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A new long acting ophthalmic formulation of Carteolol containing alginic acid

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Abstract

Alginic acid was evaluated as a potential vehicle in ophthalmic solutions for prolonging the therapeutic effect of carteolol. This anionic vehicle was expected to slow down drug elimination by the lacrimal flow, both by undergoing in-situ gel formation and by interacting with the mucus. In vitro studies indicated that carteolol is released slowly from alginic acid formulations, suggesting an ionic interaction. The adhesive behavior of alginic acid solution was better than that of another polymer, hydroxyethylcellulose (HEC). Intraocular pressure (IOP) measurements of rabbit eyes treated with a 1% carteolol formulation with or without alginic acid showed that this polymer significantly extended the duration of the pressure-reducing effect of carteolol to 8 h. The increased ocular bioavailability of 1% carteolol in the presence of alginic acid led to an equivalent concentration in the target tissue although administration was only once a day compared with twice a day for 1% carteolol alone. The overall results of this study indicate that the alginic-acid vehicle is an excellent drug carrier, well tolerated, and could be used for the development of a long-acting ophthalmic formulation of carteolol.

Keywords: Alginic acid; Carteolol; Ophthalmic delivery; Bioavailability; Intraocular pressure

1. Introduction

Ocular residence time is shortened as a consequence of rapid elimination from the corneal surface by the lacrimal flow. Treatment compliance can, therefore, be insufficient due to the high frequency of administration. As the ocular efficacy of topically applied drugs is influenced by the corneal contact time, the most common method of improving the ocular availability of drugs is to increase precorneal residence time by using hydrogels (Fitzgerald and Wilson, 1994; Edsman et al., 1996).

Several ways of prolonging the presence of drugs in the precorneal area consist of increasing the viscosity of the dosage form by adding water-soluble polymers (Thermes et al., 1992; Felt et al., 1999). An alternative approach aimed at improving bioavailability, is the use of polymeric solutions, which change to a gel as a result of

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exposure to the physiological temperature, pH or ionic composition of the lacrimal fluid. Phase transition systems (such as Gelrite®) are instilled in a liquid form and shift to the gel in the presence of mono or divalent cations (Rozier et al., 1989; Thermes et al., 1992).

Sodium alginate, the sodium salt of alginic acid, is a natural hydrophilic polysaccharide containing two types of monomers, β-D-mannuronic acid (M) and α-L-guluronic acid (G). The polymer forms three-dimensional hydrogel matrices. The high G content alginate forms a low viscosity, free-flowing liquid at concentrations suitable for gel formation in the lacrimal fluid (Cohen et al., 1997). This natural product has already found many applications in the food and pharmaceutical industries, especially in oral formulations for reflux oesophagitis and for wound dressings.

Alginic acid was chosen as a vehicle for ophthalmic formulations since it exhibits several favorable biological properties such as biodegradability and non-toxicity (Al-Shamklani et al., 1991). A prolonged precorneal residence of formulations containing alginic acid was looked for, not only based on its ability to gel in the eye but also because of its mucoadhesive properties (Smart et al., 1984; Matsumoto and Mashiko, 1990; Fuongfuchat et al., 1996).

The first objective of this work was to evaluate the rheological behavior, the interaction with carteolol and the adhesion bond strength of the carteolol–alginic formulations.

A further aim was to determine whether the addition of a mucoadhesive polymer could improve the ocular bioavailability of carteolol and prolong its therapeutic effect.

2. Materials and methods

2.1. Materials and animal testing

Alginic acid (Protacid F120, PRONOVA biopolymer, Norway), hydroxyethylcellulose (Natrosol 250M, HERCULES, France) and carteolol hydrochloride (OTSUKA, Japan) were used as received. All the components, including those obtained from standard commercial suppliers, were pharmaceutical grade.

Fauve de Bourgogne pigmented or New Zealand (NZ) albino rabbits weighing approximately 3 kg were used in the in vivo experiments. The animals were exposed to a 12-h light/12-h dark cycle and had free access to water and food. Rabbits were used and handled in accordance with the ARVO Resolution on the Use of Animals in Vision and Ophthalmic Research.

2.2. Preparation of solutions

1. Alginic acid solutions (1 or 2% carteolol–alginic): the preparation was carried out following the manufacturing process described in the patent (Maurin et al., 1998). Solutions contained 1 or 2% (w/v) carteolol hydrochloride, 1% (w/v) alginic acid and 0.005% benzalkonium chloride in phosphate buffer–sodium hydroxide pH 6.8. In previous studies (data not shown), 1% (w/v) alginic acid was determined to be the best concentration for the preparation of solutions.
2. HEC solution (1 or 2% carteolol–HEC): 1 or 2% (w/v) carteolol hydrochloride, 0.3% (w/v) HEC and 0.005% (w/v) benzalkonium chloride were dissolved in phosphate buffer.
3. Reference solutions (1 or 2% carteolol): 1 or 2% (w/v) carteolol hydrochloride and 0.005% (w/v) benzalkonium chloride were dissolved in phosphate buffer.

All solutions were sterilized by 0.2 μm filtration, except 0.3% (w/v) HEC which was prepared from 1% (w/v) HEC solution sterilized by autoclaving. The tonicity of the solutions was adjusted using sodium chloride.

2.3. Viscosity

Measurements were carried out using a VT500 rotary viscosimeter (HAAKE) equipped with a coaxial cylinder NV system thermostated at 33 ± 1°C. The shear rate was set at 1200/s.

2.4. In vitro diffusion test

Cellulose membrane tubing (Spectrapor no 6, cut-off 1000 Da, diameter 11.5 mm, thickness 18

mm, POLYLABO, France) was used for the dialysis test. Membranes, containing 2.5 ml of test solutions, were closed with plastic clamps and immersed in 1 l of water maintained at 33°C and stirred at 500 rpm. Then 3 ml samples were withdrawn into a 1 cm cell at appropriate intervals and read directly without dilution by U.V. spectrophotometry at 250 nm. Each in vitro diffusion test was repeated six times.

2.5. Adhesive properties

The contact angle and the surface tension of the different solutions were checked using a KRUSS K12 tensiometer. The method was based on the technique of Wilhelmy's plate. The apparatus was controlled by a computer which also analyzed the results. Temperature was maintained at 33°C using an HAAKE-c type thermostat. A platinum plate 19.9 mm long and 0.2 mm thick was used. The plate was immersed in the test solution, then pulled. The maximal force required to detach the plate from the solution was measured. Each result was a 30 measurement mean and each sample was tested twice (Ruyssen and Molle, 1965).

2.6. IOP studies

IOP was recorded in restrained Fauve de Bourgogne pigmented rabbits using an applanation pneumatonometer (MENTOR 30 Classic) without local anesthesia. The procedure used in the IOP assay was similar to the methods described previously (Vareilles et al., 1977; Kau and Limp, 1989). Briefly, ocular hypertension was induced in conscious rabbits by delivering, within 1 min, 70 ml/kg of warm water (37°C) by orogastric intubation. Changes in IOP were comparable in both eyes, the maximal hypertension being observed between 10 and 40 min post-waterloading. A single 25 μl drop of test solution was topically applied to one eye, the contralateral eye serving as control. Treatment was performed 1, 6 or 8 h before the induction of ocular hypertension. Initial values were measured before treatment, then just before waterloading, and every 10 min for 50 min after orogastric administration. To improve the accuracy of IOP values, at least three individual successive measurements were made at each time. Maximal hypertension (IOP_m) was determined in both eyes in relation to initial IOP (IOP_i). The inhibition of hypertension (IH) and the corresponding decrease in IOP (ΔIOP_{max}) were then calculated according to the formulae:

$$IH(\%) = \frac{(IOP_m - IOP_i)\text{control} - (IOP_m - IOP_i)\text{treated}}{(IOP_m - IOP_i)\text{control}}$$

$$\Delta IOP_{max} = (IOP_m)\ \text{control} - (IOP_m)\ \text{treated}$$

2.7. Ocular bioavailability

Bilateral instillations (25 μl) of 1% carteolol (twice a day) and 1% carteolol–alginic (once a day) were performed into the conjunctival sac of Fauve de Bourgogne pigmented rabbits for 2 weeks. Groups of six animals were sacrificed at 0–0.25–0.50–1–2–4–6–8 h after the last instillation and carteolol assayed by HPLC in the aqueous humor (AH) and in the iris/ciliary body (ICB), or by LCMS in plasma. These methods were validated (Chapuzet and Mercier, 2000).

2.7.1. Sample preparation

Almost 50 μl of AH was deproteinized with 100 μl of perchloric acid. Samples were then centrifuged for 10 min at $1740 \times g$. One ICB or 1.5 ml of plasma was incubated overnight with 1 ml NaOH (1N). Water (2 ml) was then added to ICB samples. Carteolol was extracted from samples by passing them through Extrelut® NT3 and eluting with 15 ml of diethylether. After evaporation, the residue was solubilized with 150 μl of mobile phase (ICB) or 0.05% acetic acid (plasma).

2.7.2. Chromatographic conditions

Thus supernatants (AH) or the mobile phase (ICB) (75 μl) were injected into a Waters® Spherisorb® S5 ODS2. The mobile phase was composed of methanol, acetonitrile and 0.1% triethylamine/0.1% sodium hexane sulfonate (pH 2.25) in a 4:16:80 (v/v/v) ratio. The flow rate was set at 1 ml/min and detection by spectrophotometry at a wavelength of 252 nm.

The plasma (50 μl) extracts were injected into an Uptisphere® system guard cartridge column

and eluted with acetonitrile/0.05% acetic acid 13:87 (v/v). The flow rate was set at 0.25 ml/min and detection performed by mass spectrometry with Simple Ion Monitoring 293.2.

2.8. Ocular tolerance evaluation

The ocular tolerance of 1 and 2% carteolol–alginic was evaluated over a 28-day topical application (50 μl, four times a day) in NZ albino rabbits. Histopathology of the eyes was performed at the end of the study on paraffin embedded eyes. Corneal sensitivity was evaluated with a Cochet and Bonnet esthesiometer after the first instillation.

2.9. Statistical analysis

The results were presented as mean ± S.D. The data on carteolol release from HEC or alginic vehicle were compared using Student's *t*-test or a Mann–Whitney *U*-test, according to the variance checks. Comparisons at different times of data obtained from the bioavailability experiments (carteolol concentrations) and IOP experiments (inhibition percentage) were performed using Student's *t*-test.

The variances were checked with Cochran's *C*-test. A *P*-value of less than 0.05 was considered significant.

3. Results

3.1. Viscosity and in vitro diffusion test

Viscosity values were investigated at 1200/s for three preparations, 1 and 2% carteolol–alginic solutions and 1% carteolol–HEC solution. Comparable viscosity values, close to 5 mPa.s, were obtained.

Dialysis curves (Fig. 1) showed that alginic acid excipient involves a highly statistically significant effect on the in vitro release of carteolol compared with HEC or the reference solution. The drug release pattern obtained for the 1% carteolol–alginic sample was markedly different from that of the reference solution without polymer. The time for 50% carteolol release from the alginic acid solution was 3 h. This difference was not due to the increase in viscosity of the solution because an isoviscous solution (1% carteolol–HEC solution) presented the same release kinetics as the reference 1% carteolol. The time for 50% carteolol release from these two formulations was similar and close to 1 h. This result was probably due to an ionic interaction between alginic acid and carteolol.

Similar results were obtained with the 2% carteolol solutions.

3.2. Adhesive properties

The contact angle measured between the platinum plate and all the different test solutions was zero. The adhesion work (Wa), following Young's equation, was, therefore, related to the surface tension measured (γ_{LV}); $Wa = 2\gamma_{LV}$. The wetting work (Wm) was calculated using the formula:

$$Wm = \gamma_{LV} - Wa = -\ \gamma_{LV}$$

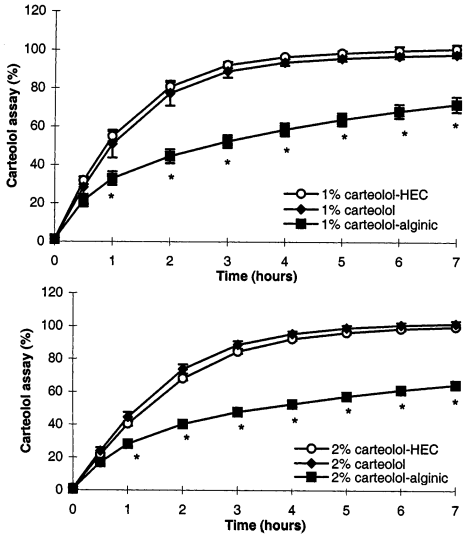

Fig. 1. In vitro diffusion test of 1 and 2% carteolol (error bars are mean ± S.D., $n = 6$). *$P < 0.05$; Student's *t*-test or Mann–Whitney test.

Table 1
Adhesion measurement

Test solution	Surface tension γ_{LV} (mN m^{-1})	Adhesion work Wa (mJ)	Wetting work Wm (mJ)
Alginic excipient	31.3	62.6	−31.3
1% carteolol–alginic	31.5	63.0	−31.5
2% carteolol–alginic	31.5	63.0	−31.5
2% carteolol–HEC	36.8	73.6	−36.8
2% carteolol	37.3	74.6	−37.3

The results in Table 1 show that the addition of alginic acid induced a decrease in surface tension and consequently a decrease in adhesion work and an increase in wetting work, which means a better adhesion between the solutions and the substrate. Moreover, carteolol concentration had no effect on the surface tension values of alginic solutions.

Furthermore, the 2% carteolol–alginic solution presented a surface tension (31.5 mN/m) lower than the critical surface tension of the mucin-coated cornea ($\gamma_c = 38$ mN/m) and practically equal to the critical surface tension of the clean cornea ($\gamma_c = 30$ mN/m). Thus, according to Zisman's theory, the adhesive behavior of such a solution on the cornea would be maximal (Rosen, 1978).

Two percent of carteolol–HEC solution gave the same results as the reference solution, without polymer, with surface tensions of 36.8 and 37.3 mN/m, respectively. The alginic acid vehicle compared with the conventional HEC vehicle used in ophthalmology presented interesting adhesive properties; the behavior of carteolol–alginic eyedrops suggests they should be able to remain in contact with the front of the eye.

3.3. IOP reduction by the carteolol formulations

Results concerning IOP measurements are indicated in Table 2. The maximal decrease in IOP was obtained 1 h after instillation for both the carteolol formulations. The inhibition of hypertension for 1% carteolol was 18.9%, corresponding to a 2.1 mmHg decrease, statistically comparable with the inhibition obtained with 1% carteolol–alginic, which was 19.2%, corresponding to a 2.2 mmHg decrease. This result is classically described for β-blocker activity in the rabbit (Kau and Limp, 1989).

However, the 1% carteolol–alginic formulation significantly extended the duration of the pressure-reducing effect of carteolol for up to 8 h after instillation. The 1% carteolol–alginic presented a hypotensive activity of 8.9% inhibition corresponding to a 1.2 mmHg decrease, significantly higher than that of 1% carteolol solution.

3.4. Ocular bioavailability

After the last instillation of 1% carteolol-alginic, the aqueous humor, iris/ciliary body and plasma levels of carteolol were compared with the values obtained after instillation of 1% carteolol. The pharmacokinetic profile (Fig. 2) was similar for both treatments.

The aqueous humor carteolol concentrations were generally higher for the alginic formulations, with a statistically significant difference ($P < 0.05$) at 1 and 2 h after the last instillation. In both cases, the carteolol concentrations reached a maximum at 1 h after the last instillation (515 ± 151 ng/ml for 1% carteolol-alginic and 353 ± 124 ng/ml for 1% carteolol). The presence of alginic acid in the formulation led to a 50% increase in the $AUC_{0\rightarrow 8\ h}$.

There was no change in iris/ciliary body carteolol concentrations after administration of the formulations, whether or not they contained alginic acid. These concentrations obtained after 15 days' administration represented steady-state levels, probably resulting from the binding of carteolol to melanin (Nagata et al., 1993; Fujio et al., 1994). The amount of the β-blocker was similar

Table 2
Effect of carteolol on IOP as a function of formulations and pretreatment time (mean ± S.D.; *n*, number of rabbits)

Inhibition of hypertension (%)	Time after instillation		
(ΔIOP max(mmHg))	1	6	8
1% carteolol	18.9 ± 4.9 (2.1 ± 0.8) (*n* = 7)	13.0 ± 4.4 (1.5 ± 0.6) (*n* = 8)	2.2 ± 4.1 (0.2 ± 0.4) (*n* = 7)
1% carteolol–alginic	19.2 ± 6.4 (2.2 ± 0.8) (*n* = 8)	12.5 ± 4.0 (1.6 ± 1.0) (*n* = 6)	8.9 ± 2.7 (1.2 ± 0.5)[a] (*n* = 8)

[a] $P < 0.05$; Student's *t*-test.

for the carteolol–alginic formulation ($AUC_{0\rightarrow 8\ h}$ = 48 618 ng/h per 70 mg of tissue) and for the carteolol formulation (56 176 ng/h per 70 mg of tissue). The relative bioavailability was 1.8 corresponding to the doses applied (twice a day for 1% carteolol and once a day for 1% carteolol–alginic).

For both the treatments, carteolol was found in plasma before the last instillation (0.12 ± 0.07 ng/ml for 1% carteolol–alginic and 0.65 ± 0.35 ng/ml for 1% carteolol). The difference between these values results from the interval between the last instillation and dosing (12 h for 1% carteolol and 24 h for 1% carteolol–alginic). The carteolol concentrations increased to attain C_{max} values of 30.69 ± 17.96 and 51.01 ± 20.80 ng/ml for 1% carteolol–alginic and 1% carteolol, respectively. The concentrations then decreased slowly in the same manner.

3.5. Ocular tolerance

Carteolol–alginic eye drops showed excellent ocular tolerance and did not present a local anesthetic effect after instillation. No local ocular irritation could be observed after 28-day topical application. Only a few signs of increased lacrimation were noted. No histopathologic changes in the eye or adnexae were observed after treatment.

4. Discussion

By visual inspection, 1 and 2% carteolol-alginic form clear, colorless solutions. Both carteolol–HEC and carteolol–alginic solutions exhibit comparable low viscosities, compatible with topical administration without risk of blurred vision and discomfort. From the point of view of patient acceptability, the liquid dosages are preferable and ideally the solution should be able to sustain

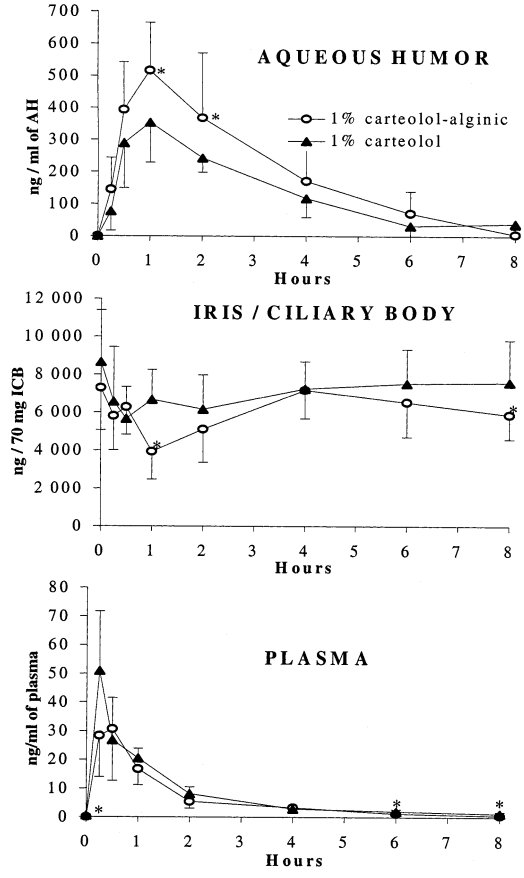

Fig. 2. Carteolol concentrations in aqueous humor, iris/ciliary body, and plasma of pigmented rabbits after 2 weeks' bilateral instillations of 25 μl of 1% carteolol formulations (mean ± S.D.; $n = 12$). *, $P < 0.05$; Student's *t*-test.

drug release and to remain in contact with the surface of the eye for an extended period of time. The in vitro release studies showed that carteolol diffuses from alginic acid much more slowly than from HEC, probably due to an ionic interaction between carteolol and alginic acid. The adhesive properties of carteolol–alginic compared with carteolol–HEC suggest that the alginic acid vehicle could be a good candidate for enhancing precorneal residence time.

Sodium alginate is known as a bioadhesive polymer in topical delivery. In a neutral medium, the mucin molecule is negatively charged ($pK_a = 2.6$) and behaves as an anionic polyelectrolyte, forming a weak viscoelastic gel, which consists of a network of linear, flexible and random coil molecules. Polymer–mucin interactions include chain interlocking, conformational changes and non-covalent bond formation. Polymers should, therefore, have functional groups that are able to form hydrogen bonds and the polymer chain should be flexible enough to form as many bonds as possible. Polymers with carboxyl groups, such as sodium alginate, exhibit a better bioadhesion (Albasini and Ludwig, 1995). Polymers are frequently used in ophthalmic solutions. The mucoadhesive properties of polymers might, therefore, influence contact times of vehicles. How well the gel stays in the eye is probably dependent not only on its mucoadhesive properties, but also on its bulk rheological properties.

The in vivo experiments demonstrated a modification of the carteolol activity and bioavailability in the presence of alginic acid. The IOP reduction studies demonstrated a significant enhancement of the duration of action of the 1% carteolol–alginic formulation. The penetration of carteolol into the aqueous humor was increased by the presence of alginic acid in the formulation. This can probably be explained by the interaction between carteolol and alginic acid as demonstrated by the dialysis study. The bioavailability increase could be due to the gelling of the solution on the ocular surface promoted by the presence of divalent cations in the lacrimal film (Cohen et al., 1997) and to the mucoadhesive properties of the alginic acid. This enhancement of β-blocker bioavailability explains why ICB levels after once daily application of carteolol–alginic were equivalent to the levels resulting from twice daily applications of carteolol. It is interesting to note that the peak plasma concentration of carteolol was lower with the formulation containing alginic acid. This could minimize β-blocker adverse effects resulting from systemic uptake, particularly as the administration is only half as frequent with carteolol–alginic as with carteolol. All these results show that alginic acid could increase the precorneal residence time of carteolol–alginic eyedrops, providing a prolonged delivery of carteolol. This should lead to a reduction in the frequency of carteolol dosing from twice to only once a day.

In conclusion, the excellent ocular tolerance and the longer action of the carteolol–alginic formulation should reduce the frequency of administrations and improve the comfort and compliance of patients during treatment by this β-blocker.

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