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Plasma concentrations and ocular effects of cyclopentolate after ocular application of three formulations

K. LAHDES, R. HUUPPONEN, T. KAILA, D. MONTI, M. F. SAETTONE & L. SALMINEN
Departments of Ophthalmology and Clinical Pharmacology, University of Turku, Turku, Finland, Department of Pharmaceutical Technology and Biopharmacy, University of Pisa, Pisa, Italy and Department of Ophthalmology, University of Tampere, Tampere, Finland

1 Eight volunteers received in randomized order two 30 μl drops of either 1% w/v cyclopentolate hydrochloride or a corresponding amount of cyclopentolate polygalacturonate in saline or in acetate buffer in one eye. Cyclopentolate concentrations in plasma were measured by a radioreceptor assay.

2 Peak plasma drug concentrations of about 3 ng ml^{-1} occurred within 30 min after all formulations. Occasionally, a second concentration peak in plasma, probably reflecting drug absorption from the gastrointestinal tract, was seen after 2 h. The mean elimination half-life of cyclopentolate was 111 min when all subjects and formulations were considered together. There were no statistically significant differences between the formulations with respect to the time-course of plasma drug concentration.

3 The maximal mydriatic effect was reached within about 15 min and was maintained for several hours, often being 1/3 of its peak value after 30 h. Similarly, an intense cycloplegic response was achieved within a few minutes, the peak changes in the near-point of vision being 9 to 10 dioptres. The cycloplegic response was more intense after one of the polygalacturonate complexes, especially at later time points.

Keywords cyclopentolate ocular therapy pharmacokinetics polygalacturonate mucoadhesion

Introduction

Cyclopentolate is widely used in ophthalmology for its intense mydriatic and cycloplegic activity. Systemic side effects, especially in the CNS, have been described both in adults and in children (Adcock, 1971; Awan, 1976; Binkhorst *et al.*, 1963; Kellner & Esser, 1989; Khurana *et al.*, 1988; Shihab, 1980) after ocular cyclopentolate. Abuse of cyclopentolate eyedrops because of CNS effects has been described recently (Sato *et al.*, 1992). Systemic drug absorption from eyedrops has been demonstrated in adults (Kaila *et al.*, 1989) as well as in children (Lahdes *et al.*, 1992).

Conjunctival and nasal membranes are assumed to be the sites of systemic drug absorption following ocular drug application (Salminen, 1990). This absorption can be reduced by promoting transcorneal drug penetration at the expense of penetration through nasal mucous membranes. An approach to this goal is to prolong the contact time of drugs with the cornea, and/or by facilitating their penetration into the eye. In rabbits the use of muco-adhesive polymers containing cyclopentolate resulted in an increased mydriatic effect relative to the application of a hydrochloride solution of the drug (Huupponen *et al.*, 1992; Saettone *et al.*, 1992), whilst accelerating ocular and diminishing systemic drug absorption (Huupponen *et al.*, 1992). Thus the polymer might act as a depot for ionically bound cyclopentolate in contact with the ocular surface.

Mucoadhesive polymers are assumed to be retained on the ocular surface longer than conventional drug formulations, thus facilitating transcorneal drug penetration. The extent of systemic drug absorption may, however, either decrease or increase; the reduced nasolacrimal drainage tends to lower systemic drug absorption from the nasal mucosa, whereas absorption through the conjunctiva may be facilitated. In humans the ratio of conjunctival to corneal area is about twofold larger than in rabbits (Watsky *et al.*, 1988), which is likely to favour systemic drug absorption through the conjunctiva at the expense of corneal penetration.

We have measured plasma concentrations of cyclo-

Correspondence: Dr Risto Huupponen, Department of Clinical Pharmacology, University of Turku, Kiinamyllynkatu 10, SF-20520 Turku, Finland

pentolate and its ocular effects after application of a standard hydrochloride salt solution in comparison with two mucoadhesive polymers with differing buffer compositions and pH values.

Methods

Subjects and medication

Eight healthy volunteers (seven females, one male) with a mean (± s.d.) age of 21 ± 0.5 years, a mean weight of 58 ± 10 kg and a mean height of 170 ± 13 cm, participated in this cross-over, randomized study after giving informed consent. The study was approved by the Joint Ethics Committee of the University of Turku and Turku University Hospital. The volunteers received either commercial 1% (w/v) cyclopentolate hydrochloride (formulation 1, pH 5.5, osmolality 428 mOsm kg^{-1}, Oftan-Syklo®, Leiras Pharmaceuticals, Tampere, Finland), or 0.89% (w/v) cyclopentolate polygalacturonate in saline (formulation 2, pH 6.6, osmolality 299 mOsm kg^{-1}) or 0.89% (w/v) cyclopentolate polygalacturonate in acetate buffer (formulation 3, pH 5.1, osmolality 272 mOsm kg^{-1}). The polygalacturonate complexes had similar mydriatic activity in rabbit eyes and they were prepared in the Department of Pharmaceutical Technology/Biopharmaceutics, University of Pisa, Italy as described by Saettone *et al.* (1992). The active drug content of the polygalacturonate complexes corresponded to 1% w/v cyclopentolate hydrochloride.

Study protocol

The medications were applied at intervals of at least 1 week as two 30 µl drops instilled over 3 min with an adjustable laboratory pipette to the lower cul-de-sac of the right eye to recumbent subjects.

Before drug administration, an intravenous cannula was inserted into an antecubital vein for blood sampling. Blood (10 ml) was collected into EDTA-tubes at 0, 3, 8, 15, 20, 30 min and at 1, 1.5, 2, 4 and 6 h after administration of the second eye drop. The cannula was kept patent with dilute heparin solution, which was discarded before the blood samples were taken. Plasma was separated promptly by centrifugation and kept frozen until analyzed.

Pupil diameter (mydriasis) in both eyes was measured at standard background illumination using a millimeter scale attached to the telescope of a Goldmann perimeter at 0, 3, 8, 15, 20, 30, 45 min at at 1, 1.5, 2, 4, 6, 24 and 30 h. The recordings were made by two experienced persons who were unaware of the cyclopentolate preparations received by the volunteers. Double checks were made regularly to ensure uniformity of the readings.

Cycloplegia was estimated in both eyes by measuring the near-point of vision using an R.A.F. near-point meter at 0, 8, 15, 30, 45 min and at 1, 1.5, 2, 4, 6, 24 and 30 h. The median of three consecutive readings was recorded at each time.

The amount of lacrimation and conjunctival hyperaemia in the treated eye after drug instillation were evaluated by the intestigator using a four-point scale. Similarly, the volunteers reported any subjective discomfort after eye drop application using a four-point scale.

Drug assay

Plasma cyclopentolate concentrations were measured by a modification of a radioreceptor assay described for the determination of ipratropium in plasma (Ensinger *et al.*, 1987). Cyclopentolate was extracted from plasma into dichlorethane which was evaporated to dryness under a nitrogen stream. The evaporated samples were equilibrated with (*N*-methyl-^{3}H)-atropine (New England Nuclear, Wilmington, USA) and with a muscarinic receptor preparation obtained from rat brain. The limit of determination of the method was 300 pg cyclopentolate ml^{-1} plasma. At a cyclopentolate concentration of 2 ng ml^{-1} the coefficient of variation of the assay was 9.3%.

Data analysis

Areas under the plasma cyclopentolate concentration-time curves were calculated using the linear trapezoidal rule, assigning a zero value to concentrations below the limit of determination. The assignment of t_{max} and C_{max} values was based on the first peak in the plasma drug concentration-time profile. The terminal elimination half-life ($t_{½,z}$) of cyclopentolate was computed from the linear portions of the semilogarithmic plots of the drug concentration-time data using at least three (median five) data points. The area from the time of the last measurable drug concentration (C_{last}) to infinity was calculated from C_{last}/λ_z and represented about 13% of the total AUC. The mydriatic and cycloplegic responses were calculated as changes from pretreatment values.

Analysis of variance (ANOVA) for repeated measures was applied to the data, allowing for formulation and subject and, where appropriate, time effects. For significant effects, the ANOVA was followed by multiple comparisons. The preservation of statistical significance after multiple comparisons was confirmed with the Bonferroni method. The non-parametric Friedman-test was used to analyse variables measured with an ordinal scale.

Results

Plasma drug concentrations

Peak plasma cyclopentolate concentrations of 2–3 ng ml^{-1} were reached within 30 min in most subjects (Table 1, Figure 1). Neither C_{max} ($P = 0.43$) nor t_{max} values ($P = 0.45$) differed between the formulations. No cyclopentolate was detected in baseline samples.

When data from all subjects and formulations were pooled the terminal elimination half-lives during the 20 analyzable treatment sessions ranged from 49 to 266 min with a mean of 111 min. In three subjects (four

Table 1 Mean (± s.d.) values of parameters describing the systemic pharmacokinetics of cyclopentolate after ocular administration to eight volunteers

Formulation	C_{max} *(ng ml^{-1})*	t_{max} *(min)*	*AUC(0, 360 min) (ng ml^{-1} min)*	*AUC*[a]	$t_{1/2,z}$[a] *(min)*
1	2.8 ± 1.3	15 ± 11	324 ± 127	447 ± 108	112 ± 23
2	2.6 ± 1.2	10 ± 9	299 ± 97	384 ± 82	98 ± 35
3	3.1 ± 1.1	13 ± 11	369 ± 118	480 ± 147	112 ± 68

Formulation 1: cyclopentolate hydrochloride, formulation 2: cyclopentolate polygalacturonate in saline buffer, formulation 3: cyclopentolate polygalacturonate in acetate buffer, a: $n = 5$.

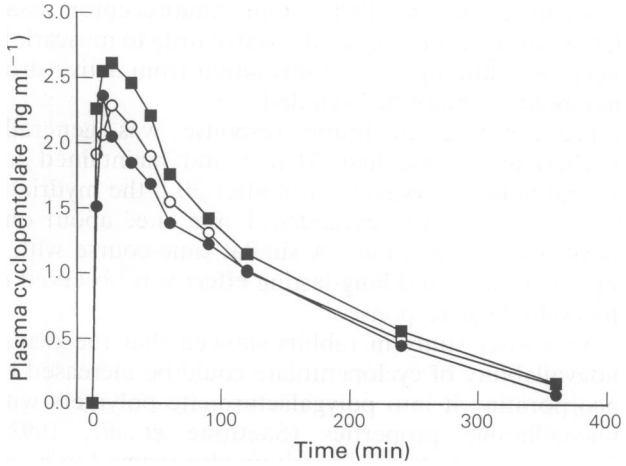

Figure 1 Mean plasma cyclopentolate concentrations after application of formulation 1 (○), formulation 2 (●) and formulation 3 (■). Two 30 μl drops were given at 3 min intervals.

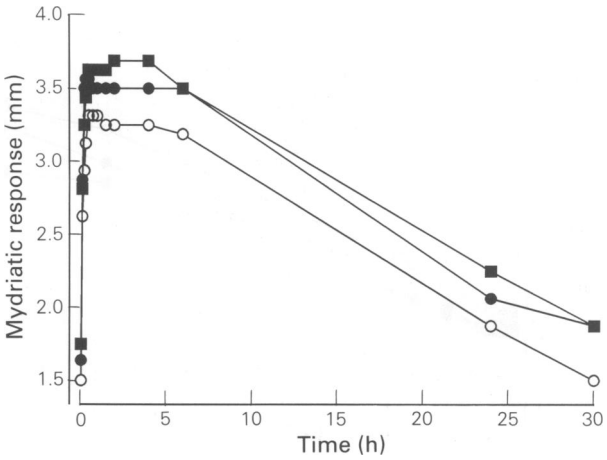

Figure 2 Mydriatic response (change from the pretreatment level) in the treated eye after application of the three cyclopentolate formulations (see Figure 1 for key).

sessions) the terminal half-life could not be calculated owing to nonlinearity of the terminal drug concentration-time slopes. The results from those subjects ($n = 5$) with complete data did not show any significant between-formulations difference in the half-life ($P = 0.85$, ANOVA, Table 1).

AUC values (Table 1) did not differ between the formulations, but variability between subjects was large (formulation effect: $P = 0.139$, subject effect: $P = 0.001$, ANOVA).

Pharmacodynamics

Conjunctival hyperaemia was prolonged after formulation 3. With the exception of lacrimation, the same trend towards a lower tolerability for this formulation was seen also in other subjective judgments, although these differences were not statistically significant (Table 2).

Differences in mydriatic response after application of the formulations were not statistically significant whether measured from all data points (formulation effect: $P = 0.091$, formulation × time interaction: $P = 0.54$, ANOVA, Figure 2) or as the integral value (Table 3). None of the formulations caused mydriasis in the untreated eye.

Data on cycloplegic response suggested differences between the formulations (formulation effect: $P = 0.057$, ANOVA, Figure 3). Furthermore, there was a significant formulation × time interaction in the change

Table 2 Tolerability of the different cyclopentolate formulations. Means ± s.d. are given

	Subjective discomfort		*Hyperaemia*	
	degree	*duration*[a]	*duration*	*intensity*
Formulation		*(s)*	*(min)*	
1	1.2 ± 0.8	47 ± 25	2.9 ± 7.0	0.25 ± 0.46
2	1.8 ± 0.7	46 ± 15	4.9 ± 7.0	0.50 ± 0.54
3	2.0 ± 0.5	57 ± 19	17.9 ± 13.8[b]	1.25 ± 0.89

The degree of discomfort and hyperaemia were measured using a four point scale: 0 = absent, 1 = mild, 2 = moderate, 3 = severe; the differences between formulations were not significant (Friedman-test). a = no difference between formulations ($P = 0.21$), differences between subjects significant ($P = 0.008$, ANOVA), b = significantly different from formulations 1 and 2 (ANOVA: $P = 0.004$, followed by contrasts with Bonferroni correction).

Table 3 Mydriatic response in the treated eye as change from the pretreatment level (means ± s.d.)

Formulation	*Maximal mydriasis (mm)*	*Integral response(0–1800 min) (mm min)*
1	3.4 ± 0.7	4492 ± 1154
2	3.6 ± 0.7	4957 ± 946
3	3.6 ± 0.7	5135 ± 1425

Neither the maximal mydriatic responses ($P = 0.29$) nor the integral responses ($P = 0.13$) differed significantly between the formulations (ANOVA).

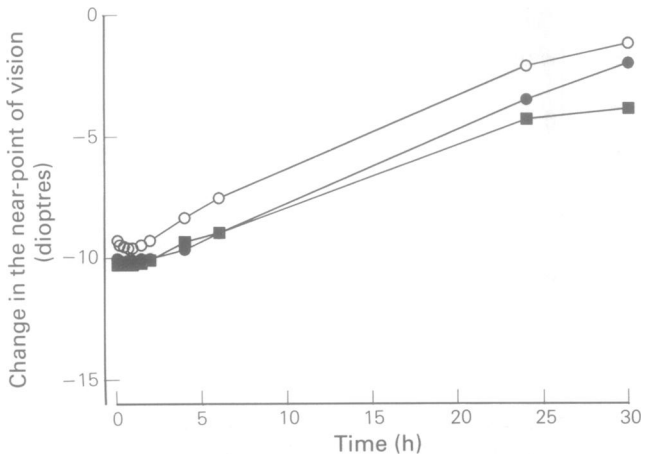

Figure 3 Cycloplegic response (change in the near-point of vision from the pretreatment level) after application of the three cyclopentolate formulations (see Figure 1 for key).

of near-point of vision ($P = 0.038$, ANOVA), indicating different shapes of the cycloplegic response curves (Figure 3). When the between-formulations differences were tested separately at the time of occurrence of the peak cycloplegic response and at the end of the observation period, the difference was statistically significant (between formulations 1 and 3, $P = 0.021$, ANOVA) at the latter time point but not at time of the peak effect ($P = 0.416$, ANOVA).

Discussion

We have shown previously that cyclopentolate is absorbed rapidly into the systemic circulation after ocular administration (Kaila *et al.*, 1989; Lahdes *et al.*, 1992) but its elimination half-life in plasma was not recorded. This study showed that cyclopentolate is eliminated from plasma with a mean half-life of about 100 min, a value which is slightly shorter than that of atropine (Kentala *et al.*, 1990).

In some subjects plasma cyclopentolate concentrations showed a second peak between 2 and 4 h, possibly reflecting absorption of swallowed drug from the gastrointestinal tract after drainage through the nasolacrimal duct to the nose and pharynx.

Cyclopentolate is used in eye drops as a racemate. However, the ocular effects are mainly due to the (−)-isomer (Smith, 1976). Our radioreceptor assay detects only the binding of the active drug to muscarinic receptors, although any contribution from active drug metabolites cannot be excluded.

The maximal mydriatic response was generally reached within the first 30 min and maintained for several hours. However, even after 30 h the mydriatic response frequently exceeded 1 mm, i.e. about one third of the peak value. A similar time-course with a rapidly ensuing and long-lasting effect was observed in the cycloplegic response.

An earlier study in rabbits showed that the ocular bioavailability of cyclopentolate could be increased by incorporating it into polygalacturonate polymers with mucoadhesive properties (Saettone *et al.*, 1992). However, the buffer composition also seemed to be an important factor, since the difference in favour of the mucoadhesive formulations was large when compared with cyclopentolate hydrochloride in physiological saline (Saettone *et al.*, 1992), but only modest when a commercial cyclopentolate hydrochloride preparation was used as a reference (Huupponen *et al.*, 1992). In particular, the borate ions present in the commercial preparations, like those used here, may increase the ocular bioavailability of cyclopentolate (Wang & Hammarlund, 1970). In this study, the polygalacturonate complexes had little or no effect on the mydriatic and cycloplegic responses to cyclopentolate.

In rabbits, the systemic absorption of cyclopentolate was reduced by the use of a polygalacturonate complex (Huupponen *et al.*, 1992). This contrasts with the present finding. Cyclopentolate complexes with more intense mydriatic effects in rabbits have recently been described (Saettone *et al.*, 1992), but their clinical applicability remains to be elucidated.

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