions, as to their effects...
Effect of log\(_10\) concentrations of dexamethasone-21-acetate on corneal wound tensile strength of treated and contralateral eyes (12 eyes per point) on day 6. Vehicle (control) = 12 eyes.

Fig. 6. Effect of log\(_10\) concentrations of dexamethasone-21-phosphate on corneal wound tensile strength of treated and contralateral eyes (12 eyes per point) on day 6. Vehicle (control) = 12 eyes.

Fig. 7.

on corneal wound healing. The route, topical ocular, was standard with a daily dosage schedule of two drops per dose for five applications per day. Systemic absorption, with inhibition of wound healing in the contralateral eye, was found for two of the three steroid derivatives. This report demonstrates corticoid effect on the contralateral eye and supports those findings that demonstrate systemic absorption of steroids following ocular application.\(^5\), \(^6\) Delayed resorption of hyphema has been observed in treated and contralateral eyes following topical treatment with 0.1 per cent betamethasone alcohol.\(^12\) Burch and Migeon\(^13\) reported a marked reduction in urinary cortisol secretory rates in man while receiving topical 0.1 per cent dexamethasone-21-phosphate (Migeon, personal communication), indicating systemic absorption. Although not observed in this test model, wasting or weight loss of animals can be observed by day six with the three dexamethasone derivatives, at 0.1 per cent, when topically applied five times per day. Other sensitive test models apparently will be needed to demonstrate a true systemic effect of topically applied corticosteroids.

Tensile strength of corneal wounds following steroid treatment for 15 days appeared to indicate a critical period from days 3 to 6. An apparent parallel healing relationship for control, steroid-treated, and contralateral eyes was noted from days 6 to 15 but not from days 3 to 6. This inhibition of corneal wound healing was most pronounced with 0.1 per cent dexamethasone alcohol. These results are consistent with earlier speculation that steroids interfere with stromal healing by inhibiting the poliferation of keratoblasts.\(^1\) However, these studies do not allow determination of the effects of these dexametha-
sone derivatives on the cellular or molecular mechanisms of wound healing.

Dexamethasone-21-phosphate is highly soluble (1 Gm. per milliliter); dexamethasone alcohol and dexamethasone-21-acetate are less soluble (12.9 x 10^-2 and 1.6 x 10^-2 mg. per milliliter, respectively) in the vehicle used. If solubility were the essential feature for expression of corticosteroid activity, one would expect dexamethasone-21-phosphate to be the most active. Since the data demonstrate dexamethasone alcohol as the most inhibitory, however, it would seem greater steroid solubility is not the most important property for steroid activity expression. Dexamethasone alcohol is reported to be released at different rates from various nonaqueous vehicles, both in vitro and in vivo. While comparative data were not extended for dexamethasone-21-phosphate and dexamethasone-21-acetate, certainly, vehicle solubility and release of steroids should be important to activity expression. The effect of other vehicles exhibiting different solubilities for these three corticoid derivatives of dexamethasone is another parameter which merits investigation.

One should also be concerned about solubility and penetration of these three dexamethasone derivatives into the corneal tissue. Insolubility has been suggested as a characteristic which allows a steroid to bind to tissues easily. Perhaps tissue solubility and penetration are critical parameters involved in the greater systemic absorption exhibited by dexamethasone alcohol and dexamethasone-21-phosphate.

Perhaps the differences observed are secondary to differences in intrinsic activity or in transport (active and/or passive) of dexamethasone alcohol, dexamethasone-21-phosphate, and dexamethasone-21-acetate. Further investigations with this technique and with other techniques need to be undertaken to elucidate any real intrinsic activity and transport differences for these three dexamethasone derivatives.

Local and/or systemic metabolism of each dexamethasone derivative that was used may be important in the activity expression of the various corticoids. If one assumes that the alcohol configuration is active (inhibitory in the case of corneal wound healing), some metabolic release by esterase activity of dexamethasone-21-phosphate and dexamethasone-21-acetate must occur. Such esterase activity has been suggested by Schlagel. The active alcohol form is vulnerable to inactivation of the 21-deoxy side chain by hepatic enzymes. Steroid degradation patterns, both local and systemic, are presently not well defined. What role metabolism plays is not known, yet metabolic patterns not readily discernible from these data may explain the differences noted for these three dexamethasone derivatives.

Two nonsteroid, anti-inflammatory drugs, indomethacin and phenylbutazone, did not decrease corneal tensile strength. Indomethacin has been reported not to affect healing of nonpenetrating corneal wounds and has been reported to have only one mode of action.

This study suggests therapeutic possibilities for steroid use in corneal wounds. The possibility of a regimen designed to minimize inhibitory effects of steroids on
Corneal wound healing by choice of the appropriate corticoid or timing of administration is suggested. In addition, numerous avenues of investigation concerning steroid solubility, metabolism, transport, intrinsic activity, and pharmacokinetic properties with respect to corneal wound healing and other activity parameters could be undertaken.

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REFERENCES