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# Preclinical Overview of Brinzolamide

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**Abstract.** The development of topically active carbonic anhydrase inhibitors (CAIs) is a significant recent achievement in glaucoma medical treatment. Brinzolamide, the newest topical CAI, exhibits selectivity, high affinity, and potent inhibitory activity for the carbonic anhydrase type II isozyme (CA-II), which is involved in aqueous humor secretion. These characteristics, along with good ocular bioavailability, make brinzolamide maximally effective in lowering intraocular pressure (IOP) by locally inhibiting CA-II in the ciliary processes and suppressing aqueous humor secretion. Notable among its attributes as a safe and efficacious glaucoma drug is brinzolamide's superior ocular comfort profile because of its optimized suspension formulation at physiologic pH. The degree of tolerability in the eye is considered an important determinant of a patient's willingness to comply with the dosing regimen for a long-term glaucoma medication. Results from the preclinical pharmacologic evaluation of brinzolamide indicated that it acts specifically to inhibit CA without significant other pharmacologic actions that could introduce undesired side effects. Moreover, the typical side effects associated with systemically administered CAIs are expected to occur at a lower incidence or not occur at all with brinzolamide, as its therapeutic dose and low systemic absorption do not produce a problematic level of systemic CA inhibition. Brinzolamide's long tissue half-life in the eye, particularly in the iris–ciliary body, favors a prolonged duration of IOP lowering. This was substantiated in clinical trials, which showed that twice-daily brinzolamide provides as significant an IOP reduction as three-times-daily brinzolamide or dorzolamide in a relatively high percentage of patients. Brinzolamide has been shown by the laser Doppler flowmetry technique to improve blood flow to the optic nerve head in pigmented rabbits after topical administration, without producing an increase of blood $pCO_2$, indicating a potential for a local vasodilatory effect involving the optic nerve head circulation. The mean concentration of brinzolamide found in the retina of pigmented rabbits (0.338 μg equivalents/g) after a single dose of $^{14}$C-brinzolamide is sufficient to inhibit CA-II. These data suggest that topical brinzolamide could improve the blood flow in the optic nerve head in humans should it inhibit carbonic anhydrase in that vascular bed. Brinzolamide is a new topically active CAI that is safe and efficacious for reducing intraocular pressure. It offers the convenience of topical dose administration and greater freedom from side effects related to the inhibition of CA seen with the systemic administration of CAIs. Its formulation has been optimized to provide greater comfort upon instillation, and this can result in a higher compliance rate by the patient. Results of studies in animals show that brinzolamide has promise for increasing blood flow to the optic nerve head; however, this requires further assessment in the clinic. Brinzolamide represents a significant technical achievement and an important addition to the medical treatment of glaucoma as both a primary and an adjunctive drug. (**Surv Ophthalmol 44 [Suppl 2]**:S119–S129, 2000. 

**Key words.** AL-4862 • brinzolamide • carbonic anhydrase inhibition • Dutch-belted pigmented rabbit • glaucoma • intraocular pressure reduction • ocular blood flow • ocular comfort • ocular hypertension • thienothiazine sulfonamide • topical carbonic anhydrase inhibitor

Carbonic anhydrase (CA) was first described 65 years ago as the enzyme in blood that catalyzes the reversible hydration of carbon dioxide involved in the formation of hydrogen ion and bicarbonate anion.[38] In the ciliary processes, bicarbonate anions move with sodium ions across the epithelial cell membrane to create the osmotic gradient that drives aqueous humor secretion. Eight years after the enzyme was discovered, sulfanilamide was reported to inhibit its activity.[29] In 1950 there were reports on the synthesis and activity of heterocyclic sulfonamides as carbonic anhydrase inhibitors (CAIs).[39,42] Acetazolamide was the first of these potent CAIs to be developed pharmaceutically. At that time it was





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suggested that reducing aqueous humor secretion might provide an effective means of lowering intraocular pressure (IOP) to treat glaucoma.[18] Shortly thereafter, other findings prepared the way for the use of the more potent sulfonamide CAIs to inhibit carbonic anhydrase in the ciliary processes and to suppress aqueous secretion.[23,49] Subsequently, the renal effects of acetazolamide were described and it became the first useful diuretic drug of this class.[33] After that, reports began to appear in the ophthalmic literature wherein acetazolamide administered systemically to glaucoma patients was observed to produce a decrease in IOP.[6,8,20] An IOP-lowering effect in both nephrectomized and normal rabbits and the accompanying decrease in the posterior chamber aqueous humor bicarbonate concentration in rabbits and guinea pigs treated with a CAI was also shown, indicating its local site of action.[4,5] Moreover, the reduction of bicarbonate entry into the posterior chamber that accompanied CA inhibition was demonstrated.[24] Sodium transport into the rabbit posterior chamber was shown again recently to be reduced by the CAI methazolamide, as was aqueous flow into the posterior chamber.[31]

Acetazolamide was the first drug of this class to be used to lower IOP by systemic administration. The ophthalmic use of other CAIs, such as methazolamide, ethoxzolamide, and dichlorphenamide, followed shortly thereafter. An impetus for the investigation of then-available CAIs for topical efficacy to lower IOP was the relatively high incidence of side effects and adverse events that resulted from their systemic administration at doses effective for lowering IOP. Fewer than one third of patients were able to tolerate systemic CAIs for longer than 1 month. It was particularly troublesome because it most commonly resulted in discontinuation of therapy and was the symptom complex consisting of malaise, fatigue, weight loss, depression, anorexia, and loss of libido.[17,19] Thus, poor patient tolerability and its effect on compliance have placed these agents in a secondary or tertiary role among drugs to treat glaucoma.

The earliest attempts to reduce IOP by administering then-available CAIs topically were unsuccessful because of insufficient enzyme inhibition and/or ocular bioavailability. Years later, a concerted effort by researchers in the pharmaceutical industry, academic scientists, and ophthalmic investigators demonstrated the feasibility of achieving topical efficacy for lowering IOP.[32] This paved the way for the discovery and development of clinically useful topical CAIs for treatment of glaucoma.[30] Dorzolamide was the first of these drugs to be marketed for ophthalmology.[15] Brinzolamide is the newest topical CAI to be successfully developed and marketed.

In general, sulfonamide CAIs inhibit the enzyme with high specificity and in a noncompetitive and reversible manner. The currently available CAIs show little or no inhibitory activity against other enzymes and do not act on a variety of receptors for neurotransmitters and hormones. CAIs vary according to their affinity (Ki) of binding to a particular CA isozyme, potency ($IC_{50}$) for inhibiting that isozyme, and physicochemical properties, which can influence their tissue distribution and scope of activity. The ocular pharmacology of inhibitors of human CA isozyme type II (CA-II), the cytosolic isozyme in the secretory ciliary epithelial cells, is to suppress aqueous humor secretion and lower the IOP.[50] Brinzolamide and dorzolamide are most potent against CA-II, with less activity against CA-IV, followed by CA-I. CA-IV is the membrane-bound isozyme of CA also found in the ciliary processes, but its contribution to aqueous humor secretion is unclear at this time.[34,35] The $IC_{50}$ values for acetazolamide, ethoxzolamide, dorzolamide, and brinzolamide against CA-IV are all similar and, generally, are an order of magnitude less than those for CA-II. The clinical significance of this relative to efficacy for lowering IOP and producing side effects is not known.

Oral acetazolamide has been demonstrated by aqueous fluorophotometry to decrease aqueous humor flow in normal subjects.[13] Subsequently, it was reported that oral acetazolamide, but not topical timolol maleate, reduces aqueous flow in subjects during sleep, when flow is physiologically decreased compared to the awake state.[37,47] Oral acetazolamide and topical dorzolamide were recently reported to reduce aqueous humor flow in human subjects by 30% and 17%, respectively.[36] There was an additional 16% decrease of flow when acetazolamide was added to dorzolamide. These results are in contrast to those from a more recent report that shows that either oral acetazolamide or topical dorzolamide alone gives maximum reduction of IOP and aqueous humor flow in patients with ocular hypertension or primary open-angle glaucoma.[43] Based on the earlier results and the fact that systemic acidosis can lower the IOP, the speculation was that the systemic acidosis produced by oral acetazolamide, but not topical dorzolamide, contributed to its greater IOP reduction. The relative efficacy of brinzolamide 1% has been compared to that of dorzolamide 2% for suppressing aqueous humor flow in normal volunteers by means of aqueous fluorophotometry. Reductions of daytime flow by brinzolamide and dorzolamide were 19% and 14%, respectively, and of nighttime flow, 16% and 8%, respectively. Thus, these two agents produce comparable reductions in aqueous humor flow when given in their respective clinical concentrations.[22]

The additivity of topical CAI to topical beta-blocker

relative to suppression of aqueous humor flow has been studied. A study in healthy volunteers comparing topical dorzolamide with topical timolol showed that dorzolamide reduced aqueous flow by 18% and timolol by 47%.[48] When either drug was added to the other, the incremental increase in flow suppression was comparable to the effect of the added drug when given alone. Thus, the flow-suppressant effects of timolol and dorzolamide given acutely were found to be additive, and together these two drugs produced a 55% decrease of flow. The effects of dorzolamide and timolol on IOP were consistent with their effects on flow. An effect of CAIs on tonographic outflow facility has not be demonstrated.[25,26,28]

## Physical and Chemical Characteristics of Brinzolamide

Brinzolamide, also known as AL-4862, is the R-(+) enantiomer of 4-ethylamino-3,4-dihydro-2-(3-methoxypropyl)-2H-thieno[3,2-e]-1,2-thiazine-6-sulfonamide, 1,1-dioxide, a thienothiazine sulfonamide with a free amine and a molecular weight of 383.51.[21] Its aqueous solubilities are 0.9% (pH 5) and 0.05% (pH 7.4). It forms a suspension at physiologic pH.[21] Brinzolamide's octanol/water (O/W) distribution coefficient at pH 5 is 0.7, whereas at the physiologic pH, 7.4, it is a relatively high 6.6. Under the same conditions (pH 7.4), the O/W distribution coefficients of dorzolamide and acetazolamide were determined to be 1.72 and 0.23, respectively, whereas their aqueous solubilities were found to be 0.67% and 0.21%, respectively.[21] Thus, brinzolamide is more lipophilic than either dorzolamide or acetazolamide at physiologic pH, where acetazolamide exists in its charged form and dorzolamide is uncharged. Higher lipophilicity favors the ability to move across lipid membrane barriers.[16] Brinzolamide's pKa values are 5.9 (amine) and 8.4 (primary sulfonamide), allowing it to act as an acid or a base (ampholyte) depending upon the pH.[21] The relatively high pKa of the primary sulfonamide is considered to render brinzolamide less reactive with glutathione under normal physiologic conditions, rendering it less likely to undergo a similar reaction with a macromolecular nucleophile, which could produce an allergen *in vivo*.[11,51] At a pH of 7.0 to 7.4, brinzolamide has very low water solubility, which results in its being a suspension at physiologic pH, making it more comfortable in the eye because less drug is in solution.

## General Pharmacology of Brinzolamide

The principal pharmacologic activity of brinzolamide is carbonic anhydrase inhibition. Brinzolamide, its other isomer (antipode) and its desethyl (major) metabolite, did not produce any significant displacement of known ligands in vitro from binding sites related to 34 common physiologic receptors and enzymes that might contribute to side effects or ancillary pharmacology.

Groups of 10 male Sprague-Dawley rats, 150–250 g, were given single doses of brinzolamide, 1, 10, and 30 mg/kg, or 0.9% saline solution, intravenously, and their behavior was observed during a 24-hour period. No neurologic signs related to treatment were observed to occur. In a separate group of four male rats, 30 mg/kg of brinzolamide intravenously (IV), produced protection from maximal electroshock in 50% of the animals, indicating an anticonvulsant effect of brinzolamide at this dose level. When administered orally to CD-1 strain male mice (10 males per group), brinzolamide dosages of 0, 1, 10, and 30 mg/kg, did not produce a neurologic deficit as measured by rotarod motor performance at 30 or 60 minutes after dose administration. Each 30-μL drop of the product delivers approximately 0.3 mg, which, in a person weighing 50 kg, equals 0.006 mg/kg. Thus, 30 mg/kg is a 5,000-fold multiple of this dose.

In anesthetized dogs given artificial respiration (two male and two female dogs weighing 9.1–11.6 kg), receiving IV infusions of 10 mg/kg of brinzolamide or 0.9% saline solution over 15 minutes, there was no significant change in the cardiovascular responses to epinephrine, norepinephrine, acetylcholine, or isoproterenol; moreover, a statistically significantly ($P \leq 0.05$) reduced blood pressure response (3–mm Hg change) to histamine was without biological significance. No biologically significant changes in blood gas $pCO_2$, $pO_2$, or blood pH occurred. No significant changes in mean arterial blood pressure or heart rate were noted in this study, nor did the lead II electrocardiogram waveform change compared with predose recordings (Alcon Research, Ltd., data on file).

The potential for effects of intravenous infusion of brinzolamide at 1 and 10 mg/kg over 15-minute periods on cardiac and circulatory function was investigated in anesthetized dogs, two males and two females weighing 9.0–11.2 kg, receiving artificial respiration. Effects were compared with pretreatment baseline values and to 0.9% saline solution control conditions. Directly measured parameters were evaluated every minute during infusion and at 5-minute intervals for a minimum of 30 minutes after infusion ended. No biologically significant effects on cardiac or circulatory function were observed after administration of saline or brinzolamide at 1 mg/kg. Brinzolamide dosages of 10 mg/kg IV, did produce an increase by more than 20% in the cardiac output, +dP/dT, and contractile force (Alcon Research Ltd., data on file).

Four groups of 10 male CD-1 mice each were given an oral dose of 0.25% methylcellulose vehicle or 1, 10, or 30 mg/kg of brinzolamide to examine the potential effects on gastrointestinal propulsion of a charcoal meal during a 30-minute period after dosing. Brinzolamide dosages of 30 mg/kg, produced a 44% reduction in intestinal charcoal meal progression, but 1 and 10 mg/kg produced 8% and 18% decreases, respectively. The 44% decrease was statistically significant ($P \leq 0.05$), as compared with the vehicle control result (Alcon Research, Ltd., data on file).

Five groups of 10 male Sprague-Dawley rats each, weighing 180–250 g, were hydrated with 25 mL/kg of saline (orally) and injected intravenously with 0.9% saline solution, 0.3, 1, 10, or 3 mg/kg of brinzolamide, or 5 mg/kg of acetazolamide. Four hours after administration of the control or drug treatment, the urine volume, pH, and urinary electrolyte concentrations were analyzed. Brinzolamide at 0.3 mg/kg was without effect on measured parameters. At 1 mg/kg, brinzolamide produced increases in urine volume, pH, $[Na^+]$, and $[K^+]$, but a decrease in $[Cl^-]$. Urine pH and $[K^+]$ increases were statistically significant ($P \leq 0.05$). Brinzolamide at 3 mg/kg produced an increase in urine volume, pH, $[Na^+]$, and $[K^+]$, a decrease in $[Cl^-]$, and an increase in $pO_2$ and $pCO_2$, but not blood pH. These changes were statistically significant ($P \leq 0.05$). Acetazolamide significantly increased urine pH, $[Na^+]$, and $[K^+]$ and decreased $[Cl^-]$. Urine volume was also increased by acetazolamide (Alcon Research, Ltd., data on file).

The potential for an effect of brinzolamide on sleep time induced by pentobarbital sodium was studied in male CD-1 mice. Oral doses of brinzolamide of 1, 10, and 30 mg/kg prolonged barbiturate sleep time by 57%, 15%, and 35%, respectively. This effect was not considered to be biologically significant, as known active agents affect sleep duration to a far greater degree (Alcon Research, Ltd., data on file).

Taken together, the results of these general pharmacologic studies imply a high order of safety and low incidence of side effects for brinzolamide, and this has been borne out in clinical trials in humans.

## Carbonic Anhydrase Inhibition and Binding In Vitro

The $IC_{50}$ values for inhibition of human CA isozymes I, II, and IV were determined by means of a pH stat assay similar to that published previously.[27,40] Compounds tested included brinzolamide, its S-(−) isomer, brinzolamide desethyl metabolite, dorzolamide, dorzolamide desethyl metabolite, and acetazolamide. Enzyme was incubated with the inhibitor for 4 minutes at 5°C and throughout the 4-minute assay, also at 5°C. Binding affinity constants ($K_i$) were determined for brinzolamide, ethoxzolamide, dorzolamide, and acetazolamide for CA-I and CA-II by means of a 5-dimethylaminonaphthalene-1-sulfonamide fluorescence competitive inhibition assay at a temperature of 37°C and pH 7.4.[7,10,45] These $IC_{50}$ and $K_i$ values are given in Table 1.

The R-(+) isomer of brinzolamide was selected as the active ingredient for the product, as it is more potent than the S-(−) isomer. The S-(−) isomer of brinzolamide has 1/35th the affinity of the R-(+) enantiomer for CA-II. Brinzolamide has its highest affinity ($K_i = 0.13$ nM) and inhibitory potency ($IC_{50} = 3.19$ nM) for CA-II, which is responsible for aqueous humor secretion. It has 246 times higher affinity and 428 times greater inhibitory potency for CA-II than for CA-I, which is present in red blood cells (RBCs). The affinity ($K_i$) values for CA-II determined for dorzolamide and acetazolamide were 0.51 nM and 33.8 nM, respectively, giving them less affinity for the enzyme compared to brinzolamide. Thus, the affinity of brinzolamide for CA-II in this assay is approximately fourfold higher than that of dorzolamide and 2.5-fold higher than that of ethoxzolamide. $K_i$ values against CA-IV were not determined; however, the $IC_{50}$ values against CA-IV were comparable for brinzolamide (45.3 nM), ethoxzolamide (22.1 nM), dorzolamide (32.0 nM), and acetazolamide (33.1 nM). Binding constants were determined at physiologic temperature and pH, and are believed to be more representative of the relative inhibitory potencies against carbonic anhydrase in vivo. This is based on the fact that the binding of brinzolamide is mostly hydrophobic, and hydrophobic interactions like these increase with temperature.[12,41]

## Intraocular Pressure Lowering In Vivo

Brinzolamide's IOP effect was studied with use of Dutch-belted pigmented rabbits and cynomolgus monkeys with ocular hypertension induced by argon laser trabeculoplasty.[14] The Dutch-belted rabbit is a strain of pigmented rabbit that, compared to New Zealand albino rabbits, exhibits spontaneous ocular hypertension and a robust IOP response to topical CAIs. The rabbit required higher doses of CAI than the monkey for a given level of IOP decrease. Monkeys were trained to undergo the IOP measurement procedure without sedation or central nervous system anesthesia. Experiments in both models employed the pneumatonometer to measure IOP under local anesthesia only, with proparacaine ophthalmic solution diluted 1:5.

When administered as two drops of 0.025 mL each, brinzolamide ophthalmic suspension lowered

TABLE 1

*Enzyme Activity of Brinzolamide and Other Carbonic Anhydrase Inhibitors*

| Assay | Enzyme | Brinzolamide | Ethoxzolamide | Acetazolamide | Dorzolamide |
|---|---|---|---|---|---|
| Binding | CAI | 32 ± 1 | 3.61 ± 0.49 | 673 ± 82 | 1,240 ± 417 |
| Ki(nM) ± SD | CAII | 0.13 ± 0.03 | 0.33 ± 0.01 | 33.8 ± 4.9 | 0.51 ± 0.09 |
| CA inhibition | CAI | 1,367 ± 82 | 164 ± 8 | 657 ± 82 | 28,032 ± 613 |
| IC50 (nM) ± range | CAII | 3.19 ± 0.3 | 1.10 ± 0.18 | 9.04 ± 0.16 | 3.74 ± 0.02 |
| | CAIV | 45.3 ± 0.3 | 22.1 ± 1.8 | 33.1 ± 13.7 | 32.0 ± 0.7 |

SD = standard deviation.

IOP in Dutch-belted pigmented rabbits in a dose-dependent manner with an onset within 0.5 hour and a peak response by 1–2 hours (Fig. 1). The following dose levels were studied: 0.5% (0.25 mg), 1% (0.5 mg), and 2% (1 mg). Mean baseline IOP ranged from 31.4 to 33 mm Hg for all experimental groups. IOP in the brinzolamide-treated eye was lowered significantly ($P < 0.05$) below that in the untreated contralateral eye at all dose levels studied and at all times throughout the 6-hour experiment. The percent change from baseline IOP (treated eye minus control eye as a percentage of baseline IOP) produced by brinzolamide 0.5% suspension was statistically significant; however, it was significantly less than the change produced by brinzolamide 2%. Peak percentage decreases (average of two experiments) in IOP from baseline were 11.8%, 21.6%, and 24.8% for 0.5%, 1%, and 2% brinzolamide, respectively. The lack of a significant IOP change in the contralateral untreated eyes implies that the amount of brinzolamide absorbed systemically was insufficient to inhibit CA in that eye. The administration of 0.5 mg of brinzolamide intravenously to this same strain of rabbits failed to produce a significant decrease in IOP. Thus, it is concluded that the IOP effect from the topical administration of at least 1 mg of brinzolamide is caused by its local action on carbonic anhydrase and not systemic absorption of the drug.

The influence of formulation viscosity on the topical efficacy of brinzolamide 1% suspension over the range of 1 to 5,000 centipoise was investigated in the rabbit model. Whereas the efficacy of brinzolamide was improved at 200 centipoise compared to 1 centipoise (23.1% versus 16.8% IOP decrease, respectively), only slight and nonsignificant improvement in efficacy was obtained with a further viscosity increase to 5,000 centipoise (23.4% IOP decrease). The viscosity of the developed formulation was selected as being optimal for efficacy and duration of action, ocular comfort, and pharmaceutical elegance (low sedimentation rate and effective redispersion of the particles in suspension). Formulating brinzolamide at pH 7 resulted in a comfortable suspension (Alcon Research, Ltd., data on file).

The laser-treated monkey responded to lower doses of brinzolamide than the pigmented rabbit with regard to IOP-lowering action.[14] Thus, experiments in the laser-treated monkey model typically used the instillation of a single 0.03-mL drop in contrast to the dual 0.025-mL drops used in the rabbit experiments. After twice-daily topical ocular treatment of monkeys with either 0.3 mg or 0.6 mg of brinzolamide, the IOP was significantly reduced from baseline IOP at the 12-hour postdose A.M. and P.M. measurement times (Fig. 2). From mean baseline IOPs of 33.0 mm Hg and 31.7 mm Hg, the peak percentage decreases after the first dose were 30.8% and 26.9% with 1% and 2% brinzolamide suspension, respectively. After the third dose, the decreases were 32.8% and 32.9% for 1% and 2% drug, respectively. Not unexpectedly, no IOP effects were observed in the contralateral untreated monkey eye, which was not laser-treated and normotensive. The onset of IOP lowering by brinzolamide in the laser-treated monkey model was seen by 1 hour after dosing, the earliest time point at which IOP was measured. Peak IOP lowering occurred in the monkey by 3 hours after dosing. The IOP decrease was usually greater at 12 hours after the previous morning dose than it was at 12 hours after the previous evening dose. The normal diurnal IOP curve of the laser-treated monkey model exhibits a higher IOP in the morning compared to the evening. In general, the IOP-lowering efficacy of brinzolamide in the monkey was observed to be greater, and usually occurred at a lower dose, than that observed in the pigmented rabbit. Mean baseline IOP was in the same low- to mid-30–mm Hg range for rabbit and monkey models used in these experiments. Compared to the clinical data, the rabbit appeared to be more predictive of the magnitude of the IOP decrease seen clinically and the monkey predictive of the dose-response relationship in the human.[9,44,46] Physiologic and anatomic differences between species are the probable explanation.

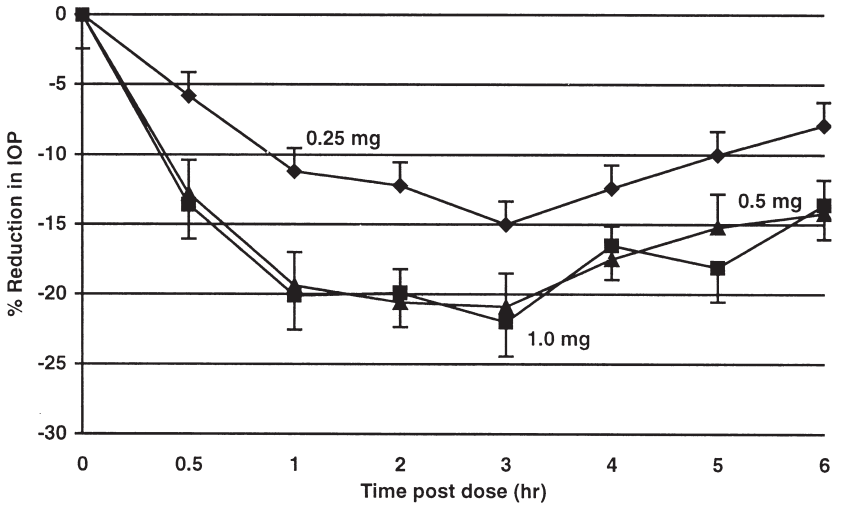


*Fig. 1.* Dose-response and time course for intraocular pressure lowering in Dutch-belted rabbits. Brinzolamide doses studied were 0.25 mg (diamonds), 0.5 mg (triangles), and 1.0 mg (squares), obtained by instilling brinzolamide suspensions containing 0.5%, 1.0%, or 2.0% drug, two drops of 0.025 mL each to one eye. Values are mean ± SEM of change in IOP (treated eye minus control eye) expressed as a percentage of the baseline IOP. All treated eye values were statistically significantly different ($P < 0.05$; Student's *t*-test) from those of the control eyes.

## Ocular Blood Flow

Carbon dioxide is a potent vasodilator involved in the metabolic aspect of autoregulation of blood flow in the eye and elsewhere in the body. The response of the microcirculation to increased $CO_2$ tension is a measure of its autoregulatory capacity and reserve. For this reason, CAIs are used clinically to assess cerebral blood flow reserve, as they conserve $CO_2$ in

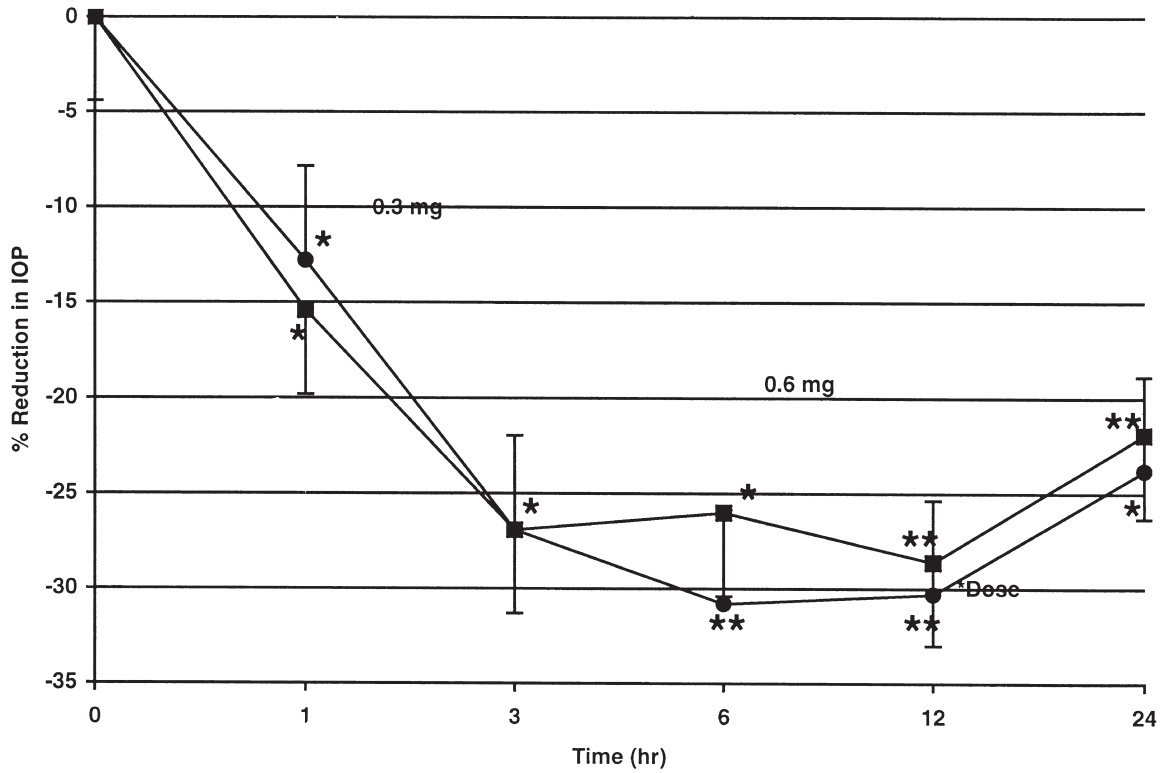


*Fig. 2.* Dose-response and time course for intraocular pressure lowering by brinzolamide in laser-treated glaucomatous cynomolgus monkeys. Brinzolamide doses studied were 0.3 mg (circles) and 0.6 mg (squares) obtained by instilling one drop of 0.03-mL volume of 1.0% or 2.0% brinzolamide suspension to one eye at time 0 and again immediately after the hour 12 IOP measurement. Asterisk indicates $P < 0.01$ and double asterisk, $P < 0.001$, by Student's *t*-test. Values are mean ± SEM.

the tissues, and the $CO_2$ can then act on blood vessels and produce vasodilatation. The autoregulatory capacity of the retinal and optic nerve head vascular beds may be impaired in glaucoma.[1] Improvement of ocular blood flow, particularly flow to the optic nerve head, is expected to be beneficial to those patients with glaucoma who have deficient optic nerve head (ONH) flow. Because brinzolamide is found in the retina (mean, 0.338 μg equivalents per gram) after topical administration to animals, has a long half-life in this tissue, and could possibly cause elevated $CO_2$ there, the response of the ocular blood flow to topical and systemic brinzolamide in animals has been investigated.[3]

Because of the suggestive nature of some preliminary blood flow data in rabbits and cats, a more extensive crossover evaluation of the blood flow response after topical ocular dosing of brinzolamide was carried out. Topical ocular administration of brinzolamide 2% suspension, one drop twice daily, in a 1-week multidose crossover study in nine tranquilized (acepromazine) Dutch-belted rabbits significantly increased blood flow to the optic nerve head as measured by the laser Doppler flowmetry (LDF).[2,3] These results are presented in greater detail elsewhere in this supplement.[2]

## Clinical and Nonclinical Pharmacokinetics and Biodisposition

The preclinical pharmacokinetics and disposition of brinzolamide have been studied in rats, rabbits, and monkeys.[21] The pharmacokinetics of brinzolamide have been characterized after both oral and topical ocular administration to human normal volunteers and glaucoma patients.[21] Topical pharmacokinetic studies were done in normal volunteers not at steady state and in glaucoma patients at steady state. In addition, a multiple-dose oral pharmacokinetic study in normal volunteers was done. Steady state is achieved more rapidly from oral than topical dosing. The metabolism in humans and in vitro plasma protein binding of brinzolamide have been characterized.

## Topical Brinzolamide Ocular Uptake and Distribution in Rabbits

Ocular uptake and distribution studies of $^{14}$C-brinzolamide were performed in New Zealand albino and pigmented Dutch-belted rabbits to study melanin binding. When administered topically as a 1% ophthalmic suspension, brinzolamide is readily absorbed into the conjunctiva, cornea, iris–ciliary body, aqueous humor, choroid, retina, and lens. Maximum concentrations of radioactivity are achieved in anterior segment tissues within 0.5–2 hours. The concentration of brinzolamide in iris–ciliary body of pigmented rabbits ($3.85 \pm 2.33$ μg equivalents per gram or 10 μM) is sufficient to inhibit the enzyme in excess of that needed to suppress aqueous humor formation and to lower IOP. Systemically absorbed drug is substantially associated with erythrocytes and other tissues that contain CA enzyme (kidney, liver, stomach, small intestine, spleen, lungs, and salivary glands). Plasma concentrations are low, as most of the drug concentrates in the RBCs. Radioactivity was eliminated from the aqueous humor and cornea with half-lives of 3 and 5 hours, respectively. Thus, brinzolamide in the cornea prolongs its delivery to the ciliary processes. Consistent with tight binding to carbonic anhydrase, the half-lives in iris–ciliary body, choroid, retina, and lens are substantially longer, being a matter of days (Alcon Research, Ltd., data on file).

Studies in pigmented Dutch-belted rabbits showed a sixfold higher peak iris–ciliary body concentration of drug than was found in albino rabbits. These results are consistent with a moderate degree of melanin binding of brinzolamide. However, the pharmacokinetics of brinzolamide appear to be influenced more by binding to carbonic anhydrase than by binding to melanin, as the terminal half-lives in albino and pigmented rabbits were very similar (Alcon Research, Ltd., data on file).

## Systemic Pharmacokinetics, Absorption, and Bioavailability

Pharmacokinetics, single- and multiple-dose tissue distribution, autoradiography, and excretion/mass balance studies were performed in rats. The metabolism of brinzolamide in vivo was determined in Sprague-Dawley and Fischer-344 rats. Whole-blood and plasma concentrations of brinzolamide and its metabolites were measured in toxicology studies in rats. The lack of chiral inversion to the S-enantiomer was demonstrated in rats, monkeys, and humans (Alcon Research, Ltd., data on file).

Low oral doses of $^{14}$C-brinzolamide administered to rats showed nearly complete absorption (>90%); however, bioavailability decreased from 70% to 45% with increasing dose. Brinzolamide preferentially binds to CA-II and saturates this isozyme in RBCs on repeated dosing, which consequently contributes to the nonlinear nature and lack of dose proportionality observed for brinzolamide pharmacokinetics after oral administration (Alcon Research, Ltd., data on file).

Studies also showed that brinzolamide was absorbed systemically after topical ocular administration and was substantially associated with CA in the erythrocytes.[21] Brinzolamide in the plasma was below

quantitation limits after topical ocular administration to rabbits. Thus, brinzolamide absorbed systemically after topical ocular administration is expected to bind to CA in the RBCs with little free drug remaining in the plasma. Despite this degree of systemic bioavailability, complete saturation of CA in erythrocytes is not achieved. Systemic acidosis or the other side effects associated with oral CAIs are therefore not expected to occur.

Brinzolamide clinical pharmacokinetics are typical of sulfonamide CAIs and are influenced by its tight binding to carbonic anhydrase in tissues, which contain relatively high enzyme concentrations, most notably the erythrocytes (RBCs). Human RBCs contain approximately 150 μM of carbonic anhydrase enzyme, of which about 20 μM is CA-II isozyme. The remainder is the CA-I isozyme.

## Topical Brinzolamide Pharmacokinetic Studies in Human Normal Volunteers

Systemic exposure to brinzolamide was studied in 15 normal male volunteers given bilateral topical ocular doses three times daily for 14 days of brinzolamide suspension 3%, three times the concentration of drug in product. Drug concentrations in plasma and whole blood were measured in samples obtained 2 hours after morning doses on days 8 and 15. On day 8, the mean whole-blood concentration 2 hours after the 8 A.M. dose was 1.42 ± 0.80 μg/mL (range, 0.606–3.35 μg/mL). Two weeks later, the maximal concentration had increased to 2.17 ± 0.85 μg/mL (range, 1.02–4.20 μg/mL). However, brinzolamide concentrations in the blood were not sufficient to saturate CA in RBCs by day 15. Brinzolamide whole-blood concentrations declined during the subsequent 8 weeks with a mean half-life of 111 ± 29 days, reflecting the tight binding of brinzolamide to carbonic anhydrase in the RBCs. Brinzolamide plasma concentrations were below the limit of quantitation at all time points because of the drug's preferential distribution to RBCs.[21]

## Oral Brinzolamide Pharmacokinetic and Metabolism Studies in Human Normal Volunteers

Seven male and seven female normal volunteers were given oral doses of 1-mg brinzolamide capsules twice daily for 32 weeks (or matching placebo capsules in a randomized 4:1 drug:placebo design), and the whole-blood and plasma pharmacokinetics of brinzolamide and its major (*N*-desethyl-brinzolamide) and minor (*O*-desmethyl- and *O*-desmethylpropyl-brinzolamide) metabolites were investigated. This dosage regimen was selected to achieve greater daily systemic exposure to brinzolamide (2 mg) than theoretically could be obtained by administering brinzolamide 1% ophthalmic suspension to both eyes three times daily (1.8 mg). Mean RBC data for brinzolamide demonstrated the rapid saturation of RBC CA isozyme II (approximately 20 μM) within 2–4 weeks. Steady-state brinzolamide concentrations in RBCs (mean, 20–25 μM) were achieved within 12 weeks. After the initial rapid increase in parent drug, the *N*-desethyl metabolite appeared at quantifiable whole-blood levels in all subjects by the sixth week, but was not found in any plasma samples. Two other active metabolites found in animals, *O*-desmethyl-brinzolamide and *N*-desmethoxypropyl-brinzolamide, were not detected in human blood. Evaluation of individual RBC concentration versus time profiles indicated that steady state for both parent drug and metabolite was achieved by week 28. By week 32, mean RBC concentrations of *N*-desethyl metabolite were 7.31 ± 1.82 μM in males and 18.2 ± 7.5 μM in females.Total carbonic anhydrase activity measurements in whole blood show a rapid fall in activity after oral administration, mostly because of decreased CA-II activity as saturation of this isoenzyme in RBCs by parent drug occurs. At the end of the study, mean total CA activity was about 30% of prestudy values, with approximately 4%–5% of residual CA-II activity remaining at steady state. The mean total CA inhibition observed in this study (about 70%) was well below that expected to result in systemic side effects.[21]

## Metabolism of Brinzolamide in Animals and Humans

The metabolism of brinzolamide in rats and monkeys primarily occurs in the liver via oxidative *O*- and *N*-dealkylation.[21] Based on retention in erythrocytes, three metabolites, *N*-desmethoxypropyl-brinzolamide, *O*-desmethyl-brinzolamide, and *N*-desethyl-brinzolamide, appear to bind to CA. These compounds were found in rat urine along with the *N*-propionic acid analog of brinzolamide. The latter also appeared in the bile but not in the blood. No inversion of one isomer to the other has been found for brinzolamide in vivo; therefore, it was assumed that metabolites derived from brinzolamide were the R-isomers and not racemic mixtures. In cynomolgus monkeys, brinzolamide is metabolized to *N*-desethyl-brinzolamide and *O*-desmethyl-brinzolamide. These metabolites, along with brinzolamide, are found in monkey whole blood after topical ocular dosing with brinzolamide ophthalmic suspension. *N*-desmethoxypropyl-brinzolamide was not detected in monkey whole blood.

Clinical studies have shown that brinzolamide and *N*-desethyl-brinzolamide are at substantial concentrations in human whole blood after topical and oral dosing. The *N*-desethyl metabolite was not detected

in plasma. In normal subjects treated orally with brinzolamide capsules twice daily for 32 weeks, CA-II saturation occurred within 2 to 4 weeks, reflecting the much higher systemic input rate from oral dosing compared to topical ocular dosing. The *N*-desethyl metabolite accumulated to steady-state levels ranging from approximately 5–25 μM in erythrocytes. The *N*-desmethoxypropyl and *O*-desmethyl metabolites were not detected in human whole blood but were present in urine as minor metabolites. Plasma concentrations of brinzolamide were generally below the quantitation limit (<7.5 ng/mL [Alcon Research, Ltd., data on file]).

In glaucoma patients, concentrations of parent drug approached erythrocyte CA-II saturation (20–25 μM in erythrocytes) typically by 6–9 months. The *N*-desethyl metabolite was also detected in some subjects at concentrations lower than those of the parent drug (Alcon Research, Ltd., data on file).

## Tissue Distribution of Brinzolamide

The tissue distribution of $^{14}$C-brinzolamide in rats was typical for this drug class, showing prolonged retention in tissues containing CA. Tissue concentrations of radioactivity were greater than those for plasma. After oral administration of 1 mg/kg of $^{14}$C-brinzolamide to rats, the highest radioactivity concentrations ($C_{max}$ values) were observed in tissues with high levels of CA, such as the liver, kidneys, spleen, stomach, small intestine, lungs, and salivary glands. Radioactivity levels in fat, skeletal muscle, skin, eyes, and testes were low. Concentrations of radioactivity in the tissues declined slowly for both dosing regimens, with half-lives ranging up to about 33 days. The half-lives were generally similar to those of blood (approximately 20 days). Because of its high affinity for CA-II, brinzolamide is extensively distributed into erythrocytes and exhibits a long half-life in whole blood. Binding to erythrocyte CA is saturable; therefore, in contrast to plasma pharmacokinetics, whole-blood pharmacokinetics of brinzolamide are nonlinear and drug concentrations are not dose proportional. Brinzolamide is moderately bound to plasma proteins.[21]

Results of whole-body autoradiography in male Fischer-344 rats after a single dose of $^{14}$C-brinzolamide (1 mg/kg) were in good agreement with results of the tissue excision study. These results also showed that low levels of radioactivity crossed the blood-brain barrier and were present in brain, presumably associated with CA (Alcon Research, Ltd., data on file).

## Plasma Protein Binding

An in vitro protein-binding study in human plasma showed binding in the range of 58.5% to 62.7%, which was independent of brinzolamide concentration over the range of 0.01 to 10 μg/g.[21] In rat and monkey plasma, the binding of $^{14}$C-brinzolamide was higher at the 0.01-μg/g level, but was concentration independent from 0.1 to 10 μg/g (rat, 74.8–79.9%; monkey, 23.8–28.5%). Because of its moderate level of protein binding, brinzolamide is not likely to interact with other drugs that bind to plasma protein.

## Elimination of Brinzolamide

After intravenous administration of 1 mg/kg of $^{14}$C-brinzolamide to male rats, 32% and 29% of the radioactivity was recovered in the urine and feces, respectively, over 14 days. The remaining radioactivity was in the carcass, presumably bound to carbonic anhydrase in blood and other tissues. In human urine, brinzolamide is eliminated principally as the unchanged drug; however, *N*-desethyl-brinzolamide also undergoes excretion.[21]

## Enterohepatic Circulation

Enterohepatic circulation was evident in the rat after the oral administration of $^{14}$C-brinzolamide. Twenty percent of a 1-mg/kg dose to bile duct–cannulated male Fischer-344 rats was excreted in the bile. Although more than 20% of this biliary radioactivity was absorbed, this amount contributed only about 1% to the total concentration of radioactivity in the blood 96 hours after administration. Thus, the contribution of enterohepatic circulation of $^{14}$C-brinzolamide–derived radioactivity to the whole-blood concentration of radioactivity in the rat was negligible (Alcon Research, Ltd., data on file).

## Excretion in Milk

Low concentrations of radioactivity were excreted in rat milk. Lactating Fischer-344 rats were given oral doses of 1 mg/kg of $^{14}$C-brinzolamide, and the concentrations in milk were substantially less than those in plasma at each collection time. The mean milk to plasma concentration ratios ranged up to 0.6 at 24 hours after administration, indicating limited transfer of radioactivity from plasma to milk (Alcon Research, Ltd., data on file).

## Fetal Absorption

Radioactivity crossed the placenta of the fetus in pregnant female rats given single oral doses of $^{14}$C-brinzolamide (1 mg/kg). The transfer of radioactivity from the dam to the fetus was greater for animals treated during the period of organ development than for animals treated when organ development was nearly complete. Concentrations of radioactivity in maternal blood were much higher than those in maternal tissues. Peak levels of radioactivity in fetal

tissues were attained 24 hours after administration. At 24 hours after administration, the fetal blood retained some radioactivity. Maternal tissues with the highest concentrations of radioactivity were those containing high levels of CA: liver, kidneys, lungs, mammary glands, and placenta (Alcon Research, Ltd., data on file).

## Conclusions

Brinzolamide is a new specific CAI of the thienothiazine sulfonamide class that exhibits selectivity, high affinity, and potent inhibitory activity for CA isozyme type II and exhibits IOP-lowering efficacy. It is comfortable on instillation. It is readily absorbed into the eye and has relatively long half-lives (days) in iris–ciliary body, choroid, retina, lens, and blood. Whereas whole-blood concentrations of brinzolamide are present after topical ocular administration, indicating systemic absorption, plasma levels of parent drug and metabolites are very low, and complete saturation of CA in erythrocytes is not achieved at steady state. Thus, systemic acidosis or the other side effects associated with oral CAIs are not expected to occur.

## Methods of Literature Search

References cited in this article were identified from a computer-based MEDLINE literature search covering the period of January 1966 to August 1999, reference lists of review articles, and ARVO abstracts (1983–1999). Key words employed were *topical carbonic anhydrase inhibitors*, *carbonic anhydrase inhibition*, *cornea*, *glutathione*, *sulfonamides*, *intraocular pressure*, *brinzolamide*, *dorzolamide*, and *aminozolamide*. No non-English papers are cited.

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