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PREPARATION AND EVALUATION OF A SUSTAINED-RELEASE OPHTHALMIC VEHICLE FOR DAPIPRAZOLE

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ABSTRACT

The present investigation is concerned with the development and "in vivo" evaluation of a long-acting ocular vehicle for the α-adrenergic blocking drug dapiprazole (DAP). The approaches tested for prolonging the activity were a) salification of the drug base with polygalacturonic acid (PGA), and b) formulation as a highly viscous hydrogel. The vehicles prepared by applying (singly or in combination) these techniques, and two reference aqueous vehicles containing DAP-HCl were submitted to a series of biological tests on rabbits (miosis and reversion of mydriasis). When compared with an aqueous solution

an aqueous solution, reconstituted prior to use from a freeze-dried formulation (marketed in Italy as Glamidolo^{R}, Angelini).

The topical administration of ophthalmic drugs from aqueous solutions (collyria) is characterized by a poor bioavailability and a short duration of action, as a result of a series of concomitant physiological factors (induced lacrimation, tear turnover, solution drainage etc.) which concur in removing the solution from the eye. These factors have been widely investigated and detailed in the relevant literature, and several approaches to extend the ocular residence time of topically applied medications have been reported (4). In the present study two such approaches, namely, a) salification of the basic drug with a polyanionic polymer and b) increased vehicle viscosity, were applied to the development of a long-acting ocular formulation for DAP. The effect of the said manipulations on the biological activity of a series of DAP vehicles was submitted to a preliminary verification "in vivo", by performing miosis and reversion of tropicamide-induced mydriasis tests in rabbits.

EXPERIMENTAL

Materials: Dapiprazole base, DAP-B (m.p. 163-164 °C) containing an equivalent amount of DAP-HCl, the

polyanionic complex DAP-base/PGA increased significantly some activity parameters (maximal miotic intensity, duration, AUC) of the drug, while the hydrogel vehicle containing DAP-HCl prolonged the apparent absorption and elimination phases of the drug, mainly by virtue of a prolonged ocular retention. The combination of the two approaches, as in the hydrogels containing the DAP/PGA complex, permitted the best control of the pharmacological activity parameters, which corresponded to a prolonged-pulse release of the drug. In the miosis test, the AUC values did not show any dose-dependent increase, while this effect was evident in the mydriasis-reversion experiments. The possible mechanism(s), by which the examined techniques may influence the activity parameters and the overall ocular bioavailability of DAP are discussed.

INTRODUCTION

Dapiprazole hydrochloride (DAP-HCl, 5,6,7,8-tetrahydro-3-[2-(4-o-tolyl-1-piperazinyl)ethyl]-s-triazolo[4,3,a]pyridine monohydrochloride) is an α-adrenergic blocking agent, mainly used for the treatment of chronic simple glaucoma in cases where a miotic effect coupled to reduction of intraocular pressure (IOP) is desired (1). Other therapeutic applications are the induction of pre-operative miosis in specified cases of eye surgery, and the reversion of pharmacologically-induced mydriasis (2,3). The drug is clinically used as

and hydrochloride, DAP-HCl (m.p. 192-193 °C) were purified samples supplied by Acraf SpA (Ancona); high molecular weight hydroxypropylcellulose, HPC (KLUCEL HF, Hercules Inc., Wilmington, Del.) and polygalacturonic acid, PGA (4.89 mEq/g, Orion Chemicals, Milano) were commercial samples, used without prior purification. Benzalkonium chloride, U.S.P. grade (BZ) was purchased from Carlo Erba S.p.A.; Nylon 6 (Capran 77C, thickness 27 ± 0.3 μ) was obtained from Allied Chem. Corp., Morristown, N.J.

Vehicles: The DAP-B/PGA ionic complex was prepared by adding portionwise, at room temperature, 0.68 g PGA to a stirred solution of DAP-B (1.0 g) in methanol (50 ml). The solvent was then evaporated to give a highly water-soluble creamy white powder, containing 59.52% DAP-B. The pH of a 1.68% w/v solution of the complex (corresponding to 1.0% DAP-B) was 5.4.

The composition of all DAP vehicles is indicated in Table I.

The gel vehicles 5-8 were prepared by adding a water solution of DAP-HCl or PGA complex to a preformed HPC gel containing BZ as the preservative. As an example, the preparation of vehicle n° 5 is described. A solution of the ionic complex DAP-B/PGA (0.84 g) in water (35 ml) was slowly added, while stirring, to a gel prepared by hydrating overnight 4.5 g HPC in water

Table I - Composition of all dapiprazole vehicles submitted to investigation

	VEHICLE N°							
	1	2	3	4	5	6	7	8
DAP-HCl	0.5	1.0					0.5	1.0
DAP-B/PGA			0.84[a]	1.68[b]	0.84[a]	1.68[b]		
HPC					4.5	4.5	4.5	4.5

All vehicles contained water, q.s. to 100 ml, and 0,01 % w/v BZ.
[a]Equivalent to 0,5% DAP-B; [b]Equivalent to 1.0% DAP-B

(60 ml) containing benzalkonium chloride (0.01 g). The mixture was then brought to the final weight, and was stored at 5 °C until used.

"In Vitro" Release Tests: These tests were performed by measuring the rate of dapiprazole release from the gel vehicles 6 and 8 to an aqueous sink, through a nonporous membrane. To this purpose, a thin nylon membrane, which had been preconditioned by extraction with ethanol (1h at 60 °C) and overnight hydration in distilled water at room temperature, was positioned between the receiving (5 ml) and the donating (4 ml) compartment of a glass "GH" flow-though diffusion cell (5), thermostated at 30 °C. At t = 0 the vehicle was

introduced into the upper section of the cell, and the stirred receiving solution (0.01 M HCl) was periodically sampled and analyzed spectrophotometrically for DAP-HCl (234 nm), using an appropriate calibration curve. The withdrawn samples were immediately replaced with equal amounts of prewarmed receiving solution.

Biological Tests: a) Miotic activity tests were carried out on non-anaesthetized, male albino rabbits weighing 2.5 - 3.0 Kg, using a standard procedure (6). The vehicle dose, which was applied into the lower conjunctival sac of one eye, was in all cases 50 µl; each vehicle was tested at least on six animals. b) Mydriasis-reversion tests were performed on vehicles 1, 5 and 6, using at least six rabbits for each vehicle. For these experiments both eyes of the rabbits were pre-treated with 10 µl of a 1.0% commercial solution of tropicamide (Visumidriatic, Merck Sharp & Dohme). After reaching the peak mydriatic effect (c. 25 min), the vehicle under study (50 µl) was applied into the lower conjunctival sac of one eye, and the measurement of the pupillary diameter was performed as indicated previously. The results were expressed as differences in diameter, in mm, between the eye treated with tropicamide alone and the eye which had received the DAP vehicle.

RESULTS

"In Vitro" Release: These tests had essentially the purpose of providing information on the extent of DAP binding in the PGA ionic complex, and on the influence of salification/complexation by PGA on DAP release from the vehicles. The study was carried out on the gel vehicles 6 and 8, containing an equivalent amount of DAP respectively as PGA complex and as hydrochloride. In both cases DAP was released with zero-order kinetics over a period of 36 h; release from vehicle 6, containing the PGA complex, was much slower with respect to vehicle 8, containing the hydrochloride: the slopes of the release plots (in mg/cm² per hour) were $4.83 \cdot 10^{-3}$ (R)' for vehicle 6, and $2.19 \cdot 10^{-2}$ (R) for vehicle 8. As demonstrated in a previous paper for a similar case (7), the ratio of the two slopes, R'/R, is equal to C_f/C_i, where C_i is the total amount of drug in the diffusing medium, and C_f is the concentration of "free" drug, i.e., in equilibrium with drug bound by the PGA complex. A simple calculation shows that in vehicle 6 only 22% of the total amount of drug was in free diffusible form. This value is to be considered indicative, and is based on some simplifying assumptions, the main of which is that no binding takes place in either vehicle between HPC, the gel-forming agent, and dapiprazole.

Table II - Summary of the miotic activity parameters of the DAP vehicles.

Vehicle n°	Peak time min	I_{max} mm	Duration min	AUC cm^2 (±95% C.L.)	K'* min^{-1} ·1000
1	30	1.87	150	28 (13)	13.7
2	30	1.75	180	33 (9)	13.0
3	120	2.50	290	102 (7)	12.6
4	120	2.50	310	93 (10)	14.1
5	60	2.10	370	86 (14)	3.5
6	60	2.40	380	100 (19)	4.0
7	90	1.60	330	70 (24)	3.2
8	85	2.00	350	98 (14)	2.6

*First order rate constant for elimination, determined from the terminal slopes of the lines of the log change in pupillary diameter vs. time plots.

"In Vivo" Data: a) Miotic activity. The results of the miotic activity tests on rabbits, carried out on the eight vehicles under investigation are summarized in Table II. For greater clarity, the data will be discussed in separate subsections.

a1) Effect of the applied dose. The preparations listed in Table II can be ideally divided into four couples

(1-2, 3-4, 5-6, 7-8), in each of which the drug was present at two concentrations, corresponding to 0.5 and 1.0% DAP. An inspection of the data shows that, within each couple, a 100% increased concentration resulted in very little or no increase of the activity parameters. Even if in some cases slightly increased I_{max} (vehicle 6 vs. 5, or 8 vs. 7) or duration values (vehicle 2 vs. 1, or 8 vs. 7) were apparent, in no case the AUC values of each couple of preparations were statistically different.

a2) Effect of complexation of DAP-B by PGA. This effect is evidenced by a comparison of the activity data within each one of two vehicle couples, 1-3 and 2-4. In each couple the vehicles contained an equivalent amount of drug (0.5 or 1.0%), but in a different form (HCl or PGA complex). Within each couple, the presence of the PGA complex resulted in a significant (c. 3 times) increase in bioavailability, associated with an increase in duration and intensity of activity. Salification of DAP-B by PGA had little effect on the apparent (Newtonian) viscosity of the vehicles (which were 1.4 and 1.7 mPa · sec for vehicles 3 and 4, vs. c. 1.0 for vehicles 1 and 2, at 30 °C) so that a possible influence of viscosity effects on the ocular retention, and hence on the activity of the PGA complex should be ruled out. The apparent elimination kinetics of

vehicles 1-4 (reflected by the K' values in Table II), which were in the same range as those of vehicles 1-2, suggest a relatively short preocular retention. This indication is apparently in contrast with the high AUC values of the vehicles.

a3) Effect of gelification by HPC. This effect can best be appraised by comparing the activity parameters within each of two groups of vehicles: 1-3-5-7 (0.5% DAP) or 2-4-6-8 (1.0% DAP). The reported data show that, within the first group, the presence of HPC (and the associated great increase of vehicle viscosity) had a positive effect on bioavailability (2.5 x) when the drug was present as the hydrochloride (vehicle 7 vs. 1). A similar AUC increase was not apparent when the drug was present in the gels as the PGA complex (vehicle 5 vs. 3). In other words, addition of the gel-forming cellulose derivative was apparently unable to increase the AUC values displayed by the liquid vehicles containing the PGA salt. Similar considerations apply to the other group of vehicles (1.0% DAP). The gel vehicles, however, appeared to exert some control on DAP release and absorption, as indicated by an increased duration of activity (Cf. e.g. vehicles 7 and 8 vs. 1 and 2, respectively), and by consistently lower K' values, corresponding to a prolongation of the apparent absorption and elimination phases of the drug in the eye.

b) Reversion of mydriasis. Administration of 0.1 mg tropicamide to both eyes of the rabbits produced an intense mydriatic effect, which began slowly to decline experiments, in these tests a 2-fold increase of the administered drug dose (vehicle 6 vs. 5) produced a significantly increased biological response. The relative AUC values for vehicles 1, 5 and 6 (calculated at 2 h, corresponding to the end of the effect of vehicle 1) were 1, 1.94 and 3.2. The corresponding relative AUC values at the end of the experiment (7 h) were 1.0, 4.5 and 8.5, respectively.

DISCUSSION

The approaches to enhance and/or prolong the ocular activity of DAP evaluated in the present study, although not unprecedented, differ in some respects from previous literature examples. Salification of pilocarpine with alginic acid (8), with poly(acrylic acid-lauryl methacrylate) (9), with poly(acrylic acid) (10, 11) etc., has been reported to prolong the activity of the drug when the salts were administered in a solid dosage form (insert) (8, 10), or when the complex itself was insoluble in water (9). The "hydrogel" approach, on the other hand, has been applied to various ophthalmic drugs, and 3-5 fold increases in penetration and activity have been

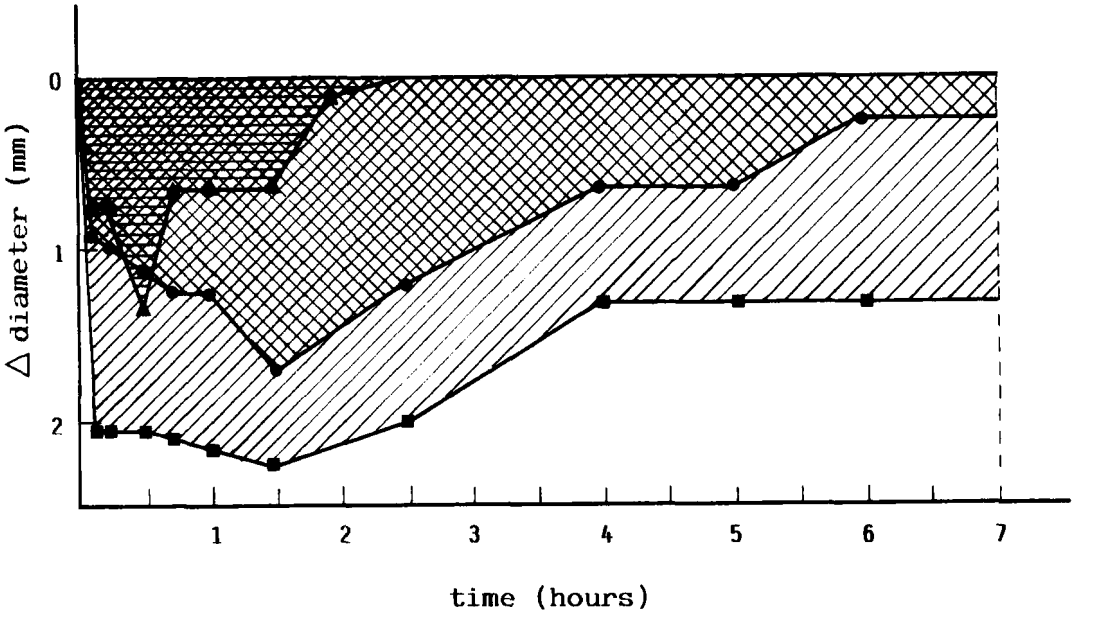

Fig. 1 - Effect of DAP vehicles N° 1 (▲), 5 (●) and 6 (■) on reversal of tropicamide-induced mydriasis.

reported. The combined effect of the two techniques has apparently never been investigated, with the possible after 90 min, but was still very evident (c. 70% of the peak intensity) after 7 h. The results of tests, in which the dapiprazole vehicles n°1 (reference), 5 and 6 were applied to one of the dilated eyes are illustrated in Fig. 1, where the areas corresponding to the reversion effect vs. time of each vehicle are outlined. In the figure, the data are expressed as differences in pupillary diameter, $\Delta \phi$, between the DAP-treated and the

dilated, contralateral eye, vs. time. As shown, vehicle 1 produced a reversion of short duration, while the effect of an equivalent dose of DAP (as PGA complex) administered in the gel-vehicle 5 was significantly greater, both for intensity and duration, and was still evident after 7 h. Contrary to the miosis exception of an example mentioned in the patent literature, concerning a poly(acrylic acid) hydrogel partially neutralized by pilocarpine base (11).

The presently tested PGA salt of DAP has shown pharmacological results (peak time shifted to a later time, increased I_{max}) corresponding to an increased contact time (12). PGA consists of linear chains of D-galacturonic acid units (pyranose form), joined in α(1-4)-glycosidic linkages, with a molecular weight ranging from 25 to 100 · 10^3. It formed a soluble salt, or polyanionic complex with the sparingly soluble DAP-B, from which the bound drug was released "in vitro" at a slow rate. The remarkable (more than 3-fold) AUC increases produced by the DAP-B/PGA vehicles, whose viscosity was low, cannot be rationalized in terms of a viscosity-induced reduction of the ocular drainage rate. Furthermore, as pointed out by Lee and Robinson (4), only moderate increases in ocular bioavailability may be observed as a result of a significant, viscosity-induced reductions of solution drainage,

partly on account of conjunctival absorption. The apparent rate constants for elimination of the DAP-B/PGA liquid vehicles, which are in the same range as those of the DAP-HCl solutions, would also point to a short ocular permanence of the former vehicles. The strong and somewhat delayed pulse of miotic activity observed with the DAP-B/PGA liquid vehicles might tentatively be explained in terms of adhesion of the polymer salt to the mucin layer at the corneal surface, leading to a temporary retention and to an improved drug penetration. There are sufficient structural affinities between PGA and the polysaccharide portion of mucin to justify this assumption, and large evidence has been gathered on the capacity of some polyanionic polymers to interact with and adhere to the mucin layer of conjunctival tissues (13).

While the PGA liquid vehicles proved capable of greatly enhancing the ocular bioavailability of DAP, their enhanced-pulse delivery might not be quite satisfactory for therapeutic purposes. High values of the peak miotic intensity, corresponding to high aqueous humour levels of the drug, are not desirable since they might intensify dose-related side effects of the drug (14). On the other hand, the hydrogel vehicles containing DAP-HCl showed activity parameters corresponding to a prolonged retention, as indicated in

particular by the low values of the elimination rate constants, reflecting a sustained absorption, distribution and elimination of the drug (15). It was speculated that the combination of the two techniques might provide an optimization of the activity. The resulting vehicles (5 an 6) appeared indeed to provide the best activity characteristics (long duration, satisfactory but not excessive peak miotic intensity), corresponding to a prolonged-pulse delivery of the drug. The highly viscous gel matrix apparently acted by assisting the ocular retention of the polyanionic complex, thus providing the best conditions for a prolonged release.

The same gel vehicles (5 and 6) also showed remarkably prolonged activity characteristics in the mydriasis-reversion tests. In these experiments, contrary to the miosis tests, significant dose-dependent effects could be observed. This phenomenon might be attributed to the more complex pharmacological situation existing in the reversal tests, where a competitive interaction might occur between the mydriatic and the miotic agent for the respective receptors.

In conclusion, the remarkable influence on DAP activity exerted by the low-viscosity PGA vehicles is an issue deserving further attention, in view of the possible implication of a muco-adhesive mechanism. A

closer study of the potential interactive properties of polyuronic acids and of their drug salts with the precorneal mucin, might led to a better understanding of the bio-adhesive properties of these and other similar anionic polymeric materials, and of their influence on ocular drug bioavailability.

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