REFERENCES


Prednisolone phosphate penetration into and through the cornea. Keith Green and Susan J. Downs.

The penetration of labeled prednisolone phosphate into the aqueous humor and cornea of rabbits was measured at times up to six hours after the addition of one drop to the cornea in various vehicles. Adsorbobase produced a marked increase in both corneal and aqueous humor content over sodium chloride solution. Benza-}

konium chloride (0.01 per cent) increased the penetration of prednisolone in Adsorbobase but not in sodium chloride solution. The use of benzalkonium chloride as a preservative in vehicles which increase corneal retention time appears to be contraindicated in steroid preparations.

The eye is highly susceptible to inflammatory and topical corticosteroids have proved to be an invaluable therapeutic aid in the treatment of inflammatory response of various etiology as well as infection of the eye.1-3 One undesirable side effect of corticosteroids, however, is their ability to induce an ocular hypertension4-15; this effect can be reduced by using a dilute solution of steroid but maintenance of the anti-inflammatory capability then becomes difficult.6,16 Recent studies with a new vehicle, Adsorbobase,16,17 indicate that there is retention of this vehicle on the eye for long periods of time. The present study was undertaken to evaluate the efficacy of the vehicle in providing a more constant low concentration source of steroid to the eye. The steroid was prednisolone phosphate sodium, which has been found to be about four times more potent than hydrocortisone in anti-inflammatory activity.18

Adult albino rabbits (2 to 4 kilograms) were used. The solutions were 3H-prednisolone phosphate sodium (New England Nuclear Corp., Boston, Mass.; 6.731H) in Adsorbobase (AB) (Burton, Parsons and Company, Inc., Washington, D. C.) or 0.9 per cent sodium chloride solution (NaCl). The prednisolone concentrations and vehicles tested were: 0.125 and 1 per cent in AB; 0.125 per cent in AB plus benzalkonium chloride (BAC, 0.01 per cent); 0.125 and 1 per cent NaCl, and 0.125 per cent in NaCl plus BAC.

Fifty microliters of the test solution were placed on the sclera at the corneoscleral junction in the 12 o'clock position. At various times after drug instillation an anterior chamber paracentesis was performed on each eye after topical anesthetic (0.5 per cent Prapracaine hydrochloride, Squibb, New York, N. Y.) and washing with 2 ml. of NaCl solution. Eighteen rabbits were used on the same day of succeeding weeks, allowing one week of recovery before proceeding to the next paracentesis. The rabbits were used randomly at the different time intervals and solutions from week to week. A 100 μl sample of aqueous humor was transferred to a glass vial and, after the addition of 0.5 ml. distilled water and 10 ml. Bray's solution, assayed for radioactivity in a Unilux III liquid scintillation counter (Searle Analytic, Des Plaines, Ill.). Samples were counted for 2 × 105 counts in 60 minutes whichever came first. The lowest count rate measured was at least twice background, ensuring adequate counting rates. Samples of the drug as applied to the eye were also counted.
Corneal steroid content was determined in another series. Six-millimeter buttons were trephined from previously removed corneas, placed in 0.5 ml of tissue solubilizer, NCS (Amersham/Searle, Des Plaines, Ill.), or 1 ml of Protosol (New England Nuclear Corp., Boston, Mass.), and digested at 50°C for 24 hours. With NCS, 10 μl of glacial acetic acid was added after solubilization was complete. Bray’s solution was added to the vial and the samples counted until constant values were obtained. No difference was found between the use of NCS or Protosol as a tissue solubilizer. Suitable blanks and duplicate samples were run simultaneously containing the same volume of NCS or Protosol alone.

Some prednisolone recovery experiments were performed. Fifty microliters of labeled 0.125 per cent prednisolone in AB was placed on each eye and 10 μl of aqueous withdrawn by paracentesis after one hour. One sample was counted immediately; the other sample was run with an isopropanol:ammonium hydroxide:water solvent system (7:1:2, v/v/v) on a thin-layer chromatogram with a nonlabeled marker in parallel for identification. The fluorescent front was scraped into 0.6 ml distilled water and the sample counted in the normal manner. The results indicate that 100 per cent of the radioactivity in the aqueous is caused by labeled prednisolone.

All eyes receiving the steroid were quiet with no conjunctival injection. There was no reaction to the administration of any solution to the eye.

The aqueous prednisolone concentration at various times for all vehicles is given in Fig. 1. One per cent prednisolone in NaCl gives an eightfold greater concentration than 0.125 per cent in NaCl at early times, a figure identical with the concentration ratio; the ratio later becomes 4:1. BAC in NaCl, 0.125 per cent, has no effect on steroid penetration. One per cent in AB causes values double those found with 1 per cent in NaCl at all time intervals; 0.125 per cent in AB gives double the values found with 0.125 per cent in NaCl until four hours; thereafter, the values are similar. BAC plus 0.125 per cent in AB gives a 50 per cent increase over 0.125 per cent in NaCl up to one hour. Similarly, 0.125 per cent in AB plus BAC produces a threefold increase over 0.125 per cent in NaCl up to four hours.

The time required for the concentration to decrease to one-half of the concentration at any time during the initial two hours is the half-time, and was calculated from Fig. 1. NaCl results in a half-time of 40 and 25 minutes for 0.125 and 1 per cent, respectively; with AB it is 54 and 37 minutes, respectively; and with AB plus BAC it is 42 minutes for 0.125 per cent (Fig. 1).

Thus, the half-time is increased by AB over other vehicles. Although more total steroid initially enters the aqueous under the influence of BAC,
the rate is not sustained and the half-time is similar to NaCl.

The corneal content of prednisolone is shown in Fig. 2; all solutions cause a peak steroid concentration at two hours. The values found with 1 per cent in AB are six times greater than 0.125 per cent in AB and twelve times greater than 0.125 per cent in NaCl. BAC plus 0.125 per cent in NaCl has no effect on the steroid content of the cornea over 0.125 per cent in NaCl. 0.125 per cent in AB gives double the values found with 0.125 per cent in NaCl at all times; at six hours the value with AB is four times greater than with NaCl. BAC plus 0.125 per cent in AB produces values three times greater than 0.125 per cent in NaCl and 50 per cent greater than those with 0.125 per cent in AB up to 2 hours; at 6 hours the values for AB and AB plus BAC are identical. The half-time of loss from the cornea with AB plus BAC is close to that found with NaCl.

Recent studies on prednisolone phosphate sodium penetration into the cornea and aqueous humor of rabbit using NaCl vehicle indicate that approximately the same amounts of the drug enter the aqueous humor and the cornea as with our NaCl vehicle. The time course of penetration differs, however, and may reflect a difference in experimental technique.

The values found with AB indicate two differences as compared to NaCl. One, that more drug (at least double in any time period up to, and including, two hours after instillation) enters the aqueous (Fig. 1) and two, more is retained in the cornea (Fig. 2). The increased steroid penetration from AB is presumably related to the longer retention time on the corneal surface.

In the presence of BAC, the corneal steroid content with AB initially increases rapidly, and the aqueous content is initially higher than with NaCl (Figs. 1 and 2). The effect of BAC in inducing greater steroid penetration is related to its known permeability enhancing effect, where there is a physical disruption of the epithelial cell membranes and a widening of intercellular spaces. When BAC is used in conjunction with any agent which extends the retention time of a drug on the cornea it would appear that the extended time allows BAC to produce its known effects on epithelial permeability. The half-time loss of prednisolone from the cornea in 0.125 AB plus BAC is similar to that found with NaCl alone. This would imply that BAC is also enhancing endothelial permeability to prednisolone.

Whether or not the levels of aqueous steroid found after AB alone are below the threshold needed to precipitate a steroid hypertension remains to be determined.

Any agent which extends the corneal contact time without enhancing corneal permeability would seem to be desirable with respect to ophthalmic steroids. The long retention time caused by Adsorbobase has been demonstrated in these studies to fulfill the requirements of a vehicle which extends the drug contact time, allowing high corneal contents to be obtained without the excessive aqueous humor concentrations found when used in conjunction with BAC, a commonly used preservative in ophthalmic steroid preparations.

We wish to thank Dr. Howard M. Leibowitz for his generosity in making available his data on prednisolone phosphate penetration studies prior to publication. Burton, Parsons and Company, Inc. supplied the titrated prednisolone phosphate sodium.

From the W. K. Kellogg Research Laboratories, The Wilmer Institute, The Johns Hopkins University School of Medicine, Baltimore, Md. This work was supported by Public Health Service Research Grant EY 00034 and Research Career Development Award K4 EY 46-354 (Dr. Green) from the National Eye Institute. Submitted for publication Dec. 17, 1974. Reprint requests: Dr. Keith Green, Departments of Ophthalmology and Physiology, Medical College of Georgia, 1459 Gwinnett St., Augusta, Ga. 30902.

Key words: steroids, prednisolone phosphate, vehicle, benzalkonium chloride, aqueous humor, cornea, rabbit.

REFERENCES
6. Armary, M. F.: Effect of corticosteroids on intraocular pressure and fluid dynamics, I.


Characteristics and pharmacologic utility of an intraocular pressure (IOP) model in unanesthetized rabbits.* R. J. Seidehamel and K. W. Duncan.

A laboratory model was designed to assess the effects of drugs on intraocular pressure (IOP) in rabbits. Its utility for evaluating the antiglaucoma potential of drugs was tested by employing 1-epinephrine, an adrenergic agent known to be effective in glaucoma. When 1-epinephrine was applied topically to the eye, it caused a concentration-dependent reduction of normal IOP and marked antagonism of elevated IOP induced by water load. These effects were of long duration. Antagonism of IOP elevation was earlier in onset than reduction of normal IOP, suggesting different underlying mechanisms. Similarities between these results and those reported for glaucomatous man establish the utility of this IOP model in the evaluation of the antiglaucoma potential of adrenergic-like compounds.

The search for improved medical therapy in glaucoma has prompted development of numerous methods for laboratory evaluation of drug effects on intraocular pressure (IOP). Investigations in our laboratory utilizing some of these methods allowed recognition of particular features which, when incorporated into a single animal model may provide a more meaningful evaluation of a drug’s antiglaucoma potential. Features of the model include the following: (1) animals are unanesthetized and thus normally responsive to drugs and stimuli; (2) normal and elevated IOP’s are provided sequentially in the same eye; (3) reproducible IOP measurements are made by electronic tonometry; (4) testing can be of sufficient duration to observe the time-course of drug action; (5) appropriate controls are available for post-drug IOP comparisons. The pharmacologic utility of this model for detecting and evaluating potential antiglaucoma agents, particularly of the adrenergic type, was examined employing l-epinephrine, an adrenergic agent with known clinical efficacy in open-angle glaucoma.

Methods. Female New Zealand-White rabbits, 1.8 to 2.5 kilograms body weight, were deprived of food for 18 hours and maintained in restrainer boxes during experimentation. IOP was measured indirectly from the corneal surface, without local anesthesia, using a Mackay-Marg Model No. 12 electronic tonometer. The tip of the tonometer probe was moistened with wetting solution (Barnes-Hind) to avoid corneal abrasion. Pupil diameter was measured to the nearest 0.5 mm. under constant illumination with a clear, straight-edge ruler.

IOP elevation was accomplished in unanesthetized rabbits using a modification of a procedure previously reported.17,18 Rabbits were administered 60 ml. per kilogram of tap water rapidly via gavage following measurement of normal or baseline IOP. IOP was measured 10, 20, and 30 minutes thereafter to determine maximal increase (elevated IOP) during this time. The procedure was repeated in the same animals 2, 4, and 6...