A bioavailability comparison in rabbits of two steroids formulated as high-viscosity gels and reference aqueous preparations

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In order to assess the bioavailability of steroids from a high-viscosity gel, rabbit cornea and aqueous humor levels were measured over 12 hr following topical instillation of a gel and reference preparation. Concentrations associated with a 1% tritiated prednisolone acetate gel were found to be approximately four times larger than those of the reference preparation. A graphical comparison of the 1% tritiated prednisolone sodium phosphate data showed the area under corneal and aqueous time curves to be five and 10 times greater, respectively, with the gel than data associated with the reference preparation. The gel vehicle is well retained in the rabbit eye and is responsible for the large increases in bioavailability.

Key words: prednisolone acetate, prednisolone sodium phosphate, ophthalmic bioavailability, vehicle, aqueous humor, cornea, rabbit

New delivery systems for ophthalmic drug preparations have received much attention over the past few years.1-4 By and large, these vehicles have been designed to increase the amount of drug that penetrates the cornea by minimizing physiological factors such as drainage, blinking, and/or tearing.5 Physiological factors, along with noncorneal absorption,6 are responsible for the relatively poor bioavailability that is associated with aqueous preparations dosed topically to the eye.

In order to minimize these factors and thus improve bioavailability, water-soluble polymers have been added to aqueous solutions of ophthalmic drugs. Polymers such as methylcellulose, hydroxypropyl methylcellulose, and polyvinyl alcohol impart a slight lowering of surface tension and an increase in viscosity. The increase in viscosity prolongs contact of the drug in the eye, thereby resisting drainage,6 and a lowering of the surface tension improves mixing with the precorneal tear film. In rabbits a twofold to threefold improvement in pilocarpine bioavailability has been found for viscous aqueous solutions.7

The following study was conducted in rabbits to determine whether a high viscosity gel would show increased bioavailability for two steroids when compared to commercially available, reference preparations. Prednisolone sodium phosphate, 1%, and prednisolone acetate, 1%, were chosen for study because of their clinical importance and also because these drugs are representative of a typical water-soluble and water-insoluble steroid, respectively.

Materials and methods

Preparations. Prednisolone acetate [6,7-3H(N)] (Amersham/Searle Corp., Arlington Heights, Ill.)
was first purified by using silicic acid (Bio-Sil A, 100-200 mesh; Bio-Rad Laboratories, Richmond, Calif.) as the support material and chloroform ethanol 9:1 as the eluant. Further purification was carried out by isothermal vacuum distillation in order to remove volatile, labile tritium. The purified, labeled drug was incorporated into U.S.P. prednisolone acetate powder (Organon, West Orange, N. J.) by recrystallization. The procedure consisted of dissolving both tritiated and nontritiated prednisolone acetate in dichloromethane/methanol 2:1. An optimally vibrating ultrasonic probe was placed into a beaker of cold (4°C) distilled water. The steroid solution was added quickly, whereupon a microfine precipitate of prednisolone acetate formed immediately. The dried microfine powder was assumed to contain tracer homogenously throughout its crystalline structure. The labeled microfine prednisolone acetate was used to prepare a 1% aqueous suspension (Econopred vehicle; Alcon Laboratories, Inc., Ft. Worth, Texas) as well as a 1% aqueous gel suspension (Carbopol 940; B. F. Goodrich, Cleveland, Ohio).

Prednisolone sodium phosphate-^3H (general labeled; New England Nuclear, Boston, Mass.) was purified by isothermal vacuum distillation for removal of volatile, labile tritium and further treated to remove other impurities by paper chromatography (Whatman 3M, St. Paul, Minn.) with isopropanol/ammonium hydroxide/water 7:1:2 used as the solvent system. The tracer was eluted from the paper with methanol/water 50:50. Upon removal of the solvent, the labeled drug was dissolved in 20 ml of 1% prednisolone sodium phosphate ophthalmic solution (Inflamase Forte; SMP International, Inc., San German, Puerto Rico). The labeled drug was also used to prepare an aqueous gel (Carbopol 934; Goodrich) containing 1% prednisolone sodium phosphate (Organon). The radiochemical purity was determined for all four preparations by thin-layer chromatography.

In vivo methodology. New Zealand white rabbits without observable eye defects, weighing 1.5 to 3.0 kg and 2 to 3 months of age, were selected for the study. Six rabbits were used at each time interval for each gel and reference prednisolone acetate preparation, and 12 rabbits were used at each sampling time for each gel and reference prednisolone sodium phosphate preparation. A larger number of rabbits were used for the latter
study because preliminary experiments showed larger variability associated with prednisolone sodium phosphate than prednisolone acetate, presumably because of the former drug’s poorer penetrability. An additional rabbit, corresponding to each formulation, received a positive nonradioactive control dose prepared identically to the radioactive formulations. The counts per minute associated with the aqueous humor and cornea of these rabbits provided for background subtraction in determining net counts per minute for each tracer sample.

For the aqueous solution and suspension dosage forms, a single 50 μl dose (Eppendorf pipette, Brinkmann Instruments, Inc., Westbury, N. Y.) was administered to the right eye. The lower lid was pulled away from the globe, and the drop was allowed to fall onto the cornea, with any excess falling into the conjunctival sac. In dosing the gel preparations, the right lower eyelid was also pulled away from the globe; however, the dose or extruded ribbon (50 μl, tuberculin syringe without needle) was carefully placed lengthwise along the lower portion of the conjunctival sac, and the eyelid was carefully returned to its normal position. The animals were randomized with respect to the formulation each received.

At each time interval, the prescribed number of rabbits was sacrificed, and aqueous humor and cornea were sampled. One hundred microliters of Aqueous humor (100 μl) was removed from the anterior chamber with a 27-gauge needle attached to a 1 ml tuberculin syringe and added directly to a counting vial. An 8 mm corneal button was removed with the use of a trephine, weighed, and immediately placed in a counting vial containing 2.0 ml of tissue solubilizer (Soluene 350, Packard Instruments, Inc., Downers Grove, Ill.), which was allowed to stand overnight. Then 10 ml of scintillation solution was added to each vial and counted (Beckman LS-230 scintillation counter; Beckman Instruments, Inc., Fullerton, Calif.)

Results

The specific activities of the reference and gel preparations of prednisolone acetate were 27.0 and 27.8 μCi/mg, and those for prednisolone sodium phosphate were 16.1 and 16.3 μCi/mg. The radiochemical purity of each tracer prior to and after preparation of each dosage form varied from 96% to 98%. A particle diameter analysis was performed (Walter C. McCrone Associates, Inc., Chicago, Ill.) on the 1% prednisolone acetate suspension; it was found to conform to the manufacturer’s specifications for topical use in the eye.
**Prednisolone acetate.** The aqueous humor levels of prednisolone acetate obtained after dosing with the 1% preparations (Fig. 1) showed much greater increases for the gel dosage form. The gel results showed an area under the curve 4.5 times greater than the reference preparation. From the 1 hr determination through 9 hr, a statistically significant difference ($p < 0.05$) was obtained for each interval. The time to peak for the gel and reference preparation was found to be 2.5 and 1.5 hr, respectively. A comparison of drug levels obtained for the cornea samples showed the area under the curve to be four times greater for the gel preparation (Fig. 2). Following the 1 hr determination through the end of the study, all values pertaining to the gel preparation are statistically higher, with the exception of the 11 hr value. The time to peak observed for the corneal data was 1.5 and 1 hr respectively, for the gel and reference dosage form.

**Prednisolone sodium phosphate.** Values were statistically higher ($p < 0.05$) for the 1% prednisolone sodium phosphate gel preparation in aqueous humor (Fig. 3) for all time intervals except at 10 and 60 min and statistically higher in the cornea (Fig. 4) for all intervals except at 12 hr. By direct comparison of the areas under each curve, the gel preparation showed a 5.5-fold increase of drug in the cornea and a 10.6-fold increase in the aqueous humor. In aqueous humor, the peak time was observed at 2 hr; however, for corneal tissue two peaks can be interpreted from the data at 20 min and 2 hr.

**Discussion**

When a drop is applied to the eye, it is diluted by reflex tearing, and the added volume in excess of the normal lacrimal volume is drained from the eye. Studies with rabbits have demonstrated that the smaller the drop, the slower the drainage rate and the more
Fig. 4. Mean prednisolone sodium phosphate concentration in 8 mm corneal buttons of rabbits (N = 12) after dosing with 50 μl of 1% gel (•) or solution (○). Each bar represents 1 S.D.

Extensive the bioavailability. By reducing the volume of the instilled drop from 50 μl, an increase of 1.8-fold in pilocarpine bioavailability was observed in rabbit eyes. An ideal dose of 1 to 5 μl, accurately measured and carefully administered, has been recommended in order to gain maximum results from an instilled drug. However, this approach is limited because of the technological difficulties involved in providing an accurate, reproducible, and inexpensive means by which a small dose can be applied on a routine basis. An alternate approach involves resisting eye washout through formulation control.

The gel preparations used in this study were formulated to resist the shearing force generated by eyelid and/or eyeball movements. As a consequence, significant quantities of gel were observed in the rabbit conjunctival sacs through 8 hr. It was also observed that the quantity of gel in the rabbit slowly diminished over time. Therefore drug is probably made available for absorption partly by diffusion through the bulk of the gel and partly by uptake from the surface of gel as it continuously erodes. In either case, the fact that the gel is well retained allows the drug to be absorbed over an extended time period, resulting in a large multiple increase in bioavailability.

A clear explanation for the double peaks cannot be given at this time. The phenomenon could be due to the use of different animals at each time interval or quite possibly due to the formation of metabolites which show different kinetics. In a study by Kupferman and Leibowitz, double peaks can be interpreted for data associated with the cornea of normal rabbits (intact corneal epithelium) at 5 and 60 min time intervals following topical dosing with a 0.125% solution of prednisolone sodium phosphate.

Leibowitz and Kupferman have presented
evidence that the bioavailability achieved with a 1% suspension of prednisolone acetate produces a maximal anti-inflammatory effect in the cornea. It was pointed out that concentrations of prednisolone acetate suspensions higher than 1.0% would have a greater potential for toxicity without offering additional therapeutic benefit. Consequently, the large increase in aqueous and corneal tissue levels observed for both steroid gel preparations may not be advantageous. However, the large increase in bioavailability makes it possible to significantly reduce the concentration of drug in the gel and still maintain a maximal therapeutic effect. A reduction approximately fourfold to fivefold in dosage is therefore permitted with this dosage form and would be expected to reduce the potential for systemic side effects.

The fact that both a soluble and a practically insoluble steroid showed significant increases in bioavailability with the gel dosage form suggests that all presently marketed steroids should likewise show similar improvements in bioavailability.

REFERENCES