L-662,583 is a topically effective ocular hypotensive carbonic anhydrase inhibitor in experimental animals

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1 L-662,583 was a potent inhibitor *in vitro* of purified, human erythrocyte carbonic anhydrase II, possessing an IC_{50} of 0.7 nM. The IC_{50} values for MK-927, acetazolamide and methazolamide were 13.0 nM, 10.8 nM and 21.2 nM, respectively.

2 A 1 h pretreatment with one 50 μ l drop of a 0.1% solution of L-662,583 blocked carbonic anhydrase activity in a homogenate of the iris + ciliary body of albino rabbits by 63%. Similar treatment with 0.1% suspensions of acetazolamide and methazolamide elicited inhibitions of 30% and 20%, respectively. This *ex vivo* model indirectly assesses the ability of an agent to enter the rabbit eye.

3 Concentrations of L-662,583 in the cornea, aqueous humour and iris + ciliary body of albino rabbits were determined by h.p.l.c. at predetermined times after the instillation (one drop of 50 μ l) of a 2% solution of L-662,583. Peak levels for cornea (47.4 μ g g⁻¹), aqueous humour (4.51 μ g ml⁻¹) and iris + ciliary body (9.61 μ g g⁻¹) occurred at 0.5, 2 and 1 h after instillation, respectively.

4 The experimentally elevated intraocular pressure of the right eye of rabbits, induced by prior intraocular injection of α -chymotrypsin, was maximally decreased by 4.5 mmHg, 6.2 mmHg and 9.8 mmHg after the instillation (one drop of 50 μ l) of 0.01%, 0.1% and 0.5% solutions of L-662,583, respectively. All three concentrations lowered intraocular pressure at all time points from 1 h up to and including 5 h, the last recorded time point. The unilateral instillation of L-662,583 (0.5%) into the contralateral, left eye failed to lower the elevated intraocular pressure of the untreated, right eye. This finding indicates that the site of action of topically applied L-662,583 in this paradigm is local. The ocular normotensive, albino rabbit was much less susceptible than the ocular hypertensive rabbit to the intraocular pressure lowering effect of topically applied L-662,583, with a 2% solution maximally decreasing intraocular pressure by 2.3 mmHg.

5 Unilateral ocular hypertension was elicited in the right eye of sedated, cynomolgus monkeys by argon laser-induced photocoagulation of the trabecular meshwork. The instillation (one drop of 50 μ l) of L-662,583 (2%) significantly lowered the elevated intraocular pressure of the right eye at all time points from 1 h up to and including 5 h. The maximum decline was 8.3 mmHg at 3 h and this represented a reduction of 23% from the corresponding baseline value of 36.8 mmHg. The intraocular pressure of the hypertensive, right eye was maximally decreased by 4.1 mmHg and 4.8 mmHg after the instillation of 0.5% and 1% solutions of L-662,583, respectively. Like the rabbit, the normotensive eye of cynomolgus monkeys was more resistant than the hypertensive eye to the ocular hypotensive action of L-662,583, as indicated by the inability of 0.5% and 1% solutions of the agent to lower intraocular pressure. L-662,583 (2%) maximally reduced the intraocular pressure of normotensive monkey eyes by 2.4 mmHg at 2 h.

6 L-662,583 is structurally different from MK-927, a carbonic anhydrase inhibitor that lowers the intraocular pressure of glaucoma patients following the instillation of a 2% solution. These preclinical observations indicate that L-662,583, like MK-927, is a water-soluble carbonic anhydrase inhibitor which, on topical administration, lowers intraocular pressure by virtue of an action confined to within the eye.

Introduction

An elevated intraocular pressure is a characteristic feature of open-angle glaucoma, the most prevalent form of glaucoma. Intraocular pressure is primarily controlled by the rates at which aqueous humour enters and leaves the eye and aqueous humour outflow is impaired in open-angle glaucoma. Reduction in elevated intraocular pressure is at present the only clinically acceptable treatment of open-angle glaucoma. Most patients are treated topically with one or more of the following classes of drugs: β -adrenoceptor antagonists, sympathomimetics, parasympathomimetics and anticholinesterases. However, local and/or systemic side effects are encountered to varying degrees with these agents (for review see Sugrue, 1989).

The bulk of aqueous humour entering the eye is produced through an active secretory process by the nonpigmented epithelial cells of the ciliary process (for review see Caprioli, 1987). Carbonic anhydrase II is present in these cells

(Wistrand & Garg, 1979; Wistrand *et al.*, 1986) and, following inhibition of the isoenzyme, bicarbonate formation is decreased which, in turn, diminishes sodium and fluid secretion into the eye (Maren, 1967; 1987). Systemically administered carbonic anhydrase inhibitors have been used to treat open-angle glaucoma for over thirty years (Becker, 1954). The agents are very effective in lowering intraocular pressure but patient compliance is often poor, with approximately 50% of patients stopping medication because of side effects. These include fatigue, gastrointestinal disturbances, anorexia, paresthesias and loss of libido; the unwanted side effects stemming from the extraocular inhibition of the enzyme (Epstein & Grant, 1977; Lichter *et al.*, 1978).

It has been long recognized that a topical carbonic anhydrase inhibitor with an efficacy profile similar to that of oral agents would represent a major advance in the treatment of ocular hypertension. However, attempts in the past to obtain such an agent have been somewhat unsuccessful (for review see Sugrue, 1989). MK-927 (d,1-5,6-dihydro-4-(2-methylpropylamino)-4H-thieno-[2,3b]thiopyran-2-sulphonamide-7,7-dioxide hydrochloride) can be formulated at 2% in aqueous

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Figure 1 Structures of L-662,583 and MK-927.

solutions and the preclinical pharmacology of the compound was such (Sugrue *et al.*, 1988; Wang *et al.*, 1988; Maren & Bar-Ilan, 1988) that the agent was selected for topical evaluation in both human volunteers (Lippa *et al.*, 1988) and glaucoma patients (Hennekes *et al.*, 1988). The instillation of one drop of a 2% solution of MK-927 elicited a significant reduction in the intraocular pressure of the latter group and it is the first reported carbonic anhydrase inhibitor to possess this profile. L-662,583 (5-(3-dimethylaminomethyl-4-hydroxyphenylsulphonyl)thiophene-2-sulphonamide hydrochloride) is a water-soluble carbonic anhydrase inhibitor that is structurally different from MK-927 (Figure 1). In this study we have shown L-662,583 to be a topically effective, ocular hypotensive agent in experimental animals. Some of the results have been presented previously to the British Pharmacological Society (Sugrue *et al.*, 1989).

Methods

Animals

Adult, female cynomolgus monkeys and adult, New Zealand albino rabbits of either sex were used. The rabbits had free access to standard laboratory diet while the monkeys were fed twice daily. Both species had free access to tap water and were housed on a 12 h light/dark cycle.

In vitro studies

Carbonic anhydrase II was isolated from lysed, human erythrocytes as described in detail elsewhere (Ponticello *et al.*, 1987). Inhibition of the purified isoenzyme was assessed by a pH stat assay. This assay measures the rate of hydration of CO_2 by determining the rate at which a standard solution of NaOH has to be added to a lightly buffered solution to maintain a constant pH as CO_2 is bubbled into the buffer. Carbonic anhydrase inhibitors depress the reaction and this depression is measured as a decrease in the volume of NaOH required to maintain a given pH. Results were expressed as the IC_{50} values which were obtained from semilog plots of % inhibition against log concentration (for experimental details see Ponticello *et al.*, 1987).

Ex vivo studies

Rabbits were killed by an i.v. overdose of sodium pentobarbitone 1 h after topical treatment. The iris + ciliary body was dissected free and homogenized with a Tekmar Tissuemizer

(setting 60, 30s) in 2 ml of chilled 0.02 M Tris HCl buffer, pH 8.4. The carbonic anhydrase activity of 0.2 ml aliquots was determined by a pH stat assay. To the 0.2 ml aliquot was added 2.8 ml of Tris HCl buffer and a mixture of 5% CO_2 /95% air was bubbled through at a rate of 50 ml min^{-1} . A solution of 0.025 N NaOH was added to keep the pH constant at pH 8.3 over a time period that was for a minimum of 3 min. The volume of NaOH delivered was recorded as a function of time. The slope of the linear portion of the curve was expressed as ml NaOH min^{-1} . A blank containing 0.2 ml of Tris HCl buffer in place of 0.2 ml of tissue was also run and blank values were subtracted from enzyme activities. Results were expressed as mean $\pm$ s.e.mean in ml NaOH min^{-1} . % inhibition was also calculated.

Ocular distribution studies

Rabbits were killed by an i.v. injection of sodium pentobarbitone at preselected times after topical treatment. Aqueous humour was withdrawn and the cornea and iris-ciliary body were separated from the remainder of the eye. Samples were frozen in liquid nitrogen and then stored at -80°C before assay.

Concentrations of L-662,583 were quantified by h.p.l.c. using a Hewlett Packard 1090 (PV5 SDS) chromatograph fitted with an automatic injector and attached to a u.v. detector (Kratos Spectroflow 783) set at 280 nm. Separation was carried out by a Supelcosil LC-18-DB 5μ column (4.6×150 mm) equipped with a Supelcosil LC-18-DB 5μ guard column (4.6×20 mm). Both columns were housed in an oven at room temperature. Elution was made under isocratic conditions with a 10/90 (v/v) mixture of acetonitrile and 0.1 M sodium phosphate, pH 3.0. The flow rate was 1.5 ml min^{-1} . The retention times for L-662, 583 and its internal standard, L-667,048, were 6.2 and 11.3 min, respectively. The limits of detection for cornea, aqueous humour and iris + ciliary body were $0.0375\mu\text{g g}^{-1}$, $0.05\mu\text{g ml}^{-1}$ and $0.1\mu\text{g g}^{-1}$, respectively. Results were expressed as $\mu\text{g ml}^{-1}$ or $\mu\text{g g}^{-1}$ and are the mean $\pm$ s.e.mean.

Intraocular pressure studies

Conscious rabbits were placed in restraint boxes to which they had been habituated and intraocular pressure was recorded by a pneumatic tonometer (Alcon Applanation Pneumatograph) without local anaesthesia (Vareilles *et al.*, 1977a). The tonometer was calibrated previously by manometry in rabbits under sodium pentobarbitone anaesthesia.

Ocular hypertension was elicited in the right eye of albino rabbits by the injection of α -chymotrypsin into the posterior chamber, as described elsewhere (Sugrue *et al.*, 1985). The intraocular pressure of the operated animal was checked after approximately four weeks, and animals with an elevated pressure of 30 mmHg or more (i.e. approx. 10 mmHg above the value observed in normal, untreated eyes) were used at least one month after the injection of α -chymotrypsin.

In experiments in which both ocular normotensive and hypertensive rabbits were used, results are expressed as mean $\pm$ s.e.mean in mmHg and are the change in intraocular pressure from the resting level (t_0) measured just before administration of L-662,583 or vehicle. The significance of the data was determined as the difference from the t_0 value by use of the two-tailed test of Dunnett (1964).

The intraocular pressure of the right eye of sedated cynomolgus monkeys was experimentally elevated by photo-coagulating the trabecular meshwork with an argon laser (Coherent model 920) as described elsewhere (Lee *et al.*, 1985). For measurements of intraocular pressure, the monkeys were kept in a sitting position in restraint chairs throughout the experiment. Animals were lightly sedated by i.m. ketamine hydrochloride ($3\text{--}5\text{ mg kg}^{-1}$) approximately 5 min before each measurement of intraocular pressure, and one drop of 0.5%

Table 1 *In vitro* inhibition of human carbonic anhydrase II by L-662,583 and other carbonic anhydrase inhibitors

Compound	IC ₅₀ (nM)
L-662,583	0.71 ± 0.13
MK-927	13.0 ± 2.0
Acetazolamide	10.8 ± 0.02
Methazolamide	21.2 ± 0.11

Inhibition of purified human erythrocyte carbonic anhydrase II was assessed by a pH stat assay. Results are expressed as the IC₅₀ values which were obtained from semilog plots of % inhibition against log concentration. Values presented are group means ± s.e.mean (n = 4–6).

proparacaine hydrochloride, a local anaesthetic, was instilled before each reading. Baseline values were obtained three days before treatment, which consisted of instilling one 50 µl drop of L-662,583 or vehicle. At least a one week washout period

Figure 2 Effect of 2% L-662,583 on the intraocular pressure (IOP) of normotensive, conscious, albino rabbits. L-662,583 was instilled (one drop, 50 µl) into both eyes. Results are expressed as the difference in mmHg from the before treatment value (21.3 ± 0.4 mmHg) and each point is the group mean (n = 12); vertical lines show s.e.mean. *P < 0.05, significantly different from the before treatment value.

Table 2 *Ex vivo* inhibition of carbonic anhydrase activity (CA) in a homogenate of the iris + ciliary body of the albino rabbit at 1 h post-treatment

Treatment	Dose (%)	CA activity (ml NaOH min ⁻¹)		% inhibition
		Vehicle	Treated	
L-662,583	0.5	0.816 ± 0.042	0.035 ± 0.017	96
L-662,583	0.1	0.813 ± 0.056	0.303 ± 0.018	63
Acetazolamide	0.1	0.727 ± 0.017	0.508 ± 0.036	30
Methazolamide	0.1	0.727 ± 0.017	0.583 ± 0.056	20

Compound or vehicle was instilled (one drop, 50 µl) into both eyes 1 h before the removal of the iris + ciliary body. Carbonic anhydrase activity was assessed by a pH stat assay. The volume of NaOH added was recorded as a function of time and the slope of the linear portion of the curve was expressed as ml NaOH min⁻¹. Values presented are group means ± s.e.mean (n = 6).

Table 3 Concentrations of L-662,583 in the cornea, aqueous humour and iris + ciliary body of the albino rabbit after the instillation of a 2% solution of L-662,583

Sampling Time (h)	Concentrations of L-662,583		
	Cornea (µg g ⁻¹)	Aqueous humour (µg ml ⁻¹)	Iris + ciliary body (µg g ⁻¹)
0.5	47.4 ± 5.8	2.41 ± 0.52	5.90 ± 0.62
1	46.8 ± 5.9	3.42 ± 0.99	9.61 ± 1.07
2	23.4 ± 2.0	4.51 ± 1.30	7.30 ± 1.29
4	9.4 ± 0.7	1.29 ± 0.16	3.87 ± 0.55

L-662,583 (2%) was instilled (one drop, 50 µl) into both eyes and samples were taken at times indicated for h.p.l.c. analysis. Values presented are group means ± s.e.mean (n = 12).

Figure 3 Effect of L-662,583 on the unilateral, α -chymotrypsin-induced elevation of intraocular pressure (IOP) in albino rabbits. Rabbits were pretreated with α -chymotrypsin in the right eye at least one month previously. L-662,583, at 0.01% (○), 0.1% (●) and 0.5% (□), was instilled (one drop, 50 µl) into both eyes. Results are expressed as the difference in mmHg from the corresponding before treatment value (42.7 ± 2.2 – 44.3 ± 2.2 mmHg) and each point is the group mean (n = 6); vertical lines show s.e.mean. *P < 0.05, significantly different from the before treatment value.

was employed before testing on the same animal. The normotensive left eye was treated in an exactly similar manner to the hypertensive right eye for readings of intraocular pressure. Values for the intraocular pressure of both eyes were compared to the corresponding baseline values at the same time point by a paired Student's *t* test (two-tailed). Results are expressed as mean ± s.e.mean.

Drugs

The following drugs were used: L-662,583, MK-927, acetazolamide, methazolamide (all synthesized by Merck Sharp and Dohme Research Laboratories), ketamine hydrochloride (Ketalar, Parke-Davis), proparacaine hydrochloride (Ophthetic, Allergan) and sodium pentobarbitone (generic). Topical doses of L-662,583 are expressed as a free base.

The vehicle used for topical administration was 0.5% aq. hydroxyethylcellulose (HEC). L-662,583 was in solution in

0.5% HEC (pH approximately 4.5) whereas acetazolamide and methazolamide were in suspension. One drop of 50 μ l was used in all topical studies.

Results

In vitro studies

The IC_{50} of L-662,583 against carbonic anhydrase II purified from human erythrocytes was 0.7 nM (Table 1). The corresponding values for MK-927, acetazolamide and methazolamide were 13.0 nM, 10.8 nM and 21.2 nM, respectively.

Ex vivo studies

A 1 h pretreatment with a 0.5% solution of L-662,583 blocked carbonic anhydrase activity in a homogenate of the iris + ciliary body of the albino rabbit by 96% (Table 2). Lowering the dose instilled to 0.1% resulted in a 63% inhibition. The instillation of 0.1% suspensions of acetazolamide and methazolamide blocked enzyme activity by 30% and 20%, respectively.

Ocular distribution studies

The instillation of a 2% solution of L-662,583 resulted in a peak concentration of 47.4 μ g g⁻¹ of L-662,583 being present in the cornea of the albino rabbit at 0.5 h post-administration (Table 3). Peak levels in the aqueous humour and the iris + ciliary body occurred at 2 and 1 h and were 4.51 μ g ml⁻¹ and 9.61 μ g g⁻¹, respectively.

Intraocular pressure studies

The instillation of one 50 μ l drop of a 2% solution of L-662,583 significantly decreased the intraocular pressure of normotensive, albino rabbits at 0.5, 1 and 2 h with the maximum decline of 2.3 mmHg occurring at 2 h (Figure 2). Vehicle (0.5% HEC) had no effect on intraocular pressure (data not shown).

The elevated intraocular pressure of rabbits pretreated with α -chymotrypsin was significantly lowered at all time points from 1 h up to and including 5 h, the last recorded time point, after the instillation of 0.01%, 0.1% and 0.5% solutions of L-662,583 (Figure 3). The maximum reductions elicited by 0.01%, 0.1% and 0.5% solutions of L-662,583 were dose-related, being 4.5 mmHg, 6.2 mmHg and 9.8 mmHg, respectively. The unilateral instillation of a 0.5% solution of L-662,583 into the left, i.e. normotensive eye of these animals failed to lower the intraocular pressure of the right, i.e. hypertensive eye (Figure 4). In contrast, the unilateral instillation of 0.5% L-662,583 into the right eye significantly decreased intraocular pressure of that eye at all time points from 1 h up to and including 5 h, with the maximum decline of 9.3 mmHg occurring at 3 h. In all experiments, vehicle (0.5% HEC) had no effect on the intraocular pressure of the hypertensive, right eye (data not shown).

The instillation of a 2% solution of L-662,583 significantly lowered the intraocular pressure of unilateral, ocular hypertensive, cynomolgus monkeys at all time points from 1 h up to and including 5 h (Figure 5). The maximum fall of 8.3 mmHg occurred at 3 h and this represented a reduction of 23% from the corresponding baseline value of 36.8 mmHg. Vehicle (0.5% HEC) had no effect on intraocular pressure (data not shown). The peak decline of 4.8 mmHg in intraocular pressure occurred at 3 h after the instillation of a 1% solution of L-662,583. Small, but significant reductions in intraocular pressure were present from 2 h up to and including 6 h after 0.5% L-662,583 with the nadir of 4.1 occurring at 2 h. The intraocular pressure of the normotensive, left eye of cynomolgus

Figure 4 Effect of the unilateral instillation of 0.5% L-662,583 on the unilateral, α -chymotrypsin-induced elevation of intraocular pressure (IOP) in albino rabbits. L-662,583 was instilled (one drop, 50 μ l) into either the contralateral, i.e. left eye (○) or into the hypertensive, right eye (●). Results for the hypertensive, right eye are expressed as the difference in mmHg from the corresponding before treatment value (42.8 ± 1.8 and 43.2 ± 1.6 mmHg) and each point is the group mean ($n = 6$); vertical lines show s.e.mean. * $P < 0.05$, significantly different from the before treatment value.

monkeys was significantly decreased at 1, 2 and 3 h after 2% L-662,583; intraocular pressures declining from 19.2 ± 0.7 mmHg, 20.8 ± 0.6 mmHg and 20.4 mmHg to 17.8 ± 0.4 mmHg, 18.4 ± 0.4 mmHg and 18.5 ± 0.5 mmHg, respectively ($n = 16-24$). Both 0.5% and 1% solutions of L-662,583 failed to reduce significantly the intraocular pressure of normotensive, cynomolgus monkeys (data not shown).

Discussion

Preclinical and clinical studies (see Introduction for references) have shown that the topical administration of MK-927, a carbonic anhydrase inhibitor, to the eye results in a decrease in intraocular pressure. The objective of this study was the identification of an agent exhibiting a preclinical profile comparable to that of MK-927 but possessing a different chemical structure.

A comparison of L-662,583, MK-927, acetazolamide and methazolamide revealed that L-662,583 was the most potent of the four agents in blocking carbonic anhydrase II isolated

Figure 5 Effect of L-662,583 on the intraocular pressure (IOP) of unilateral, ocular hypertensive, cynomolgus monkeys. Ocular hypertension was induced in the right eye of sedated cynomolgus monkeys by argon laser-induced photocoagulation of the trabecular meshwork. Baseline IOP readings were taken three days before the instillation (one drop, 50 μ l) of 0.5% (○), 1% (●) or 2% solutions (□) of L-662,583 into both eyes. Results are expressed as the difference in mmHg from the corresponding baseline value (33.1 ± 1.5 – 40.1 ± 2.0 mmHg) and each point is the group mean ($n = 16-24$); vertical lines show s.e.mean. * $P < 0.05$, significantly different from the corresponding baseline value.

from human erythrocytes. The inhibition of carbonic anhydrase activity in a homogenate of the iris + ciliary body of the albino rabbit, after the topical administration of an agent, provides an indirect reflection of the concentration of the compound present in the homogenate and the assay is useful from a comparative viewpoint. After a dose of 0.1%, carbonic anhydrase activity at 1 h was suppressed much more after L-662,583 than after acetazolamide or methazolamide; thus, indicating better ocular penetration. L-662,583 appears to be less active than MK-927 in this model, as reflected in the 90% inhibition of carbonic anhydrase activity at 1 h after the instillation of a 0.1% solution of MK-927 (Sugrue *et al.*, 1988). It is important to note that a tissue homogenate is used in this paradigm, and it must be emphasized that the model does not reflect the inhibition of carbonic anhydrase activity under *in vivo* conditions.

Concentrations of L-662,583 in the cornea, aqueous humour and iris + ciliary body of albino rabbits were measured by h.p.l.c. at predetermined times after the instillation of a 2% solution of L-662,583. This dose was chosen because it lowers the intraocular pressure of normotensive, albino rabbits (*vide infra*). The peak concentration of L-662,583 in the iris + ciliary body (i.e. $9.61 \mu\text{g g}^{-1}$) of normal, albino rabbits was present at 1 h. This is equivalent to $25.6 \mu\text{M}$. The concentration of carbonic anhydrase in the rabbit ciliary process has been estimated to be $0.3 \mu\text{M}$ (Ballantine & Maren, 1955) and, if the assumption is made that the concentration of L-662,583 in the iris + ciliary body of the albino rabbit is a valid reflection of the concentration present in the ciliary process, it is apparent that the concentration of the agent is vastly in excess of that of the enzyme. By using the concept of Maren (1967), which permits the determination of the fractional inhibition of the enzyme (i) based on the equation:

$$i = \frac{M}{M + K_i}$$

where M is the molar drug concentration in the tissue, it can be calculated that the enzyme present in the ciliary process of the rabbit would be essentially inhibited 100% by $25.6 \mu\text{M}$ L-662,583. A pharmacological response in the ciliary process would be anticipated when the enzyme is blocked by 99% or more (Maren, 1967). Consonant with this is the observed reduction in the intraocular pressure of normotensive, albino rabbits after the acute instillation of a 2% solution of L-662,583 (*vide infra*).

The persistently elevated intraocular pressure of rabbits pretreated with α -chymotrypsin was very responsive to topically applied L-662,583, with 0.01%, 0.1% and 0.5% solutions significantly lowering intraocular pressure in a dose-dependent manner. The α -chymotrypsin-induced elevation in intraocular pressure in the rabbit is, in all probability, a consequence of impaired aqueous humour outflow (Sears & Sears, 1974). This model is very responsive to topically applied ocular hypotensive agents such as timolol (Vareilles *et al.*, 1977b) and carbonic anhydrase inhibitors such as L-645,151 (Sugrue *et al.*, 1985), aminozolamide (Putnam *et al.*, 1987) and MK-927 (Sugrue *et al.*, 1988). The paradigm is good for screening compounds for ocular hypotensive activity. However, the extrapolation of ocular hypotensive activity in these rabbits to man is unwarranted. This is indicated by the fact that topically administered L-650,719 (6-hydroxybenzo[b]thiophene-2-sulphonamide) manifested ocular hypotensive activity both in this model and in normotensive albino rabbits (unpublished observations) and yet was inactive in man (Werner *et al.*, 1987). The most likely explanation for this disparity is related to differences in ocular penetration. Striking

differences exist between rabbit and man regarding processes that have a negative effect on ocular penetration. For example, man blinks every 5 s in contrast to every 15 min in the rabbit and tear turnover rate is 50% lower in rabbit than in man (Lee & Robinson, 1986). In addition, the corneal surface area is smaller in man than in the rabbit (Watsky *et al.*, 1988). In a study in which methazolamide was applied topically to rabbits and humans scheduled for cataract surgery, appreciably more compound was present at 8 min and 1 h in aqueous humour samples from rabbits than from humans (Edelhauser & Maren, 1988). Notwithstanding its apparent lack of predicting ocular hypotensive activity in man, the ocular hypertensive rabbit is very useful in attempting to uncover the site of action of an ocular hypotensive agent. This is due to the fact that only the intraocular pressure of the right eye of these animals was experimentally elevated. The unilateral instillation of 0.5% L-662,583 into the left eye of these rabbits failed to lower significantly the elevated intraocular pressure of the right eye. In contrast, a similar treatment to the hypertensive, right eye profoundly reduced intraocular pressure. This observation indicates that the ability of L-662,583 to lower intraocular pressure is, in fact, due to a local action and is not the result of the absorption of the agent into the circulation followed by a subsequent redistribution. The magnitude and duration of the ocular hypotensive effect of L-662,583 was appreciably less in normotensive, albino rabbits than in ocular hypertensive rabbits, as reflected in the changes in the intraocular pressure of these animals after a dose of 2%. However, a direct comparison of the two models is invalid because there is no *a priori* reason to assume that the physiological regulation of intraocular pressure in the ocular hypertensive eye mimics that of the normal eye, a topic that has been discussed previously (Langham & Lee, 1957).

The changes to the optic nerve head in cynomolgus monkeys following argon laser-induced ocular hypertension are similar to those in human eyes with glaucoma (Radius & Pederson, 1984; Marx *et al.*, 1988). The intraocular pressure of unilateral, ocular hypertensive, cynomolgus monkeys is decreased by the acute (Lee *et al.*, 1985) and repeated instillation (Lee *et al.*, 1987) of a range of clinically used ocular hypotensive agents. Moreover, the doses shown to be active are the same as those used to treat glaucoma patients. The acute instillation of a 2% solution of L-662,583 elicited a peak reduction of 23% in the intraocular pressure of ocular hypertensive monkeys. The intraocular pressure of ocular hypertensive cynomolgus monkeys was reduced by 37% after 2% MK-927 in a separate study (Wang *et al.*, 1988). First impressions would suggest that MK-927 is more active than L-662,583 in this model. However, this conclusion may be clouded by the fact that the two experiments have been done by separate groups with different monkeys.

In summary, the carbonic anhydrase inhibitor L-662,583 is structurally different from MK-927, a compound that, on topical administration, lowers the intraocular pressure of glaucoma patients. L-662,583 is more potent than MK-927 at inhibiting human carbonic anhydrase II *in vitro*, but its ability to penetrate the rabbit eye may be somewhat less than that of MK-927. Topically administered L-662,583, like MK-927, is an ocular hypotensive agent in a variety of pharmacological models.

Thanks are extended to the following for technical assistance: Mrs D. Alix, Mr P. DePhillips, Mrs M. Dukic, Mrs R. Evans, Ms H. Funk, Ms C.A. Hunt, Mrs C. Klotzbuecher, Mr C. Ludden, Mr S. Michelson, Mrs E. Reiss, Mr J. Sondey and Mrs S. Volksdorf, and to Mrs M. Olkowski for typing the manuscript.

References

- BALLINTINE, E.J. & MAREN, T.H. (1955). Carbonic anhydrase activity and the distribution of Diamox in the rabbit eye. *Am. J. Ophthalmol.*, **40**, 148–154.
- BECKER, B. (1954). Decrease in intraocular pressure in man by a carbonic anhydrase inhibitor, Diamox. *Am. J. Ophthalmol.*, **37**, 13–14.
- CAPRIOLI, J. (1987). The ciliary epithelia and aqueous humor. In *Adler's Physiology of the Eye—Clinical Application*. ed. Moses, R.A. & Hart, W.M. pp. 204–222. St Louis: The C.V. Mosby Company.
- DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. *Biometrics*, **20**, 482–491.
- EDELHAUSER, H.F. & MAREN, T.H. (1988). Permeability of human cornea and sclera to sulfonamide carbonic anhydrase inhibitors. *Arch. Ophthalmol.*, **106**, 1110–1115.
- EPSTEIN, D.L. & GRANT, W.M. (1977). Carbonic anhydrase inhibitor side effects; serum chemical analysis. *Arch. Ophthalmol.*, **95**, 1378–1382.
- HENNEKES, R., PFEIFFER, N., LIPPA, E., GARUS, H., GREHN, F. & JAEGER, A. (1988). MK-927: an active topical carbonic anhydrase inhibitor in patients. *Invest. Ophthalmol. Vis. Sci.*, **29** (suppl.), 82.
- LANGHAM, M.E. & LEE, P.M. (1957). Action of Diamox and ammonium chloride on formation of aqueous humor. *Br. J. Ophthalmol.*, **41**, 65–92.
- LEE, P.-Y., PODOS, S.M., HOWARD-WILLIAMS, J.R., SEVERIN, C.H., ROSE, A.D. & SIEGEL, M.J. (1985). Pharmacological testing in the laser-induced monkey glaucoma model. *Curr. Eye Res.*, **4**, 775–781.
- LEE, P.-Y., PODOS, S.M., SERLE, J.B., CAMRAS, C.B. & SEVERIN, C.H. (1987). Intraocular pressure effects of multiple doses of drugs applied to glaucomatous monkey eyes. *Arch. Ophthalmol.*, **105**, 249–252.
- LEE, V.H.L. & ROBINSON, J.R. (1986). Review: topical ocular drug delivery: recent developments and future challenges. *J. Ocular Pharmacol.*, **2**, 67–108.
- LICHTER, P.R., NEWMAN, L.P., WHEELER, N.C. & BEALL, O.V. (1978). Patient tolerance to carbonic anhydrase inhibitors. *Am. J. Ophthalmol.*, **85**, 495–502.
- LIPPA, E.A., VON DENFFER, H.A., HOFMANN, H.M. & BRUNNER-FERBER, F.L. (1988). Local tolerance and activity of MK-927, a novel topical carbonic anhydrase inhibitor. *Arch. Ophthalmol.*, **106**, 1694–1696.
- MAREN, T.H. (1967). Carbonic anhydrase: chemistry, physiology and inhibition. *Physiol. Rev.*, **47**, 595–781.
- MAREN, T.H. (1987). Carbonic anhydrase: general perspectives and advances in glaucoma research. *Drug. Development Res.*, **10**, 255–276.
- MAREN, T.H. & BAR-ILAN, A. (1988). Ocular pharmacology and hypotensive activity of a topically active carbonic anhydrase (CA) inhibitor, a 4-alkylaminothienothienopyran-2-sulfonamide, MK-927. *Invest. Ophthalmol. Vis. Sci.*, **29** (suppl.), 16.
- MARX, M.S., PODOS, S.M., BODIS-WOLLNER, I., LEE, P.-Y., WANG, R.-F. & SEVERIN, C. (1988). Signs of early damage to glaucomatous monkey eyes: low spacial frequency losses in the pattern ERG and VEP. *Exp. Eye Res.*, **46**, 173–184.
- PONTICELLO, G.S., FREEDMAN, M.B., HABECKER, C.N., LYLE, P.A., SCHWAM, H., VARGA, S.L., CHRISTY, M.E., RANDALL, W.C. & BALDWIN, J.J. (1987). Thienothienopyran-2-sulfonamides: a novel class of water-soluble carbonic anhydrase inhibitors. *J. Med. Chem.*, **30**, 591–597.
- PUTNAM, M.L., SCHOENWALD, R.D., DUFFEL, M.W., BARFKNECHT, C.F., SEGARRA, T.M. & CAMPBELL, D.A. (1987). Ocular disposition of aminozolamide in the rabbit eye. *Invest. Ophthalmol. Vis. Sci.*, **28**, 1373–1382.
- RADIUS, R.L. & PEDERSON, J.E. (1984). Laser-induced primate glaucoma. II. Histopathology. *Arch. Ophthalmol.*, **102**, 1693–1698.
- SEARS, D. & SEARS, M. (1974). Blood-aqueous barrier and alpha-chymotrypsin glaucoma in rabbits. *Am. J. Ophthalmol.*, **77**, 378–383.
- SUGRUE, M.F. (1989). The pharmacology of anti-glaucoma drugs. *Pharmacol. Ther.*, **43**, 91–138.
- SUGRUE, M.F., GAUTHERON, P., GROVE, J., MALLORGA, P., SCHWAM, H., VIADER, M.P., BALDWIN, J.J. & PONTICELLO, G.S. (1988). MK-927: a topically effective ocular hypotensive carbonic anhydrase (CA) inhibitor in rabbits. *Invest. Ophthalmol. Vis. Sci.*, **29** (suppl.), 81.
- SUGRUE, M.F., GAUTHERON, P., MALLORGA, P., GRAHAM, S.L., SCHWAM, H., SHEPARD, K.L. & SMITH, R.L. (1989). L-662,583: a topically effective ocular hypotensive carbonic anhydrase inhibitor in experimental animals. *Br. J. Pharmacol.*, **96**, 18P.
- SUGRUE, M.F., GAUTHERON, P., SCHMITT, C., VIADER, M.P., CONQUET, P., SMITH, R.L., SHARE, N.N. & STONE, C.A. (1985). On the pharmacology of L-645,151: a topically effective ocular hypotensive carbonic anhydrase inhibitor. *J. Pharmacol. Exp. Ther.*, **232**, 534–540.
- VAREILLES, P., CONQUET, P. & LE DOUAREC, J.-C. (1977a). A method for the routine intraocular pressure (IOP) measurement in the rabbit: range of IOP variations in this species. *Exp. Eye Res.*, **24**, 369–375.
- VAREILLES, P., SILVERSTONE, P., PLAZONNET, B., LE DOUAREC, J.-C., SEARS, M.L. & STONE, C.A. (1977b). Comparison of the effects of timolol and other adrenergic agents on intraocular pressure in the rabbit. *Invest. Ophthalmol. Vis. Sci.*, **16**, 967–996.
- WANG, R.-F., SERLE, J.B., PODOS, S.M. & SUGRUE, M.F. (1988). The ocular hypotensive effect of the topical carbonic anhydrase (CA) inhibitor MK-927 in glaucomatous monkeys. *Invest. Ophthalmol. Vis. Sci.*, **29** (suppl.), 16.
- WATSKY, M.A., JABLONSKI, M.M. & EDELHAUSER, H.F. (1988). Comparison of conjunctival and corneal surface areas in rabbit and human. *Curr. Eye Res.*, **7**, 483–486.
- WERNER, E.B., GERBER, D.S. & YODER, Y.J. (1987). Effect of a topical carbonic anhydrase inhibitor, 6-hydroxybenzo[b]thiophene-2-sulfonamide, on intraocular pressure in normotensive subjects. *Can. J. Ophthalmol.*, **22**, 316–319.
- WISTRAND, P.J. & GARG, L.C. (1979). Evidence of a high-activity C type of carbonic anhydrase in human ciliary processes. *Invest. Ophthalmol. Vis. Sci.*, **18**, 802–806.
- WISTRAND, P.J., SCHENHOLM, M. & LONNERHOLM, G. (1986). Carbonic anhydrase isoenzymes CA I and CA II in the human eye. *Invest. Ophthalmol. Vis. Sci.*, **27**, 419–428.

(Received March 23, 1989)

Revised July 3, 1989

Accepted August 29, 1989