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Semisolid ophthalmic vehicles. III. An evaluation of four organic hydrogels containing pilocarpine

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Summary

The validity of four hydrogels, based on synthetic polymers, as vehicles for topical ophthalmic drugs was evaluated by determining, in rabbits and in humans, the miotic response induced by pilocarpine. The hydrogels were prepared with two different types of poly(acrylic acid), PAA-1 and PAA-2; with poly(acrylamide), PAAm, and with ethylene maleic anhydride, EMA. Their biological effect was compared with that induced by an aqueous solution of the drug (AS). The rheological characteristics of the vehicles were investigated: PAA-1, PAA-2 and EMA displayed a plastic type of flow, while PAAm was pseudo-plastic. In rabbits, administration of pilocarpine in the hydrogel vehicles doubled the drug bioavailability (as expressed by the area under the miotic response vs time curve, AUC) with respect to AS; however, no statistical differences were apparent among the AUC values of the hydrogels. In humans, the same vehicles showed more pronounced activity differences. While PAA-2 and PAAm produced an approximately 3-fold bioavailability increase with respect to AS, PAA-1 only doubled the bioavailability, and EMA showed activity parameters not different from those of the aqueous solution. Possible correlations between the viscosity parameters of the vehicles and ophthalmic bioavailability in the two species are discussed. The present results appear to confirm previous observations on the poor validity of rabbits for studies on the influence of vehicle viscosity on bioavailability of topical ophthalmic drugs.

Introduction

Vehicles based on natural, semisynthetic or synthetic hydrogels have often been reported to improve the bioavailability of topical ophthalmic drugs (Krohn and Breitfeller, 1976; Schoenwald et al., 1978; Yamauchi and Matsuzawa, 1979; Goldberger et al., 1979; Saettone et al., 1980, 1982; March et al., 1982). In spite of some drawbacks, such as difficulty of sterilization and easy bacterial contamination, that have thus far limited their large scale production and clinical use, these vehicles present highly positive characteristics, such as, for example, a high viscosity, and a better miscibility with the lacrimal fluid with respect to the standard ophthalmic ointments. The former factor may ensure a prolonged retention of the medication in the eye and an increased bioavailability (Patton and Robinson, 1978), while the latter is of relevance to release of water-soluble drugs, and to patient acceptance. The potential advantages presented by hydrogels with respect to

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TABLE 1

COMPOSITION OF THE VEHICLES TESTED IN THE STUDY *

Vehicle	Composition
AS	Isotonic, buffered (Sørensen pH 5.5 phosphate buffer) aqueous solution
PAA-1	Poly(acrylic acid)-1, 1.5% w/v, adjusted to pH 6.0 with NaOH
PAA-2	Poly(acrylic acid)-2, 0.6% w/v, adjusted to pH 6.0 with NaOH
PAAm	Poly(acrylamide), 4% w/v
EMA	Ethylene maleic anhydride, 0.8% w/v, adjusted to pH 6.0 with NaOH after hydration

* All vehicles contained 0.13% w/v pilocarpine nitrate.

paraffin-based ointments may warrant further research aimed at improving and optimizing their physicochemical and biological properties.

The capacity of a poly(acrylic acid) hydrogel to augment significantly the ocular bioavailability of tropicamide in humans, both with respect to a viscous solution and to a paraffin ointment, was described in a previous paper (Saettone et al., 1980). Rabbits were found, in a parallel study, to be much less sensitive than humans to variations of type and viscosity of vehicles (Saettone et al., 1982). The present report is concerned with a preliminary evaluation, in rabbits and in humans, of four hydrogels based on commercial synthetic polymers, containing pilocarpine. Pilocarpine, a widely used drug for the treatment of chronic simple, or wide angle glaucoma, requires several daily instillations when administered in eyedrops, and would greatly benefit from a vehicle ensuring longer ocular retention times, and hence, less frequent applications. In particular, this investigation had the double aim: (a) of verifying the validity of the different polymeric formulations, by evaluating their capacity of improving the miotic activity parameters of the drug with respect to a standard aqueous vehicle; and (b) of comparing the responses of the two species, in order to gain additional information on the validity of rabbits for predictive studies on semisolid ophthalmic vehicles.

Materials and Methods

Materials

The following materials were used as received: two types of poly(acrylic acid), PAA-1 and PAA-2 [1]; poly(acrylamide), PAAm [2]; ethylene maleic anhydride, EMA [3]; and pilocarpine nitrate, $PiNO_3$, USP grade [4]. Water was doubly distilled from an all-glass apparatus. All other materials were USP or BP grade.

Vehicles

The gel fomulations listed in Table 1 were prepared as follows. PAA-1 and PAA-2: the polymers were dispersed in water by stirring at room temperature. The appropriate amount of pilocarpine nitrate ($PiNO_3$) was then added and the mixture was adjusted to pH 6.0 with 1.0 N NaOH to yield a clear gel. PAAm: the gel was obtained by prolonged stirring at room temperature of a dispersion of the polymer, also containing the appropriate amount of $PiNO_3$. EMA: a dispersion of the polymer was heated at 95°C, while stirring, for 20 min. Stirring was then continued at room temperature overnight. After addition of $PiNO_3$, the mixture was brought to pH 6.0 with NaOH to yield a clear gel.

All preparations, stored in glass containers, were sterilized by autoclaving at 2 bars for 20 min. The pH of AS after sterilization was unchanged; the pH of the sterilized hydrogels ranged from 6.05 to 6.25. The pilocarpine content of the sterilized preparations, determined by HPLC (Dunn et al., 1981), showed an average 0.15% decrease with respect to the theoretical amount.

Rheological measurements on the sterilized hy-

[1] Carbopol 910 and 940, respectively; Goodrich Chemical Co.
[2] Gelamide 250, American Cyanamid Co.
[3] EMA 91, Monsanto Co.
[4] E. Merck, Darmstadt.

drogels were carried out at 30 °C using a Rotovisco RV 12 viscometer [5], equipped with a MV II P measuring system, at rates of shear ranging from 0 to 30 s^{-1}.

Biological studies

(a) Rabbit tests. Male, albino New Zealand rabbits (2.5–3.0 kg) were used throughout. 24 h prior to testing, the palpebral rims of the animals were carefully epilated in order to facilitate the administration of the medications, and the subsequent measurements. Before each experiment, the animals, in restraining boxes, were acclimated for 30 min in a laboratory whose constant lighting was such as to give a baseline pupillary diameter in the range 5.9–6.3 mm. The applied dose was in all cases 50 μl. The application was made on the everted lower lid of the right eye using a microsyringe (for AS) or a 1 ml tuberculin syringe; the other eye, untreated, served as control. After dosing, the lids were gently held together for a few seconds to avoid blinking and loss of vehicle.

All measurements of pupillary diameter were made to the nearest 0.1 mm with a micrometer held at a fixed distance from the rabbits' eyes, by the same operator. Each vehicle was tested at least on 10 rabbits; no animal was used more than once. The results are presented as average variation of pupillary diameter, with respect to the basal diameter (±95% CL) vs time.

(b) Human tests. The tests were carried out, for each vehicle, on groups of at least 10 healthy Caucasian informed volunteers of either sex, aged 18–65 years, free from ocular lesions or disorders. Tests were carried out under medical supervision. Subjects receiving more than one treatment were allowed at least 3 weeks of rest between treatments.

All experiments were carried out in the same room, with constant artificial lighting. The dosing (50 μl) of one eye, and the subsequent measurement of pupillary diameter were carried out as described in the previous section. After application of the medications, the patients were instructed to shut their eyes for 30 s, in order to avoid blinking and reduced reflex lacrimation. As in the case of rabbits, baseline diameters were measured prior to dosing; afterwards, measurements were made at appropriate intervals until the pupillary diameter returned to the baseline value. The results are presented as the average variation of pupillary diameter with respect to basal diameter (±95% CL) vs time.

[5] Haake, Karlsruhe, F.R.G.

Results and Discussion

Biological data

The results of the miosis studies carried out on all vehicles are presented in graphical form in Fig. 1 (rabbit data) and in Fig. 2 (human data). The main activity parameters (peak miosis intensity, I_{max}; time to peak, T_p; duration, D; and area of the response vs time curve, AUC) are summarized in Table 2.

(a) Rabbits. In rabbits, the duration of activity induced by all gel vehicles (~ 300 min) was doubled with respect to the aqueous solution, AS (~ 160 min). The peak miotic response, I_{max}, of AS (1.52) was intermediate between the I_{max} of PAA-1, PAA-2 and PAAm (all giving practically identical values, 1.83) and the I_{max} of EMA (1.33). The differences, however, were not statistically significant at the 95% probability level. The areas under response vs time curves (AUC) calculated for the hydrogels were roughly doubled with respect to that of AS; the AUC corresponding to EMA was lower, but not statistically different from those of the other three gel vehicles. The peak time associated with EMA (60 min) was longer with respect to those observed for the other vehicles, including AS (30–40 min).

(b) Humans. In humans, the same vehicles showed more pronounced activity differences. As shown graphically in Fig. 2, and numerically in Table 2, the I_{max} produced by PAA-2 (1.85) and PAAm (1.65) was higher, and significantly different with respect to the I_{max} of AS (0.95), while the I_{max} produced by PAA-1 was lower with respect to those of the other hydrogels (1.35), and not significantly different from that of AS.

The EMA vehicle, although apparently well-tolerated in rabbits, induced a stinging sensation,

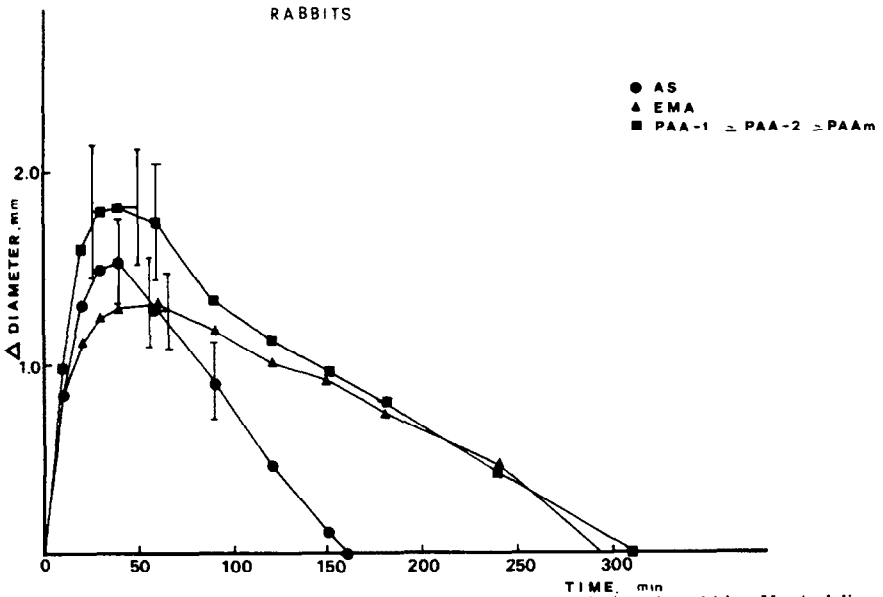

Fig. 1. Miotic effect of the hydrogels, and of the reference solution, in rabbits. Vertical lines indicate 95% C.L. All preparations contained 0.13% w/v pilocarpine nitrate.

accompanied by lacrimation, in some human subjects. This was a possible reason for the lower activity parameters observed with this vehicle (I_{max} and AUC not significantly different from AS, and relatively short duration of miotic activity).

The two most active vehicles, PAA-2 and PAAm, showed peak times (60 min) of the same order as AS, while greater values were shown by PAA-1 and EMA (90 min).

The human AUC values were in the (decreas-

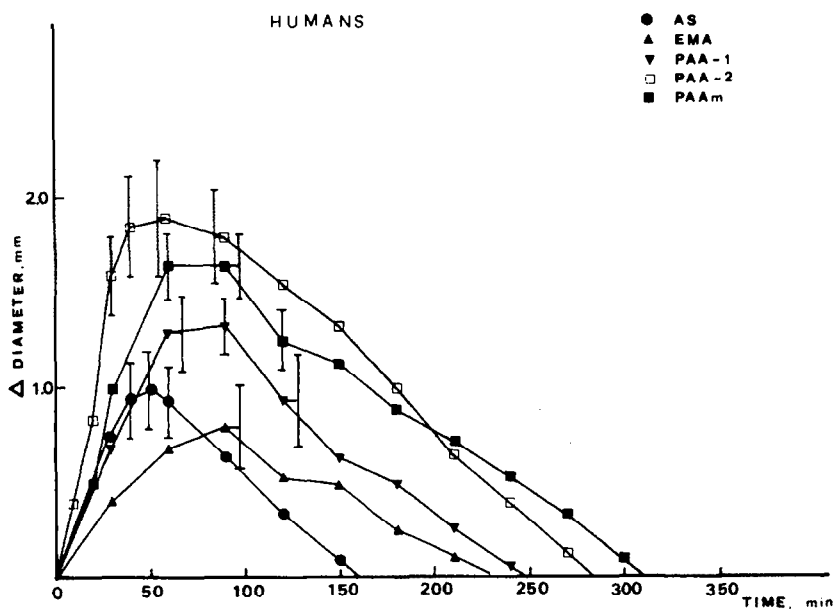

Fig. 2. Miotic effect of the hydrogels, and of the reference solution, in humans. Vertical lines indicate 95% C.L. All preparations contained 0.13% w/v pilocarpine nitrate.

TABLE 2

SUMMARY OF THE ACTIVITY PARAMETERS IN HUMANS (H) AND IN RABBITS (R) OF PILOCARPINE NITRATE (0.13% w/v) IN DIFFERENT VEHICLES

Vehicle	I_{max}		T_p		D		AUC		Relative AUC	
	H	R	H	R	H	R	H	R	H	R
AS	0.95(0.2)	1.52(0.22)	50	40	150 ± 15	160 ± 14	86.8 ± 28	133.1 ± 30	1	1
PAA-1	1.35(0.17)	1.83(0.31)	90	30	240 ± 11	310 ± 18	171.4 ± 57	279.4 ± 65	1.97	2.1
PAA-2	1.85(0.34)	1.84(0.31)	60	40	270 ± 28	300 ± 16	311.3 ± 70	279.8 ± 70	3.59	2.1
PAAm	1.65(0.15)	1.82(0.2)	60	30	300 ± 15	300 ± 15	275.4 ± 49	256.3 ± 50	3.17	1.92
EMA	0.8 (0.19)	1.33(0.25)	90	60	210 ± 15	290 ± 14	97.8 ± 33	235.7 ± 47	1.13	1.77

I_{max} = maximal miotic response (mm ± 95% CL); T_p = time to peak (min); D = duration of activity (time for the pupillary diameter to return to the baseline value) (min ± 95% CL); AUC = area under the activity vs time curve (cm^2 ± 95% CL).

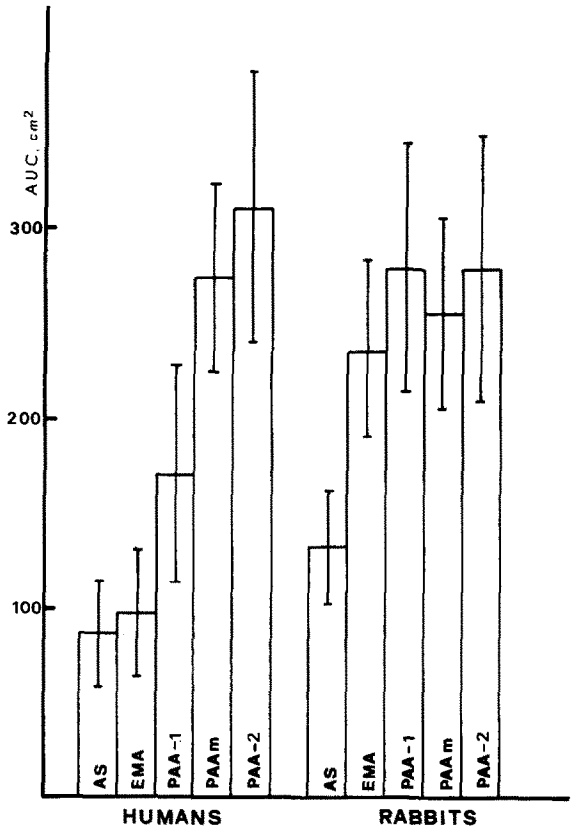

Fig. 3. Areas under the miotic response vs time curves (AUC) obtained in the two species with the vehicles under study. Vertical lines over bars indicate 95% C.L.

ing) order: PAA-2 > PAAm > PAA-1 > EMA > AS. PAA-2 and PAAm produced an approximately 3-fold increase of AUC with respect to AS, while the AUC corresponding to PAA-1 was intermediate between those of the two former vehicles and those of AS and EMA. The AUC values for all vehicles and for both species are represented graphically, with the corresponding 95% confidence limits, in Fig. 3. This figure, which allows a clearer comparison of the relative values of this parameter, and of the statistical differences, provides evidence for the dissimilar bioavailability of pilocarpine from the same preparations in the two species.

Rheological data

The rheological characteristics of the hydrogels are summarized in Table 3. PAA-1, PAA-2 and EMA showed a simple, plastic type of flow, while PAAm was pseudo-plastic. The apparent viscosity at a rate of shear of 16 s^{-1} was also calculated for all vehicles. Although of limited value, this parameter was considered of some use for correlation purposes. Correlations between yield value of the three plastic vehicles and duration of activity are presented in Fig. 4. The graph clearly shows that, in rabbits, an increase of yield value from 140 to 706 did not bring about any increase of duration. In humans, the effect on duration of increased yield value was rather small. A similar correlation between yield values and AUC, presented in Fig. 5, again shows that no bioavailability increase resulted in rabbits from an augmented yield value, while in humans such an increase was clearly apparent. The correlation between AUC and apparent viscosity, presented in Fig. 6, also shows that the latter parameter had no influence on bioavailability in rabbits. In humans, a dependence of bioavailability on apparent viscosity was evident for the plastic vehicles, while the pseudo-plastic one (PAAm) fell out of the correlation line, showing an AUC value much higher than expected on the basis of its relatively low apparent viscosity.

TABLE 3

RHEOLOGICAL CHARACTERISTICS OF THE HYDROGELS

Vehicle	Type of flow	Pastic viscosity (cps)	Plastic yield value (dynes/cm^2)	Apparent viscosity at 16 s^{-1} (cps)
PAA-1	plastic	27.5	140	51.6
PAA-2	plastic	29.7	706	87.5
EMA	plastic	11.8	170	35.3
PAAm	pseudo-plastic	–	–	41.9

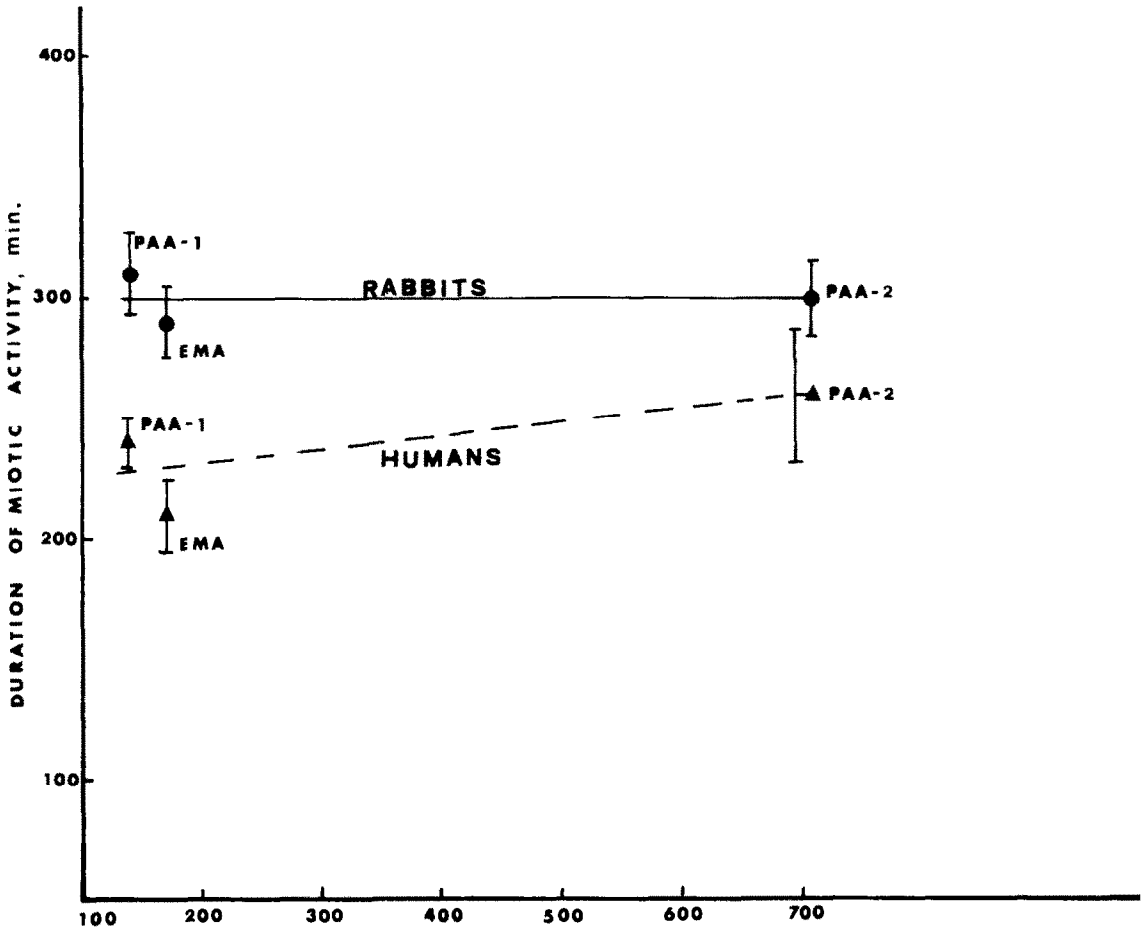

Fig. 4. Correlations between plastic yield values of the hydrogels and duration of miotic activity in the two species.

The reported data appear to confirm in general the previous indications that hydrogel vehicles can improve the ocular bioavailabilty of pilocarpine with respect to an aqueous solution, presumably as the result of a prolonged retention in the eye. Although the activity enhancement was evident in both species, some important differences in the response of rabbits and humans to the same viscous vehicles were readily apparent.

Rabbits responded to the hydrogel vehicles with an increased duration of action, not accompanied by a concomitant increase of I_{max}, and gave practically the same response to vehicles showing different rheological characteristics and chemical structure of the polymeric matrix (cf. Fig. 1). An inspection of Fig. 3 shows that, in these animals, the ocular bioavailability of the drug from all four hydrogels was practically doubled with respect to the reference solution, AS, and that no significant differences existed among the AUC values of these vehicles.

In humans, however, marked bioavailability differences could be detected among the hydrogels, and the maximum bioavailability increase was 3.6 times with respect to AS. The correlations found between duration, or AUC, and some viscosity parameters of the hydrogels (Figs. 4–6) seem to indicate viscous effects as responsible for the observed activity differences in humans, at least for the plastic vehicles. A greater sensitivity of the human eye to viscous effects was described in previous reports (Saettone et al., 1982, 1984),

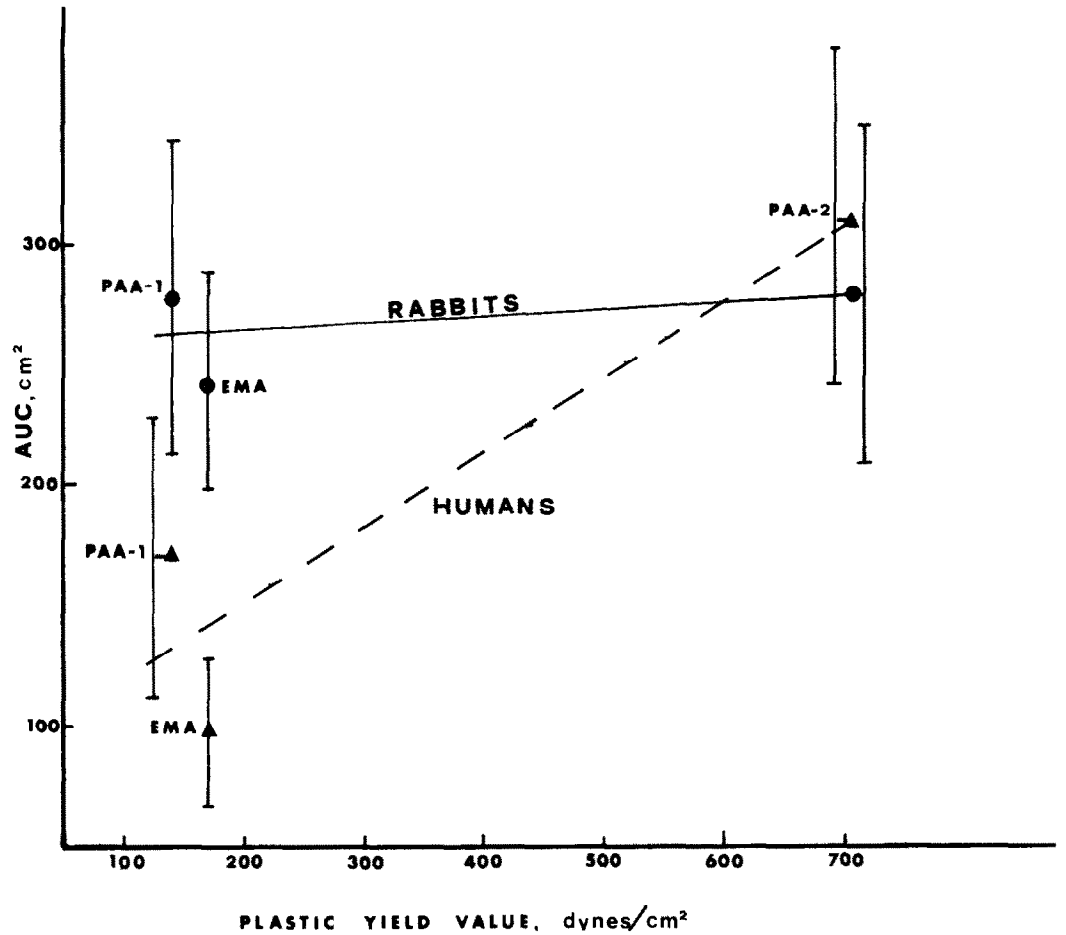

Fig. 5. Correlations between plastic yield values of the hydrogels and AUC values of the hydrogels in the two species.

and was attributed to the different ocular dynamics of instilled vehicles, resulting from the different human ocular anatomy and physiology.

The particular behaviour of EMA in humans deserves some comment. This vehicle, on account of its gel-like consistency and plastic viscosity, should have been better retained in the eye compared to AS, but it failed to show an increased bioavailability with respect to the reference aqueous vehicle. As indicated before, the poor performance of EMA in humans is probably to be attributed to a slightly irritant effect, which induced lacrimation in many subjects thus favouring a rapid elimination from the eye.

The high bioavailability of pilocarpine produced by PAAm, on the other hand, cannot easily be explained on the basis of the rheological behaviour (pseudo-plastic) of this vehicle, nor of its apparent viscosity, that was relatively low, and intermediate between those of EMA and PAA-1. According to Patton and Robinson (1978), if a pseudo-plastic system undergoes shear in the eye it will thin and drain from the eye, thus being poorly retained. The present results, on the contrary, seem to indicate that pseudo-plasticity, i.e. the capacity of a gel to flowing under very low shear stresses, may not be a negative factor per se. It can be speculated that a pseudo-plastic vehicle, such as PAAm, might flow and spread over the precorneal area more easily than a plastic one, that behaves as a solid until the shear stress exceeds the plastic yield value.

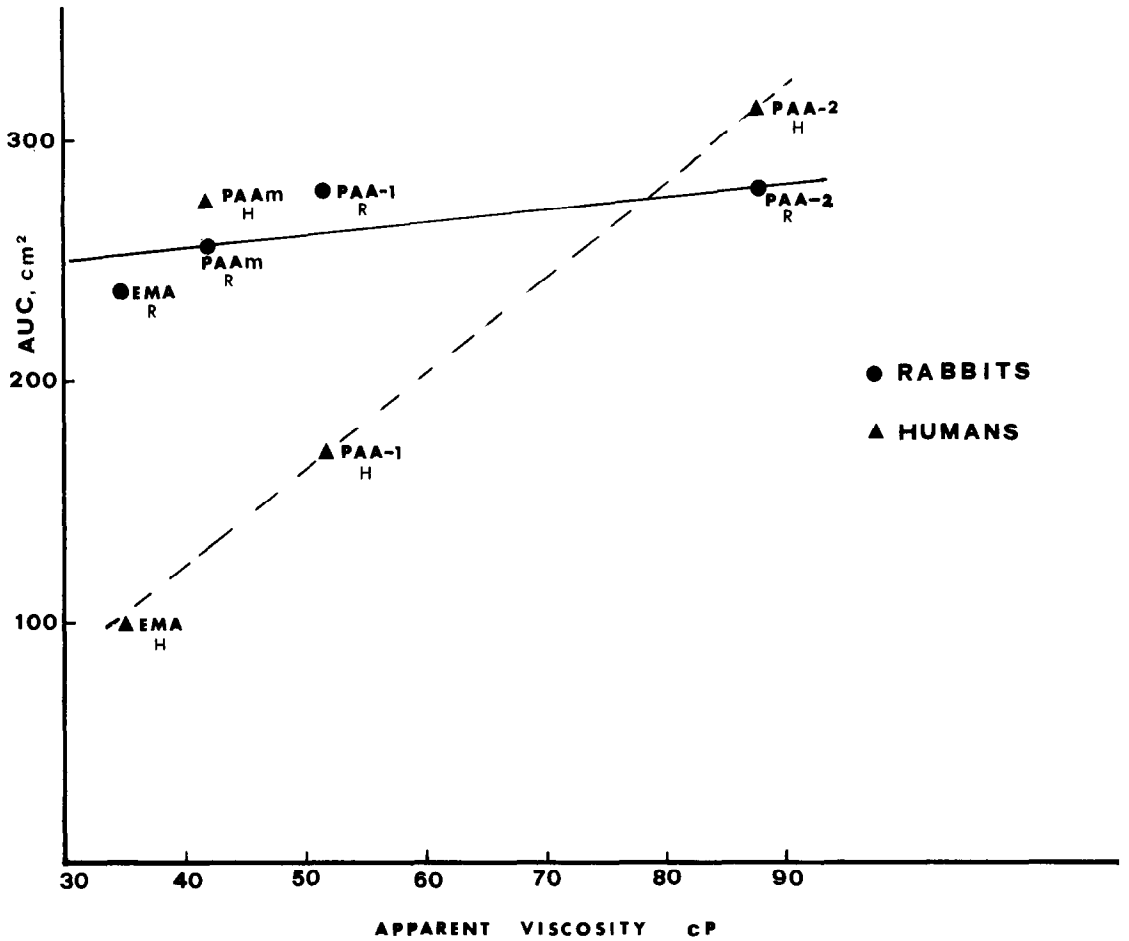

Fig. 6. Correlations between apparent viscosity of all hydrogels and AUC values in the two species.

In general, the hydrogels under investigation appeared to exert little, or no control of pilocarpine release. According to Schoenwald et al. (1978), in the case of improved retention and bioavailability, an indication of controlled release is a decrease in I_{max} with a delay in T_p, while an increase in I_{max} and no change in T_p indicate that release is rapid and uncontrolled. The delayed peak times shown by PAAm and by PAA-1 with respect to PAA-2 might indicate some degree of control, particularly for the former vehicle that also showed the longest duration of activity. In no case, however, was a statistically significant decrease in I_{max} observed in humans with respect to the reference solution.

In conclusion, the present preliminary evaluation has confirmed the positive properties of some organic hydrogels, particularly the poly(acrylic) derivatives PAA-2 and PAAm, as vehicles for pilocarpine. These two vehicles performed in humans, as regards bioavailability and duration of activity, much better than the other two hydrogels, while in rabbits the differences went unnoticed. This indicates once more that rabbits, although valid as experimental models for a preliminary screening of prospective ophthalmic drugs and vehicles, do not adequately simulate the behaviour of viscous vehicles in the human eye.

As a final remark, it should be pointed out that in the present explorative study the vehicles contained a pilocarpine concentration (0.13% w/v) which was much lower with respect to the usual

therapeutic concentrations. A threshold dose, i.e., the minimum amount of drug still giving a measurable miotic response, was applied in order for any increase in bioavailability brought about by the hydrogel vehicles to be readily apparent. The much higher concentrations (2–4%) required to elicit the complex response, resulting in facilitation of the aqueous outflow and reduction of the intra-ocular pressure, usually induce an intense and long-lasting miosis that severely complicates or hinders the interpretation of vehicle effects. Following a similar line of reasoning, Lee and Hammarlund (1975) used very dilute solutions of mydriatics to evaluate vehicle effects on transcorneal penetration of homatropine and tropicamide. It is hoped that further tests on the same vehicles containing therapeutic concentrations of pilocarpine, carried out on glaucoma patients, will validate the present results, that, at this stage, are only indicative of the relative capacity of the hydrogels to be retained in the eye and to deliver sub-therapeutic amounts of the drug.

Acknowledgement

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