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Effect of ocular pigmentation on pilocarpine pharmacology in the rabbit eye. I. Drug distribution and metabolism

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Summary

Systemic absorption, ocular metabolism and ocular distribution of pilocarpine were studied after ocular application of 2% solution of [^{3}H]pilocarpine in albino and pigmented rabbits. Systemic absorption of ocularly applied pilocarpine was rapid. In the eye pilocarpine was efficiently and equally metabolized to pilocarpic acid in albino and pigmented rabbits. Pilocarpine accumulated in the pigmented iris, ciliary body, and choroid and retina more than in the corresponding albino tissues. Pretreatment with non-labelled pilocarpine did not affect the concentration of [^{3}H]pilocarpine in the pigmented iris, ciliary body or choroid and retina, indicating a high capacity for drug binding in these tissues.

Introduction

Pigmented iris, ciliary body and ciliary muscle of rabbit and monkey take up pilocarpine more than the corresponding albino tissues. After incubation in vitro in pilocarpine solution the difference in pilocarpine concentration of pigmented and albino iris was 2–3-fold (Lyons and Krohn, 1973; Newsome and Stein, 1974; Lazare and Horlington, 1975). The miotic response induced by pilocarpine was more rapidly washed off from albino than pigmented iris in vitro (Ohara, 1977). As far as concentration is concerned, the wash-off of pilocarpine from the ocular tissues has

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not been studied. Recently, in vivo uptake of pilocarpine by the pigmented anterior uvea was studied after application of low concentration, i.e. 0.1, 0.2, 0.4 and 0.8%, eye drops (Lee and Robinson, 1982). According to areas under the curve (AUC) of drug concentration vs time pilocarpine accumulated 10 times more in the pigmented iris-ciliary body than in the corresponding albino tissue. The pigment uptake of pilocarpine was concentration dependent and showed no saturation with the above doses.

Since uneven distribution of pilocarpine may have consequences in ophthalmic drug therapy, we studied the distribution of 2% pilocarpine eye drops in albino and pigmented rabbit eyes. Some of the eyes had been pretreated with non-labelled pilocarpine. The amount of pilocarpine metabolites in the aqueous humor was also observed, since it has been found that there is a difference in this respect between albino and pigmented rabbits (Lee et al., 1980).

Materials and Methods

Animals

11 albino rabbits, (New Zealand Whites) (NZW), 2.5–3.7 kg, and 10 mixed-bred pigmented rabbits, 2.0–3.6 kg, were used. Prior to the test, the animals were housed singly in cages under standard laboratory conditions: 10 h dark/14 h light cycle, 20.0 ± 0.5°C temperature, 55–75% relative air humidity. The animals had no restrictions as to food or water.

Drugs

[^{3}H-G]Pilocarpine alkaloid in alcohol with a specific activity of 10.0 Ci/mmol was obtained commercially (New England Nuclear, Boston, MA) and evaporated to dryness by vacuum distillation. [^{3}H]Pilocarpine was dissolved in distilled water 3 times, and the solvent evaporated each time. Finally the labelled material was dissolved in a 2% pilocarpine base solution which had been buffered to pH 6.4 with phosphates. The addition of [^{3}H]pilocarpine had no appreciable effect on the molarity of the drug solution. The radioactivity of the solution was 0.29 μCi/μl. The purity of the tracer (97%) was established using thin-layer chromatography (TLC). Tritiated pilocarpic acid was prepared according to Repta and Higuchi (1971).

Drug distribution studies

During the experiment the rabbits were kept in wooden restraint boxes. A 30 μl volume of the labelled pilocarpine solution was instilled onto the upper corneoscleral limbus of both eyes. During instillation the upper lid was gently pulled away from the globe. Five pigmented and 4 albino rabbits received 30 μl of unlabelled 2% solution of pilocarpine into one eye 30 min before the labelled drop.

The animals were sacrificed with i.v. injection of Nembutal at 30 or 180 min after tritiated pilocarpine instillation. Aqueous humor samples were aspirated from the anterior chamber of the enucleated eyes and the eyes were promptly frozen in liquid nitrogen. Part of the aqueous humor was used for the TLC-analysis of pilocarpic

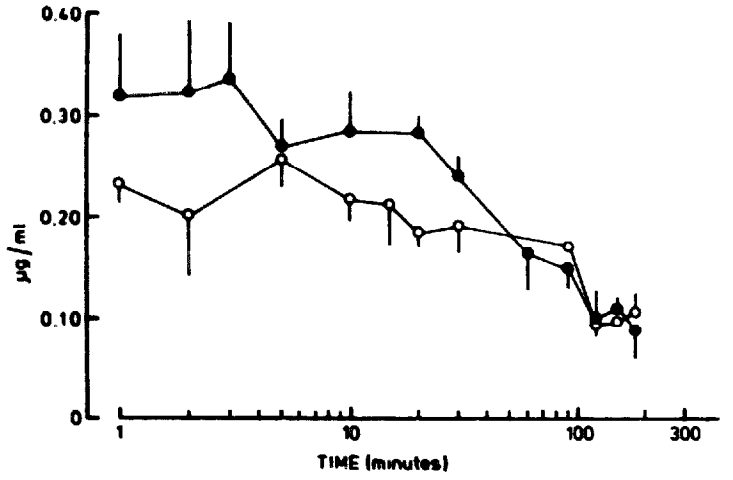

Fig. 1. Equivalent of pilocarpine (µg/ml) in rabbit plasma after application of 30 µl of 2% [^{3}H]pilocarpine into both eyes of 7 albino (open circles) and 9 pigmented (closed circles) rabbits. Vertical bars represent S.E.s.

TABLE 1

PERCENTAGE OF TOTAL DRUG DUE TO PILOCARPIC ACID IN AQUEOUS HUMOR OF ALBINO AND PIGMENTED RABBITS AFTER OCULAR APPLICATION OF 30 µl OF 2% [^{3}H]PILOCARPINE. EACH VALUE REPRESENTS THE MEAN ± S.E. OF 4 SAMPLES

Animals	Time after instillation	
	30 min	180 min
Albino	30.2 ± 6.8	70.4 ± 11.8
Pigmented	24.2 ± 3.0	64.0 ± 6.9

TABLE 2

RADIOACTIVE MATERIAL DISTRIBUTION IN ALBINO AND PIGMENTED RABBIT EYE 30 AND 180 min AFTER APPLICATION OF 30 µl OF 2% [^{3}H]PILOCARPINE. VALUES SHOW THE MEANS ± S.E. OF 9 ALBINO AND 8 PIGMENTED EYES

Tissue	Equivalent of pilocarpine (µg/g of tissue)			
	30 min		180 min	
	Albino	Pigmented	Albino	Pigmented
Cornea	20.83 ± 5.52	38.11 ± 5.74 **	2.43 ± 0.39	5.54 ± 1.57
Aqueous humor	8.01 ± 1.50	13.88 ± 2.02 **	1.19 ± 0.14	2.09 ± 0.29 *
Iris	5.36 ± 1.88	14.64 ± 2.37 **	1.79 ± 0.47	11.85 ± 2.92 ***
Ciliary body	3.48 ± 1.00	10.86 ± 2.15 **	0.85 ± 0.14	8.77 ± 3.13 ***
Conjunctiva	4.56 ± 2.08	7.84 ± 2.69	1.85 ± 1.33	2.01 ± 0.53
Anterior sclera	3.96 ± 0.62	9.29 ± 1.65 **	0.70 ± 0.28	2.23 ± 0.38 **
Lens	0.25 ± 0.03	0.22 ± 0.03	0.114 ± 0.007	0.125 ± 0.006
Vitreous	0.068 ± 0.009	0.084 ± 0.013	0.147 ± 0.099	0.068 ± 0.003 *
Choroid and retina	0.66 ± 0.16	2.14 ± 0.33 ***	0.18 ± 0.08	2.04 ± 0.80 ***

* $P < 0.05$ vs albino eyes.
** $P < 0.01$ vs albino eyes.
*** $P < 0.001$ vs albino eyes.

acid. The eyes were stored at −20°C until the dissection of the tissues, which was carried out as described previously (Salminen, 1978).

Blood samples were collected during the test from a precannulated ear artery. The samples were centrifuged at 2300 rpm for 15 min and 200 μl plasma samples were pipetted into the scintillation vials.

The radioactivity of the samples was determined by liquid scintillation counting. The tissue samples were digested with a solubilizer (Lumasolve, Lumac B.V., Schaesberg, The Netherlands) in glass vials at 40–50°C. Scintillation liquid (Lipoluma, Lumac) was added and samples were counted when the chemiluminescence had vanished. The scintillation liquid (Lumagel, Lumac) was added directly to the aqueous humor and plasma samples. All the samples were counted at 9°C in an LKB Wallac Rackbeta 1215 liquid scintillation counter for 10 min or up to 10,000 counts.

Metabolic studies

The proportional amounts of pilocarpine and pilocarpic acid were analyzed according to Lee et al. (1980). Portions (4 × 10 μl) of aqueous humor samples were applied to Whatman Linear LK5-D preadsorbent silica-gel plates (Pierce Chemicals, Rockford, IL). The plates were developed in *n*-butanol saturated with 14.8 M NH_4OH and allowed to air dry. The path of the solvent front was divided into 10 equal sections, which were scraped off the plate, transferred to scintillation cocktails (Lipoluma) and counted as described earlier. The approximate R_f-values of pilocarpine and pilocarpic acid were 0.85 and 0.34.

Analysis of the results

The results are expressed as means ± S.E. of pilocarpine radioactivity equivalents (μg/g of tissue wet weight), denoted total pilocarpine concentration in the text. Statistical significances of the differences were tested with Mann-Whitney's U-test. A *P*-value of less than 0.05 was considered to be significant.

Results

Ocularly applied pilocarpine rapidly entered the systemic blood circulation (Fig. 1). High plasma concentrations of total pilocarpine were achieved at 1 min and peak concentrations within 3–5 min.

The metabolism of pilocarpine was considerable in both albino and pigmented rabbits (Table 1). Significant differences in the relative amounts of pilocarpic acid were not observed in this study between the two rabbit strains.

At 30 min, the pigmented iris, ciliary body and choroid and retina, in this order, contained more radioactivity than the corresponding albino tissues (Table 2). The proportional distribution of total pilocarpine in these tissues was the same in both rabbit strains, but the concentrations were 2–3-fold higher in the pigmented than in the albino tissues. The concentrations of total pilocarpine in pigmented rabbits also exceeded those of albino rabbits in the cornea, aqueous humor and anterior sclera.

TABLE 3

RADIOACTIVE MATERIAL DISTRIBUTION IN ALBINO AND PIGMENTED RABBIT EYES 30 min AFTER APPLICATION OF 30 μl OF 2% [^{3}H]PILOCARPINE. THE EYES WERE PRE-TREATED WITH 30 μl of 2% NON-LABELLED PILOCARPINE BEFORE APPLICATION OF RADIOACTIVE EYE DROP. VALUES SHOW THE MEANS ± S.E. OF 5 ALBINO AND 4 PIGMENTED EYES

Tissue	Equivalent of pilocarpine (μg/g of tissue)	
	Albino	Pigmented
Cornea	15.48 ± 2.69	28.40 ± 4.56 *
Aqueous humor	7.21 ± 1.44	10.02 ± 1.74
Iris	3.68 ± 0.67	14.80 ± 4.00 **
Ciliary body	2.81 ± 0.31	7.93 ± 2.34 **
Conjunctiva	3.6[illegible] ± 1.25	5.18 ± 1.44
Anterior sclera	3.18 ± 0.82	6.56 ± 1.18 *
Lens	0.28 ± 0.04	0.20 ± 0.02
Vitreous	0.11 ± 0.03	0.15 ± 0.05
Choroid and retina	0.47 ± 0.12	1.13 ± 0.19 *

* $P < 0.05$ vs albino eyes.
** $P < 0.01$ vs albino eyes.

Pretreatment of the eyes with non-labelled 2% solution of pilocarpine did not cause statistically significant changes in the radioactivity of the ocular tissues (Table 3).

In the cornea, aqueous humor and anterior sclera of pigmented and albino rabbits, and in the choroid and retina and anterior uvea of albino rabbits, the concentrations of total pilocarpine at 180 min were only 11–40% of the values in these tissues at 30 min (Table 2), indicating the rapid elimination of radioactivity from these tissues. In pigmented tissues the levels at 180 min were 81–95% of those at 30 min, indicating slow elimination of the total pilocarpine.

Discussion

The plasma concentrations of total pilocarpine after ocular application found in this study corresponded well to the values of Patton and Francouer (1978), which included only data points of 15, 30 and 60 min. The rapid conjunctival absorption of ocular pilocarpine into the systemic circulation was probably due to the conjunctival vasodilatation caued by the drug (Ehlers, 1977). Systemic absorption contributed to the ocular concentration of total pilocarpinc via the blood stream. On the other hand, conjunctival absorption accelerated the precorneal loss of pilocarpine, thus decreasing the ocular bioavailability of the drug (Lee and Robinson, 1979).

The metabolism of pilocarpine in the tissues of albino rabbit eyes has previously been studied in vitro (Makoid and Robinson, 1979) and after long-term therapy in vivo (Sendelbeck et al., 1975). In vitro metabolism was minimal; in vivo, after 4 days' treatment, 65% of the drug was in the form of pilocarpic acid and 5% as isopilocarpine. In the eyes of pigmented rabbits, extensive corneal metabolism of

pilocarpine in vivo has been reported by Lee et al. (1980). According to our in vivo results, obtained using a similar method to that of Lee et al. (1980) pilocarpine was extensively metabolized in the eyes of both albino and pigmented rabbits. Our results are consistent with the reported esterase activities of the eyes of albino and pigmented rabbits (Lee et al., 1983). Total esterase activity in the cornea, aqueous humor, and iris-ciliary body of albino and pigmented rabbits was about equal, with a high α-naphthyl acetate substrate concentration (6×10^{-5} M) in the oldest (12 weeks) rabbit group of the study. In our experiment the rabbits were more than 12 weeks old and the pilocarpine concentrations in the aqueous humor at 30 min were $4–7 \times 10^{-5}$ M.

In albino rabbits pilocarpine forms a reservoir in the corneal epithelium; from here it is released into the aqueous chamber compartment, which includes the aqueous humor, iris and ciliary body (Makoid and Robinson, 1979). Thus the rate of elimination of pilocarpine from the aqueous humor and anterior uvea is about equal. In our study pilocarpine was rapidly eliminated from the aqueous humor, iris and ciliary body of albino rabbits. The proportionate distribution of ocularly applied pilocarpine corresponded to the results obtained by Lazare and Horlington (1975), Sieg and Robinson (1976), Makoid and Robinson (1979), and Salminen (1979).

In the anterior structures of the pigmented rabbit eye, the relative distribution of total pilocarpine was characterized by accumulation of the drug in the iris and ciliary body. At 30 min, 2–3 times more radioactivity was present in the pigmented than in the albino anterior uvea. In the elimination phase (at 180 min), the difference was 6–10-fold. The concentration of pilocarpine in the choroid and retina was also related to ocular pigmentation. The difference in radioactivity between pigmented and albino choroid and retina increased from 3-fold at 30 min to 11-fold at 180 min. The increase in the difference between total pilocarpine concentrations in pigmented and albino tissues during the experiment is caused by the slower elimination of the drug from pigmented tissues.

The cornea, aqueous humor and anterior sclera of pigmented rabbits likewise contained more radioactivity after ocular application than the corresponding tissues of the albino rabbits. Lazare and Horlington (1975) have reported differences in the penetration of pilocarpine into the aqueous humor between 4 rabbit strains. These differences may be caused by different precorneal rates of drug loss in the different strains. This is supported by our preliminary results, which show that the rate of precorneal loss of pilocarpine in pigmented rabbits is slower than in albino rabbits. Another explanation of the differences in the concentrations of the anterior sclera is the unavoidable contamination of the sclera by the pigment of the adjacent anterior uvea during dissection. The higher concentration of pilocarpine in the aqueous humor of pigmented than albino rabbit eye does not, however, explain the difference in drug concentrations in the iris and ciliary body in these animals. The ratio of iris and ciliary body pilocarpine concentrations to the concentration in the aqueous humor was greater ($P < 0.05$) in pigmented than in albino rabbits at 30 min and at 180 min.

Topical pretreatment of the eyes with non-labelled pilocarpine caused no change in the concentrations of labelled pilocarpine in the albino and pigmented eyes. The

capacity of the albino anterior uvea to bind pilocarpine in vitro was 2–4 μg/mg and that of corresponding pigmented tissues 7–8 μg/mg (Lyons and Krohn, 1973). These concentrations are about 1000 times higher than the concentrations of pilocarpine in the iris and ciliary body in the present study. In the in vitro incubation study of Lyons and Krohn (1973), the wash-off of pilocarpine was not studied and the concentration of the drug in the incubation medium was high (500 μg/ml) compared to that found in the aqueous humor in our study (8–14 μg/ml). The elimination of pilocarpine from the aqueous humor, anterior uvea and choroid and retina in the eyes of albino rabbits is fast and the drug levels after multiple dosing depend strongly on the interval between applications of the drug (Makoid and Robinson, 1979). After frequent administration (30 min interval) pilocarpine levels are increased even in albino rabbit eyes (Makoid and Robinson, 1979), but after treatment at 6-h intervals with 2% pilocarpine nitrate eye drops for 8 days the concentrations of the drug are about equal to those of our acute experiment (Sendelbeck et al., 1975). The pigmented tissues showed virtually no decrease in the drug concentration during 180 min after application. In addition the peak concentration of pilocarpine was achieved later (90 min) in pigmented than in albino (20 min) anterior uvea (Lee and Robinson, 1982). Consequently the real total pilocarpine concentration of pigmented anterior uvea of the pretreated eyes of this study was probably 2–3-fold compared to the nominal total concentrations of Table 3.

Since in our findings pilocarpine was slowly eliminated from the pigmented tissues, and the capacity of these tissues to bind pilocarpine has been shown to be high (Lyons and Krohn, 1973) it is probable that considerable drug accumulation occurs during long-term treatment in humans. The accumulation of ophthalmic pilocarpine in pigmented ocular tissues may explain the relative resistance to the ocular hypotensive response to pilocarpine in patients with heavily pigmented eyes (Melikian et al., 1971; Harris and Galin, 1971; Sherman, 1977).

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