International Journal of Pharmaceutics, 19 (1984) 53–61
Elsevier

IJP 00642

Effect of ocular pigmentation on pilocarpine pharmacology in the rabbit eye. II. Drug response

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(Received September 1st, 1983)
(Accepted October 28th, 1983)

Summary

The time course of the miotic response of pilocarpine in albino and pigmented rabbit eyes was studied after ocular application of 0.11, 0.43, 0.85 and 2.30 mg doses in eye drops and 0.85 and 2.30 mg doses in polymer matrices. When administered in eye drops ocular pigmentation delayed the onset of the peak effect of the 3 smallest pilocarpine doses. The magnitude of the peak effect was lower in pigmented than in albino eyes after 0.11 and 0.43 mg doses, but equal after larger doses. Ocular pigmentation increased the relative biophasic availability of 0.85 and 2.30 mg doses of pilocarpine. This was due to the slower elimination rate of pilocarpine from pigmented tissues. The relative biophasic availability of 0.11 and 0.43 mg doses of pilocarpine was not affected by the ocular pigmentation, because of the opposite effects of lower peak effect and slower elimination rate on biophasic availability in pigmented eyes. The administration of pilocarpine in polymer matrices increased the relative biophasic availability of the drug. When administered in polymer matrices, pilocarpine showed a typical time course of prolonged pulse-entry of the drug into the eye.

Introduction

The pigmented irides of rabbits and rhesus monkeys bind pilocarpine more extensively than albino irides in vitro (Lyons and Krohn, 1973; Lazare and Horling-

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ton, 1975). Ocularly administered pilocarpine is also accumulated in the pigmented tissues of the eye (Lazare and Horlington, 1975; Lee and Robinson, 1982; Salminen et al., 1983b). As shown by the areas under the curves of tissue concentration vs time, 10 times more pilocarpine is located in the iris-ciliary body of pigmented than of albino rabbits (Lee and Robinson, 1982). The difference in the availability of pilocarpine between the anterior uvea of pigmented and albino rabbits was due to the greater uptake and slower rate of drug elimination from the pigmented anterior uvea (Lee and Robinson, 1982; Salminen et al., 1983b).

Constriction of the iris sphincter muscle induced by pilocarpine was prolonged in pigmented irides compared to albino ones in vitro (Ohara, 1977). Comparisons of the miotic effect of pilocarpine in the albino and pigmented rabbit eyes have not been made in intact eyes. In this study we have compared the time course of miosis after ocular application of various pilocarpine doses in albino and pigmented rabbit eyes. The drug was administered in aqueous eye drops and polymer matrices. The latter dosage form is intended to prolong the duration of the drug action in the eye.

Materials and Methods

Animals

Five to six New Zealand White albino rabbits (3.1–3.9 kg) and mixed-breed pigmented rabbits (2.7–3.6 kg) were used. Before 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 and water.

Pilocarpine doses and dosage forms

Pilocarpine was administered in the form of a hydrochloride. Four different aqueous pilocarpine hydrochloride solutions were used: 0.5% and 2.0% in Sorensen's phosphate buffer (pH 6.4), 4.0% commercial eye drops with 1.4% of poly(vinyl alcohol) (PVA) (Oftan Pilocarpin, Pharmaceutical Manufacturers Star, Tampere, Finland; pH 4.9), and a 10.8% solution with 1.4% of PVA (pH 7.0). The commercial solution contained a preservative (0.004% of benzalkonium chloride). The test solutions were prepared immediately before each experiment. The volume applied was in all cases 25 μl, with corresponding pilocarpine doses (expressed as base equivalents) of 0.11, 0.43, 0.85 and 2.30 mg.

Doses of 0.85 and 2.30 mg (pilocarpine base equivalents) were also administered in polymer matrices. The matrices contained a mixture of poly(acrylamide) and copolymer of acrylamide, N-vinylpyrrolidone and ethyl acrylate (Khromov et al., 1976). The inserts were 4.5 mm wide, 0.35 mm thick and 3.3 mm (0.85 mg of the drug) or 9 mm (drug content 2.3 mg) long.

Test procedure

Rabbits were acclimatized to the wooden restraint boxes and dim lighting for 2 h prior to the experiments. The eye drops were pipetted onto the upper corneoscleral limbus of the eye. During the instillation the upper lid was slightly pulled away from

the globe. The inserts were placed in the inferior conjunctival fornix. The surface of the polymer matrix softened within a few minutes due to the rapid tear fluid uptake of the polymer. In the course of the experiment, the matrices dissolved in the tear fluid. Only one eye of a rabbit was used in the test procedure. An interval of at least one week was held between two tests with the same animal.

After application of the drug the eyes were photographed from a constant distance at fixed times. The negatives were enlarged with a microfilm reflector. The pupillary area was measured from the magnifications with a planimeter and the diameters of the pupils were calculated as though the pupils were circular.

Analysis of the time course of miosis

The biophasic pharmacokinetics of pilocarpine were analyzed according to Yoshida and Mishima (1975). The analysis is based on a response parameter (RP) which is related to the drug concentration surrounding the receptors (Wagner, 1968; Ohara, 1977). The changes in pupillary diameter were converted to values of RP according to the equation of Yoshida and Mishima (1975): $RP = (D_0 - D)/(D - D_{min})$, where D_0 = the original pupillary diameter, D = the diameter at a given time and D_{min} = the minimum attainable diameter (maximal response). The value for the minimum attainable diameter was attained by ocular application of 35 μl of 0.5% physostigmine salicylate twice with a 2-min interval between the instillations (Loewenfeld and Newsome, 1971). The minimum attainable pupillary diameter was 2.16 mm, corresponding to a reduction of about 5 mm in the diameter. This was in accordance with the results of Mikkelson et al. (1973), obtained after instillation of 5.2 mg pilocarpine in aqueous solution with 0.02% of cetylpyridinium chloride.

Yoshida and Mishima (1975) used a two-compartment model for the calculation of pharmacokinetic parameters from the values of the response parameter. This model is based on the fact that the amount of pilocarpine in the corneal epithelium reaches its peak levels within 2 min after instillation of the drug (Sieg and Robinson, 1976). After that, drug levels of the corneal epithelium fall exponentially as the drug diffuses into the aqueous chamber compartment (Yoshida and Mishima, 1975). The biophase of the iris is included in the aqueous chamber compartment. In the case of the polymer matrices, however, the dosage form is a third compartment, from which pilocarpine is slowly released and taken up by the corneal epithelium. The amount of pilocarpine does not decrease exponentially in the corneal epithelium, since the pilocarpine is at the same time absorbed into and released from this tissue. This leads to a prolonged absorption phase and to deviations from the kinetics of the two-compartment model. Instead of fitting our data to the model, we used numerical methods in our calculations. The values of RP were plotted against time. Actual data points were used to evaluate the magnitude of the peak response and its time delay. Relative biophasic availability (AUC) was measured as the area under the RP vs time curve, using a trapezoidal rule with extrapolation to infinite time according to Gibaldi (1977). The apparent elimination rate constant was determined with linear regression analysis from the ln(RP) vs time representation. Data points after the maximum of ln(RP) were used when eye drops were concerned. In the case of polymer matrices data points after the plateau phase were used.

The statistical significance of the differences in pharmacokinetic parameters between pigmented and albino rabbits was tested using Mann-Whitney's U-test and that between polymer matrices and aqueous solutions using Wilcoxon matched-pairs ranked-signs test. $P < 0.05$ was considered to be a significant difference.

Results

The pharmacokinetic constants of miosis induced by pilocarpine were calculated separately for each experiment, and the means ± S.E. of these separate constants are presented in Table 1. In Figs. 1 and 2 the mean responses ± S.E. are shown for each time. Consequently the differences between the magnitudes of the peak response in Figs. 1 and 2 and Table 1 result from the different methods of data treatment.

The time delay of the onset of the peak miotic response to pilocarpine was longer in pigmented than in albino eyes with the drug doses of 0.11, 0.43 and 0.85 mg in aqueous solutions, but not with the dose of 2.30 mg (Table 1, Fig. 1). With the doses of 0.11 and 0.43 mg the magnitude of the peak miotic response to pilocarpine was smaller in pigmented than in albino eyes. The higher drug doses (0.85 and 2.30 mg)

TABLE 1

PHARMACOKINETIC PARAMETERS OF THE MIOTIC RESPONSE INDUCED BY PILOCARPINE IN THE EYES OF ALBINO AND PIGMENTED RABBITS

Dosage form and dose (mg)		Peak effect		AUC (RP·h)	Apparent elimination rate constant (h^{-1})	n
		Time delay (min)	Magnitude (RP) [a]			
Aqueous solutions						
0.11	(A) [b]	21.0 ± 1.9	0.94 ± 0.09	1.37 ± 0.09	0.69 ± 0.08	5
	(P) [c]	64.0 ± 6.8 **	0.51 ± 0.09 **	1.62 ± 0.39	0.31 ± 0.08 **	5
0.43	(A)	21.2 ± 2.0	1.12 ± 0.19	1.18 ± 0.18	0.58 ± 0.06	5
	(P)	48.0 ± 7.8 **	0.67 ± 0.08 *	2.18 ± 0.55	0.42 ± 0.07	5
0.85	(A)	20.4 ± 2.8	1.51 ± 0.17	1.60 ± 0.15	0.79 ± 0.09	5
	(P)	45.0 ± 0.0 **	1.85 ± 0.26	5.22 ± 0.57 **	0.43 ± 0.04 **	6
2.30	(A)	20.0 ± 2.7	1.44 ± 0.08	2.45 ± 0.17	0.81 ± 0.05	5
	(P)	21.7 ± 2.1	1.73 ± 0.18	5.12 ± 0.33 **	0.38 ± 0.02 **	6
Inserts						
0.85	(A)	15–35 [d]	2.05 ± 0.36	5.66 ± 0.86	0.89 ± 0.08	6
	(P)	15–30 [d]	2.43 ± 0.24	12.26 ± 2.25 *	0.33 ± 0.04 **	6
2.30	(A)	15–25 [d]	3.04 ± 0.46	7.43 ± 1.43	0.88 ± 0.04	5
	(P)	25–210 [d]	2.75 ± 0.26	18.28 ± 2.78 **	0.31 ± 0.07 **	6

[a] RP = response parameter = (original pupillary diameter − pupillary diameter)/(pupillary diameter − 2.16 mm).

[b] A = albino rabbits.

[c] P = pigmented rabbits.

[d] Approximate starting point of plateau phase.

* $P < 0.05$, ** $P < 0.01$ pigmented vs albino eyes (Mann-Whitney's U-test).

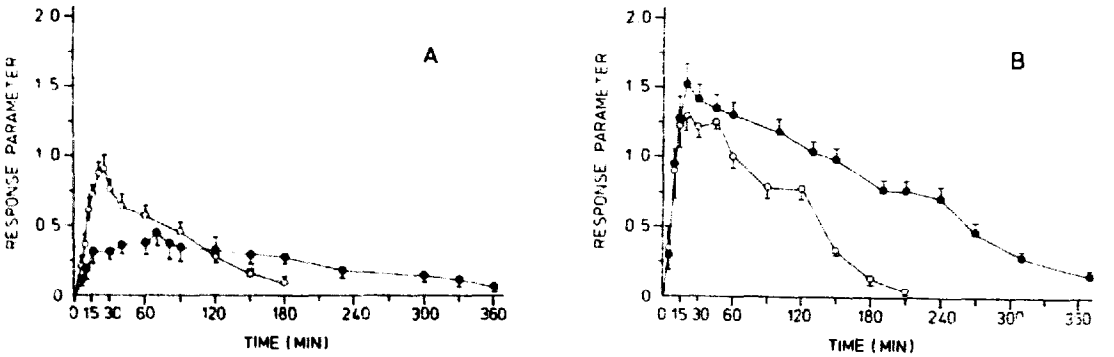

Fig. 1. Mean miotic response ± S.E. to pilocarpine (A: 0.11 mg; and B: 2.30 mg) after administration of aqueous eye drops in the eyes of albino (○) and pigmented (●) rabbits. Corresponding apparent pharmacokinetic parameters ± S.E. are presented in Table 1.

caused roughly equal peak responses in the eyes of pigmented and albino rabbits. The AUC increased with ocular pigmentation for pilocarpine doses of 0.85 and 2.30 mg, whether the drug was administered as a solution or in polymer matrix. With 0.11 and 0.43 mg doses of pilocarpine, the AUC values did not differ between pigmented and albino rabbits. The constants for the apparent rate of drug elimination from the biophase of the iris were smaller in pigmented than in albino eyes.

When pilocarpine was administered in polymer matrices, RP vs time curves plateaued. Consequently, in this case Table 1 gives the approximate starting point of the plateau phase instead of the time delay of the peak response. The onset of the miotic response after application of pilocarpine ocularly in a polymer matrix was as fast as after eye drop administration. The application of pilocarpine in a polymer matrix increased the mean of magnitude of the peak response, but the increase was only in one case (0.85 mg dose in pigmented eyes) statistically significant ($P < 0.05$). A typical time course of the response after ophthalmic administration of pilocarpine

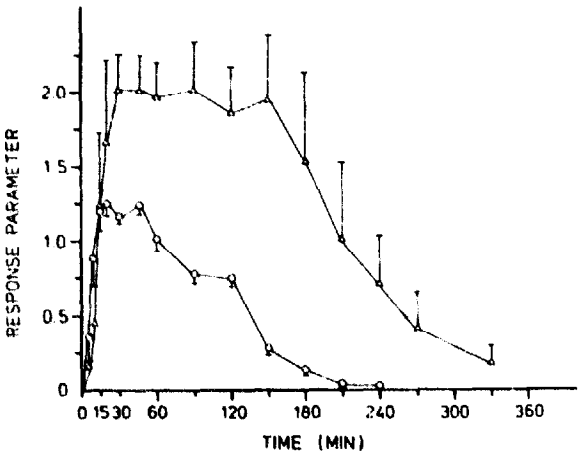

Fig. 2. Mean miotic response ± S.E. to 2.30 mg of pilocarpine in the eyes of albino rabbits after ocular application in aqueous solutions (○) and in polymer matrices (△). The corresponding apparent pharmacokinetic parameters ± S.E. of the data are shown in Table 1.

in the two dosage forms is shown in Fig. 2. Administration of pilocarpine in a polymer matrix increased the AUC of the drug in all cases compared to aqueous pilocarpine solutions ($P < 0.05$). The apparent elimination rate constants of pilocarpine did not differ between eye drop and polymer matrix groups.

Discussion

Anatomically the iris is a porous tissue which allows the rapid penetration of pilocarpine into its biophase (Smelser and Ishikawa, 1962). In the eyes of albino rabbits the peak concentration of pilocarpine in the aqueous humor and the time delay of the peak miotic response are equal, i.e. 20 min (Chrai and Robinson, 1974; Makoid and Robinson, 1979). In this study too the peak miotic response in the eyes of albino rabbits was reached at 20 min after administration of eye drops. The time delay of the peak miotic response in the eyes of pigmented rabbits was longer and dependent on the pilocarpine dose. An increase in the dose shortened the time delay of peak miosis in the eyes of the pigmented rabbits (Table 1, Fig. 1). Compared to the albino eyes, ocular pigmentation reduced the magnitude of the peak effect with small doses (0.11 and 0.43 mg) of pilocarpine but not with large doses (0.85 and 2.30 mg) (Table 1, Fig. 1). This behavior is explained by the binding of pilocarpine to the ocular pigmentation. The binding of the drug to the pigment retards the access of pilocarpine to the receptors and decreases the concentration of the free drug, thus delaying the onset of peak effect and reducing its magnitude. Alternatively this difference in the time course of the drug action may be explained by differences in the absorption and metabolism of pilocarpine. In the first part of our study (Salminen et al., 1983b), however, no metabolic differences were observed between albino and pigmented rabbit strains and ages, which were the same as those used here. With very small doses (0.05 mg), differences of metabolism (Makoid and Robinson, 1979; Lee et al., 1980) may partly explain the differences in the peak effect. Since total radioactivity in the aqueous humor after administration of 0.6 mg dose of tritiated pilocarpine was higher in pigmented than in albino eyes (Salminen et al., 1983b) and after 0.05 mg dose the aqueous humor radioactivities were equal in the rabbit strains (Lee and Robinson, 1982), differences in corneal drug penetration likewise do not explain the delayed and reduced peak responses to pilocarpine. In human eyes increased ocular pigmentation delayed the onset of peak miosis of 0.36 mg of pilocarpine but did not affect its magnitude (Smith et al., 1978).

With doses of 0.85 and 2.30 mg, no reduction of the peak response by ocular pigmentation was observed. Increase of the dose from 0.85 to 2.30 mg did not increase the magnitude of the peak response. This unexplained phenomenon, which earlier has been observed with the doses above 0.4 mg (Erb, 1977) may explain why ocular pigmentation lacked an effect on the magnitude of the peak response with high pilocarpine doses.

Between pigmented and albino rabbits Lee and Robinson (1982) found a 10-fold difference in the availability of pilocarpine calculated from iris-ciliary body concentration vs time curves. Most probably the binding of pilocarpine by ocular pigmentation decreased the free drug available to receptor binding, and conse-

quently AUC values of RP vs time curves were no higher than 3-fold in pigmented compared to albino rabbits. With the lowest pilocarpine doses (0.11 and 0.43 mg), the slower elimination rate and lower peak effect in pigmented eyes resulted in AUC values equal to those in albino eyes. With higher instilled doses (0.85 and 2.30 mg), AUC values increased with ocular pigmentation because of the equal onset of drug action and the slower elimination rate of pilocarpine from pigmented than albino iris. Thus the miotic effects of pilocarpine are prolonged in the pigmented rabbit eyes compared to albino eyes (Fig. 1). Pigmentation of the anterior uvea may form a reservoir of pilocarpine from which the drug is released during the elimination phase of the drug action, resulting in prolonged duration of action. Prolongation of the mydriatic effects of atropine in pigmented irides has also been reported (Salazar et al., 1976).

The apparent elimination rate constants of miotic response in the eyes of pigmented rabbits were smaller than in the eyes of albino rabbits and about equal to the constants calculated from human data by Mishima (1981). Since pilocarpine is slowly released from the corneal epithelium to the aqueous chamber compartment, the first-order elimination rate constants of this study reflect both drug distribution and elimination (Yoshida and Mishima, 1975; Makoid and Robinson, 1979). Elimination in turn is a compilation of many rate processes, including elimination of the drug via aqueous turnover and uveal blood circulation, metabolism of the drug and its distribution in the tissues (Makoid and Robinson, 1979).

The polymer matrix is a solid drug delivery system which when administered ocularly releases the drug in the lower conjunctival fornix to the tear fluid. Prolonged duration of drug action has been achieved with polymer matrices in many studies (Maichuk, 1975; Bensinger et al., 1976; Salminen et al., 1983a). When the precorneal contact time of drug is prolonged using a vehicle with high viscosity and the release of the drug from the vehicle is not the rate-determining phase of drug absorption, concentration of the drug in the aqueous chamber compartment of the eye is initially increased as in eye drop administration (Sieg and Robinson, 1977). With prolonged precorneal contact time, drug absorption into the corneal epithelium is prolonged and thus drug release into the aqueous chamber compartment is shifted to a later time. Thus the absorption profile is extended and the magnitude of the peak effect is increased. From Fig. 2 it is evident that the vehicle did not determine the penetration rate of pilocarpine to the receptor sites. The increased AUC with polymer matrices was due to the prolonged precorneal contact, and the matrices showed a prolonged pulse-entry of pilocarpine, thus differing from the modest pulse-entry with eye drops and controlled release system described previously (Sendelbeck et al., 1975). The reason why the polymer matrices used in this study show prolonged pulse-entry of pilocarpine is the rapid release of the drug from the matrix. This is caused by the hydrophilic character of the polymer and the high water-solubility of pilocarpine hydrochloride. Tear fluid penetrates rapidly into the hydrophilic support material and consequently a water-soluble drug is leached out. On the basis of the results of Mishima (1976) and Loucas and Haddad (1972) pilocarpine impregnated contact lenses and pilocarpine alginate matrices also deliver pilocarpine as a prolonged pulse-entry.

The clinical importance of the binding of pilocarpine to the ocular pigmentation has been demonstrated. In normal volunteers, the intraocular pressure of darkly pigmented eyes responded to a single dose of pilocarpine less than that of lightly pigmented eyes (Melikian et al., 1971). In a multiple-dose study, darkly pigmented glaucomatous eyes showed a relative resistance to pilocarpine compared to lightly pigmented eyes (Harris and Galin, 1971). Our observations on the effect of ocular pigmentation on the pharmacokinetic parameters of pilocarpine are consistent with these findings.

Acknowledgements

This study was supported by grants from the Academy of Finland. The authors wish to thank Mrs. Tuula Rissanen for her technical assistance.

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