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Vehicle effects on ophthalmic bioavailability: the influence of different polymers on the activity of pilocarpine in rabbit and man

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Viscosity-increasing polymers are usually added to ophthalmic drug solutions, on the premise that an increased vehicle viscosity should correspond a slower elimination from the preocular area, and hence a greater transcorneal penetration of the drug into the anterior chamber. Many polymers have been screened in the attempt to determine the ideal polymer and viscosity, and to establish quantitative relationships between vehicle viscosity, retention time and ophthalmic bioavailability. The experiments are usually carried out by the different authors on rabbits or man, and this has generated some confusing and contradictory data, since the two species show important differences in ocular anatomy and physiology (e.g., different rates of blinking, tear secretion and turnover, drainage of instilled fluid, etc) that may produce different responses to vehicle viscosity. In spite of some diverging reports as the effect, in man and in rabbits, of the viscosity of the vehicle on bioavailability, there appears to exist an implicit agreement on the thesis that, within each species, vehicles prepared with different polymers should behave identically when compared on an equal viscosity basis (Patton & Robinson 1975). In the present study, three Newtonian equiviscous vehicles and one pseudoplastic vehicle, all prepared with different polymers and containing pilocarpine were tested on rabbits and man. The aims of the investigation were (a) to verify the equal viscosity-equal activity assumption, i.e., the alleged lack of influence on activity of the type of polymer; (b) to define species differences in the biological response to the same vehicles; and (c) to assess the relevance to activity of the type of flow of the vehicle (Newtonian *vs* pseudoplastic).

All preparations tested were made by adding the appropriate amount of polymer to an aqueous isotonic buffered (Sørensen 0·2 M phosphate buffer, pH 5·5) $2 \cdot 10^{-2}$ M solution of pilocarpine nitrate (AS) serving as reference. The following polymers, at the indicated w/v concentrations, were used: hydroxypropylcellulose, low molecular weight (HPCL, Klucel LF, Hercules Inc.), 5·0%; hydroxypropylcellulose, medium molecular weight (HPCM, Klucel MF, Hercules Inc.), 1·2%; polyvinyl alcohol, high (90·000) molecular weight (PVA, Polyviol W 48/20, Wacker Chemie), 5·0%; polyvinylpyrrolidone (PVP, Plasdone K 90, GAF), 6·0%. All solutions were sterilized by autoclaving at 2 bars for 20 min; their pH after sterilization was virtually unchanged, while the pilocarpine content (h.p.l.c.) showed an average 0·15% decrease. Viscosity determinations on the sterilized vehicles, made at 30°C on a Rheomat 30 rotary viscometer (Contraves AG) indicated for HPCL, PVA and PVP a Newtonian behaviour (rate of shear up to 700 s^{-1}) and a viscosity of 73 ± 2·5 cps.

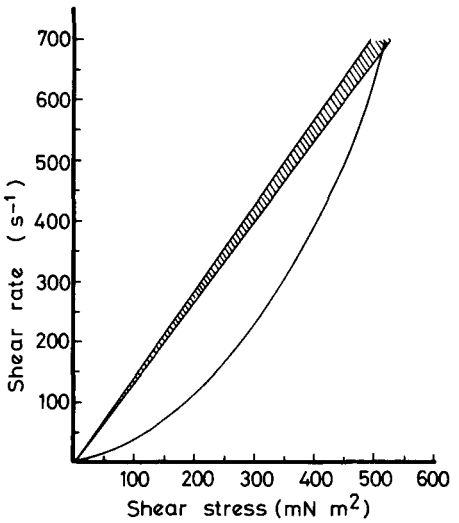

FIG. 1. Rheograms (30°) of the vehicles tested in the present study.

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Table 1. Summary of rabbit (R) and human (H) activity data of pilocarpine nitrate in different vehicles.*

Vehicle	I_{max} R	I_{max} H	TP R	TP H	D R	D H	AUC R	AUC H
AS	1·66 ± 0·12	1·66 ± 0·14	30	60	300 ± 20	225 ± 15	64·4 ± 11·8	57·0 ± 8·0
PVP	1·90 ± 0·30	2·80 ± 0·17	40	80	320 ± 21	220 ± 11	68·8 ± 14·8	86·8 ± 10·0
HPCL	1·85 ± 0·65	2·40 ± 0·20	40	80	320 ± 21	280 ± 12	68·1 ± 15·2	98·4 ± 13·0
HPCM	1·91 ± 0·26	2·20 ± 0·32	40	60	360 ± 18	200 ± 15	73·3 ± 17·2	56·1 ± 10·9
PVA	1·96 ± 0·22	2·95 ± 0·17	40	80	400 ± 24	340 ± 10	96·4 ± 16·5	136·1 ± 19·2

* In order of increasing AUC in humans. I_{max} = peak height, mm ± 95% CL; TP = time to peak, min; D = duration of activity, min ± 95% CL; AUC = area under activity-time curve, cm^2 ± 95% CL.

The HPCM solution was pseudoplastic, with an apparent viscosity of 73 cps (the same as the other solutions) at a rate of shear of 700 s^{-1}. The rheograms of the vehicles are illustrated in Fig. 1.

Miosis-time studies on rabbits (male albino, 2–2·5 kg) were conducted on unanaesthetized, preconditioned animals under standardized lighting conditions, and at least eight separate determinations were made for each solution. The preparation (50 µl) was instilled into one eye of the animal, the other eye serving as control. The horizontal diameter of the pupil was estimated to the nearest 0·1 mm with a micrometer held always at the same distance from the eye, by the same operator. The measurements were made at intervals, commencing immediately postinstillation, and continued until the pupil size returned to its normal value. Miosis-time studies on man were carried out on healthy volunteers of either sex, aged 20–65 years; patients with conjunctival or corneal abrasions and disorders were excluded. Each preparation was tested on groups of at least eight patients, by instilling 50 µl into one eye, while the other served as control. Measurements of pupil size were made as already indicated; all vehicles were well tolerated by the patients, and did not produce visual disturbances. The following main activity parameters were evaluated: I_{max}, or peak height (maximum change in pupillary diameter), time to peak (TP), duration of activity (time for pupil to return back to normal) and area under the miosis-time curve (AUC). Within each species, the results were submitted to statistical analysis to test differences among vehicles and between each vehicle and the standard aqueous solution (AS). A summary of the human and rabbit data is presented in Table 1; the AUC data, with the associated 95% confidence limits, are also shown in Fig. 2.

Inspection of the data shows that, in the rabbit experiments, the I_{max} and the TP of the viscous vehicles were slightly larger than those of AS, but the difference was not significant. The duration of activity and the AUC values were also larger for the viscous vehicles respect to AS, but the difference was significant only for the PVA solution, whose AUC and duration were 1·5 and 1·33 times greater, respectively, than those of AS ($P < 0·05$).

In humans all viscous vehicles, with the exception of the pseudoplastic one, HPCM, exhibited activity parameters significantly greater ($P < 0·05$) than those of the non-viscous solution, AS. Only PVP showed a duration of activity practically identical with that of AS, but all other parameters were significantly increased over those of the reference solution. The human AUC values, considering the value obtained for AS equal to 1, were 1·52, 1·72 and 2·38 for PVP, HPCL and PVA, respectively. A significant difference was found between the activity parameters of PVA and those of the other two Newtonian viscous vehicles, HPCL, and PVP. Thus, PVA appeared significantly more active in both species in increasing the transcorneal penetration of pilocarpine. The overall increase of bioavailability produced by PVA with respect to AS, as measured by the ratio of the AUC values (which should reflect the aqueous humour concentration of the drug), amounted to 1·5 times (rabbits) and 2·38 times (humans). This was in satisfactory agreement with existing literature data (Chrai & Robinson 1974) which indicated that the maximum improvement in drug activity in rabbits, resulting from an increased viscosity (up to 100-fold), is about twice that of an aqueous solution. The poor performance of the other Newtonian viscous solutions, PVP

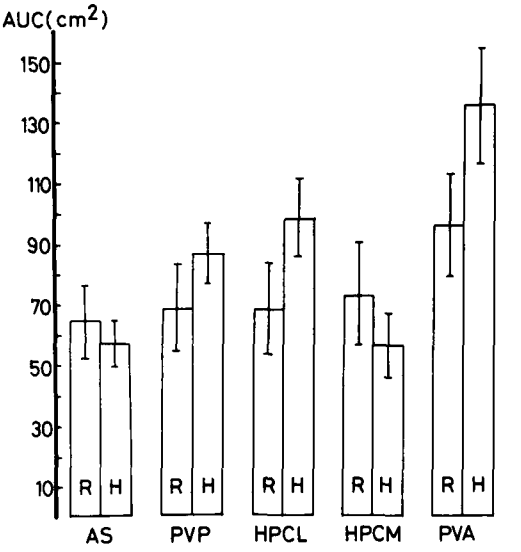

FIG. 2. Areas under the miotic response – time curves (AUC) obtained in humans and in rabbits with the preparations under study. Vertical lines over bars indicate 95% confidence limits.

and HPCL, with respect to PVA is noteworthy, particularly considering that all three vehicles should have, according to the current theories, identical drainage rates within each species, and consequently identical effects on the contact time of the drug with the precorneal area. The inferior results observed in humans with the pseudoplastic vehicle, HPCM, might possibly be explained assuming that the shear created by blinking and eyeball movement would cause the vehicle to thin, thus decreasing its viscosity to approximate that of the non-viscous solution. The observation that in rabbits, whose blinking rate is very low (4 times h^{-1}), the pseudoplastic vehicle performed as well as HPCL and PVP (even if all three vehicles were not significantly different from AS) gives some support to this hypothesis.

The presently reported differences in ocular bioavailability observed with vehicles containing the same amount of drug, and showing practically identical viscosity characteristics, appear unprecedented in the rabbit and human ophthalmic literature, with perhaps one exception. Adler et al (1971) reported that equiviscous (and presumably Newtonian, although the type of flow was not reported) solutions of methylcellulose (MC) and of two different grades of PVA, showed substantial differences in promoting transcorneal penetration of fluorescein. While two solutions (MC and a high-molecular weight PVA produced a moderate increase of penetration, the other (low-molecular weight PVA) had little, if any effect on the penetration of the dye with respect to a saline solution. Two hypotheses were advanced to rationalize the observed penetration increase: (a) a greater initial dye saturation of the tear film or (b) a direct effect of some polymers on the corneal permeability. Either hypothesis might apply to the present results, although the first one appears perhaps more likely. The possibility of a greater initial saturation of the tear film receives support from an investigation by Benedetto et al (1975), who found that a high-molecular weight PVA solution of 120 cps viscosity produced, up to 6 min after instillation, a significantly greater thickness of the precorneal tear film relative to a hydroxypropylmethylcellulose solution of the same viscosity. These authors demonstrated that the retention of a drug in the precorneal tear film is not strictly related to the viscosity of the vehicle, or to its surface properties, but rather to the surface spreading characteristics of the vehicle, and to the ability of a polymer to drag water as the vehicle spreads over the ocular surface with each blink. Another possible cause for the different drug availability from equiviscous polymer solutions might reside in binding phenomena: these would decrease the activity of the diffusible species in the tear film, thus producing the effect of a reduced dose. Thus, in order to rationalize the present results, a much greater binding capacity for pilocarpine should be admitted for the HPCL, HPCM and PVP solutions, with respect to PVA. However, this hypothesis appears unlikely, also on account of the results of Ticho et al (1979), who found by dialysis experiments that pilocarpine binding by a cellulose derivative (hydroxyethylcellulose) was insignificant.

The possibility that the increased bioavailability of the drug from the PVA solution might be due to surface effects was also considered in the present study. Indeed, a reduced surface tension, as well as a decreased interfacial tension between the vehicle and the outer (oily) layer of the precorneal tear film, might favour vehicle spreading and mixing with the film constituents, thus possibly improving the contact between the drug and the corneal epithelium. This hypothesis, however, was disproved by the observation that, while PVA showed a much lower surface and interfacial tension (against perhydrosqualene) with respect to AS, the other two less efficient vehicles, HPCL and HPCM, showed values which were even lower than those of PVA. The surface and interfacial tension (against perhydrosqualene) values of the present vehicles, in dynes cm^{-1}, measured on a Cenco-DuNouy tensiometer at 30 °C, were the following: AS, 68·7 and 40·8; HPCL, 43·9 and 16·2; HPCM, 44·5 and 16·9; PVA, 48·2 and 18·9; PVP, 66·6 and 35·1. These data clearly indicate, in agreement with the observations of Benedetto et al (1975), that the surface properties imparted by a polymer to a vehicle, though probably helpful, are insufficient in themselves to secure an increased bioavailability.

The following considerations may be offered as conclusion. The present study has shown the interesting properties of a high-molecular weight PVA solution as an ophthalmic vehicle, pointing to non-viscous effects in ophthalmic bioavailability, and warranting further work (e.g. studies of polymer surface spreading and water-dragging characteristics, investigations on drug-polymer binding, etc) on this and other polymers. A lower sensitivity of rabbits, with respect to man, to vehicle-mediated effects influencing transcorneal penetration of pilocarpine has also been observed. Therefore, assumptions of general validity, and extrapolations to other species of ophthalmic bioavailability data obtained with this animal may not prove entirely correct, although rabbits can be used as satisfactory models in other fields of ophthalmic research. The reported data may also shed some light on controversial reports existing in the literature about the so called 'vehicle effect' in occular bioavailability. Clearly, since this effect may be influenced not only by viscosity but by the species of the test animal, the chemical structure and physicochemical characteristics of the polymer, the type of flow of the vehicle, etc, widely different results may be observed unless all possible sources of variation are identified, duly considered, and carefully controlled.

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