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Ocular disposition of pilocarpine in the pigmented rabbit: site(s) of metabolism

Ray W. Wood * and Joseph R. Robinson

School of Pharmacy, University of Wisconsin, Madison, WI (U.S.A.)

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Summary

The ocular disposition and metabolism of pilocarpine in the pigmented rabbit was examined following topical application. The data suggest that, of the anterior segment tissues sampled, the major sites of metabolism are the iris-ciliary body complex and the cornea. Significant metabolism of pilocarpine appears to occur in the corneal stroma-endothelium but not in the aqueous humor. This observation suggests that, unlike the albino rabbit eye, the stroma-endothelium and aqueous humor cannot be considered as a single homogeneous compartment when describing the ocular disposition of pilocarpine in the pigmented rabbit.

Introduction

The albino rabbit has been commonly used in ocular drug disposition studies, in part because extensive data has been collected regarding its biochemistry and physiology. In order to elucidate mechanisms of drug movement into and through the eye, the ocular disposition of pilocarpine in the albino rabbit has been systematically examined (Patton and Robinson, 1975, 1976; Sieg and Robinson, 1977; Lee and Robinson, 1979; Makoid and Robinson, 1979). With the premise that binding could affect the kinetics of drug movement through the eye, Lee and Robinson (1980, 1982) investigated the ocular disposition of pilocarpine in the pigmented rabbit using radiotracer techniques. These preliminary studies indicated that in addition to binding differences, significant metabolism of pilocarpine occurred in the eye of the pigmented rabbit following topical application of a pilocarpine solution. These results were in contrast to findings in the albino rabbit, which suggested that the rate and extent of ocular metabolism is negligible (Makoid and Robinson, 1979; Sendelbeck et al., 1975).

The present study was initiated to further explore the disposition of pilocarpine in the pigmented rabbit in anticipation of identifying possible sites of metabolism as well as further quantifying mechanisms of pilocarpine movement through the pigmented rabbit eye.

Materials and Methods

Materials

Water was double-distilled from alkaline per-

* Present address: Travenol Laboratories, Morton Grove, IL, U.S.A.

Correspondence: J.R. Robinson, School of Pharmacy, University of Wisconsin, Madison, WI, U.S.A.

manganate solution in an all-glass apparatus. Pilocarpine nitrate was obtained commercially (J.T. Baker Chemicals, Phillipsburg, N.J) and was used without further purification. Male, mixed breed rabbits (Klubertanz, Edgerton, WI) weighing between 2 and 2.5 kg were used throughout the studies. They were individually housed in standard laboratory cages and were fed a regular diet with no restrictions on food or water. Lighting was maintained on a 24 h basis in the caging facilities.

Methods

Preparation of pilocarpine solutions. Drug solutions utilized for topical application to the rabbit eye were prepared by adding sufficient pilocarpine nitrate to Sorensen's buffer to obtain a 5×10^{-2} M concentration and a final pH of 6.24. The resulting solution was rendered isotonic with NaCl. These solutions were prepared fresh prior to each experiment.

Preparation of pilocarpic acid solutions. Solutions of pilocarpic acid for topical application were prepared by adding 10 ml of 0.1 N NaOH to 4 ml of a 1% aqueous solution of pilocarpine nitrate and allowing the mixture to stir for 2–4 h. A portion of this solution, 9.6 ml was adjusted to pH 7 with 3.5 ml of 0.1 N HCl and the resulting solution lyophilized. The residue was then reconstituted in 1.4 ml of phosphate buffer at pH 7.4.

Tritiated pilocarpic acid was prepared by the addition of 100 μl of 0.1 N NaOH to purified tritiated pilocarpine alkaloid, 4.1 Ci/mmol, (New England Nuclear, Boston, MA) and the resulting solution allowed to stir for 2 h. The tritiated pilocarpic acid solution was then purified by vacuum distillation and reconstituted with 1.4 ml of the solution of non-radioactive pilocarpic acid just prior to the start of the experiments. The final concentration of pilocarpic acid used in these experiments was approximately 0.64 M and each 25 μl aliquot contained approximately 500,000 cpm. The final pilocarpic acid solution was hypertonic.

The yield and purity of pilocarpic acid from the alkaline hydrolysis of pilocarpine has been discussed in detail in a previous report (Wood and Robinson, 1984). Briefly, the hydrolysis of pilocarpine is quantitative as indicated by high-performance liquid chromatography. However, the hydrolysis resulted in two products corresponding to both pilocarpic acid and isopilocarpic acid based on retention times. The composition of a hydrolyzed solution of pilocarpine, under the reaction conditions described, was found to be 83.5% pilocarpic acid and 16.5% isopilocarpic acid. Differences in ocular disposition of the two isomers were assumed to be negligible.

Ocular tissue and aqueous humor concentration versus time profiles of pilocarpine and pilocarpic acid following topical application of pilocarpine. Test animals were placed in restraining boxes to minimize movement. The normal upright posture of the animals was maintained at all times and care was taken to permit normal eye and head movement.

Twenty-five μl of pilocarpine nitrate solution was instilled onto the rabbit cornea. For instillation, the lower lid was gently pulled away from the globe to form a pocket but was immediately returned to the normal position after drug instillation. At various times post-instillation, rabbits were sacrificed by rapid injection of a lethal dose of sodium pentobarbital into a marginal ear vein. A single puncture was then made, through the corneal-scleral junction into the anterior chamber and a 200 μl aliquot of aqueous humor was transferred quantitatively to a holding vial immersed in a dry-ice–isopropanol bath prior to sample work-up.

Following removal of aqueous humor, the eye was proptosed, the cornea was excised with a scalpel and the iris and ciliary body removed as a single entity with the aid of forceps. These tissues were gently rinsed with doubly distilled water and blotted dry. The wet weights of these tissues were obtained and the tissues were then immediately placed in holding vials immersed in a dry-ice–isopropanol bath.

The aqueous humor, cornea and iris-ciliary body samples were then worked up and assayed as previously described (Wood and Robinson, 1984). Briefly, tissue was homogenized at 4°C, treated with trichloroacetic acid and centrifuged. The supernatant was transferred to a culture tube and lyophilized. The resulting residue was then reconstituted in water and extracted with hexane. The hexane phase was discarded and the aqueous phase

was extracted with chloroform following pH adjustment to approximately 9. The pH of the remaining aqueous phase was adjusted to approximately 4 and extracted with *n*-butanol. The two extracts were then combined, dried under nitrogen at 40°C, reconstituted in 60 μl of doubly distilled water and assayed for pilocarpine and pilocarpic acid content by high-performance liquid chromatography. The HPLC system consisted of a solvent pump; a fixed volume (20 μl) sample injection valve, a 5 μm particle size reversed-phase octadecyl column equipped with precolumn and a variable UV detector set at 215 nm. The mobile phase consisted of 97% (v/v) doubly distilled water, 3% (v/v) methanol, and 5% potassium dihydrogen orthophosphate. There was no pH adjustment. The flow rate was 1.0 ml/min, and an external standard curve method was used to quantitate pilocarpine and pilocarpic acid utilizing peak heights. Tissues from three eyes were pooled, extracted and assayed for each determination of pilocarpine and pilocarpic acid.

In some experiments, the corneal epithelium was removed prior to dosing with pilocarpine. In order to facilitate removal of the corneal epithelium, two drops of a 0.5% solution of proparacaine-HCl (Ophthetic, Allergan Pharmaceuticals, Irvine, CA) were instilled into the eyes of experimental animals. After 5 min, the eye was proptosed and the epithelial layer removed in toto by scraping with a scalpel. The eye was then carefully blotted with moist tissue to remove any loose fragments of epithelium from the precorneal area and returned to its normal position.

Ocular tissue and aqueous humor concentration versus time profiles of pilocarpic acid following a topical dose of pilocarpic acid. Twenty-five μl doses of pilocarpic acid solution, prepared as previously described, were topically instilled, animals sacrificed and surgery performed in the manner previously described. Two-hundred μl samples of aqueous humor were transferred to liquid scintillation counting vials (Research Products International, Elk Grove, IL) and 20 ml of liquid scintillation solution (Aquasol, New England Nuclear, Boston, MA) were added. Samples were stored in the dark at room temperature for at least 24 h prior to counting to minimize chemiluminescence. Ten minute counts of each sample were made using a liquid scintillation spectrometer (Packard Model 2002, Packard Instruments, Downers Grove, IL). The error associated with the mean cpm (counts per minute) for a 10 min count was 10% for each tissue studied. The final count rate obtained for each sample was then converted to μg of pilocarpic acid per ml of aqueous humor.

After obtaining the wet weights of the cornea and iris-ciliary body, the tissues were transferred to liquid scintillation vials and 0.1 ml of a tissue solubilizer (Protosol, New England Nuclear, Boston, MA) was added. The tissues were solubilized in an oven at approximately 50°C for 24 h, were then removed and 100 μl of hydrogen peroxide added. The mixture was allowed to cool for 30 min and 20 ml of scintillation fluid was added to each vial. The samples were stored at room temperature for 24 h and counted as previously described. The final count rate obtained for each sample was then converted to mg of pilocarpic acid per g of tissue. Concentration–time profiles of pilocarpic acid in the cornea and iris-ciliary body were then constructed.

Results and Discussion

In order to fully describe the ocular disposition of pilocarpine in the pigmented rabbit, sites of pilocarpine metabolism need to be established. Two ocular tissues, namely the cornea and iris-ciliary body, together with aqueous humor, were sampled in the present studies. Concentration–time profiles of pilocarpine and pilocarpic acid in these sampling compartments following topical instillation of pilocarpine are shown in Figs. 1–3. These profiles indicate that pilocarpic acid contributes a significant fraction to the total drug observed in these tissues throughout the entire time course of the study and is consistent with results of a previous study (Lee et al., 1980).

The data shown in Figs. 1 and 2 were subjected to one-compartment pharmacokinetic analysis in order to obtain apparent elimination rate constants for pilocarpine. The apparent elimination rate constants obtained were then compared to apparent elimination rate constants previously re-

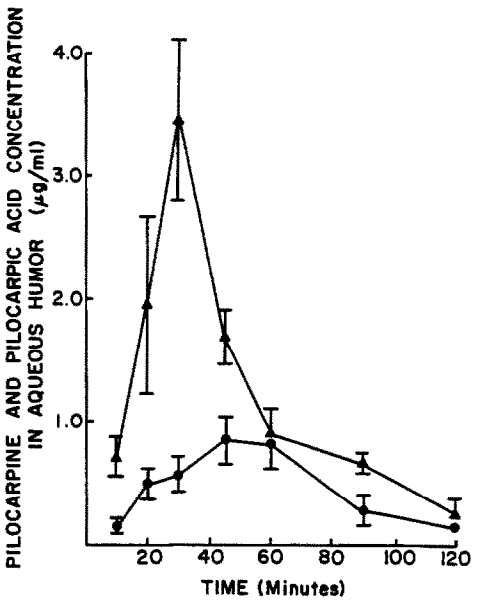

Fig. 1. Concentration of pilocarpine (▲———▲) and pilocarpic acid (●———●) in the aqueous humor of the pigmented rabbit following topical application of 5×10^{-2} M pilocarpine solution in the intact eye. Error bars represent standard error of the mean; $n \geq 7$.

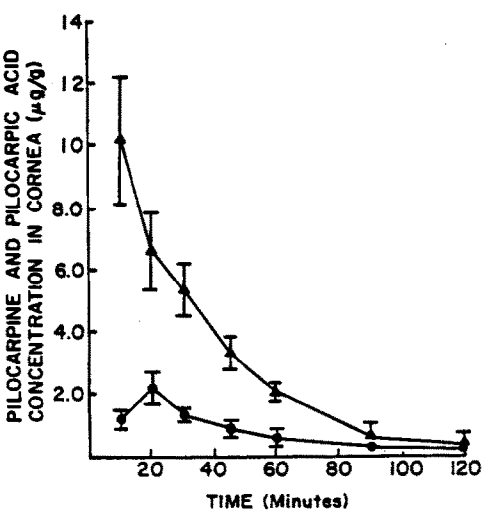

Fig. 2. Concentration of pilocarpine (▲———▲) and pilocarpic acid (●———●) in the cornea of the pigmented rabbit following topical application of 5×10^{-2} M pilocarpine solution in the intact eye. Errors bars represent standard error of the mean; $n \geq 7$.

ported for the albino rabbit (Sieg and Robinson, 1976) for which the rate of pilocarpine metabolism was found to be negligible. In this latter report, concentration–time profiles of pilocarpine in the cornea and aqueous humor were similarly treated by one-compartment pharmacokinetic analysis. These comparisons are shown in Table 1 and indicate that the apparent elimination rate constant describing the loss of pilocarpine from the cornea is significantly higher in the pigmented rabbit as compared to the albino rabbit. Conversely, the apparent elimination rate constants describing decline of pilocarpine from the aqueous humor are similar for both the pigmented and albino rabbit. The rate constants were compared by an *F*-test using the General Linear Model as described in Statistical Analysis Systems (SAS Users Guide: Statistics, 1982).

Inspection of Fig. 1 shows that pilocarpic acid appears in the aqueous humor in significant quan-

TABLE 1

APPARENT ELIMINATION RATE CONSTANTS OBTAINED FROM CONCENTRATION VERSUS TIME PROFILES OF PILOCARPINE IN THE CORNEA AND AQUEOUS HUMOR OF ALBINO AND PIGMENTED RABBITS

Species	k_{eli} (Intact cornea)	k_{el} (Abraded cornea)
Corneal elimination of pilocarpine:		
Albino [a]	0.02 min^{-1} (0.004) [b,c]	0.02 min^{-1} (0.002) [d]
	r [e] = 1.0	r = 0.98
Pigmented [f]	0.03 min^{-1} (0.002) [c]	0.03 min^{-1} (0.002) [d]
	r = 0.97	r = 0.97
Aqueous humor elimination of pilocarpine:		
Albino [a]	0.02 min^{-1} (0.002)	0.02 min^{-1} (0.002)
	r = 1.0	r = 0.98
Pigmented [f]	0.02 min^{-1} (0.003)	0.02 min^{-1} (0.002)
	r = 0.99	r = 0.98

[a] Data obtained from Sieg and Robinson (1976).
[b] Numbers in parentheses are standard errors of the estimated rate constant.
[c] Statistical difference between k_{el} (Intact cornea) of albino and k_{el} (Intact cornea) of pigmented at $P = 0.05$ for corneal elimination of pilocarpine.
[d] Statistical difference between k_{el} (Abraded cornea) of albino and k_{el} (Abraded cornea) of pigmented at $P = 0.05$ for corneal elimination of pilocarpine.
[e] r is correlation coefficient.
[f] $n \geq 7$.

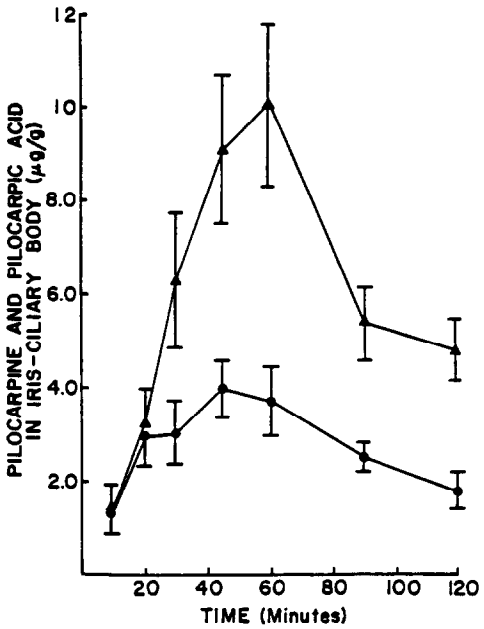

Fig. 3. Concentration of pilocarpine (▲———▲) and pilocarpic acid (●———●) in the iris-ciliary body of the pigmented rabbit following topical application of 5×10^{-2} M pilocarpine solution in the intact eye. Error bars represent standard error of the mean; $n \geqslant 7$.

tities following dosing with pilocarpine. There are two possible sources of the metabolite which appears in the aqueous humor. First, once intact pilocarpine is transferred across the cornea and enters the aqueous humor, there may be sufficient enzyme activity in this fluid to convert significant quantities of pilocarpine to pilocarpic acid. Second, pilocarpic acid may be formed in surrounding tissues such as the iris-ciliary body or cornea and subsequently transported to the aqueous humor. Elimination of pilocarpine from the aqueous humor arises primarily from normal aqueous humor turnover which has an associated rate constant of 0.01–0.02 min^{-1} (Sieg and Robinson, 1976) and absorption of pilocarpine into anterior segment tissue such as the iris-ciliary body. Moreover, metabolism of pilocarpine in the aqueous humor of the pigmented rabbit may additionally contribute to the overall elimination from this compartment. Assuming that the loss of pilocarpine from aqueous humor due to normal turnover and absorption by surrounding tissues are similar for both the albino and pigmented rabbit, then a significantly higher rate constant describing elimination of pilocarpine from the aqueous humor of the pigmented rabbit would indicate significant metabolism occurring in this fluid. However, an examination of Table 1 shows that the apparent rate constants describing elimination of pilocarpine from the aqueous humor in both the albino and pigmented animal are all of a similar magnitude, approximately 0.02 min^{-1}. This suggests, therefore, that metabolism of pilocarpine in the aqueous humor of the pigmented rabbit is insignificant and does not contribute to the overall elimination from this compartment. Rather, the appearance of pilocarpic acid in this fluid is due to its transport from the surrounding tissues. Minimal conversion of pilocarpine to pilocarpic acid in the aqueous humor is not surprising based on data generated by previous work. For example, Lee et al. (1983) found that esterase activity in the aqueous humor of the pigmented rabbit contributed only approximately 1% of the total esterase activity. Schonberg and Ellis (1969) incubated aqueous humor and serum of the albino rabbit with pilocarpine and were able to detect degradation of the substrate in the serum but reported no degradation of pilocarpine in the presence of aqueous humor.

Using an argument similar to that used in the case of aqueous humor, it may be determined whether the cornea is a site of metabolism for pilocarpine. From Table 1, it can be seen that the rate constants describing the apparent elimination of pilocarpine from the cornea in the albino rabbit, where epithelial cells have been removed and where the cornea remains intact prior to dosing, are similar, having a value of approximately 0.02 min^{-1}. However, the rate constants describing the apparent elimination of pilocarpine from the cornea of the pigmented rabbit in the two cases are approximately 0.03 min^{-1}. This increase in the magnitude of the rate constant must be attributed to an additional elimination route which is present in the pigmented rabbit but not present in the albino rabbit. If all other elimination routes are assumed to be similar in the two species and since pilocarpic acid is detectable in the cornea of the

pigmented rabbit, then metabolism of pilocarpine must be occurring in the cornea of the pigmented rabbit. In fact, the apparent rate constant describing the metabolism of pilocarpine in the cornea can be obtained by taking the difference between the elimination rate constants of pilocarpine in the pigmented and albino rabbit and it is found to be approximately 0.01 min^{-1}, which is in excellent agreement with the estimated value obtained by Lee et al. (1980). Additional work by Lee et al. (1983) supports the conclusion that metabolism of pilocarpine occurs in the cornea. These workers found approximately 32% of total esterase activity in ocular tissue and aqueous humor of the pigmented rabbit attributable to the cornea.

To examine the possibility of the corneal epithelium being a major site of pilocarpine metabolism in the cornea, the corneal epithelium was removed prior to dosing with pilocarpine. Fig. 4 shows that levels of pilocarpic acid in the cornea which has been abraded prior to dosing are similar to those obtained when the cornea remained intact. If the corneal epithelium is the sole site of corneal metabolism of pilocarpine, then removal of the corneal epithelium prior to dosing with pilocarpine should result in significantly reduced

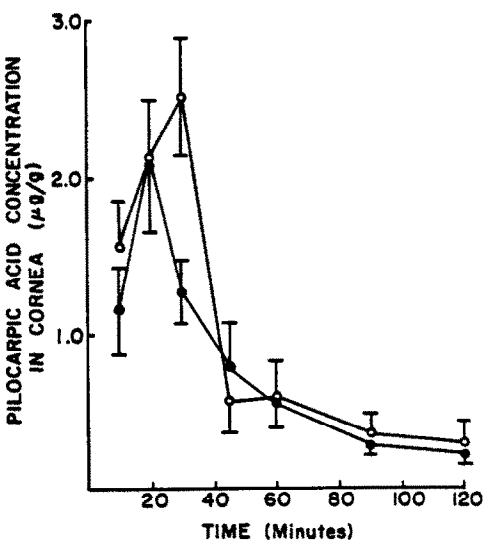

Fig. 4. Corneal concentration of pilocarpic acid in pigmented eyes following topical administration of 5×10^{-2} M solution of pilocarpine in abraded eyes (○) and intact eyes (●). Error bars represent standard error of the mean; $n \geq 7$.

levels of pilocarpic acid in the cornea. Consistent with the findings of Sieg and Robinson (1976), it was observed that upon removal of the corneal epithelium the extent of pilocarpine absorption was significantly increased. It could be argued therefore that if saturation of enzymatic activity is not being approached, then one would expect to observe higher concentrations of metabolite in the stroma simply due to the higher concentration of substrate present. Therefore the data presented in Fig. 4 does not warrant conclusions regarding relative contributions of the corneal epithelium and stroma to pilocarpine metabolism but they do suggest that the epithelium is not the sole site of corneal metabolism. This observation warrants further discussion. It has been established, based on similar pharmacokinetic parameters describing concentration versus time profiles of pilocarpine in the corneal stroma-endothelium compartment and the aqueous humor of the albino rabbit, that the endothelium does not present itself as a barrier for movement from the corneal stroma to the aqueous humor (Sieg and Robinson, 1976). However, it is interesting to note that in the pigmented rabbit, k_{el} for the stroma-endothelium is 0.03 min^{-1} whereas k_{el} for the aqueous humor is 0.02 min^{-1}. As has been discussed, the higher k_{el} for the stroma-endothelium of the pigmented rabbit as compared to the albino rabbit can be ascribed to metabolism. Nevertheless, if the stroma-endothelium and aqueous humor are to be considered as a single homogeneous compartment, as was done in the case of the albino rabbit, then the magnitude of k_{el} for the aqueous humor should approach that of the stroma-endothelium. This difference suggests that in the pigmented rabbit, the enzyme system responsible for the metabolism of pilocarpine resides in the corneal stroma-endothelium, among other sites, but not in the aqueous humor. If this is true, then the stroma-endothelium and the aqueous humor should not be considered as a single compartment when describing the overall disposition of pilocarpine in the pigmented rabbit.

There were insufficient data to abstract elimination rate constants of pilocarpine from the iris-ciliary body concentration–time profiles primarily due to the apparent binding and accumulation of pilocarpine in this tissue (Lee and Robinson, 1982).

TABLE 2

CONCENTRATION OF PILOCARPIC ACID IN THE AQUEOUS HUMOR AND IRIS-CILIARY BODY OF PIGMENTED EYES FOLLOWING TOPICAL APPLICATION OF EITHER 0.64 M PILOCARPIC ACID OR 0.05 M PILOCARPINE SOLUTIONS

Time (min)	Concentration following topical [a] application of pilocarpic acid		Concentration following topical [a] application of pilocarpine	
	mg/μl of aqueous humor	mg/g of iris-ciliary body	mg/μl of aqueous humor	mg/g of iris-ciliary
10	0.26 (0.12) [b]	0.76 (0.06)	0.14 (0.06)	1.28 (0.43)
20	0.37 (0.12)	0.82 (0.11)	0.48 (0.12)	2.96 (0.61)
30	0.62 (0.22)	0.80 (0.12)	0.56 (0.13)	3.04 (0.66)
45	0.46 (0.15)	0.55 (0.09)	0.84 (0.19)	3.97 (0.60)
60	0.33 (0.13)	0.62 (0.11)	0.81 (0.20)	3.72 (0.76)
90	0.13 (0.04)	0.52 (0.10)	0.27 (0.12)	2.48 (0.35)
120	0.07 (0.01)	0.38 (0.07)	0.14 (0.02)	1.83 (0.39)

[a] $n \geq 7$.
[b] Numbers in parentheses represent standard error of the mean.

However, an examination of pilocarpic acid disposition in the pigmented rabbit following either topical dosing with pilocarpic acid or topical dosing with pilocarpine lends insight into whether the iris-ciliary body is a site of pilocarpine metabolism.

Table 2 presents concentrations of pilocarpic acid in the aqueous humor and iris-ciliary body at various times post-instillation of either a pilocarpine or pilocarpic acid solution. Regardless of its relative concentration in the aqueous humor, concentration of pilocarpic acid in the iris-ciliary body is consistently higher following topical instillation of pilocarpine as compared to topical instillation of pilocarpic acid. This observation is expressed as ratios in Table 3. If one assumes that no metabolism of pilocarpine occurs in the iris-ciliary body following its transport to this tissue from the aqueous humor, then the ratio of pilocarpic acid concentration in the iris-ciliary body to its concentration in aqueous humor should be similar following either topical instillation of pilocarpine or pilocarpic acid. However, Table 3 indicates that the ratio obtained following topical instillation of pilocarpine is significantly higher than that obtained following dosing with pilocarpic acid at all time points monitored. This consistently higher ratio suggests that perhaps pilocarpine is converted to pilocarpic acid once pilocarpine permeates the iris-ciliary body. The observed higher ratio obtained following topical instillation of pilocarpine could be alternatively explained by different disposition kinetics of pilocarpic acid depending upon its source, (i.e. topical dosing versus metabolism) and/or binding of pilocarpic acid to the pigmented iris-ciliary body. Table 4 presents apparent elimination rate constants ob-

TABLE 3

RATIOS [a] OF PILOCARPIC ACID CONCENTRATION (IRIS-CILIARY BODY:AQUEOUS HUMOR) FOLLOWING TOPICAL APPLICATION OF PILOCARPINE AND PILOCARPIC ACID SOLUTIONS

Time (min)	$\frac{[\text{Iris-Ciliary Body}]}{[\text{Aqueous Humor}]}$ [b]	$\frac{[\text{Iris-Ciliary Body}]}{[\text{Aqueous Humor}]}$ [c]
10	2.92	9.14
20	2.22	6.17
30	1.29	5.43
45	1.20	4.73
60	1.88	4.59
90	4.00	9.19
120	5.43	13.07

[a] Ratios are based on mean data; standard error in all cases is < 50% of the mean.
[b] Ratio obtained following topical dosing of pilocarpic acid.
[c] Ratio obtained following topical dosing of pilocarpine.

TABLE 4

APPARENT ELIMINATION RATE CONSTANTS OBTAINED FROM CONCENTRATION VERSUS TIME PROFILES OF PILOCARPIC ACID IN THE AQUEOUS HUMOR AND IRIS-CILIARY BODY OF PIGMENTED RABBITS

Instilled solution	k_{el} for aqueous humor profiles (Intact cornea)	k_{el} for iris-ciliary humor (Intact cornea)
25 μl of 0.05 M pilocarpine	0.03 min^{-1} (0.002) [a] r [b] = 1.0	0.01 min^{-1} (0.001) r = 0.94
25 μl of 0.64 M pilocarpic acid	0.03 min^{-1} (0.003) r = 0.99	0.01 min^{-1} (0.002) r = 0.99

[a] Numbers in parentheses are standard errors of the estimated rate constant.
[b] r is correlation coefficient.

tained from one-compartment pharmacokinetic analysis of pilocarpic acid concentration versus time profiles following either a topical dose of pilocarpic acid or pilocarpine. These data suggest that elimination kinetics of pilocarpic acid following its appearance in either the aqueous humor or iris-ciliary body is similar regardless of its source, i.e. topical dosing vs pilocarpine metabolism. Moreover, the data shown in Table 2 indicate that accumulation of pilocarpic acid in the iris-ciliary body, following its topical instillation, does not appear to occur to the extent observed for other compounds (Lee and Robinson, 1982; Salazar and Patil, 1976; Salazar et al., 1976). In fact, it has been shown that in vitro, pilocarpic acid has a low affinity for synthetic melanin (Wood, 1984). Therefore, based upon the available data, it appears that the iris-ciliary body is an additional site of pilocarpine metabolism in the pigmented rabbit. Additional support for concluding that the iris-ciliary body is a site of pilocarpine metabolism comes from the work of Lee et al. (1983). These workers have shown the esterase activity in the iris-ciliary body of the adult pigmented rabbit is 2–3 times higher than esterase activity in the cornea and approximately 40 times higher than esterase activity in the aqueous humor.

The present studies have raised several issues which need to be addressed. For example, some of the conclusions of this report are based on previous work that has indicated that the rate of metabolism of pilocarpine in the albino rabbit is negligible. However, recent work (Salminen et al., 1984) has indicated that pilocarpine is significantly metabolized in the albino rabbit eye. The reason for this apparent discrepancy is not clear. However, since the influence of age on ocular drug disposition is well documented (Lee et al., 1983; Miller and Patton, 1981; Miller and Patton, 1982; Francoeur et al., 1983), it is possible that animal age may have contributed to the difference.

In addition, in the present studies, ocular tissues and fluids in which pilocarpine and pilocarpic acid concentrations were monitored were limited to the cornea, iris-ciliary body and aqueous humor. Therefore, it remains to be seen whether metabolism of pilocarpine occurs at sites other than the cornea and iris-ciliary body. For example, prior to corneal permeation of pilocarpine it may be possible for metabolism to occur in the precorneal area, i.e. conjunctiva or tear fluid. Although apparent esterase activity has been detected in the tear fluid (Wood et al., 1985), it is expected that only a small fraction of pilocarpic acid detected in the cornea is contributed from the precorneal area because of the observed low corneal permeability of pilocarpic acid.

In summary, it has been found that pilocarpine, upon topical instillation, is subject to metabolism in the cornea and iris-ciliary body of the pigmented rabbit but not the aqueous humor. Significant corneal metabolism appears to occur in the corneal stroma-endothelium in addition to the corneal epithelium. Unlike the albino rabbit eye, the stroma-endothelium and aqueous humor cannot be considered as a single homogeneous compartment when describing the ocular disposition of pilocarpine in the pigmented rabbit. This is due to observed differences in metabolism in the two compartments of the pigmented rabbits.

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