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Disposition of topically applied vitamin A in the albino rabbit eye

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Summary

Using radiotracer techniques, the disposition of vitamin A in the albino rabbit eye was studied following instillation of its solution in arachis oil in tear chamber. Early peak times were observed in the conjunctiva and the cornea suggesting rapid uptake of the drug by these tissues. Perhaps because of their proximity to the tear pool, these two tissues possessed the highest concentration of vitamin A. Sustained drug concentration was evident in all the ocular tissues studied—conjunctiva, cornea, aqueous humor and iris-ciliary body—beginning at 30 min post-instillation. This presumably was due to a reservoir for vitamin A in the precorneal area and perhaps the corneal epithelium; the nature of this reservoir has yet to be resolved. The significance was that vitamin A was available to the conjunctiva and the cornea from topical dosing, the two tissues known to undergo changes in vitamin A deficiency and dry-eye states. Unfortunately, these data as yet do not permit the formulation of a dosage regimen for topically applied vitamin A in the treatment of xerophthalmia.

Introduction

Dryness of the conjunctiva and the cornea, a condition known as xerophthalmia, is among the earliest manifestations of vitamin A deficiency (Sommer et al., 1976). In the short-term xerophthalmia interferes with tear film stability; in the long-term it can lead to blindness (Van Horn et al., 1980). Indeed, xerophthalmia is the leading cause of childhood blindness in many developing countries (World Health Organization, 1976). Fortunately, cases involving mild to moderate degeneration of the xerophthalmic cornea and conjunctiva can be reversed upon topical instillation of an oil solution of vitamin A or of its metabolite, retinoic acid (Pirie, 1977; Sommer and

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Emran, 1978; Van Horn et al., 1981). However, the distribution of these retinoids in the eye following topical instillation has not been studied and quantitated. As a result, it is not clear whether a correlation exists between the concentration of retinoids in the conjunctiva and cornea and the density of their binding proteins (RBP) known to be present in these tissues (Wiggert et al., 1977; Wiggert et al., 1978; Asahara, 1980; Rask et al., 1980). Likewise, it is not clear what the optimal dose size and dosing frequency should be. In order to resolve these uncertainties it is necessary to establish the time course of vitamin A disposition in the eye. This report presents preliminary data on vitamin A disposition in the cornea, conjunctiva, iris-ciliary body and aqueous humor in healthy albino rabbits at various times following topical instillation of single doses of a 0.1% solution of vitamin A in arachis oil. Arachis oil was chosen over other oils like castor and mineral oils because: (1) it seemed to be well tolerated by the rabbit eye; (2) it was not too viscous to be instilled; and (3) it tended to release vitamin A into the tear film more readily than the other two oils because the oil/water partition coefficient of vitamin A was the lowest in this oil.

Materials and methods

All trans [^{3}H]vitamin A (spec. act. 5 Ci/mmol) was obtained from New England Nuclear (Boston, MA), while non-radioactive vitamin A was obtained from Sigma Chemicals (St. Louis, MO). The purity of both was checked prior to each experiment by thin-layer chromatography on silica gel using the solvent system ethanol: methylene chloride (1:99) and found to be 97.5–99% pure. They were used without further purification.

Solutions of vitamin A (0.1 mg · ml^{-1}) in arachis oil were prepared fresh prior to each experiment. It was determined that 0.25 μCi of [^{3}H]vitamin A per μl of dosing solution provided the counting efficiency required. The dosing solutions were kept in the dark when not in use.

All rabbits were male, New Zealand albinos, 2–3 kg in weight. During the experiment, they were kept in restraining boxes in a normal upright posture. A 25-μl volume of drug solution was instilled directly onto the cornea of the test animal. During instillation the lids were withdrawn from the globe of the eye but were immediately returned to their normal position after instillation. Both eyes of the test animal were used, but the dosing time was staggered so that one animal might, for example, be used for a 10- and 30-min time point. Rabbits were sacrificed at various times by an intravenous injection of a 30% sodium phenobarbital solution into a marginal ear vein. The corneal and conjunctival surfaces were thoroughly rinsed with normal saline and blotted dry. The anterior segment samples—conjunctiva, aqueous humor, cornea and iris-ciliary body—were obtained in that order.

The aqueous humor samples were transferred to vials (BioVials, Beckman, Irvine, CA) containing 4 ml of prerefrigerated scintillation cocktail (Aquasol-2, New England Nuclear, Boston, MA). Each of the tissue samples was digested at 55°C for 18 h in 1.5 ml of a tissue solubilizer (Protosol, New England Nuclear, Boston, MA)

contained in a glass scintillation vial (CMS, Fountain Valley, CA) followed by decolorization in 100 μl of hydrogen peroxide and addition of 10 ml of a scintillation cocktail (Econofluor, New England Nuclear, Boston, MA). All samples were stored in the dark for 24 h prior to counting in a liquid scintillation spectrometer (Beckman Model 7500). After correcting for background the data in counts per minute were converted to micrograms through the use of standards.

Results and discussion

Because no attempt was made in the present study to evaluate the metabolism of vitamin A in the various ocular tissues samples, the concentration–time profiles displayed in Fig. 1 should, strictly speaking, be interpreted as those for total vitamin A, i.e. intact drug plus metabolites that might be formed. Nonetheless, that vitamin

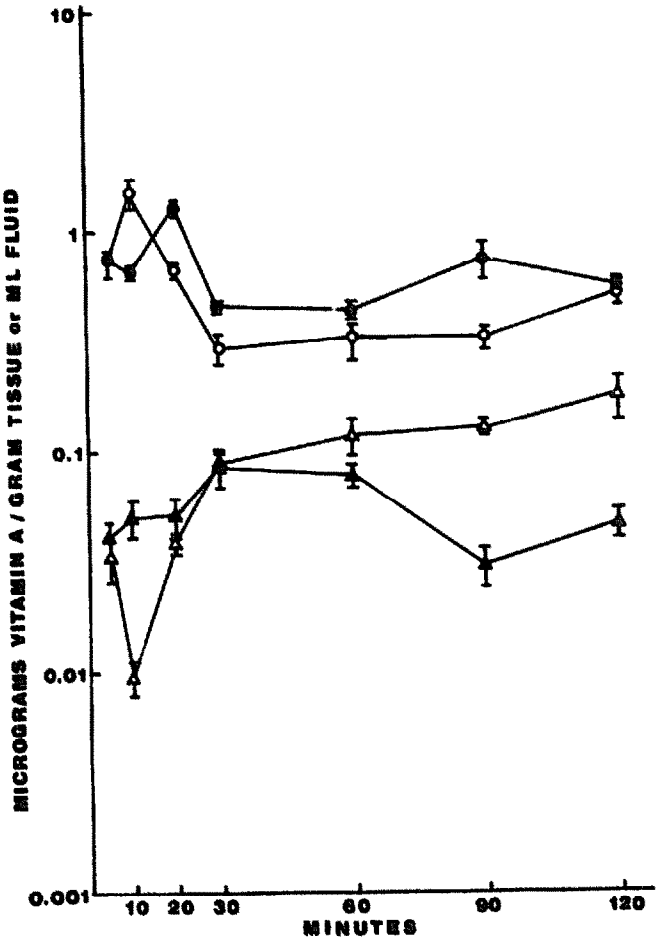

Fig. 1. Concentration–time profile of vitamin A in the conjunctiva (○), cornea (●), aqueous humor (△) and iris-ciliary body (▲) of the albino rabbit eye. Between 8 and 12 eyes were used for each time point. The error bars represent standard error of the mean.

A can be detected in the cornea following topical application of a therapeutic dose is significant because it casts doubt on the suggestion by Rask et al. (1980) that RBP-bound vitamin A is required to ensure uptake of the drug by the xerophthalmic cornea. As shown in Fig. 1, the highest concentration of vitamin A was found in the cornea and the conjunctiva, the two tissues in direct contact with the tear pool into which doses of the drug in arachis oil were placed. Peak drug levels were reached somewhat sooner in the conjunctiva than the cornea, 10 min as opposed to 20 min. Possibly, the later peak time observed in the cornea was due to redistribution of the drug between its receptors found in the epithelium, stroma and endothelium (Wiggert et al., 1977) as the drug was traversing these successive layers of the cornea.

This early peak time seen in the cornea with an oil solution of vitamin A resembles that seen with aqueous solutions of drugs like pilocarpine (Sieg and Robinson, 1976) and fluorometholone (Sieg and Robinson, 1981). In the case of aqueous solutions, the early peak time has been attributed to the combined influence of tear flow, corneal and conjunctival absorption, and drainage of the instilled solution away from the precorneal area. It is apparent from the present study that the reduced drainage loss expected of a more viscous oil solution, relative to an aqueous solution, has minimal impact in prolonging the peak time in the cornea. This is in accord with the small contribution made by drainage to the early peak time seen in an epithelial pilocarpine concentration vs time profile (Lee and Robinson, 1979).

While the cornea and conjunctiva virtually showed the same concentration of vitamin A, there actually was more drug in the conjunctiva than the cornea partly because the conjunctiva weighed more. Mechanistically, that accumulation of vitamin A occurred in the conjunctiva despite its vascularity implies tenacious binding of the drug to this tissue. However, it is not known whether this binding mainly represents non-specific tissue binding or specific binding to receptors for vitamin A, which now have been identified in a number of ocular tissues including the conjunctiva (Wiggert et al., 1978; Asahara, 1980). Similarly, because there has been no information on the relative number of receptors in the cornea and conjunctiva and on the energetics of binding of vitamin A to them, no definitive statement can be made on the cause underlying the different amount of vitamin A found in these two tissues at this time.

In addition to the early peak times seen in the cornea and the conjunctiva, an important feature shown in Fig. 1 is the relatively constant level of drug in all the tissues studied at times beyond 30 min post-instillation. At least in the aqueous humor, this pattern mimics that seen with another oil-soluble drug, fluorometholone, administered in an ointment but not in an aqueous solution (Sieg and Robinson, 1975). This suggests that the oil solution behaves like an ointment by being retained in the precorneal area via a mechanism yet to be revealed. Nevertheless, it can be speculated that the oil containing the drug is physically deposited onto the corneal and conjunctival surfaces, but this is not likely in view of the absence of sustained levels of pilocarpine, a water-soluble drug, also administered in an ointment (Sieg and Robinson, 1977). Interestingly, should this mechanism be a viable one, the tear film would have its already reduced stability further compromised as the corneal

surfaces would now be rendered hydrophobic, and therefore not readily wettable, by the physical presence of an oily layer.

As an alternative to physical deposition of the oil solution onto the corneal and conjunctival surfaces, a portion of it could be incorporated into the phospholipid layer of the tear film. The drug contained in it is then releasd either through partitioning into the bulk of the tear film whereupon it diffuses to the cornea and the conjunctiva, or by being physically transferred to the cornea and conjunctiva through blinking. There is no experimental evidence to either support or refute this possibility at this time.

There is yet a third possibility. This concerns the instantaneous, sequential partitioning of the drug from oil into the tear and then the lipophilic epithelium. Once in the corneal epithelium the drug serves to sustain levels in the remaining intraocular tissues including the corneal stroma and endothelium, iris-ciliary body and aqueous humor. This seems to be a reasonable mechanism in view of the drug's lipophilicity, but has to be proven in another experiment involving rabbits whose corneal epithelia have been removed prior to dosing.

Returning to the early portion of the concentration–time profile, the aqueous humor seemed to display peak and valley type behavior. At first glance, this behavior could be interpreted as differential diffusion of vitamin A and its metabolites, which may be formed in the tear fluids and/or the cornea, through the cornea into the aqueous humor. But this has been refuted by computer simulations (data not shown) using various combination of rates of drug absorption and elimination and of metabolite formation and elimination. Nevertheless, Fig. 1 shows that prior to 30 min, the concentration of vitamin A in the iris-ciliary body was higher than the aqueous humor, which presumably supplied the iris-ciliary body itself with the drug. This is not surprising in view of the low protein content in the aqueous humor that ordinarily will bind some of the drug, and of the likely existence of vitamin A receptors or binding proteins in the iris-ciliary body (Asahara, 1980). Eventually, the binding sites in the iris-ciliary body are saturated, thereby allowing vitamin A levels in the aqueous humor to increase and exceed those in the iris-ciliary body.

In conclusion, sustained concentration of vitamin A is detected in the rabbit eye following topical instillation of its solution in oil. While this finding should be considered in the frequency of topical administration of this drug, no dosage regimen can be recommended as yet pending both single and multiple dose studies on the time course of vitamin A concentration in the rabbit eye for times beyond 120 min, the last time point in the present study. Admittedly, another limitation of the present study is that metabolism of vitamin A following ocular absorption has not been examined. Nonetheless, progress is underway: (1) to evaluate the utilization of vitamin A, including metabolism, by the cornea and conjunctiva following uptake; and (2) to confirm the role of the oily layer in the tear film and of the corneal epithelium in the disposition of vitamin A in both healthy and vitamin A-deficient rabbit eyes.

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