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Gamma scintigraphic study of precorneal drainage and assessment of miotic response in rabbits of various ophthalmic formulations containing pilocarpine

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Summary

Topical drug delivery to the eye is generally characterized by poor intraocular bioavailability. Hydrocolloids are used to increase viscosity and hence to slow down drug dilution and elimination by the lacrimal flow. The purpose of this work was to monitor corneal contact time by gamma scintigraphy and to compare the miotic response induced by viscosified vehicles containing pilocarpine nitrate. New Zealand White rabbits were used as test animals. The polysaccharides tested were hydroxyethylcellulose (HEC), xanthan gum and gellan gum (Gelrite®). The present comparative study using a solution containing no viscosifying agent as reference showed that Gelrite® and xanthan gum prolonged significantly the constriction of the pupil, whereas no prolongation of the miotic response could be shown with HEC. The clearance of the solutions labelled with ^{99m}Tc-DTPA was measured over a 10 min period and was shown to follow a two-phase pattern. The zero-order rate constant was determined for the initial 2 min and was significantly different for all the viscosified formulations in comparison with the reference solution.

Introduction

The intraocular penetration of topically applied drugs is influenced by corneal contact time. After instillation, tear flow drains out the drug from the corneal surface, following substantially a two-phase pattern (Chrai et al., 1973; Greaves et al., 1990). An increase in viscosity is generally acknowledged as being a factor leading to a slower elimination rate (Chrai and Robinson, 1974), and hence to a greater intraocular bioavailability. In order to monitor this increased bioavailability, it is possible either to perform assays for the drugs in the aqueous humor or to measure some pharmacological response directly related to the amount of drug at the receptor site. As the former technique implies the use of a large number of animals to obtain a valid set of data, and only one time point can be obtained with one eye, we made use of the latter one, i.e., the measurement of pupil size vs time after the administration of various pilocarpine nitrate solutions (Gurny et al., 1987; Saettone et al., 1992). Pilocarpine is used

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for the treatment of open angle glaucoma by topical administration to ensure a decreased intra-ocular pressure. Owing to its pharmacodynamic properties, several daily applications of the eye drops are needed. The introduction of a vehicle ensuring a longer ocular retention time, and hence a prolonged duration of action would be greatly appreciated by patients using the treatment on a long term basis. In the present study, we have taken advantage of a well known and readily measurable side-effect of pilocarpine: the pupil contraction.

The corneal contact time can be assessed by various non-invasive methods such as gamma scintigraphy. This method was first used in ophthalmology to evaluate the drainage system and in particular the in vivo lacrimal drainage dynamics (Rossomondo et al., 1972). During the preceding 20 years, gamma scintigraphy has become an established technique in pharmaceutical research (Wilson and Washington, 1988; Greaves et al., 1992).

Materials and Methods

Materials

Hydroxyethylcellulose (HEC QP 52000H, Union Carbide, U.S.A.), xanthan gum (Keltrol 1000, Kelco, Division of Merck & Co. Inc., U.S.A.), and Gelrite® gellan gum (Kelco, Division of Merck & Co Inc., U.S.A.) were sterilised by autoclaving a 2%, a 4%, and a 0.8% aqueous solution, respectively.

These concentrated solutions were diluted by mixing pilocarpine nitrate solution with Tris-nitrate buffer (pH 6.5–6.8; 0.019 M) to give a final concentration of 2% pilocarpine nitrate and 0.325 or 0.5% HEC, or 0.3% xanthan gum, or 0.6% Gelrite®. Solution tonicity was adjusted by addition of mannitol to obtain an iso-osmotic solution and an antiseptic preservative (quaternary ammonium) was added. These solutions were compared with a reference solution containing 2% pilocarpine nitrate and the same additives, except for the viscosifying agent which was omitted. Pilocarpine nitrate was obtained as a lyophilized powder (Chibro-Pilocarpine®, Merck, Sharp & Dohme, Chibret, France). All the other additives were pharmaceutical grade products and were used as obtained from the usual suppliers.

Radiopharmaceuticals

Technetium-99m (^{99m}Tc) was prepared, in the Department of Nuclear Medicine at the University Hospital of Geneva, by elution of a commercially available ^{99}Mo-^{99m}Tc generator (Mallinckrodt, The Netherlands) as pertechnetate ions. A kit (Oryx Pharmaceutica, Switzerland) was used for the preparation of ^{99m}Tc-labelled diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) from the eluate. The final solution was prepared by adding 0.15 ml of the chelate to 0.85 ml of the preparations to yield a final concentration of 0.325 or 0.5% HEC or 0.3% xanthan gum or 0.6% Gelrite®. These four solutions were compared to the same reference solution as above. All solutions contained 2% (w/v) pilocarpine nitrate, and their activity ranged from 2.78 MBq (= 75 μCi) to 1.96 MBq (= 53 μCi) per 25 μl dose, depending on the time of administration.

Methods

Male and female albino rabbits were used as test animals. All procedures were performed according to the ARVO statement for the use of animals in ophthalmic and vision research and to Swiss federal laws.

Miosis determination

The unanesthetized rabbits were kept in restraining boxes during the experiments and without their heads being hindered, so that all normal eye movements were retained. Prior to the experiments, the rabbits were acclimated in the laboratory for 30 min (standard white light). Each formulation was usually tested on five to six different rabbits. Baseline values were measured (seven time points) for 1 h prior to the administration. A 25 μl drop was applied to the top of the scleral region of one eye by slightly pulling the eyelids open, the other eye remaining untreated. The eyelids were immediately returned to their normal position after administration. After the ad-

ministration, measurements were carried out every 5 min during the first hour, then every 10 min until pupil size returned to normal. The miotic effect (%) was calculated as the difference between the pupil diameter induced by the pilocarpine and the individual average baseline values (Ibrahim, 1989). The determination of the pupil response was carried out with a video monitoring equipment consisting of a video camera (Sony CCD type DXC 101P) connected to a video tape recorder (Sony Betamax SLHF 950). The measurements of the pupil diameter and of a scaling standard were performed on the monitor screen (Sony KX27PS1). The scaling standard is used to determine the magnification factor of the camera lenses.

The evaluated parameters were: (i) intensity of the miotic effect at peak time (E_{max}); (ii) time to reach the maximum miotic effect (T_{max}); (iii) persistence of the miotic response (PMR), expressed as the time elapsed from instillation to when the pupil size had returned to baseline values; and (iv) area under miotic effect vs time curve (AUC).

Gamma scintigraphy

Each formulation was tested on five to seven rabbits. Each animal received the same formulation on three separate days. 25 μl of the solutions were instilled onto the left corneal surface (at the 12 o'clock position) of the rabbit to determine clearance of the radionuclide. The rabbit was positioned on a table without the restraining box, its head being gently supported by the hand with its left eye at 6 cm from the collimator aperture. A 25 μl aliquot of the solution to be tested was placed in a plastic tube, which was taped between the ears of the rabbit, and used as a position marker. The precorneal clearance was measured using a gamma camera (Toshiba GCA 602A) tuned to detect the 140 keV radiation of ^{99m}Tc and fitted with a 4 mm pinhole collimator. Dynamic images were recorded using a 128×128 pixel matrix for 10 min using 36×5 s frames followed by 12×10 s frames and 15×20 s frames. The recording was started exactly 5 s after instillation (time needed to place the rabbit in front of the collimator). The division of the overall scinti-

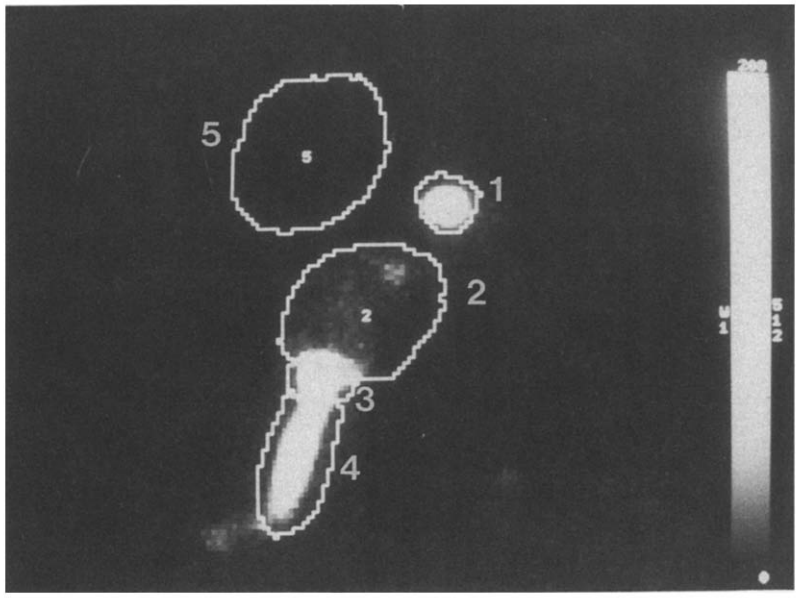

Fig. 1. Typical definition of the regions of interest (ROIs). 1, position reference; 2, precorneal surface; 3, inner canthus; 4, nasolacrimal duct; 5, background.

TABLE 1

Summary of the miotic activity parameters of the vehicles containing 2% pilocarpine nitrate in rabbits (mean ± standard deviation)

	Reference solution ($n = 6$)	HEC 0.325% ($n = 5$)	HEC 0.5% ($n = 6$)	Xanthan 0.3% ($n = 6$)	Gelrite® 0.6% ($n = 6$)
E_{max} (%)	32 ± 5	33 ± 6	32 ± 5	36 ± 4	35 ± 4
T_{max} (min)	28 ± 13	22 ± 12	28 ± 8	17 ± 5	17 ± 9
PMR (min)	148 ± 19	167 ± 31	146 ± 22	200 ± 12 [b]	182 ± 27 [a]
AUC (% min)	3161 ± 848	3545 ± 1131	3272 ± 937	4601 ± 858 [a]	4048 ± 1080

The significance of differences from the reference solution was assessed using a Student's t-test ([a] $P < 0.05$; [b] $P < 0.01$).

graphic image into five regions of interest (ROIs) is shown in Fig. 1. The total activity in the three anatomical ROIs (ROIs 2, 3 and 4) in the first frame is assumed to be 100% of the instilled dose. The remaining activity in the precorneal ROI at each time point is calculated as a percentage of the initial activity.

The evaluated parameters were: (i) activity remaining in the precorneal ROI; (ii) AUC, area under the percentage of activity remaining in the precorneal ROI vs time curve; and (iii) linear regression analysis for the initial 125 s of the elimination curve, yielding a zero-order rate constant (k_1) as well as the half life ($t_{1/2}$).

Statistical testing

The evaluated parameters were tested for goodness of fit with the normal distribution using the Kolmogorov-Smirnov test. The differences between the results obtained with an excipient were compared to those of the reference solution using a Student's t-test.

Results and Discussion

Miotic activity

The results of the miotic activity study in rabbits are summarized in Table 1, and Fig. 2 illustrates the difference in miotic response to the vehicles, which all contained 2% pilocarpine nitrate.

When compared with the reference solution, xanthan gum 0.3% showed a significantly increased AUC ($P < 0.05$) and even a highly significant prolongation of the persistence of the miotic response ($P < 0.01$). Gelrite® 0.6% showed a significant prolongation of the persistence of the miotic response ($P < 0.05$). Neither of the two

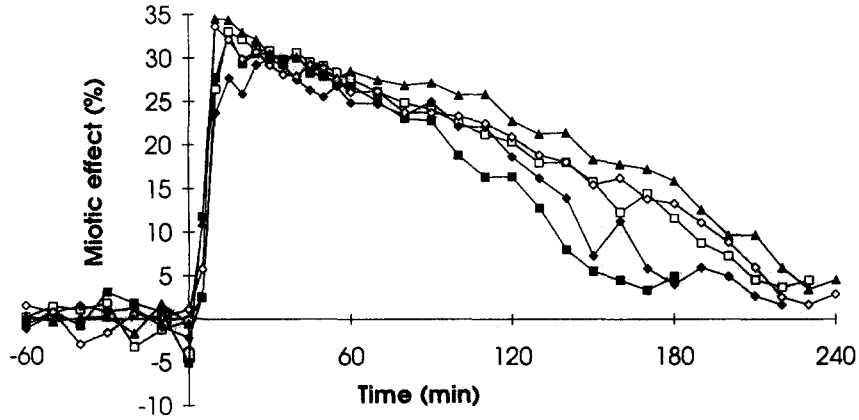

Fig. 2. Miotic response after instillation of 25 μl of various formulations containing 2% pilocarpine nitrate. (■) Reference solution ($n = 6$), (□) HEC 0.325% ($n = 5$), (♦) HEC 0.5% ($n = 6$), (◇) xanthan gum 0.3% ($n = 6$), (▲) Gelrite® 0.6% ($n = 6$).

TABLE 2

Clearance of solutions containing ^{99m}Tc-DTPA incorporated into vehicles containing pilocarpine nitrate 2% (mean ± standard deviation)

Time (min)	% remaining in the precorneal region of interest				
	Reference solution ($n = 13$)	HEC 0.325% ($n = 9$)	HEC 0.5% ($n = 8$)	Xanthan 0.3% ($n = 9$)	Gelrite® 0.6% ($n = 9$)
1	22 ± 15	56 ± 30 [b]	69 ± 32 [b]	77 ± 26 [b]	94 ± 9 [b]
2	12 ± 9	43 ± 33 [a]	58 ± 29 [a]	69 ± 32 [b]	88 ± 16 [b]
3	8 ± 7	32 ± 31	50 ± 41 [a]	51 ± 33 [b]	80 ± 18 [b]
4	6 ± 3	26 ± 27	46 ± 39 [a]	44 ± 31 [b]	69 ± 15 [b]
5	5 ± 3	24 ± 25	42 ± 39 [a]	38 ± 30 [a]	64 ± 15 [b]
6	5 ± 3	21 ± 22	40 ± 37 [a]	34 ± 27 [a]	59 ± 16 [b]
7	5 ± 3	20 ± 23	33 ± 34	30 ± 24 [a]	49 ± 20 [b]
8	4 ± 2	18 ± 23	32 ± 35	27 ± 22 [a]	41 ± 19 [b]
9	4 ± 2	16 ± 20	31 ± 35	24 ± 19 [a]	34 ± 19 [b]

The significance of differences from the reference solution was assessed using a Student's t-test ([a] $P < 0.05$; [b] $P < 0.01$).

formulations based on HEC showed any significant difference in the four evaluated parameters.

Scintigraphic evaluation of the retention time

The curves of the activity remaining in the corneal ROI vs time are shown in Fig. 3; Table 2 presents the activity remaining on the cornea at different time points and Table 3 summarizes the mean values of the evaluated parameters.

Gelrite® 0.6% and xanthan gum 0.3% showed at all times a significantly higher retention of the radioactive tracer in the corneal ROI in comparison with the reference solution. HEC showed an increase in remaining activity for 6 min after instillation of the higher concentration (0.5%) and for 2 min for the lower concentration (0.325%). The dispersion of the results was so

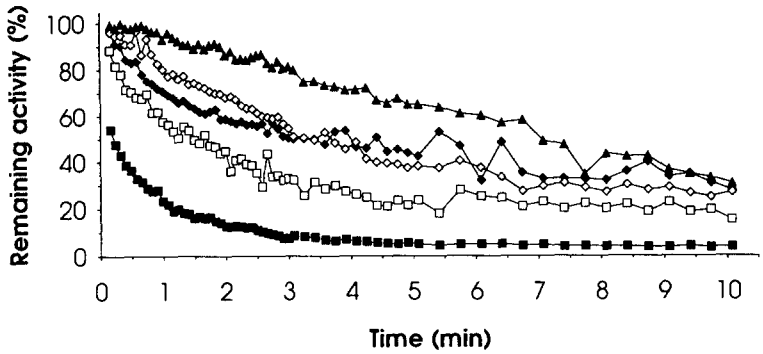

Fig. 3. Precorneal drainage of ^{99m}Tc-DTPA incorporated into various formulations containing pilocarpine nitrate 2%. (■) Reference solution ($n = 13$), (□) HEC 0.325% ($n = 9$), (♦) HEC 0.5% ($n = 8$), (◇) xanthan gum 0.3% ($n = 9$), (▲) Gelrite® 0.6% ($n = 9$).

TABLE 3

Precorneal drainage parameters (mean ± standard deviation for AUC, and mean ± standard error for k_1)

	Reference solution ($n = 13$)	HEC 0.325% ($n = 9$)	HEC 0.5% ($n = 8$)	Xanthan 0.3% ($n = 9$)	Gelrite® 0.6% ($n = 9$)
AUC (%min)	88 ± 49	295 ± 244 [a]	455 ± 355 [a]	445 ± 254 [b]	636 ± 131 [b]
k_1 (min $^{-1}$)	0.77 ± 0.03	0.37 ± 0.02 [b]	0.26 ± 0.01 [b]	0.20 ± 0.01 [b]	0.07 ± 0.01 [b]
$t_{1/2}$ (min)	0.91	1.86	2.69	3.45	9.66

The significance of differences from the reference solution was assessed using a Student's t-test ([a] $P < 0.05$; [b] $P < 0.01$).

wide that no conclusions could be drawn regarding the significance of the difference in the corneal residence time found in these two cases.

When compared with the reference solution, all formulations showed an increased AUC, i.e., HEC 0.325% ($P < 0.05$), HEC 0.5% ($P < 0.05$), Gelrite® ($P < 0.01$) and xanthan gum 0.3% ($P < 0.01$). The drainage rate constants were significantly different for all the formulations tested in comparison with the reference solution ($P < 0.01$).

Conclusion

According to previously published results (Gurny et al., 1987, 1990), clearance of all studied formulations was found to follow two-phase kinetics, although individual patterns of drainage may differ from the general picture.

Xanthan gum 0.3% and Gelrite® 0.6% prolonged the corneal contact time as measured by gamma scintigraphy in comparison with a reference solution with no viscosifying agent added ($P < 0.01$). As calculated from the drainage rate constant for the first phase, the elimination was decreased by a factor of 4 when using xanthan gum and by a factor of 10 when using Gelrite®. Both xanthan gum and Gelrite® prolonged the miotic response when compared to a reference solution ($P < 0.01$ and $P < 0.05$, respectively).

Both dilutions of HEC increased the corneal contact time as evaluated by gamma scintigraphy ($P < 0.05$). At the same time, the drainage rate constant calculated for the first phase was decreased by a factor of 2 for HEC 0.325% and by a factor of 3 for HEC 0.5%. It was also observed that the dispersion of individual results was much wider for the two HEC preparations than for xanthan gum or the reference solution.

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References

Chrai, S.S, Patton, T.F., Mehta, A. and Robinson, J.R., Lacrimal and instilled fluid dynamics in rabbit eyes. *J. Pharm. Sci.*, 62 (1973) 1112–1121.

Chrai, S.S and Robinson, J.R., Ocular evaluation of methylcellulose vehicle in albino rabbits. *J. Pharm. Sci.*, 63 (1974) 1218–1223.

Greaves, J.L., Olejnik, O. and Wilson, C.G., Polymers and the precorneal tear film. *STP Pharm. Sci.*, 2 (1992) 13–33.

Greaves, J.L., Wilson, C.G., Rozier, A., Grove, J. and Plazonnet, B., Scintigraphic assessment of an ophthalmic gelling vehicle in man and rabbit. *Curr. Eye Res.*, 9 (1990) 415–420.

Gurny, R., Ibrahim, H., Aebi, A., Buri, P., Wilson, C.G., Washington, N., Edman, P. and Camber, O., Design and evaluation of controlled release systems for the eye. *J. Controlled Release,* 6 (1987) 367–373.

Gurny, R., Ryser, J.E., Tabatabay, C., Martenet, M., Edman, P. and Camber, O., Precorneal residence time in humans of sodium hyaluronate as measured by gamma scintigraphy. *Graefe's Arch. Clin. Exp. Ophthalmol.*, 228 (1990) 510–512.

Ibrahim, H., Concept et évaluation de systèmes polymériques dispersés (pseudo-latex) à usage ophtalmique, Ph.D. Thesis No. 2369, Université de Genève, Imprimerie Nationale, Genève, 1989, p. 172.

Rossomondo, R.M., Carlton, W.H., Trueblood, J.H. and Thomas, R.P., A new method of evaluating lacrimal drainage. *Arch. Ophthalmol.*, 88 (1972) 523–525.

Saettone, M.F., Torracca, M.T., Pagano, A., Giannaccini, B., Rodriguez, L. and Cini, M., Controlled release of pilocarpine from coated polymeric ophthalmic inserts prepared by extrusion. *Int. J. Pharm.*, 86 (1992) 159–166.

Wilson, C.G. and Washington, N., Assessment of disintegration and dissolution of dosage forms in vivo using gamma scintigraphy. *Drug Dev. Ind. Pharm.*, 14 (1988) 211–281.