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Evaluation of muco-adhesive properties and in vivo activity of ophthalmic vehicles based on hyaluronic acid

Marco F. Saettone, Patrizia Chetoni, Maria Tilde Torracca, Susi Burgalassi and Boris Giannaccini

Laboratorio di Tecnologie Farmaceutiche-Biofarmacia, Università di Pisa, Pisa (Italy)

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Summary

A series of prospective ophthalmic vehicles based on hyaluronic acid (HA) and on polyacrylic acid (PAA) (solutions, gels, matrices prepared by compression and by casting) containing pilocarpine (Pi) or tropicamide (Tr) was evaluated for muco-adhesion, for ocular retention and for biological activity (miosis, mydriasis) in rabbits. The muco-adhesive properties were investigated in vitro using a tensile apparatus with mucin-coated surfaces, while the ocular behaviour was estimated visually, using vehicles containing a fluorescent marker. Good to excellent muco-adhesive properties were detected in the HA preparations. The bioavailability-enhancing effect, however, was not very satisfactory with Pi, probably on account of the high solubility and diffusivity of the drug. The effect was more evident with the less soluble drug Tr. The validity of the method used for evaluating bioadhesion, and the relevance of the physicochemical characteristics of the drug to a muco-adhesive ocular delivery system are discussed.

Introduction

Although several natural and synthetic polymers have been screened for their capacity of adhering to mucin–epithelial surfaces, little attention seems to have been dedicated to ocular applications after the pioneering work of Hui and Robinson (1985), who reported that the ocular bioavailability of progesterone was significantly increased by a bioadhesive polymer. Indeed, the development of vehicles endowed with the capacity of adhering to the conjunctival tissue and/or to its mucin coating might constitute an interesting alternative approach to the improvement of the bioavailability of ophthalmic medications.

Previous reports from this laboratory (Saettone et al., 1985, 1986a) have shown that some low-viscosity ophthalmic preparations based on hyaluronic acid (HA) are capable of enhancing the bioavailability of pilocarpine, and that some vehicles based on this natural polymer show strong bioadhesive properties in vitro (Saettone et al. 1987). These results appeared to agree with an earlier report by Park and Robinson (1984), who had observed, using a fluorescent in vitro technique, a strong binding of HA to isolated human conjunctival cells. Recent reports by Camber et al.

Correspondence: M.F. Saettone, Laboratorio di Tecnologie Farmaceutiche-Biofarmacia, Università di Pisa, Via Bonanno 33, 56100 Pisa, Italy.

(1987) and by Gurny et al. (1987) have confirmed the positive influence of sodium hyaluronate vehicles on the miotic effect of pilocarpine in rabbits and in humans. All these findings warranted a more accurate investigation on the muco-adhesive properties of HA salts and derivatives, in view of potential ophthalmic applications.

The aims of the present investigation were: (a) to prepare a series of HA-based semi-solid and solid vehicles containing representative ophthalmic drugs (pilocarpine or tropicamide); (b) to evaluate their ocular retention in rabbit eyes and their muco-adhesive properties in vitro, in comparison with reference polymeric preparations; and (c) to test their in vivo activity, by measuring the pupillary responses induced in rabbits.

Experimental

Materials

The following materials were used as received: four poly(acrylic acid) polymers (Carbomers), denominated PAA-1, PAA-2, PAA-3 and PAA-4 (Carbopol 910, 934, 940 and 941, respectively, Goodrich Chemical Corp.); two HA sodium salt fractions and one partial ethyl ester of HA, denominated HA1-Na, HA2-Na and HAE, respectively (Fidia SpA); high-viscosity porcine gastric mucin (PGM, Biochemo SpA); pilocarpine nitrate (PiN), m.p. 176–178 °C (Merck). Tropicamide (Tr), m.p. 96–97 °C (Prodotti Roche), was micronized prior to use (Fryma JMRS-80 jet mill) to yield particles of average diameter (geom., microscopic analysis) 4.5 μm.

Some essential characteristics of the material are as follows: PAA-1 to -4: mol.wt. 7.5×10^5; 3.0×10^6 and 4.0×10^6 and 1.25×10^6, respectively; HA1-Na: mol.wt. $134\,000 \pm 9000$, $[\eta] = 3.2$–4.0 dl/g; HA2-Na: mol.wt. $620\,000 \pm 50\,000$ $[\eta] = 10.8$ dl/g; HAE: mol.wt. 160 000, carboxyl groups 75% esterified by ethanol and 25% salified by pilocarpine base (PiB, theoretical PiB content 11.5%); PGM: η (1.0% w/w water solution) = 3.2 mPa · s, UV_{max} (0.05% w/w water solution) 258 nm, E = 1050, weight loss on drying (75 °C, 48 h) 9.43%.

Vehicles

(A) Vehicles for the bioadhesion study in vitro. PAA gels were prepared by neutralizing with 1 N NaOH 10% w/w water dispersions of the polymers, and subsequently diluting with water to obtain a final 5.0% w/w concentration of polymers. Solutions of HA1-Na (15.0% w/w) and of HA2-Na (5.0% w/w) were prepared by dissolving the polymers in distilled water. HA films were prepared by slow evaporation (40 °C) in Petri dishes of appropriate amounts of HA1-Na (5.0% w/w) or HA2-Na solutions (2.0% w/w). Circular matrices (diameter 12.0 mm, thickness 0.16 mm, average weight 17.0 mg) were cut from the dry films. Matrices of similar size and weight were prepared by compressing HAE, HA1-Na or HA2-Na by means of a hydraulic press, under a compression force of 3.0×10^8 Pa. Due to the composition of the material, the HAE matrices contained 11.5% w/w PiB.

(B) Vehicles for the ocular retention study. For an investigation on the behaviour in rabbits' eyes, 1% w/w sodium fluorescein was added to some of the above vehicles (PAA-3 and PAA-4 gels, HAE, HA1-Na and HA2-Na matrices, prepared both by casting and by compression). In order to obtain homogeneous dispersions of the fluorescent marker in the matrices prepared by compression, solutions of the HA polymers containing fluorescein (1.0% with respect to the dry polymer weight) were freeze-dried. The resulting mixtures were pulverized and compressed.

(C) Medicated vehicles for the in vivo tests. PAA gels and HA solutions identical with those described in (A), and containing 2.0% w/w PiN (dissolved) or 1.0% Tr (partly suspended) were used. Pilocarpine-HA films were prepared by slowly evaporating HA1-Na or HA2-Na solutions, at the concentrations indicated in (A), containing 1.56% w/w PiN. From the films were cut, using a punch, circular inserts (diameter 4.0 mm) of average weight 4.5 mg, each containing 1.0 mg PiN. Inserts of similar size and drug content were prepared by compressing appropriate mixtures of HA1-Na or HA2-Na and PiN. HAE was compressed directly in a 4.0 mm die to give an insert of thickness 0.4 mm and weight 6.7 mg, containing 0.77 mg PiB (equivalent to 1.0 mg PiN). Tr inserts

were prepared by compressing appropriate mixtures of the drug plus HA1-Na or HA2-Na. Each insert (average weight 4.0 mg, diameter 4.0 mm, thickness 0.25 mm) contained 0.5 mg Tr.

The drug identity and content of all preparations was monitored by HPLC in the case of PiN (Dunn et al., 1981), and spectrophotometrically (λ_{max} 256 nm) in the case of Tr, after appropriate extraction procedures.

Methods

(A) In vitro evaluation of the muco-adhesive properties. The muco-adhesive properties of the PAA gels, and of the HA solutions and matrices were evaluated by measuring the force required to separate two mucin-coated surfaces, between which were placed the samples. The apparatus was a modification of the one originally described by Ch'ng et al. (1985), and consisted essentially of a testing cell, and of an electronic digital microbalance connected to a recorder through a digital–analogue converter. The testing cell (Fig. 1) consisted of two cylindrical teflon sections: the lower one was weighted and placed in the bottom of a jacketed beaker, while the upper one (weight = 6.0 g) was suspended by means of a thin steel wire under the balance pan. Two wet filter-paper disks (MN 6/7, Macherey Nagel) were tightly secured to both sections, and 0.125 ml of a mucin gel (consisting of a 15.0% w/v dispersion of PGM in water) was spread in a uniform layer on each disk. Following application, the mucin layers were superficially dried for 5 min with cold air blown by a hair dryer. Alternatively, sections of rabbit stomach, mucosal side out, could be affixed and secured to each cell section. In both cases (mucin, or tissue) the area available for contact in each cell section was 1.13 cm^2. For the measurements, the weighted lower cell section was completely immersed in the thermostatted (30 °C) saline or buffer solution contained in the beaker. The upper cell section was equally immersed in the solution, and the balance was zeroed with the mucous layers of both cells at the same depth. The beaker, which was placed on a motorized platform, was then slowly raised until both mucous surfaces came into contact with each other, the contact being made with the force of the upper section (6.0 g). After 1 min of contact, the beaker was lowered at constant speed (1.75 mm/min), and the force required to detach the mucin-covered surfaces was recorded. For the measurement of the bioadhesive properties of the formulations, the semisolid vehicles (0.05 ml) or the solid matrices were applied on the upper cell section, freshly coated with mucin (or containing a fresh tissue section), and the procedure described before was repeated, using each time a fresh mucin (or tissue) layer also on the lower cell section. The solid matrices were hydrated before application onto the mucin, by dipping them for a few seconds in distilled water. The following buffer solutions, besides 0.9% NaCl, were used as immersion media: 0.1 M acid potassium phthalate, pH 3.45; 0.1 M phosphate, pH 6.50; and 1.33 mM isotonic phosphate, pH 7.40.

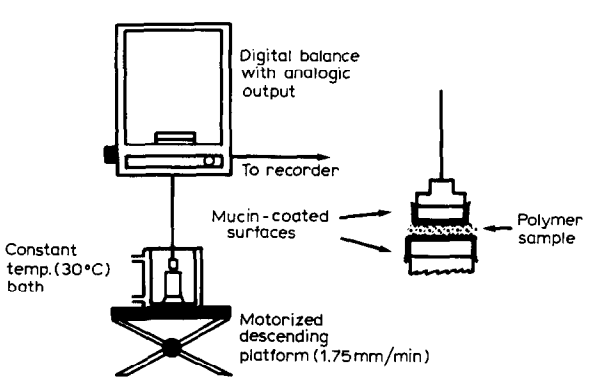

Fig. 1. Diagram of the apparatus used for the evaluation of the muco-adhesive properties of the vehicles.

(B) Evaluation of the time of residence of the formulations in rabbit eyes. The behaviour and the retention time of the fluorescein-containing preparations described under *Vehicles* part (B) were assessed by applying 50 μl of the gels, or one insert, into the lower conjunctival sac of both eyes of the animals. The corneal area was subsequently inspected at intervals, under illumination with a long-wave fluorescent lamp, and the time during which a fluorescent layer was present over the corneal surface was noted. Each vehicle was tested at least on 5 different animals; all measurements were made by the same operator.

(C) Activity tests in rabbits. Miotic (PiN) and mydriatic (Tr) activity tests were carried out on 2.0–2.5 kg New Zealand albino rabbits by stan-

dardized procedures (cf. Saettone et al. 1986b for the miosis tests, and Saettone et al., 1982 for the mydriasis tests). Each vehicle was tested on groups of at least 6 animals; the administered dose in the case of the semi-solid preparations was 50 μl. Commercial 2.0% aqueous PiN eyedrops (Pilocarpina 2%, Farmigea) and 1.0% aqueous Tr eyedrops (Visumidriatic 1%, Merck Sharp and Dohme) were used as reference vehicles.

Results and Discussion

The present prospective ophthalmic vehicles were of two types: semisolids and solids. The semisolid ones consisted of poly(acrylic acid) (PAA) hydrogels, and of hyaluronic acid (HA) solutions. The latter vehicles are defined as "solutions" because, although displaying the physical appearance of gels with a viscoelastic rheological behaviour, they are "true liquids", maintaining their characteristics on dilution (Gibbs et al., 1968; Balasz and Band, 1984). The solid preparations were HA matrices, prepared by casting from aqueous solution or by compression. The bioadhesion study was carried out using non-medicated vehicles: preliminary tests performed both on medicated and on drug-free vehicles proved that the presence of the drug (pilocarpine or tropicamide) had no significant influence on their adhesive behaviour.

Other preliminary tests were directed at validating the apparatus and the technique used for the measurement of muco-adhesion. To this purpose, a series of experiments were carried out using freshly excised rabbit stomach as the mucosal surface in the testing cell, and a PAA-3 gel and a HA-2 solution (both 5.0%) as bioadhesive vehicles. The observed mean detachment forces at pH 7.4 (in dyne/cm^2 ± 95% C.L., $n = 10$) were 656 (198) and 732 (262). These values were considered to be in satisfactory agreement with those observed by Ch'ng et al. (1985) for polycarbophil (ca. 1200 and ca. 300 dyne/cm^2 at pH 6 and 7, respectively) using a similar apparatus. The ex vivo experiments, however, were characterized by a very high variability, due to the inherent fragility, instability and sensitivity to environmental factors of the biological material. An extreme fragility of the mucous layer associated with biological membranes was also remarked on by Robert et al. (1988), who put forward a new experimental method for evaluating bioadhesion, based on mucin-free epithelium. The use of porcine gastric mucin (PGM) absorbed on filter paper instead of the excised tissue was thus adopted as the standard procedure for the present muco-adhesive study. The method proved simpler and the results were more reproducible, although the observed muco-adhesion values were 2–3 times higher with respect to those measured with rabbit stomach tissue.

TABLE 1

Evaluation of the muco-adhesive properties in vitro of semisolid and solid vehicles

Vehicle		Detachment force/area, dyne/cm^2 (± 95% C.L.)		
		pH 3.45	pH 6.50	pH 7.40
(1) PAA-1	gel	1607 (530)	1401 (250)	1110 (350)
(2) PAA-2	gel	1828 (440)	1700 (460)	1430 (390)
(3) PAA-3	gel	1812 (440)	1436 (170)	1132 (120)
(4) PAA-4	gel	2548 (320)	1764 (380)	1523 (100)
(5) HA1-Na	sol.	2025 (310)	1831 (260)	1804 (260)
(6) HA2-Na	sol.	2890 (560)	2691 (180)	2482 (500)
(7) HA1-Na	(E)	3637 (370)	3190 (810)	2607 (420)
(8) HA1-Na	(C)	2948 (450)	2335 (220)	2081 (330)
(9) HA2-Na	(E)	5677 (700)	6560 (1290)	6729 (1180)
(10) HA2-Na	(C)	4759 (940)	4084 (830)	3720 (800)
(11) HAE	(C)	4550 (630)	5247 (400)	4141 (840)

E = matrix prepared by casting; C = matrix prepared by compression.

Muco-adhesion study

The results of the tests on the PAA and on the HA vehicles, carried out using PGM-coated surfaces are summarized in Table 1.

The data in Table 1 may be commented on as follows.

(a) pH effects. The detachment forces showed in almost all cases a decreasing trend with increasing pH: the values at the 3 pH levels (3.45, 6.50 and 7.40) for each polymeric preparation, however, were never statistically different from one another. In contrast, Ch'ng et al. (1985) reported

for their bioadhesion experiments ex vivo with polycarbophil a maximum adhesion at pH 6, while at lower and higher pH (2–3 and 7, respectively) the bioadhesion of the polymer to rabbit stomach tissue was significantly reduced. These authors attributed this effect to pH-induced physicochemical changes in the mucus layer (altered rheology, ionization) and/or in the polymer (degree of swelling). The low sensitivity to pH effects of the present system is presumably to be attributed to the simpler structure of the mucin gel, not associated with a living epithelium.

(b) Effect on muco-adhesion of polymer and type of formulation. The present data appear to confirm the good adhesive properties of the poly(acrylic acid) polymers, already observed (e.g. by Smart et al., 1984) in the case of Carbopol 934 (PAA-2). Within the examined PAA series, PAA-4 (Carbopol 941) showed the best adhesive performance at all pH values. Of the two HA polymers, one (HA2-Na 5%) performed distinctly better than PAA-4, while the lower-molecular weight HA1-Na, tested at the 15% w/w concentration to bring its apparent consistency to the same level as that of the other preparations, showed essentially the same values as PAA-4. The same HA polymers were also tested as solid matrices, prepared by evaporation of aqueous solutions (E) or by direct compression (C). In all cases the higher-mol.wt. derivative HA2 performed better than the corresponding lower-mol.wt. polymer HA1, and the HA2 E matrices were distinctly more adhesive with respect to the C matrices, particularly at pH 6.50 and 7.40: at the higher pH the HA2-Na E matrix showed the highest mucoadhesive force of the whole group of formulations (6729 dyne/cm^2). The significant differences in muco-adhesion observed at pH 6.50 and 7.40 between the HA2 C and E matrices might be attributed to dissimilarities in the surface structure. Indeed, as pointed out by Peppas and Buri (1985), since the bioadhesive action of a (solid) device occurs at its interface with the mucus, the surface structure, which depends on the preparative technique, may assume a foremost importance in promoting the formation of a strong interfacial bond. However, an influence on muco-adhesion of the rate and extent of hydration of the matrices cannot be discounted, following the observation, reported in a subsequent section, that the C and E inserts showed a different hydration behaviour in vivo.

The HA ethyl ester (HAE) matrix, which could only be prepared by compression due to its limited water solubility, also displayed very good muco-adhesive properties, not significantly different from those shown by the HA2-Na C matrix.

In conclusion, the in vitro tests, while confirming the good muco-adhesive performance of PAA gels, also evidenced outstanding adhesive properties in some HA formulations. These properties were particularly evident in the HAE ester derivative and in the higher-mol.wt. HA2-Na. The solid matrices prepared by casting with the latter material were distinctly more adhesive at higher pH than those prepared by compression.

(c) Site of breaking of the muco-adhesive bond. An important point in a system such as the one used for the present tests, is the localization of the phase, or of the site, where the fracture occurs when the detachment force is applied. Such a fracture, or interaction failure, could occur: (i) within the mucin layer; (ii) at the polymer–mucous interface; or (iii) within the polymer phase. A visual inspection of the cell after each test pointed to the occurrence of separation at one of the mucin–polymer interfaces; it was, however, decided to gather additional data in order to further clarify this point. Thus, the cohesive (or "autohesive") force of the polymeric PAA gels and HA solutions was evaluated at pH 7.40, by substituting the mucin layer in the two sections of the cell with a layer of polymer absorbed on filter paper, and by measuring the detachment force after placing in the cell a sample of vehicle prepared with the same polymer. A comparison of the results, reported in Table 2, with the muco-adhesion data in Table 1 shows that the "cohesive" interaction force was in all cases greater than the corresponding muco-adhesive force, with a statistically significant difference in the case of PAA-2, -3, -4 and of HA1-Na. This rules out the possibility of a failure occurring within the polymer phase in the muco-adhesion tests. On the other hand, had the failure taken place within the mucin network, the apparent muco-adhesive force would have been the same in all the experiments. Thus, the mea-

TABLE 2

Evaluation of the "cohesive" forces of the polymeric semisolid formulations at pH 7.40

Vehicle		Detachment force/area (dyne/cm^2) (±95% C.L.)
PAA-1	5%	1300 (73)
PAA-2	5%	4359 (852)
PAA-3	5%	4040 (870)
PAA-4	5%	2029 (179)
HA1-Na	15%	3447 (580)
HA2-Na	5%	2870 (450)

sured detachment forces should pertain to a separation occurring at one of the two mucin–polymer interfaces, as already anticipated by visual inspection.

Ocular retention study

The behaviour in rabbit eyes of some representative fluorescein-containing vehicles is described in Table 3. The inclusion of a fluorescent marker in polymeric ophthalmic vehicles has been resorted to in many instances (cf. Benedetto et al., 1975) for investigations on the retention time, and on the influence of polymers on the thickness of precorneal tear film. These factors are considered of great importance in an ophthalmic vehicle, since they can ensure a high and constant drug concentration in the tear film, and hence a high ocular bioavailability (cf. Maurice and Mishima, 1984).

An inspection of Table 3 reveals some significant differences in behaviour between the PAA-3 and PAA-4 gels: the latter vehicle was retained longer, and formed a stable, long-lasting (120 min) precorneal film, evidenced by the fluorescent marker. Both HA1-Na inserts (E and C) underwent a fast hydration and apparently disappeared rapidly from the eye; the E insert, however, took a longer time to hydrate (10 vs 2 min), and formed a longer-lasting fluorescent film over the cornea (45 vs 10 min for the C insert). The higher-mol.wt. HA2-Na inserts performed better with respect to the previously mentioned ones: the E insert also in this case underwent a slower hydration (30 vs 10 min), and both formed a stable corneal film lasting 60 min. The HA1-Na and HA2-Na solutions behaved essentially as the corresponding C inserts. The HAE insert did not swell forming a gel-like mass like the other matrices: it underwent instead a slow erosion (6 h) while adhering strongly to the conjunctiva. Although well retained, however, the HAE insert did not promote the formation of a uniform precorneal fluorescent film. In conclusion, some differences among the present vehicles, already observed in the muco-adhesion tests, also emerged from the tests in vivo. The correlations between in vivo ocular behaviour and in vitro

TABLE 3

Evaluation of the behaviour of the semisolid and solid vehicles in rabbit eyes

Vehicle	Remarks
(1) PAA-3 gel	The gel is diluted by the lacrimal fluid, and overflows from the fornyx after 30 min. No uniform corneal film is observed.
(2) PAA-4 gel	The gel is diluted and overflows after 90 min. A corneal film (not very uniform) lasts 120 min.
(3) HA1-Na sol.	The solution is diluted, and overflows after 10 min. A weakly fluorescent film lasts 10 min.
(4) HA2-Na sol.	The solution undergoes slow dilution, with no apparent overflow. A fluorescent corneal film lasts 45 min.
(5) HA1-Na E	The insert hydrates within 10 min forming a gel-like mass. An overflow is observed after 15 min. A uniform corneal film lasts 45 min.
(6) HA1-Na C	The insert hydrates quickly (2 min) forming a gel-like mass. A non-uniform, weakly fluorescent corneal film lasts 15 min.
(7) HA2-Na E	The insert undergoes a slow hydration (30 min) forming a gel-like mass. A fluorescent corneal film lasts 60 min.
(8) HA2-Na C	The insert undergoes a fast hydration (10 min) forming a gel-like mass. A corneal film lasts 60 min.
(9) HAE C	The insert undergoes a slow hydration, then, 90 min after application, it undergoes a slow erosion, which terminates after 6 h. During this time, it clings tenaciously to the scleral conjunctiva. No uniform corneal film is observed, probably on account of a low dissolution rate and insufficient release of fluorescein.

TABLE 4

Summary of the miotic activity parameters in rabbits of some representative vehicles containing pilocarpine

Vehicle	I_{max} [a] (mm) (95% CL)	T_{max} [b] (min)	D [c] (min) (95% CL)	AUC [d] (cm^2) (95% CL)	Relative AUC
AS [e]	2.3 (0.5)	30	150 (18)	39.1 (6.7)	1.00
PAA-4 gel	2.8 (0.2)	40	210 (20)	65.1 (8.8)	1.66
HA1-Na sol.	3.8 (0.3)	15	270 (20)	107.7 (21.5)	2.75
HA2-Na sol.	3.3 (0.3)	30	270 (30)	112.0 (17.3)	2.86
HA1-Na (E)	2.9 (0.4)	30	270 (28)	89.4 (13.8)	2.28
HA2-Na (E)	2.7 (0.4)	40	300 (25)	111.8 (14.2)	2.86
HAE (C)	2.2 (0.5)	90	360 (25)	108.6 (17.8)	2.77

[a] Maximal miotic response.
[b] Peak time.
[c] Duration of activity (time for the pupillary diameter to return to the baseline value).
[d] Area under the miotic activity vs. time curve.
[e] Isotonic, buffered (pH 5.5) aqueous solution containing 2.0% w/w pilocarpine nitrate (Pilocarpina 2%, Farmigea).

mucoadhesion observed for the low- and high-mol.wt. HA inserts, and for the C and E inserts are noteworthy: to a higher mol.wt. and to a slower hydration (the latter depending also on the manufacturing technique) seem to correspond a better muco-adhesion and an improved retention in the eye.

Activity studies in rabbits

(a) Pilocarpine (Pi) vehicles. Table 4 summarizes the results of experiments, in which some representative vehicles were tested for miotic activity on albino rabbits. The vehicles were chosen among those which had shown the best muco-adhesive and retention performances, and contained PiN (or PiB in the case of HAE) in the amounts specified in the experimental section. The administered dose was in all cases 1.0 mg PiN, or the corresponding amount (0.77 mg) of PiB.

All polymeric vehicles were capable of increasing the bioavailability of Pi to a statistically significant extent with respect to the standard aqueous vehicle (AS). The relative AUC increases ranged from 1.66 times for PAA-4 to 2.28–2.86 times for all HA vehicles. It can be observed that the HA vehicles, while significantly increasing the bioavailability of Pi with respect to the PAA-4 gel, did not show any statistically significant AUC difference among themselves, in spite of their different physical structure and chemical characteris-

TABLE 5

Summary of the mydriatic activity parameters in rabbits of some representative vehicles containing tropicamide

Vehicle	I_{max} [a] (mm) (95% CL)	T_{max} [b] (min)	D [c] (min) (95% CL)	AUC [d] (cm^2) (95% CL)	Relative AUC
AS (e)	2.7 (0.3)	30	330 (30)	80 (14)	1.00
PAA-4 gel	2.1 (0.2)	120	600 (58)	174 (25)	2.17
HA-1 Na sol.	2.6 (0.3)	180	900 (85)	289 (52)	3.61
HA2-Na sol.	2.4 (0.2)	120	960 (90)	295 (48)	3.68
HA1-Na (C)	2.5 (0.3)	30	960 (87)	293 (55)	3.66
HA2-Na (C)	2.6 (0.3)	60	1020 (103)	330 (60)	4.12

[a] Maximal mydriatic response.
[b] Peak time.
[c] Duration of activity (time for the pupillary diameter to return to the baseline value).
[d] Area under the mydriatic activity vs time curve.
[e] Aqueous solution containing 1.0% w/w tropicamide (Visumidriatic 1%, M S & D).

tics. A closer inspection of the activity parameters listed in Table 4, however, indicates a rather fast and uncontrolled release (characterized by a high I_{max} value, a short peak time, a relatively short duration) for the HA1-Na sol. and, to a lesser extent, for the HA2-Na sol.; and controlled release (or sustained activity) characteristics for the HAE matrix, which showed a I_{max} lower than that of AS, a delayed peak time (90 min) and the longest duration of activity (6 h) within all the examined preparations. The in vivo behaviour data of the HAE matrix reported in Table 3, which indicate a strong adhesion and a slow erosion, appear in very good agreement with the miotic activity parameters.

(b) Tropicamide (Tr) vehicles. The results of the mydriatic activity tests are reported in Table 5. The rationale for evaluating the activity of Tr in the prospective bioadhesive vehicles was the lower solubility of this drug with respect to Pi. It was reasoned that Pi, on account of its high solubility, might be rapidly leached out of the bioadhesive vehicles by the tear fluid, thus partially voiding any effect due to vehicle-mediated prolongation of the residence time in the eye. Due to the limited water solubility of Tr (570 mg/100 ml), the drug was partially suspended in the semisolid vehicles, and the HA1-Na and HA2-Na matrices could be prepared only by compression. Likewise, an HAE matrix containing ionically bound drug, such as the one containing PiB, could not be prepared on account of the lower basicity of Tr with respect to PiB. The comparison was thus restricted to 6 vehicles, of which in all cases a dose corresponding to 0.5 mg of Tr was administered. An inspection of the mydriatic activity data reported in Table 5 shows that the PAA-4 gel produced a 2-fold, statistically significant AUC increase over the aqueous solution (AS); a further, also significant increase over the PAA-4 gel was produced by the 4 HA vehicles, whose AUC values, as already observed in the case of the corresponding Pi vehicles, did not differ statistically among themselves. The greatest AUC increase (4 ×) was provided by the HA2-Na insert, that also induced a 3-fold increased duration of activity.

A comparison of the data observed with the corresponding Pi and Tr vehicles is provided in Fig. 2, where the AUC values observed for Pi are plotted vs the Tr values. Although in two cases the vehicles were not strictly the same (E inserts for Pi, and C inserts for Tr), the graph shows that the relative bioavailability increases were greater when the vehicles contained Tr: the dotted line represents the result that would be expected if the relative AUC increases produced by the Tr vehicles equalled those produced by the Pi vehicles.

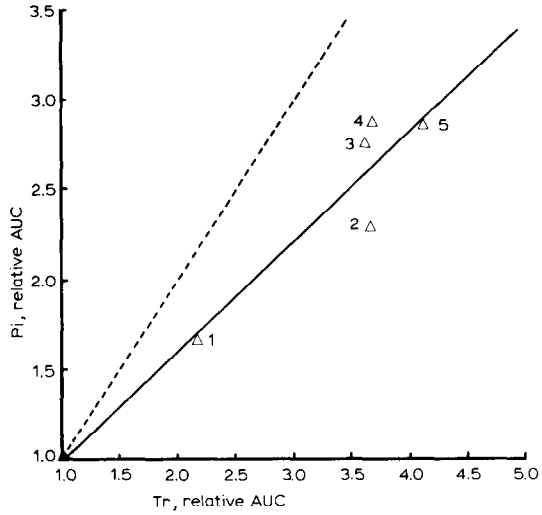

Fig. 2. Comparison of the relative AUC values observed with the pilocarpine and with the tropicamide vehicles. Key: (1) PAA-4 gel; (2) HA1-Na matrices (C, E); (3) HA1-Na sol.; (4) HA2-Na sol.; (5) HA2-Na matrices (C, E). The dotted line represents the results to be expected if the vehicles would produce the same relative bioavailability increases with the two drugs.

This point can also be evidenced in the graphs shown in Fig. 3, illustrating the relationship found, for each of the Pi and Tr vehicles, between "bioadhesion" (detachment force measured at pH 7.40) and AUC values. In spite of a great increase of bioadhesion, the AUC increases observed for the Pi delivery systems were relatively poor in comparison with those observed for Tr. These results, which presumably descend from the previously anticipated "solubility" effect, stress the importance of the permanence of the drug in a bioadhesive ocular vehicle. To profit most from the characteristics of these vehicles, a drug should possess appropriate physicochemical properties, such as a reduced solubility (and/or diffusivity).

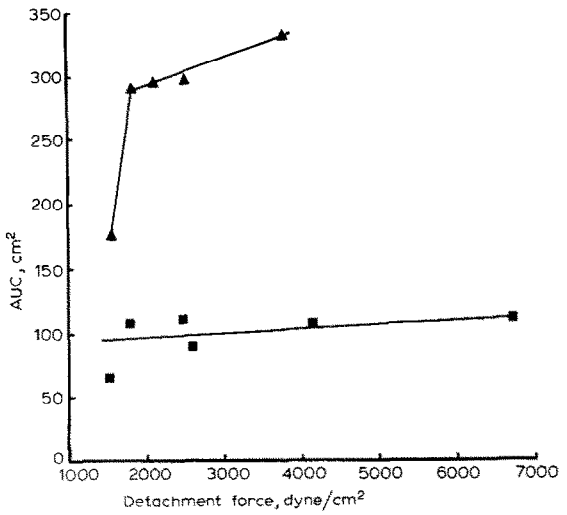

Fig. 3. Graph illustrating the relationship existing between AUC values and muco-adhesive force for the pilocarpine (■) and for the tropicamide (▲) vehicles.

This would ensure for the drug a reasonably prolonged time of permanence in the vehicle, while the vehicle would take care of a prolonged time of residence of itself in the eye.

Conclusions

The present investigation was undertaken in the attempt to identify and evaluate new prospective bioadhesive materials, and dosage forms, to be used for ocular drug administration. The authors are aware of the limitations of the method used for evaluating bioadhesion, and of its only partial relevance to the in vivo situation. Nonetheless, the method yielded useful information on the mucoadhesive properties of some formulations, and this information could be implemented by a direct observation of the behaviour of the vehicles in rabbit eyes. Hyaluronic acid emerged in this context as a very promising and interesting material, as also testified by the results obtained with the less soluble drug, tropicamide. The biological activity data, in particular, showed the relevance of the physicochemical characteristics of the drug to the efficacy of a bioadhesive delivery system. It is hoped that further tests on human volunteers, now in progress, may confirm the present data and shed additional light on the issue of bioadhesion in ophthalmic vehicles.

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