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MUCO-ADHESIVE LIQUID OPHTHALMIC VEHICLES - EVALUATION OF MACROMOLECULAR IONIC COMPLEXES OF PILOCARPINE

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ABSTRACT

Pilocarpine (Pi), a widely used anti-glaucoma drug, is characterized by a very low bioavailability, due to poor corneal penetration and extensive precorneal loss. Purpose of the present study was the preparation and "in vivo" evaluation of a series of liquid formulations containing salts (or ionic complexes) of Pi with soluble polyanionic polymers of natural, synthetic or semi-synthetic origin. It was speculated that since to some of the polymers have been attributed muco-adhesive properties, they might favour the preocular retention of the ionically bound drug, and enhance its bioavailability. The polymers submitted to investigation were a) hyaluronic acid (HA); b) poly-(galacturonic acid) (PGA); c) Mesoglycan (MG, a complex mixture of mucopolysaccharides); d)

Carboxymethylchitin (CMCh) and d) two poly(acrylic acids) of different molecular weight (PAA1 and PAA2). Aqueous solutions of the Pi polymer salts, each containing 1.53% w/w Pi base (equivalent to 2.0% Pi nitrate) were tested for miotic activity in albino rabbits, using as reference an aqueous, 2% solution of Pi nitrate, either as such or viscosized with 1.5 and 5.0% poly(vinyl alcohol), (PVA). All polymeric solutions enhanced, in some cases to a statistically significant extent, the bioavailability of the drug with respect to the reference solutions. The relevance of viscosity effects, and of possible muco-adhesive phenomena to the bioavailability of Pi from the salt-vehicles are discussed.

INTRODUCTION

In spite of a rising demand for beta-adrenergic blocking drugs in the therapy of glaucoma, pilocarpine (Pi) still continues to be the medicament of choice in a variety of cases. This drug, however, is characterized by particular physicochemical properties, which influence negatively its transcorneal absorption. As a result, only 1-2% of the Pi dose applied as an aqueous solution penetrates the cornea and gains access to the aqueous humour (1). Several attempts to circumvent this disadvantage of Pi and to increase its

bioavailability and duration of action have been reported (2). Most of these approaches rely on extending the time during which the drug remains in contact with the cornea, and essentially consist of increasing the viscosity of the vehicle (up to that of a semisolid ointment or hydrogel), thus opposing the physiological ocular drainage processes.

The alternative approach tested in the present investigation consists of administering a solution in which the drug is ionically bound, via its basic imidazole moiety, to a polycarboxylic polymeric carrier. Two comparable approaches have been reported in the past. In one case Pi was ionically bound to alginic acid; the compound, however, was intended to be administered as a solid-state ophthalmic device (3). In the second case the synthetic polymeric carrier [poly(acrylic acid-lauryl methacrylate), denominated Piloplex], was insoluble in water, and gave rise to an "emulsion" type of vehicle, which precipitated in the tear fluid forming a drug depot in the cul-de-sac (4).

The present evaluation of "soluble" Pi polymer salts was prompted by the consideration that certain polymers containing carboxyl groups, both of synthetic and natural origin [such as e.g. some poly(acrylic acid) derivatives and glycosaminoglycans] have been shown capable of good muco-adhesion in the eye (5,6).

It was speculated that the muco-adhesive polymer would be retained in the precorneal area, while delivering the ionically bound drug to the target tissue at a sustained rate, in analogy with sustained release of drugs bound to ion-exchange resins. This might potentially provide the basis for a liquid controlled/sustained delivery system.

This report describes a series of vehicles formulated on the above assumptions, and discusses their "in vivo" performance (miosis tests in rabbits).

EXPERIMENTAL

a) Materials. The following materials were used as received: hyaluronic acid, HA, MW 113.000, [η] 3.5 dl/g (20°C), 2.56 mEq/g (Fidia, Abano T., Italy); poly(galacturonic acid), PGA, 4.89 mEq/g (Orion Chemicals, Milano, Italy); low-MW (750,000) poly(acrylic acid), PAA1, and high-MW ($4 \cdot 10^6$) poly(acrylic acid), PAA2, both 10.0 mEq/g (Carbopol^R 910 and 940, respectively, Goodrich Chem. Co., Cleveland, U.S.A.); poly(vinyl alcohol), PVA, (Polyviol W 48/20, Wacker Chemie, Burghausen , F.R.G.).

Pilocarpine base, PiB, was obtained from the nitrate (m.p. 176-78°C, Sigma Chem. Co., St. Louis, U.S.A.) by extraction of the alkalinized solution. Mesoglycan, MG, (Mediolanum S.p.A., Milano, a purified mixture of acid muco-polysaccharides extracted from

bovine aorta, consisting essentially of dermatan sulfate and of heparan sulfate) and carboxymethylchitin, CMCh, (Ichimaru Pharcos Co., Takatomi, Japan), both supplied as sodium salts, were transformed into the corresponding acid forms, denominated MGA and CMChA respectively, by passing their aqueous solutions over an ion-exchange resin (Amberlite IR 118 H, Polysciences Inc., Warrington, U.S.A.). Titration of the resulting materials gave values of 2.59 mEq/g for MGA and 1.43 for CMChA.

b) Vehicles. The Pi polymer salt vehicles were prepared by neutralizing PiB solutions with solutions of the acidic polymeric materials, whose amounts were calculated from the respective neutralization equivalents. The final PiB concentration was in all cases 1.53%, equivalent to 2.0% pilocarpine nitrate ($PiNO_3$). Three 2.0% $PiNO_3$ solutions were used as reference standards for the biological tests: one (denominated AS) consisted of commercial, non-viscosized aqueous eyedrops, the others (denominated PVA1 and PVA2) were 2.0% aqueous solutions of $PiNO_3$, viscosized with 1.5 and 5.0% PVA, respectively. The rheological data of all preparations were determined at 30 °C, using a Rotovisco RV 12 viscometer (Haake, Karlsruhe, F.R.G). The composition and some relevant physicochemical data of all polymeric vehicles are listed in Table I.

Table I - Composition and physicochemical data of the polymeric vehicles containing ionically bound pilocarpine, and of the reference PVA vehicles.

Polymer	Conc., g % w/w	Viscosity mPa•sec	pH
HA	2.94	2.3	4.5
PGA	1.5	3.7	6.1
MGA	2.83	2.0	6.3
CMChA	5.1	5.3	6.6
PAA1	0.73	54.0*	6.5
PAA2	0.73	97.0*	6.5
PVA1	1.50	3.6	4.9
PVA2	5.00	62.0	4.8

All vehicles (with the exception of PVA1 and 2) contained 1.53% w/w PiB (equivalent to 2.0% $PiNO_3$).
*Pseudoplastic. The apparent viscosity was calculated at a value of D (rate of shear) of 80.3 sec^{-1}.

c) Biological studies. Miotic activity tests were carried out on non-anaesthetized, male albino rabbits weighing 2.5-3.0 Kg, using a standard procedure (7). The applied dose was in all cases 50 µl; each vehicle was tested at least on six rabbits. The results were calculated as the average variation of pupillary diameter, with respect to the basal diameter, vs. time.

Table II - Pharmacological parameters of the vehicles*

Vehicle	I_{max} mm	Peak time, min	duration min	K_e**	AUC cm²	AUC (rel.)
AS	2.3(0.24)	30	150	4.3	39(6)	1.00
PVA1	2.5(0.20)	30	180	4.0	53(10)	1.36
PVA2	2.8(0.22)	30	210	3.4	65(9)	1.66
HA	3.0(0.33)	30	210	2.9	64(5)	1.65
PGA	3.4(0.51)	40	210	3.2	70(14)	1.80
MGA	3.8(0.27)	30	240	3.6	82(12)	2.10
CMChA	4.2(0.63)	30	240	3.8	84(14)	2.15
PAA1	3.4(0.39)	20	240	2.6	77(17)	1.98
PAA2	3.0(0.33)	20	270	2.2	95(14)	2.44

*The numbers in parentheses are ± 95% confidence limits.
**First order rate constant for apparent elimination, determined from the terminal slopes of the lines of log change in pupillary diameter vs. time plots. Units are $min^{-1} \cdot 10^3$.

RESULTS

The results of the miotic activity study carried out on all Pi vehicles are summarized in Table II. When compared with AS, the polymeric Pi salts showed increased bioavailability (1.65- to 2.44-fold) and I_{max} values, with statistically significant differences in all cases. In no case, however, a true sustained

activity pattern was observed, but rather a prolonged-pulse type of miotic activity. This was evident from the miotic activity vs. time graphs (not reported in this paper), but it can also be easily deduced from some of the pharmacological parameters listed in Table II, such as the peak times, which were short in all cases, the high I_{max} values, and the values of the rate constants for apparent elimination of the drug from the eye, K_e, which were only slightly lower than the value corresponding to AS (with the possible exception of PAA2). The two "viscous" vehicles containing $PiNO_3$, PVA1 and PVA2 also showed increased activity parameters with respect to AS, with a statistically significant difference only in the case of the more viscous vehicle, PVA2. When compared on a similar viscosity basis with the polymeric Pi salts, the PVA vehicles showed in many cases inferior performances. This is best evidenced in Fig. 1, where the AUC values of all vehicles, with the relevant 95% confidence limits, are plotted vs. the logarithm of the viscosity of the solutions.

The PVA1 vehicle, whose viscosity (3.6 mPa·s) was in the same range as those of MGA, HA, PGA and CMChA (2.5 - 5.3 mPa·s) was significantly less active than MGA and CMChA, while the more viscous vehicle PVA2 (62 mPa·s) was significantly less active than PAA2.

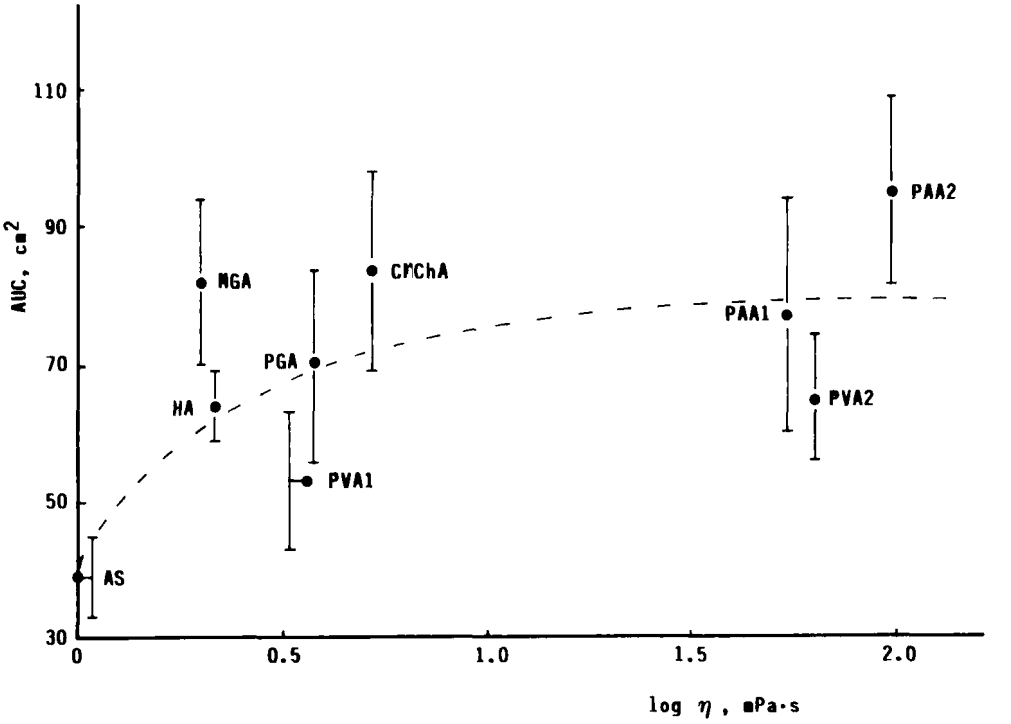

Fig. 1 - Plot illustrating the relationship existing between AUC values and apparent viscosity of the vehicles. Vertical lines indicate 95% confidence limits.

DISCUSSION

In a discussion of the present results, a preliminary distinction should be made between two main factors potentially leading to an increased bioavailability of Pi from the polymeric vehicles, namely, viscous and muco-adhesive effects. Among the vehicles, the reference ones, PVA1 and PVA2, can be reasonably assumed to be devoid of muco-adhesive

properties, and to owe their bioavailability-enhancing activity only to viscous effects. In fact, although PVA has been indicated to possess some structural requirements desirable for bioadhesion (8), it is usually considered non muco-adhesive, and it has been used by other investigators in a similar study as a reference vehicle displaying only viscous properties (9).

The data in Fig. 1 illustrate the possible bioavailability gains that can be obtained in rabbits by increasing the vehicle viscosity with PVA: a 1.36-fold AUC increase (over AS) was obtained with a viscosity increase from 1.0 to 3.6 mPa·s (PVA1). A further viscosity augmentation to 62 mPa·s (PVA2) did not cause a proportional bioavailability gain, since the AUC increase of PVA2 over AS was only 1.66-fold. Furthermore, the AUC values obtained with the two reference PVA vehicles of quite different viscosity were not statistically different from one another. These results confirm the findings of Patton and Robinson, who, in a basic paper on the effect of PVA vehicles in rabbits (10), indicated that to viscosity increases beyond 12-15 mPa·s do not correspond further significative increases of Pi concentration in the aqueous humour.

When evaluating the results obtained with the prospective muco-adhesive vehicles, it should be

remembered that their overall activity might result from a combination of viscous and of muco-adhesive effects. Thus, the muco-adhesive properties should emerge from a comparison of the Pi salt vehicles with reference vehicles of similar viscosity. A further effect potentially influencing bioavailability, as already stated in the introduction, might consist of sustained release of the ionically bound drug. This factor however, as it will be discussed in a further paragraph, was found to play a minor role in the case of the present Pi vehicles.

Within the "low viscosity" series (HA, MGA, PGA and CMChA) all vehicles showed higher AUC values with respect to PVA1, but only in the case of MGA and of CMChA the differences were statistically significant. The results obtained with MGA appear particularly interesting, on consideration of the relatively low viscosity of the solution (2.0 mPa·s). The 1.55-fold AUC increase observed for MGA over the reference vehicle PVA1 (slightly more viscous) should thus be attributed to muco-adhesive effects, resulting from the particular structure and composition of the polymeric carrier.

The increased bioavailability produced by the viscous PAA1 and -2 vehicles with respect to their reference vehicle PVA2, might also be reasonably

attributed to bioadhesive effects. The results, which were particularly good in the case of the high-MW poly(acrylic acid) PVA2, appear to confirm the muco-adhesive properties already reported for this class of polymers (5,11,12).

It should be observed, however, that the AUC value corresponding to PAA2 (95) was not statistically different from those of MGA and of CMChA (82 and 84, respectively), the only possible advantage of the more viscous (97 mPa·s) PAA2 being a slightly increased duration of activity (270 vs. 240 min). These data indicate clearly that viscosity may not be relevant to ocular muco-adhesion, and appear to agree with the main criteria for bioadhesion indicated by Kinloch (13), which include a zero o near zero contact angle (i.e., good wetting properties), a relatively low viscosity and a good contact between the bioadhesive and the substrate.

In conclusion, some of the investigated polymers appear as interesting materials for the realization of improved ophthalmic vehicles. They probably owe their characteristics to the mucopolysaccharide (HA, MGA, CMChA) or homopolysaccharide (PGA) structure, which may favour the development of strong interactions with the glycoproteinic network of the mucus, which itself is a complex mixture of mucopolysaccharides, glycoproteins and related materials (8).

It should be noted in this context that Pi is presumably not the best model drug for muco-adhesive vehicles. Indeed the drug, even if ionically bound to the polymers, showed at most a prolonged-pulse type of activity. This indicates that it probably diffused out of the vehicles (after being released in the lacrimal fluid as a result of ionic equilibria) more rapidly than the vehicles themselves were cleared from the precorneal area. A similar situation has been denounced in the case of Pi-containing nanoparticles or liposomes (2). The hypothesis of a fast diffusion of Pi from the Pi-salt vehicles is also substantiated by the observation that the relative AUC values obtained with PAA1 and PAA2 with respect to AS and to PVA2 agree satisfactorily with those calculated from the data of Davies et al. (9), who used in a similar study a PAA vehicle containing $PiNO_3$, not ionically bound to the polymeric carrier.

It ought to be finally pointed out that the present results, although interesting per se, should be assumed valid only for rabbits. These animals show a different sensitivity to viscous effects with respect to humans, a phenomenon attributable to significative differences in ocular physiology and lacrimal dynamics existing between the two species (14), and the same

inter-species difference might exist for muco-adhesive effects. Thus, further comparative studies on humans will be necessary in order to fully assess the potentiality of the present delivery system.

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