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The effect of vehicle viscosity on the ocular bioavailability of L-653,328

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Summary

The effect of the viscosity of an ophthalmic vehicle on ocular drug penetration has been investigated. Ocular concentrations of L-652,698 have been measured using HPLC and fluorescence detection, in the cornea, aqueous humor and iris + ciliary body of rabbits after instillation of 1% solutions of L-653,328 in 0, 0.2, 0.25, 0.3, 0.35, 0.4 and 0.5% hydroxyethyl cellulose (HEC). Maximum drug concentrations in all three ocular sites increased concomitantly with increase in viscosity. The correlation coefficients between ocular bioavailability, assessed by AUC (0–4 h), and with HEC viscosity were 0.93, 0.96 and 0.83 in cornea, aqueous humor and iris + ciliary body, respectively. When isoviscous solutions containing polyvinyl alcohol were examined, ocular bioavailability was similar in cornea and aqueous humor but reduced by 50% in the iris + ciliary body when compared to the equivalent HEC solution.

Introduction

Much research has been carried out on the effect of the addition of viscosifying agents to ophthalmic vehicles (Blaug and Canada, 1965; Adler et al., 1971; Chrai and Robinson, 1974; Patton and Robinson, 1975; Li and Robinson, 1989). It was argued that a viscosifying agent would reduce the drainage of a drug solution from the conjunctival sac via the nasolacrimal canal. The resulting augmentation in residence time of the ophthalmic agent in the precorneal area would then cause an increase in the drug's ocular bioavailability.

Intuitively, this concept would appear to be logical. However, little improvement in drug bioavailability above 15 cps was reported (Eriksen, 1980; Lee and Robinson, 1986). In contrast, it is well documented that drainage time and viscosity increase concomitantly (Adler et al., 1971; Chrai and Robinson, 1974). To add further confusion, if viscosity affects drainage, then isoviscous solutions should produce similar changes in bioavailability, an observation not made by Saettone et al. (1982a).

Often, ocular bioavailability has been assessed indirectly, using precorneal residence time (Blaug and Canada, 1965; Adler et al., 1971), tear fluid drug concentrations (Adler et al., 1971; Chrai and Robinson, 1974; Patton and Robinson, 1985), miosis (Adler et al., 1971) and mydriasis. Limited attention has been paid to detailed intra-ocular drug concentrations.

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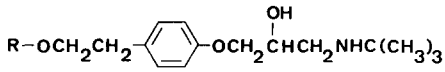

L-652,698 R = H
L-653,328 R = CH_3CO

Fig. 1. Structures of L-653,328 and L-652,698.

In seeking to optimize the ocular bioavailability of ophthalmic formulations, it seemed to us that a plateau effect did not appear to operate at 15 cps (Lee and Robinson, 1986) or 22.5 cps (Blaug and Canada, 1965). In numerous experiments, it was evident that ocular drug concentrations were invariably increased with the addition of a viscosifying agent, when compared to those obtained with an aqueous solution. Herein are our findings using the recently described beta-blocker L-653,328 as probe drug (Sugrue et al., 1988). L-653,328 is the acetate ester of L-652,698 ((*S*)-3-*tert*-butylamino-1-[4-[2(hydroxy)ethyl]2-propanol) (Fig. 1) and is a substrate for the ocular esterases which liberate the active moiety, L-652,698 during transcorneal penetration.

Materials and Methods

L-653,328 and L-652,698 were synthesized by the group of Dr J.J. Baldwin, Merck, Sharp & Dohme Research Laboratories, West Point, PA. Hydroxyethyl cellulose (HEC) was supplied by Union Carbide Co., as Cellosize, QP 52000H and polyvinyl alcohol (PVA) by Wacker-Chemie, Germany as Polyviol W48/20. Other chemicals were of analytical grade and purchased commercially.

New Zealand rabbits were obtained from either Charles River or Picosson, France. They were housed individually with a 12 h light/dark cycle and had free access to food and water.

Preparation of instillates

Sterile preparations containing 0.4, 0.5, 0.7, 0.8 and 1% (w/w) of HEC were obtained by autoclaving. Each solution was subsequently diluted with an equal weight of a 2% solution of L-653,328 to give a range of 1% solutions varying in viscosity. The viscosity of each preparation was measured at 20°C using an Ostwald viscometer. PVA solutions were made in a similar manner.

Treatment of animals

Rabbits were placed in wooden restraining boxes and bilateral instillations of one drop of 50 μl of the test material were made into the conjunctival sac and the lower lid was brought gently up to meet the upper.

The animals were sacrificed at designated times by rapid injection of a lethal dose of sodium pentobarbital into the marginal ear vein. Aqueous humor, cornea and iris + ciliary body were sampled as previously described (Grove et al., 1988).

Preparation of extracts for HPLC

Aqueous humor (120 μl) was deproteinized by the addition of 18 μl of 20% trichloroacetic acid. The sample tube was placed in a refrigerator (4°C) for 30 min to aid precipitation and then centrifuged for 2 min in an Eppendorf 5412 Centrifuge. The supernatant was then examined by HPLC.

Corneas were solubilized by the addition of 1 ml of 0.5 M KOH and heating for 30 min at 70°C in a Thermolyne Dri-bath (Pierce). After cooling, a known quantity of metoprolol was added as internal standard and the clear solution extracted by shaking for 5 min with 2 × 3.5 ml of ethyl acetate. After each extraction, the tubes were centrifuged and the organic phase transferred to another tube. The ethyl acetate layers were pooled and evaporated to dryness with gentle heating under a stream of nitrogen. The residue was reconstituted in 150 μl of 0.01 M HCl and an aliquot examined by HPLC.

The iris + ciliary body was homogenized using a glass/glass Kontes homogenizer with 0.5 ml of water. The tube was rinsed with 2 × 0.5 ml of water and after alkalinization with KOH, the pooled homogenate and rinsings were extracted with 2 × 4 ml ethyl acetate, as described for the cornea.

Calibration graphs were constructed by spiking aqueous humor or tissues from controls with known amounts of L-652,698 (the corresponding phenol formed from the prodrug L-653,328) and carrying them through the procedure.

Chromatography

A Hewlett Packard Model 1084 fitted with an automatic injector and an integrator was used with a Shimadzu RF-530 fluorimeter as detector. The excitation and emission wavelengths were 272 and 302 nm, respectively. Separation was effected by reverse-phase chromatography, as previously described (Sugrue et al., 1988).

Results

Ocular concentrations after instillation of HEC solutions

L-653,328 is a beta-adrenergic ocular hypotensive agent with modest beta-receptor blocking activity (Sugrue et al., 1988). Corneal esterases hydrolyse the acetate moiety of L-653,328 to the alcohol L-652,698. As reported previously, unchanged L-653,328 was not detected in aqueous humor (Sugrue et al., 1989) and it can be concluded that only L-652,698 reaches the iris + ciliary body. The alkaline conditions under which the corneas were solubilized favoured the formation of L-652,698. Hence all drug concentrations are reported as the alcohol.

Tables 1–3 list the mean concentrations of L-652,698 found in the cornea, aqueous humor and iris + ciliary body, respectively, after instillation of solutions of 1% L-653,328 in 0, 0.2, 0.25, 0.3, 0.35, 0.4 and 0.5% HEC. The viscosity of the instillates ranged from 1 to 100 cps (Table 4).

The maximum concentrations occurred generally at 10 or 30 min in the cornea or at 1 h post-instillation in the aqueous humor and iris + ciliary body. There was a clear linear relationship between the maximum concentrations observed in all three ocular sites and increase in vehicle viscosity (see Table 4). The concentrations at individual sampling times, obtained with viscous solutions, were not always statistically different from the corresponding values with 0% HEC. This was probably due to the small number of samples assayed. When bioavailability was assessed by measurement of the AUC (0–4 h) concentration/time profiles in the various tissues, there was again a concomitant increase with increase in the viscosity of the instillate (Fig. 2).

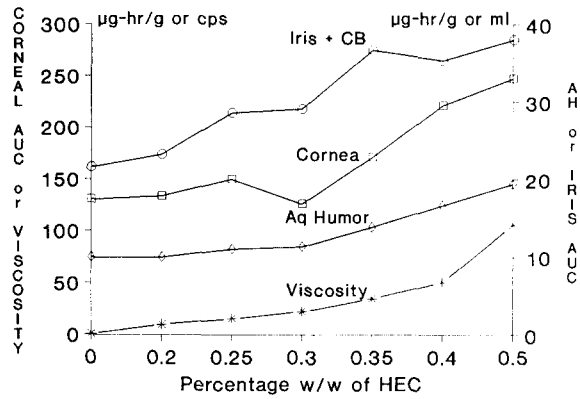

Fig. 2. The change in viscosity of different HEC solutions and the AUC (0–4 h) of the concentration/time profiles of L-652,698 in cornea, aqueous humor and iris + ciliary body.

Ocular concentration after treatment with PVA

PVA was substituted for HEC such that 3.0 and 4.5% PVA gave a solution isoviscous with 0.25 and 0.35% HEC (15 and 35 cps), respectively. When these solutions were instilled in a manner

TABLE 1

μg/g of L-652,698 in the rabbit cornea after instillation of 1% L-653,328 in various concentrations of HEC[a]

Sampling time	Concentration of HEC (% w/w)						
	0	0.2	0.25	0.3	0.35	0.4	0.5
10 min	66.4 ± 12.7	64.3 ± 9.9	84.3 ± 5.3	86.5 ± 2.7	103.0 ± 9.2[b]	92.8 ± 4.9	98.7 ± 4.9[b]
30 min	73.7 ± 11.6	64.5 ± 7.3	64.4 ± 6.3	63.7 ± 5.5	94.1 ± 10.7	113.5 ± 12.3[b]	93.9 ± 5.9
1 h	30.0 ± 3.2	36.9 ± 7.3	46.7 ± 7.2	48.7 ± 8.2	54.0 ± 6.7[b]	70.3 ± 6.5[b]	80.0 ± 3.4[b]
2 h	29.1 ± 4.9	35.5 ± 4.8	33.9 ± 2.8	17.8 ± 1.6	33.5 ± 2.2	49.4 ± 0.7[b]	65.2 ± 12.7[b]
4 h	17.1 ± 2.9	9.7 ± 2.3	16.2 ± 3.0	14.4 ± 3.9	15.9 ± 1.9	24.6 ± 3.0	25.9 ± 2.5[b]

[a]Results are expressed as mean ± SE. The number of samples was 8.
[b]Significantly different ($P<0.05$) from corresponding value without HEC.

TABLE 2

μg/ml of L-652,698 in the aqueous humor after instillation of 1% L-653,328 in various concentrations of HEC[a]

Sampling time	Concentration of HEC (% w/w)						
	0	0.2	0.25	0.3	0.35	0.4	0.5
10 min	1.07 ± 0.28	0.65 ± 0.06	1.11 ± 0.15	0.76 ± 0.08	0.72 ± 0.07	0.92 ± 0.03	1.14 ± 0.14
30 min	4.68 ± 0.62	3.41 ± 0.38	3.38 ± 0.54	3.91 ± 0.33	5.68 ± 0.81	6.33 ± 1.00	4.88 ± 0.53
1 h	2.83 ± 0.45	4.22 ± 0.91	4.23 ± 0.78	5.69 ± 1.16	5.24 ± 0.47[b]	5.76 ± 0.76[b]	7.93 ± 0.72[b]
2 h	3.29 ± 0.66	2.90 ± 0.23	3.44 ± 0.38	2.62 ± 0.18	3.73 ± 0.18	4.67 ± 0.44	6.05 ± 1.22
4 h	0.76 ± 0.12	0.89 ± 0.05	0.99 ± 0.11	1.33 ± 0.27	1.80 ± 0.31[b]	2.43 ± 0.40[b]	2.02 ± 0.39[b]

[a]Results are expressed as mean ± SE. The number of samples was 8.
[b]Significantly different ($P<0.05$) from corresponding value without HEC.

TABLE 3

μg/g of L-652,698 in the iris + ciliary body after instillation of 1% L-653,328 in various concentrations of HEC[a]

Sampling time	Concentration of HEC (% w/w)						
	0	0.2	0.25	0.3	0.35	0.4	0.5
10 min	4.73 ± 0.79	4.60 ± 1.01	6.19 ± 0.62	8.78 ± 1.35[b]	6.05 ± 1.35	5.46 ± 0.86	7.45 ± 1.89
30 min	7.99 ± 1.04	8.79 ± 1.61	8.64 ± 1.27	12.32 ± 1.42[b]	12.10 ± 1.72	11.58 ± 1.00[b]	12.28 ± 1.10[b]
1 h	5.35 ± 0.66	6.76 ± 1.09	10.45 ± 2.22	7.94 ± 1.01	11.41 ± 0.98[b]	11.60 ± 1.07[b]	15.89 ± 1.85[b]
2 h	5.02 ± 0.44	5.97 ± 0.52	7.87 ± 1.58	5.78 ± 0.99	12.05 ± 2.00[b]	9.56 ± 1.66[b]	10.05 ± 1.56[b]
4 h	5.57 ± 1.59	4.38 ± 1.28	3.76 ± 0.39	7.16 ± 2.50	3.55 ± 0.62	6.19 ± 1.07	4.01 ± 0.63

[a]Results are expressed as mean ± SE. The number of samples was 8.
[b]Significantly different ($P<0.05$) from corresponding value without HEC.

TABLE 4

Maximum concentrations of L-652,698 observed in rabbit ocular sites after instillation of 1% L-653,328 in HEC solutions of different viscosities

% HEC	Viscosity (cps)	Cornea (μg/g)	Aqueous humor (μg/ml)	Iris + ciliary body (μg/g)
0	1	73.7	4.69	7.99
0.2	9.8	64.5	4.22[a]	8.79
0.25	15.6	84.3[b]	4.23	10.45
0.3	22.5	86.5[b]	5.69	12.32
0.35	35.2	103.0[b]	5.68	12.10
0.4	51.1	113.5	6.33[a]	11.60
0.5	105.6	98.7[b]	7.93	15.89[a]

[a]Maximum at 1 h.
[b]Maximum at 10 min.
All other values occurred at 0.5 h post-instillation.

TABLE 5

Comparison of ocular bioavailability after instillation of isoviscous HEC and PVA solutions

Solution	Viscosity (cps)	AUC (0–4 h) in μg-h/g or ml		
		Cornea	Aqueous humor	Iris + CB
0.25% HEC	15.6	150.0	11.0	28.6
3% PVA	14.0	127.7	13.1	16.7
0.35% HEC	35.2	171.6	13.9	36.7
4.5% PVA	38.4	132.8	13.8	15.9[a]

[a]Significantly different ($P<0.05$) from corresponding value with isoviscous HEC.

identical to that of HEC preparations, the AUCs (0–4 h) of the cornea and aqueous humor were observed similar to those of the equivalent HEC solution (Table 5). Despite this apparent parallel behaviour, the amount of compound found in the iris + ciliary body was greatly reduced and only 50% of the AUC (0–4 h) of L-652,698 was found compared to the corresponding HEC treatments (see Table 5). This difference was statistically significant ($p < 0.05$) between the higher viscosity solutions of 0.35% HEC and 4.5% PVA.

Discussion

A recent review concluded that 'increasing solution viscosity had a limited utility in causing a marked improvement in the amount of drug ab-

sorbed' into the eye (Lee and Robinson, 1986). Indeed, for many years the addition of a viscous polymer to achieve an increase in the penetration of an ophthalmic drug has been claimed to be maximum at about 15–20 cps (Blaug and Canada, 1965; Adler et al., 1971; Patton and Robinson, 1975). On the other hand, Li and Robinson (1989) consider that it is undesirable to increase viscosity of an instilled solution above 1000 cps.

In seeking to find support for the above conclusions, we looked at the evidence of intra-ocular drug measurements which corroborate these findings. It was limited. Conclusions that are based on, for example, pre-corneal tear fluid assays, aqueous humor concentrations at two time points, or fluorophotometric determinations in the aqueous humor 1–2 h after repeated dosing of fluorescein, are not the best assessment of ocular drug bioavailability.

Therefore, systematic evaluation of the effect of the viscosity of an ophthalmic vehicle on the drug concentrations of L-652,698 after administration of its acetate prodrug L-653,328 to rabbits was undertaken. Our results demonstrate a clear, concomitant increase in ocular drug bioavailability with increase in viscosity in the cornea, aqueous humor and iris + ciliary body (Fig. 2). This trend has also been confirmed with MK-927, a topical anhydrase inhibitor (Bron et al., 1989) after administration to rabbits of 1% solutions containing 0, 0.35 and 0.5% HEC (data not shown).

The correlation coefficients between L-652,698 AUC (0–4 h) values and viscosity of the instillate were 0.93, 0.96 and 0.83 for the cornea, aqueous humor and iris + ciliary body, respectively. The maximum increases in bioavailability, obtained with 0.5% HEC, were about 2-fold at the three ocular sites. This increase in bioavailability is in agreement with observations of aqueous humor pilocarpine measurements after administration in 1 and 100 cps methylcellulose (Chrai and Robinson, 1974) and in 0 and 5% PVA (Patton and Robinson, 1975). Grass and Robinson (1984) predicted that the time to peak concentration in the aqueous humor would be increased with a decrease in pre-corneal parallel loss (i.e. increased viscosity, contact time). Our values do not provide consistent evidence of an influence of viscosity on the time to peak concentrations (see Table 4). In general, the highest corneal concentrations were at 10–30 min, the aqueous humor concentrations were at 30–60 min and the iris + ciliary body concentrations were at 60 min, reflecting the distance of each site from the pre-corneal area, rather than an effect of viscosity. These authors also claim that increasing vehicle viscosity from 1 to 90 cps should not and does not improve the bioavailability of compounds whose log partition coefficients ranged from 1 to 4, i.e. propranolol. The data we present are at variance with these authors, as indeed are those seen for timolol (Rozier et al., 1989).

Isoviscous ophthalmic vehicles have been considered to produce similar effects in the eye since their drainage characteristics and flow properties are the same (Patton and Robinson, 1975). PVA and HEC have non-Newtonian flow properties, but diluted solutions exhibit approximate Newtonian behaviour (Patton and Robinson, 1975; Saettone et al., 1982b). A single viscosity measurement does not truly establish isoviscosity, but the slight differences due to changes in flow characteristics should not radically influence our observations.

Saettone et al. (1982b) examined the influence of different isoviscous polymers on the activity of pilocarpine in rabbit and man. They found that, using the AUC of the miosis response curve, PVA appeared significantly more active in both species when compared to aqueous, polyvinyl pyrrolidone or hydroxypropyl cellulose solutions. In contrast, our results are somewhat surprising. Isoviscous solutions of PVA and HEC produced similar AUC (0–4 h) in both cornea and aqueous humor (see Table 5), but the iris + ciliary body AUC was reduced by one half in the case of 3 and 4.5% PVA solutions. This may be due to the transport of the beta-blocker to the iris by a route other than transcorneal penetration. We have observed that PVA can both increase and decrease ocular bioavailability of certain compounds. Differences in the non-corneal access routes may explain these inconsistencies.

The results from our experiments (Fig. 2) do not allow us to conclude that bioavailability plateaus at 20 cps or even at 100 cps. Higher concen-

trations of HEC were not studied, since it is generally agreed that patient acceptance of preparations with much higher viscosities is doubtful.

An argument for not exceeding 20 cps is based on drainage of vehicles from the conjunctival sac, since at higher viscosities the drainage rate did not change very much from methylcellulose (Chrai and Robinson, 1974) or PVA (Adler et al., 1971). However, these authors pointed out that contact time is not proportional to drug bioavailability (Adler et al., 1971). Since the ocular bioavailability of an ophthalmic drug is extremely low (in the order of 1% of the administered dose), it would appear that even a 2-fold increase is considerable.

Saettone et al. (1982b) reported that rabbits were much less sensitive than man to moderate increases of the vehicle viscosity. They found with tropicamide in various vehicles that while their rabbit data were in-line with previous findings (i.e. 2-fold improvement), their work in humans produced a 4-fold increase in maximum bioavailability. If their findings are not drug-specific, and may be extended to other compounds, then vehicle viscosity may be much more important in ophthalmic drug delivery than thought hitherto.

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