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Evaluation of high- and low-molecular-weight fractions of sodium hyaluronate and an ionic complex as adjuvants for topical ophthalmic vehicles containing pilocarpine

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Summary

Two low-molecular-weight fractions of sodium hyaluronate (Na-HA), denominated Hyalastin® and Hyalectin®, were investigated as potential adjuvants for ophthalmic vehicles containing pilocarpine nitrate (PiN). Tests were also performed on an ionic complex (HA/PiB) prepared from hyaluronic acid (derived from Hyalastin®) and pilocarpine base. The performance of the vehicles under study was verified by miosis and ocular retention tests carried out on albino rabbits, against a series of reference vehicles, three of which contained a high-molecular-weight fraction of Na-HA (Healon®). The group of 14 reference and test preparations exhibited Newtonian or pseudoplastic flow characteristics and encompassed a wide range of apparent viscosities (1 to 1054 mPa s). The results indicate that the HA/PiB salt and the high-MW Na-HA can significantly increase the bioavailability of pilocarpine with respect to reference vehicles of comparable viscosity: an effect that can be reasonably attributed to muco-adhesive effects. Conversely, in the present rabbit tests, the low-MW fractions of Na-HA performed poorly as adjuvants for the PiN solutions.

Introduction

Hyaluronic acid (HA), first discovered in bovine vitreous by Meyer and Palmer (1934), is a natural polysaccharide belonging to the class of glycosaminoglycans (GAGs). HA is an important component of the extracellular matrix of connective tissues such as vitreous, subcutaneous tissue, cartilage, umbilical cord, synovial fluid and tissues, etc. (Balasz and Gibbs, 1970), and has attracted the attention of several researchers, in both the pharmaceutical and cosmetic fields, owing peculiar water-binding and rheological properties. Solutions of sodium hyaluronate (Na-HA) form hydrated polymeric networks (matrices) which display a combination of coherence, elasticity, viscosity and pseudoplasticity that is unique for a water-soluble polymer at low concentration (Band, 1985). In addition, HA, as well as other GAGs, is biocompatible, nonimmunogenic and biodegradable by enzymatic means normal to the host (Sparer et al., 1983).

Applications of HA in the ophthalmic field have certainly been inspired by the presence of

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this material in the eye, where it plays an essential role as a basic component of the vitreous body (Lütjen-Drecoll et al., 1990). Preparations of high-molecular-weight Na-HA were first used during anterior segment surgery to maintain the depth of the anterior chamber and to protect the corneal endothelium, and are now indispensable aids in the intraocular lens implantation after cataract surgey. Their widespread use has originated the new concept of 'viscosurgery' (Balasz, 1983). Na-Ha solutions have also been successfully used as tear replacements for the treatment of severe keratitis sicca (Polack and McNiece, 1982). Other investigations have been prompted both by the singular physico-chemical properties of Na-HA solutions, which render the polymer an attractive adjuvant for ophthalmic vehicles, and by the muco-adhesive properties of HA, possibly deriving from structural and functional similarities existing between HA and mucopolysaccharides. In particular, data on the 'in vitro' bio-adhesive properties of HA, first published by Park and Robinson (1984) and by Saettone et al. (1987), were subsequently substantiated by in vivo animal data (Saettone et al., 1989). The capability of Na-Ha solutions to enhance the ocular bioavailability of pilocarpine (Pi), first reported by Saettone et al. (1985, 1986a), has been subsequently corroborated by the findings of Camber et al. (1987) and Camber and Edman (1989). Analogous results have been reported by Chang et al. (1988) for timolol.

Some information on the available pharmaceutical grades of Na-HA may be of relevance here. There are distinct differences in the degree of polymerisation of HA prepared from different sources, such as human umbilical cord, bovine vitreous, bovine or human synovial fluid, rooster comb, etc. Molecular weights in the range 0.115–14×10^6 have been reported (Laurent, 1970). Rooster combs have been identified as a reliable and reproducible source of HA: the highly purified polymer obtained from this material is a large molecule with an MW of $2–5 \times 10^6$. It was initially shown that sterile, high-MW Na-HA, even after extensive purification to remove proteins, nucleic acids and other GAGs, could still contain a component causing inflammation when injected into various tissue compartments (Constable and Swann, 1972). A noninflammatory, high-MW ($1.2–3 \times 10^6$) fraction (NIF) was isolated by Balasz (1979). A material of this type (MW $2–4 \times 10^6$), denominated Healon® and manufactured by Pharmacia AB (Sweden), is now available on the market for viscosurgery purposes. Two low-MW NIF fractions of Na-HA, denominated Hyalastin® (MW 1.45×10^5) and Hyalectin® (MW 5.9×10^5), were subsequently isolated and patented by Della Valle and Romeo (1983); Hyalectin, under the trade name of IAL® (Fidia S.p.A., Italy) is marketed as a viscosurgery aid.

The purpose of the present investigation was to test the potentiality of low-MW Na-HA fractions as adjuvants for topical ophthalmic formulations containing pilocarpine. A study in this direction was considered of interest, since other investigations on HA as an ophthalmic vehicle have been concerned with Healon® (Camber et al., 1987; Chang et al., 1988) or with some lower-MW Na-HA fractions prepared from this material (Camber and Edman, 1989).

The investigation was carried out on albino rabbits as a preliminary to further studies in humans.

Experimental

Materials

The following materials were used as received: two HA sodium salt fractions, denominated Na-HA1 and Na-HA2 (Hyalastin and Hyalectin, respectively); hyaluronic acid, HA, obtained from Na-HA1 by ion exchange (Fidia S.p.A, Abano T., Italy); Healon®, injectable 1% solution of Na-HA (Pharmacia S.p.A., Milano, Italy); poly(vinyl alcohol), PVA, (Polyviol W 48/20, Wacker Chemie, Burghausen, Germany); pilocarpine nitrate (PiN), m.p. 176–178 °C (Sigma, St. Louis, U.S.A.); sodium fluorescein (Carlo Erba, Milano, Italy). Pilocarpine base, PiB, was obtained from the nitrate by extraction of the alkalinized solution. Some essential characteristics of the materials (as communicated by Fidia S.p.A.) are as follows: Na-HA1: MW $134\,000 \pm 9\,000$, $[\eta] = 3.2–4.0$ dl/g; Na-HA2: MW $620\,000 \pm 50\,000$, $[\eta] = 10.8$ dl/g; HA: MW 113 000, $[\eta] = 3.5$ dl/g, 2.56 mEq./g.

Vehicles

A 2.0% w/w solution of PiN made isotonic with NaCl was used as reference standard as such (RS), and after addition of 1.5% or 5.0% w/w PVA (RS/PVA1 and Rs/PVA2, respectively). Solutions containing 2.0% w/w PiN and 0.25%, 0.50 and 0.75% Healon® (Na-HE 0.25%, 0.50% and 0.75%, respectively) were prepared by diluting the commercial 1% Healon® with solutions containing the appropriate amount of PiN. Solutions containing 2.0% w/w PiN and 1.0% (or 2.0%) w/w Na-Ha1 or Na-Ha2 were prepared by adding the appropriate amount of freeze-dried, sterile polymers to RS. The Pi salt of HA (HA/PiB) was prepared by adding to a solution of PiB the appropriate amount of Ha, calculated from the neutralization equivalent of the polymer (cf. Saettone et al., 1989b); the resulting solution was stored in freeze-dried form. For the tests, a solution containing 4.47% w/w salt (corresponding to 1.53% w/w PiB, and to 2.0% w/w PiN) was prepared. This solution, denominated HA/PiB, was tested as such and after addition of 1.0% (or 2.0%) w/w Na-HA2, or 1.5% w/w PVA. For the ocular behaviour study, 0.1% w/w Na-fluorescein was added to the vehicles.

Although sterility was ensured in all cases by appropriate measures (filtration through Millex GS 0.22 μm filters, Millipore, and/or manipulation under laminar airflow), all vehicles contained 0.1% w/w methyl *p*-hydroxybenzoate as preservative, and were tested immediately after preparation.

All preparations, numbered in sequence, are listed in Table 1 together with the relevant rheological data, determined at 30 °C using a Rotovisco RV 12 viscometer (Haake, Karlsruhe, Germany). All the reported data are the average of three determinations. The pH values of all vehicles were in the range 4.9–5.2.

Biological studies

Rabbit tests Miotic activity tests were carried out on non-anaesthetized, male albino rabbits weighing 2.5–3.0 kg, using a standard method (Saettone et al., 1986b). All procedures were performed in accordance with the ARVO resolution on the use of animals in research. The applied dose was in all cases 50 μl; each vehicle was tested on at least six rabbits. The results were calculated as the average variation of pupillary diameter with respect to the basal diameter vs time.

Studies on the ocular residence time and behaviour of the formulations were performed by examining with a slit lamp, under UV (366 nm) illumination, the eyes into which the fluorescein-containing vehicles had been instilled (50 μl). Each vehicle was tested in both eyes of at least six different animals; and all measurements were made by the same operator.

TABLE 1

Composition and rheological data of the vehicles

Vehicle No.	Vehicle type	Type of flow [a]	Viscosity [b] (mPa s)
1	RS	N	1.0
2	RS/PVA1	N	3.6
3	RS/PVA2	N	62.0
4	Na-HE 0.25%	P	167.8
5	Na-HE 0.50%	P	260.7
6	Na-HE 0.75%	P	503.5
7	Na-HA1 1%	N	7.2
8	Na-HA1 2%	P	34.3
9	Na-HA2 1%	P	242.7
10	Na-HA2 2%	P	923.5
11	HA/PiB	N	2.3
12	HA/PiB + 1% Na-HA2	P	264.2
13	HAPiB + 2% NaHA2	P	1054.0
14	HA/PiB + 1.5% PVA	N	33.3

Vehicles 1–10 contained 2.0% w/w PiN; vehicles 11–14 contained an equivalent amount (1.53% w/w) of PiB.

[a] N, Newtonian; P, pseudoplastic.

[b] All values are the average of three determinations. The value indicated for the vehicles exhibiting a pseudoplastic flow is the apparent viscosity calculated at a rate of shear of 280 s^{-1}.

Results

Vehicles

All vehicles submitted to investigation are listed in Table 1. They contained 2.0% w/w PiN (nos 1–10) or an equivalent amount of PiB (nos 11–14). Nos 1–6 can be considered as reference standards for a comparative evaluation of the low-MW HA vehicles under study (nos 7–14). No. 1 consisted

of an aqueous solution of PiN in isotonic saline, while Nos. 2 and 3 resulted from addition to no. 1 of 1.5% and 5.0% w/w PVA, an adjuvant widely used to increase the viscosity of collyria. The (Newtonian) viscosity of vehicles 2 and 3 was 3.6 and 62.0 mPa s, respectively. The three reference vehicles (nos 4–6) containing different percentages of high-MW HA (0.25–0.75%) were pseudoplastic, with apparent viscosities in the range 168–503 mPa s: the apparent viscosity values reported for these and for other vesicles showing the same type of flow were calculated at a rate of shear of 280 s^{-1}. Addition of 1.0 and 2.0% w/w Na-HA1 (Hyalastin) to vehicle 1 produced vehicles 7 and 8, respectively, while addition of 1.0 and 2.0% w/w Na-HA2 (Hyalectin) to vehicle 1 gave vesicles 9 and 10, respectively. Due to the relatively lower MW of Na-HA1, the addition of this polymer in the indicated percentages resulted in a relatively low increase of the apparent viscosity of the final preparations: the relevant values for vehicles 7 and 8 being 7.2 and 34.3 mPa s, respectively. Vehicles 8, 9 and 10 exhibited a pseudoplastic flow, with apparent viscosity values of 34.3, 242.7 and 923.5 mPa s, respectively. Vehicle no. 11 consisted of a solution of the PiB salt of HA, whose content of drug base (1.53% w/w) was equivalent to that of a 2.0% w/w solution of PiN. This vehicle, whose characteristics have been reported in a previous paper (Saettone et al., 1989b), exhibited a (Newtonian) viscosity of 2.3 mPa s. Addition to vehicle 11 of 1.0 or 2.0% w/w Na-HA2 gave the highly viscous, apparently pseudoplastic vehicles 12 and 13 ($\eta = 264.2$ and 1054.0 mPa s, respectively), while addition to 11 of 1.5% w/w PVA gave vehicle 14, exhibiting a Newtonian type of flow and a viscosity of 33.3 mPa s.

In summary, the present set of experimental and reference vehicles exhibited a wide range of viscosities (1–1054 mPa s); all vehicles containing HA derivatives showed an apparent pseudoplastic flow, with the exception of no. 7 (containing 1% of Na-Hal) and of 11, which contained the HA salt of PiB.

Miotic activity

The results of the miotic activity study in rabbits are summarized in Table 2. When compared with the reference solution 1, all vehicles, with the exception of no. 2 (1.5% PVA), nos 7 and 8 (Na-HA1 1% and 2%, respectively) showed increased AUC values, with statistically significant differences ($P < 0.05$). When compared with the reference PVA vehicle no. 2, only nos 5, 6, 10 and 13 showed significantly increased AUC values. When compared with the more viscous PVA vehicle 3, only nos 5, 6 and 13 showed significantly increased AUC values. However, none of the HA vehicles under test (7–14) showed AUC values statistially greater than those of the HE reference vehicles (nos 4–6), which also appeared to increase considerably the duration of activity of Pi both with respect to the aqeous solution 1 and to all HA vehicles under test. Vehicles 10 and 13 (both containing Na-HA2 2%) appeared to be the best performers of the group. They possibly owed their characteristics to a high viscosity, as discussed below.

TABLE 2

Summary of the miotic activity data of the vehicles

Vehicle no.	I_{max} [a] (mm)	Peak time [b] (min)	Duration [c] (min)	AUC [d] (cm^2)	AUC, relative to 1
1	2.3 (0.24)	20	150	39.1 (6.7)	1.0
2	2.5 (0.20)	30	180	53.3 (10.1)	1.4
3	2.8 (0.22)	25	210	65.1 (8.8)	1.7
4	4.0 (0.11)	20	270	77.3 (27.7)	2.0
5	2.8 (0.81)	20	300	108.8 (19.9)	2.8
6	3.4 (0.56)	20	360	106.0 (31.5)	2.7
7	2.2 (0.24)	30	180	41.9 (7.5)	1.1
8	2.6 (0.22)	30	180	53.7 (8.7)	1.4
9	2.6 (0.22)	30	240	66.0 (10.0)	1.7
10	2.8 (0.24)	30	240	83.1 (12.0)	2.1
11	3.0 (0.33)	30	210	64.6 (8.3)	1.6
12	2.6 (0.22)	30	210	67.6 (8.0)	1.7
13	3.3 (0.25)	40	240	92.4 (10.0)	2.4
14	2.9 (0.21)	30	210	70.7 (21.4)	1.8

[a] Maximum miotic effect, ±95% confidence limits.
[b] Time to reach I_{max}.
[c] Time required for the pupil to return to baseline conditions.
[d] Area under the miosis vs time curve, ±95% confidence limits.

Ocular retention study

The results of the ocular behaviour study of the fluorescein-containing vehicles are summarized in

TABLE 3

Summary of the preocular behaviour data of the vehicles

Vehicle no.	T1	T2	T3
1	*	100.5 (13.1)	2.5 (1.7)
2	35.0 (6.7)	105.2 (11.7)	5.5 (3.2)
3	80.8 (7.8)	151.6 (12.9)	13.7 (2.9)
4	300.0 (15.1)	> 360	60.0 (10.3)
5	330.0 (14.6)	> 360	60.5 (12.7)
6	200.5 (18.3)	290.0 (18.6)	90.6 (5.8)
7	43.0 (9.6)	108.5 (9.9)	4.0 (1.5)
8	76.3 (6.2)	130.5 (7.6)	7.0 (2.4)
9	97.8 (7.9)	148.5 (19.3)	19.6 (3.4)
10	120.8 (9.7)	179.3 (20.8)	55.1 (7.3)
11	100.5 (8.9)	151.2 (17.3)	5.2 (1.9)
12	116.2 (9.3)	178.7 (20.7)	20.4 (4.2)
13	142.5 (9.8)	210.2 (18.4)	49.7 (6.5)
14	95.8 (8.1)	190.8 (22.9)	10.2 (4.6)

T1, time (min) during which a uniform, fluorescent film was present over the precorneal area. * This solution did not form a uniform corneal film. T2, time (min) required for complete disappearance of fluorescence from the eye. T3, time (min) at which the fluorescent solution was discharged from the nose. Data in parentheses are 95% confidence limits.

Table 3. The reported parameters are the time of permanence of a homogeneous fluorescent film over the corneal area (T1), the time required for complete disappearance of fluorescence from the eye (T2), and the time post-instillation at which the fluorescent solution was first discharged from the nose of the animals (T3). Inspection of Table 3 reveals that the aqueous reference solution no. 1 showed the shortest T2-T3 values. Furthermore, as indicated in Table 3, the solution mixed very poorly with the tear film, and did not form at any time a completely uniform layer over the corneal area. Thus, no T1 values could be assigned to this vehicle. The HE vehicles (4–6) showed the greatest T1-T3 values, in agreement with previous literature reports on corneal permanence of high-MW hyaluronic acid solutions (Camber and Edman, 1989). In particular, vehicles 4 and 5 still showed a strong fluorescence when the observation was discontinued, so that the relevant T2 values (indicated as > 360 min) are to be considered only as a conservative estimate. The relatively more viscous HE vehicle no. 6 formed small lumps in the eye of the animals, showing probably for this reason a slightly inferior performance with respect to the other two Na-HE solutions. All the HA vehicles under test and the reference vehicle no. 3 formed more or less stable corneal films, and were retained to different degrees in the eye: none of them, however, showed retention times comparable with those of the high-MW HE reference solutions. The relevance to ocular retention of viscosity will be discussed in the next section.

Discussion

Bioavailability / viscosity correlations

It is well established that, in order to optimize ocular drug bioavailability, it is necessary to minimize the negative influences exerted by the eye (i) on precorneal drug retention and (ii) on corneal drug penetration (Lee and Robinson, 1986). The traditional approach to improve ocular drug retention relies upon increasing the viscosity of the vehicle. Earlier studies on rabbits by Robinson and associates (Chrai and Robinson, 1974; Patton and Robinson, 1975) have definitely established that the rate of drainage from the eye of an instilled solution is significantly retarded as the viscosity of the solution is increased. As a consequence, the drug saturation of the tear film and of the corneal epithelium is improved, and the overall drug bioavailability is increased. Another, more recent, approach to improve precorneal retention is based on muco-adhesion, and consists of realizing polymeric vehicles whose prolonged retention in the eye depends on interactions with the mucin layer coating the corneal surface, rather than on viscous effects alone. Since some polymers (e.g. HA and other GAGs) may display at the same time viscous and muco-adhesive effects, a critical evaluation of the viscosity-bioavailability relationships is necessary in order to assess if one or both mechanisms are actually operative.

A plot of the logarithm of the viscosity of the present vehicles vs their respective AUC values is presented in Fig. 1. The use of the logarithm of the viscosity was resorted to in order to arrange conveniently a wide range of values on the horizontal axis, thus permitting a clearer comparison of the data. A linear relationship between AUC

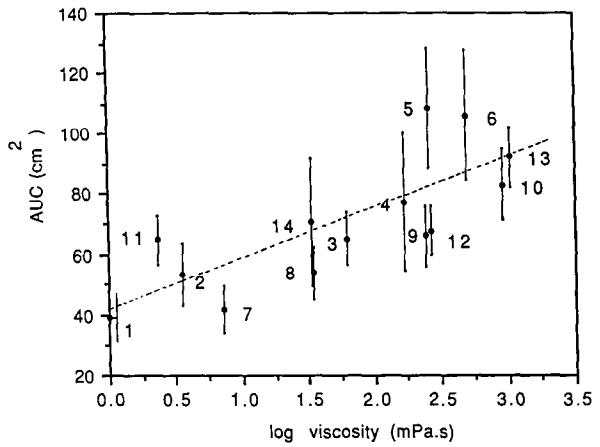

Fig. 1. Plot illustrating the relationship existing between the AUC values and the logarithm of the apparent viscosity of the vehicles under study. Each vehicle is identified by its number (cf. Table 1). Vertical lines represent 95% confidence limits.

and log viscosity is apparent: the line of best fit through the data (correlation coefficient, 0.603) is reported in the graph. The physical significance of the observed linear relationship is still unclear, and will not be further discussed, but it might deserve future attention.

The vehicles in the graph might be ideally divided into three groups: the first including the low-viscosity vehicles 1, 2, 7 and 11 (1–7.2 mPa s), the second consisting of vehicles 3, 8 and 14, of intermediate viscosity (33–62 mPa s), and a third group comprising the high-viscosity vehicles 4–6, 9, 10, 12 and 13 (167–1054 mPa s). It can be observed that, within the first group, vehicle 11 (containing the HA-salt of Pi) showed the best bioavailability characteristics in spite of a comparatively low viscosity (2.3 mPa s). The AUC value of vehicle no. 11 was 1.6 and 1.54 times greater than those of vehicles 1 and 7, respectively, with a statistically significant difference at the 95% confidence level, in spite of the fact that vehicle 7 was comparatively more viscous (7.2 mPa s). This behaviour of vehicle 11, reported previously (Saettone et al., 1989b), has been attributed to possible muco-adhesive phenomena. It is also evident from the graph that addition of 1% Na-HA2 or 1.5% PVA to vehicle 11 to give vehicles 12 and 14, respectively, did not produce any further bioavailability increase, in spite of a substantially augmented viscosity. Only the highly viscous no. 13 (1054 mPa s) was more active than 11 at a significant level. Within the 'high-viscosity' group, the Na-HE vehicles 5 and 6 showed the highest AUC values. Interestingly, they were significantly more active than nos 9 and 12, of comparable viscosity. In particular, vehicle 5 (Na-HE 0.5%) was the most active of the whole group.

If the relatively 'anomalous' vehicles 11, 5 and 6 are excluded from the comparison, it is clearly apparent that increasing the viscosity (either with Na-HE, Na-HA1 or PVA) of the aqueous reference solution 1 up to approx. 65 mPa s (vehicle 3) produces a moderate but significant (1.7-fold) increase of the AUC: further substantial viscosity increases up to 240–260 mPa s (vehicles of intermediate viscosity 9 and 12) do not bring about any further AUC increase. Only the highly viscous Na-HA2 vehicles 10 and 13 show a further small AUC increase over the members of th low-viscosity group (2.1–2.4-fold improvement with respect to vehicle 1). These data appear to agree with the mentioned findings of Chrai and Robinson (1974) and of Patton and Robinson (1975), who reported that increasing the solution viscosity from 1 to 100 mPa s through the incorporation of methylcellulose or PVA, while reducing the drainage rate 10 times, caused only a 2-fold increase in pilocarpine concentration in the aqueous humour of albino rabbit. The role of factors other than viscosity alone in determining the high activity of some of the examined vehicles, such as 11, 5 and 6, should then be appropriately assessed.

Bioavailability / ocular retention correlations

A plot of the AUC data of the individual vehicles vs the respective T1 and T2 values is presented in Fig. 2. As shown in the graph, a fair linear relationship appears to exist between the T1-T2 and the AUC values, up to retention times of approx. 200 min. The observed relationship, which includes all vehicles with the exception of nos 4 and 5, stresses the relevance to ocular bioavailability of the time of precorneal residence of the vehicle, in agreement with the current theories on ocular drug absorption. The presence of the vehicle as a continuous (T1) or discontinuous (T2) layer does not appear to have a critical influence

on bioavailability, the discontinuous precorneal film forming evidently a reservoir from which the drug is efficiently released and absorbed. The behaviour of the Na-HE vehicles 4 and 5 is noteworthy: they stand out of the correlation line, on account of much longer residence times than expected on the basis of their AUC data. In other words, these vehicles seem to remain on the precorneal area even after complete release of the drug, a phenomenon that is understandable on the basis of the high solubility and fast diffusivity of pilocarpine. A similar behaviour was reported in the case of other long-residence (or bioadhesive) vehicles containing pilocarpine (Saettone et al., 1989a).

Previous work (Saettone et al., 1982, 1984) has indicated the possible relevance to T1 of surface spreading effects, determined by the nature but not by the viscosity of the polymeric solution. The second (T2) parameter, on the other hand, might be related partly to viscosity and partly to the muco-adhesive characteristics of the solution applied to the eye: both effects potentially counteracting the elimination due to lacrimal drainage. Finally, the latter parameter (T3) might be of relevance to absorption of the drug into the systemic circulation. It is well known that large amounts (theoretically over 90%) of the drug dose applied topically to the eye can be absorbed systemically, both via the palpebral conjunctiva or

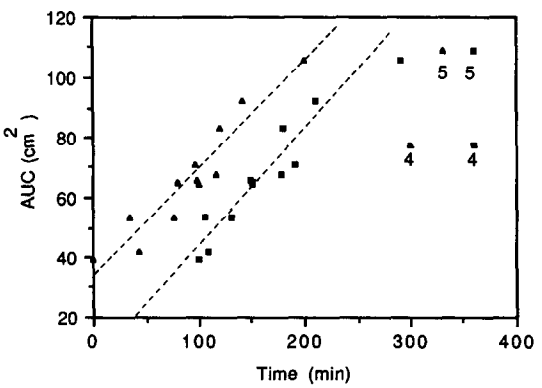

Fig. 2. Plot illustrating the relationship existing between the AUC values and the ocular retention times T1 (triangles) and T2 (open squares; cf. Table 3). The vehicles are not individually identified, with the exception of nos 4 and 5, which stood distinctly outside the correlation lines.

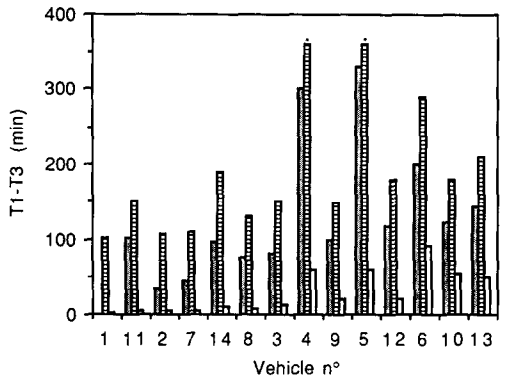

Fig. 3. T1–T3 values (in min) for all vehicles arranged in order of increasing apparent viscosity. Asterisks over the T2 bars of vehicles 4 and 5 indicate that the permanence exceed 360 min, time at which the observation was discontinued.

the nasal mucosa (Salminen, 1990). A delayed appearance of the solution at the nasal opening might correspond to a prolonged contact with the nasal mucosa, hence to an enhanced systemic absorption.

In Fig. 3, the T1-T3 values for the vehicles, arranged in order of increasing viscosity, are reported. The lack of correlation between viscosity and retention times is clearly apparent. In particular, within the low-viscosity group, the HA-PiB vehicles 11 and 14 show longer T1 and T2 times than their neighbours (even the more viscous ones 2, 3, 7 and 8). The same performance is shown, to a higher degree, by the intermediate-viscosity Na-HE vehicles 4 and 5, which show much longer T1-T2 times than other vehicles of comparable or higher viscosity.

As Table 3 also shows, some vehicles (4, 5, 6, 10, 13) showed high T3 values, indicative of a prolonged residence in the naso-lacrimal duct. It might be of interest to investigate if to these higher T3 times would correspond higher blood levels of the drug.

Conclusions

The present investigation has confirmed that one essential requirement for a prolonged ocular permanence of hyaluronic acid, and hence for a good performance of this material as an ophthal-

mic vehicle, is a high molecular weight, as offered by Na-HE.

Long ocular residence times, corresponding to a high bioavailability of Pi, were observed in the case of all Na-HE vehicles. The performance of these vehicles, which was viscosity-unrelated and particularly good at the lower examined concentrations (0.25–0.50%), is quite reasonably to be attributed to muco-adhesive effects.

Among the lower-MW HA vehicles, the HA-PiB vehicle no. 11 confirmed in the present comparative experiments its positive properties. The peculiar interest of this preparation lies in its low viscosity, which allows sterile filtration and dispensing as eyedrops: two important factors in ophthalmic drug formulation. The fact that this vehicle provided higher AUC values with respect to vehicles of much higher viscosity (even if significantly lower AUC values than those shown by the Na-HE vehicles 5 and 6), confirms that lower-MW fractions of HA can be used with advantage as salts (or ionic complexes) with basic drugs, a form in which they may display interesting bioavailability-enhancing properties.

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