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# Comprehensive Ocular and Systemic Pharmacokinetics of Brinzolamide in Rabbits After Intracameral, Topical, and Intravenous Administration

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ABSTRACT

Brinzolamide is a topical carbonic anhydrase inhibitor which reduces the production of aqueous humor in the ciliary body, thereby reducing intra-ocular pressure. It is formulated as an ophthalmic suspension. The pharmacokinetics of ocular suspensions is not well understood. The objective of this study was to characterize the pharmacokinetics of brinzolamide in rabbit aqueous humor, iris-ciliary body, plasma, and whole blood. New Zealand White rabbits were dosed via intracameral, topical and intravenous administration. After intracameral administration (4.5 μg) of solubilized brinzolamide, aqueous humor concentrations were described with a two-compartment model, the estimated clearance was 4.12 μL/min, apparent volume of distribution at steady-state 673 μL, and terminal half-life 3.4 h. After topical administration of 1% brinzolamide suspension (500 μg), absolute bioavailability based on aqueous humor $AUC_{0-\infty}$ was 0.10%. After intravenous administration of brinzolamide solution (0.75 mg/kg) elimination half-life in plasma and whole blood appeared to be over two weeks. The ratios of the measured concentrations of irisciliary body to whole blood, to plasma, and to aqueous humor concentrations enabled direct comparisons, and helped identify the significant contribution of the conjunctival-scleral pathways of absorption to the ciliary body. This study shows for the first-time the absolute bioavailability in aqueous humor and provides comprehensive pharmacokinetic parameters following administration of a topical suspension.



## Introduction

Vision loss in many forms of glaucoma is related to elevated intraocular pressure (IOP) with further injury to the optic nerve. IOP is determined by the dynamic equilibrium between the production (inflow) of aqueous humor in the ciliary body and its outflow, through the trabecular meshwork and Schlemm's canal in humans, and through intrascleral venous plexus in rabbit.[1,2] Aqueous humor production is reduced by both topical and systemic carbonic anhydrase inhibitors (CAIs).[3] Topically effective CAIs have been developed in the past two decades.[4,5] In 1998, a topical CAI, brinzolamide (AZOPT® Alcon Laboratories, Inc, Ft. Worth, Texas, USA), was approved for clinical use in the USA.[6] AZOPT® is 1% brinzolamide ophthalmic suspension which is formulated at a pH equivalent to that of human tears and thereby designed to be more comfortable to enhance patient compliance.

*Abbreviations:* AIC, Akaike information criterion; AH, aqueous humor; AUC, area under the curve; CAI, carbonic anhydrase inhibitor; CL, clearance; $C_{max}$, maximum drug concentration; CV, coefficient of variation; ICB, iris-ciliary body; IOP, intraocular pressure; LLOQ, Lower Limit of Quantitation; $LogD_{7.4}$, the logarithm of the octanol-water distribution coefficient at pH 7.4; NCA, non-compartmental analysis; PBS, phosphate-buffered saline; PEG-400, polyethylene glycol 400; PD, pharmacodynamic; PK, pharmacokinetic; PSA, polar surface area; PVDF, polyvinylidene fluoride; QC, quality control; RED, rapid equilibrium dialysis; $t_{1/2}$, half-life; Vss, apparent volume of distribution at steady state; WB, whole blood.

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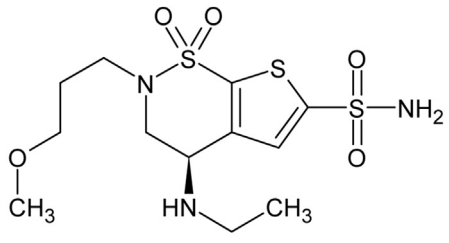
0022-3549/

The chemical structure, and some physicochemical characteristics of brinzolamide are presented in Fig. 1. Brinzolamide's $pK_a$ values allows it to act as an acid or a base depending on the pH. At a pH of 7.0–7.4, brinzolamide has low water solubility (~0.4 mg/mL), which results in it being a suspension at neutral pH when formulated at 1% (10 mg/mL). Brinzolamide's experimental LogD (the logarithm of the octanol-water distribution coefficient) at pH 7.4 is 0.82[7]. Brinzolamide has fairly high polar area surface (PSA) of 163.8,[8] and its apparent permeability coefficient through porcine cornea and conjunctiva have been reported to be $1.36 \times 10^{-7}$ cm/s and $1.36 \times 10^{-6}$ cm/s, respectively.[9,10] These values are slightly lower than the respective ones for atenolol ($1.72 \times 10^{-7}$ cm/s and $1.51 \times 10^{-6}$ cm/s),[9,10] a hydrophilic beta-blocker, indicating that brinzolamide has a fairly low permeability across biological membranes. Rabbit corneal and conjunctival permeability studies have also been performed with reported values of $0.91 \times 10^{-6}$ cm/s and $5.15 \times 10^{-6}$ cm/s respectively.[11]

To achieve pharmacological effects, almost complete inhibition of CA-II, the cytosolic isozyme in the secretory ciliary epithelial cells is required. After ocular instillation, brinzolamide must have access to the anterior and posterior chambers of the eye and accumulate in the ciliary body at adequate concentrations. Brinzolamide's IOP lowering effect has been studied in rabbits, dogs, cats, horses, and primates.[12–15] After administration topically as a 1% ophthalmic suspension, absorption into the conjunctiva, cornea, iris–ciliary body, aqueous humor, choroid, retina, and lens has been described.[16]

Delivery of ocular drugs to the intraocular tissues of the eye after topical dosing requires drug penetration through the anterior structural barriers of the cornea, conjunctiva, and sclera. Penetration across the cornea is proposed as the primary pathway by which lipophilic drugs reach aqueous humor after topical ocular administration.[17,18] The conjunctiva-sclera route of drug penetration contributes very little to drug concentrations in aqueous humor[19] but it may be important for drug access to the ciliary body.[20,21]

While topical studies of $^{14}$C-brinzolamide have been cited[16] in rabbits, there is no published data on the intraocular clearance and ocular bioavailability of the topical suspension product (AZOPT®). In addition, there is no information about ocular bioavailability of any topical ocular suspension and the extent of suspension particle dissolution in the tear fluid is still unclear. Topical application of brinzolamide suspension, combined with intracameral and intravenous dosing of brinzolamide solution, followed by sampling of aqueous humor and iris-ciliary body compartments could provide information on the pharmacokinetic (PK) parameters of the topical drug product, and help elucidate the ocular bioavailability and pathways for the ocular absorption, distribution, and elimination of brinzolamide. The potential absorption and elimination pathways following topical and intracameral administrations are highlighted in Fig. 2.



| | |
|---|---|
| Molecular weight | 383.51 |
| pKa | 6.78 (base) and 9.62 (acid) |
| $LogD_{7.4}$ | 0.82 |
| PSA | 163.8 |

**Fig. 1.** Brinzolamide structure and properties (ACDlabs® software, version 12; Advanced Chemistry Development, Inc, Toronto, Canada and [7]).

In this study the detailed ocular pharmacokinetics of brinzolamide in aqueous humor, iris-ciliary body, plasma, and whole blood was investigated following topical, intracameral, and intravenous administration. The data was explored by compartmental and non-compartmental analyses (NCA). To our knowledge this is the first published study that provides the absolute aqueous humor bioavailability for any ophthalmic suspension. The results also report comprehensive pharmacokinetic data of brinzolamide in rabbit ocular tissues, whole blood and plasma. The data from this study is expected to pave the way for an improved understanding of the formulation characteristics of brinzolamide and support the development of pharmacokinetic and pharmacodynamic (PD) models that could be used for human PK and PD predictions.

## Materials and Methods

### *In Vivo Animal Experiments*

Female New Zealand White rabbits weighing between 3 and 5.5 kg (*source: Western Oregon Research*) were used on the studies. Prior to the study, the animals underwent an ophthalmic examination (slit-lamp biomicroscopy and indirect ophthalmoscopy) and only those animals with zero ocular findings according to a modified McDonald-Shadduck Scoring System were included in the study. Treatment of the animals were in accordance with Standard Operating Procedures and the conditions specified in the Guide for Care and Use of Laboratory Animals (ILAR publication, 2011, National Academy Press). The protocol and any amendments or procedures involving the care or use of animals in this study were reviewed and approved by Institutional Animal Care and Use Committee prior to the start of the study. All study animals were acclimated to their designated housing for at least 5 days prior to test article administration.

### *Topical Administration*

AZOPT® Brinzolamide ophthalmic suspension (1%) was administered into both eyes of 16 rabbits (n = 2/time point) via a single topical ophthalmic administration at a volume of 50 μL (equivalent to 500 μg of brinzolamide dose/eye). At eight designated time points (15, 30 min, 1, 2, 4, 8, 12- and 24-h post dose) the animals were euthanized by an intravenous injection of a commercial barbiturate-based euthanasia solution for tissue collection. Aqueous humor sample were collected using paracentesis and iris-ciliary body was subsequently dissected. The samples were kept separate without pooling and the volume of aqueous humor and weight of iris-ciliary body samples were recorded. Samples were flash frozen in liquid nitrogen and placed on dry ice until storage in a freezer set to maintain −60 to −80 °C.

### *Intracameral Administration*

A saturated solution of brinzolamide was prepared in pH 7.4 phosphate-buffered saline (PBS) by transferring ~5 mg amount of brinzolamide into a vial and adding ~25 mL of pH 7.4 PBS. The vial was repeatedly swirled and shaken vigorously over the course of up to 30 min to ensure completed dissolution. It was filtered through a 0.22 μM polyvinylidene fluoride (PVDF) filter via syringe filtration. The dosing solution following filtration was measured by LC-MS/MS analysis and dose concentration was determined to be 0.18 mg/mL.

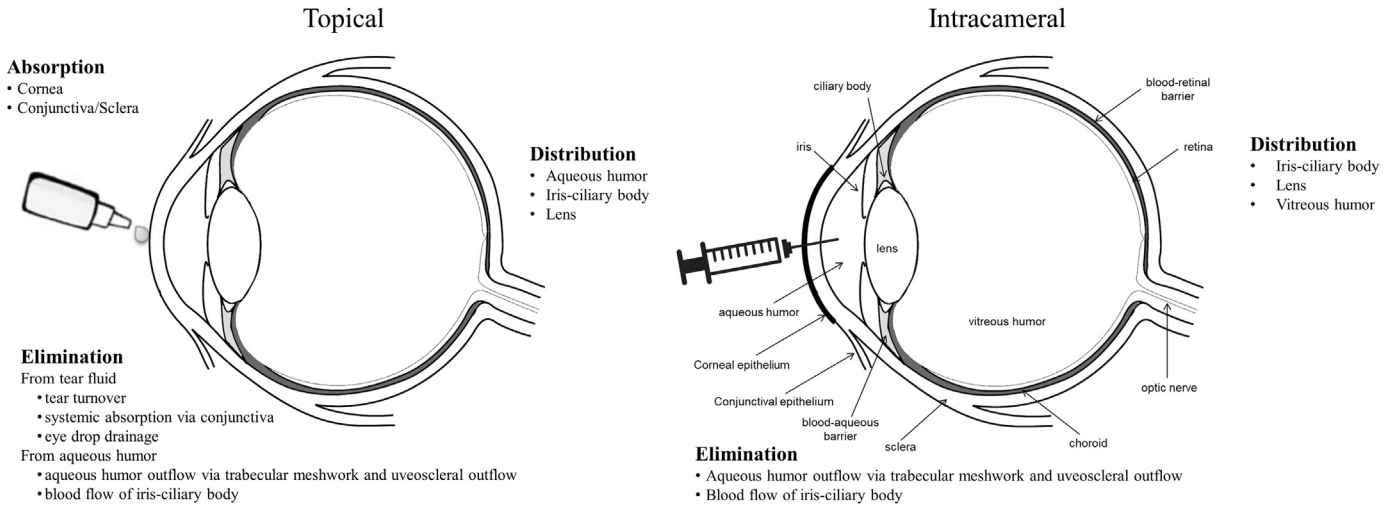



**Fig. 2.** Ocular drug disposition following topical and intracameral administration.

Animals were anesthetized with intramuscular injections of ketamine hydrochloride (25 mg/kg) and xylazine (5 mg/kg). After surgical preparation of eyes, one to two drops of topical proparacaine hydrochloride anesthetic (0.5%) and betadine (5%) was applied to the eyes of the animals. A volume of 25 μL of the test formulation (4.5 μg of brinzolamide per eye) was injected into the anterior chamber of both eyes of 9 rabbits (n = 2 eyes/timepoint). The volume of aqueous humor in a rabbit eye is 250–300 μL[22] which in turn is very comparable to the volume of aqueous humor in the human eye which is 261–310 μL.[23] The 25 μL injection volume represents 10% of this volume and is the standard volume for intracameral injection routinely used in rabbit studies. The animals were euthanized, and tissues collected from both eyes separately as described above at the time points 5, 15, and 30 min and 1, 2, 4, 6, 8, and 24 h post dose.

*Intravenous Administration*

A 2 mg/mL brinzolamide solution was prepared in 15% ethanol, 50% polyethylene glycol 400 (PEG-400), and 35% saline. The resulting solution was filtered through a 0.22 μm PVDF filter via syringe filtration. Test formulations (0.375 mL/kg, 0.75 mg/kg) were injected into the ear veins of 3 rabbits and blood samples were collected serially at time points of 5, 15, and 30 min and 1, 2, 4, 6, 8, 12, 24, 32, 48, 56, 72, 168, 240, and 336 h post dose. Aqueous humor and iris-ciliary body were dissected after euthanasia of the animals at 336 h following the procedures described above. The blood samples were collected into separate pre-chilled tubes with and without $K_2$EDTA as the anticoagulant. The sample without anti-coagulant was stored as whole blood while the blood sample in $K_2$EDTA was centrifuged to generate plasma. Blood and plasma samples were snap frozen on dry ice and stored frozen at −60 to −80 °C until analysis.

*In Vitro Plasma Protein Binding*

The fractional binding of brinzolamide to plasma proteins was measured in the incurred rabbit plasma samples using rapid equilibrium dialysis (RED). Plasma samples from the early time points of the intravenous study (5, 15, 30, and 60-min samples) were pooled for each rabbit in this experiment. Equilibrium dialysis of the rabbit plasma samples was performed against phosphate buffered saline using the RED plate. The RED base plate was placed on a thermomixer which was pre-warmed to 37 °C. Chambers reached equilibrium while shaking at 300 rpm for 4 h after which aliquots were removed from the sample chamber (donor) and from the buffer chamber (receiver) for analysis. The samples were diluted to achieve the same analytical matrix and analyzed using LC-MS/MS without analytical standards. The percent binding to plasma proteins was calculated as follows:

[(Average area response in donor samples − Average area response in receiver samples)/ (Average area response in donor samples)] x 100%

*Bioanalytical Method Development*

LC-MS/MS methods for the determination of brinzolamide in New Zealand White rabbit aqueous humor, iris-ciliary body, whole blood, and plasma were developed and qualified. Analytical stock solutions (1.00 mg/mL of the free drug) were prepared in DMSO. Working solutions were prepared in 50:50 acetonitrile: water (v: v) and then added to blank matrix to make calibration standards to final concentrations of 1000, 500, 250, 100, 50, 25, 10 and 5 ng/mL and quality control samples to final concentrations 500, 100, and 15 ng/mL. Standards and quality control (QC) samples were prepared in New Zealand White rabbit homogenized iris-ciliary body, aqueous humor, whole blood, or plasma matrices. All operations were performed under yellow light due to the potential light sensitivity of the analyte. The incurred samples were extracted via methanol precipitation and stored frozen until analysis. Iris-ciliary body samples were homogenized with collagenase buffer (5 mg/mL collagenase in PBS buffer with 0.5 mM $CaCl_2$) prior to extraction. The specificity, accuracy, and precision of the developed analytical methods was evaluated via a single-run qualification. At least 75% of standards and QC's were expected to have accuracy within ±15%, except at the lower limit of quantitation (LLOQ) where ±20% was considered acceptable. For iris-ciliary body, accuracy and precision was determined at three levels, while the accuracy and precision of QCs in the individual matrices of aqueous humor, whole blood and plasma was determined at the low and high QC levels.

*Pharmacokinetic Analysis*

Compartmental analysis of the naïve pooled data in aqueous humor following intracameral administration was performed using Phoenix WinNonlin® 8.1. software (Certara L.P.). Initial exploratory analysis of the mean concentration data was performed to visualize the observed concentration versus time profiles and to identify if

mono-exponential or bi-exponential profiles were obtained. Different weighting schemes such as uniform, 1/concentration predicted (1/Yhat), and 1/(concentration predicted)$^2$ (1/Yhat$^2$) were used for curve fitting. Residual plots, % coefficient of variation (CV%, an estimate of the precision of the estimated parameter), and standard errors were compared between the three weighting schemes. Objective function values and Akaike information criterion (AIC) were also used to compare compartment models with the same weighting. Intracameral clearance (CL), apparent volume of distribution at steady state ($V_{ss}$), and terminal half-life ($t_{1/2}$) were obtained for aqueous humor kinetics.

NCA of the average concentrations of aqueous humor and iris-ciliary body data after topical and intracameral administration using the linear up log down calculation method was then performed to obtain the PK parameters: peak concentration ($C_{max}$), time to peak concentration ($t_{max}$), terminal half-life ($t_{1/2}$) and area under the curve to the last measured concentration ($AUC_{0\text{-last}}$) and extrapolated to infinity ($AUC_{0\text{-}\infty}$). The absolute aqueous humor bioavailability was calculated with Equation (1):

$$\text{Bioavailability } (\%) = 100 \times \frac{(Topical\ AUC_{0-\infty} \times Intracameral\ dose)}{(Intracameral\ AUC_{0-\infty} \times Topical\ dose)} \quad (1)$$

Additionally, the concentration ratio between iris-ciliary body and aqueous humor after intracameral and topical administration was calculated. For the intravenous dose group for whole blood and plasma compartments, the measured concentrations for iris-ciliary body and aqueous humor at the terminal sampling time point (336 h) were used to calculate mean iris-ciliary body/aqueous humor, iris-ciliary body/whole blood, and iris-ciliary body/plasma concentration ratios.

## Results

### *Intracameral and Topical Pharmacokinetics*

After intracameral administration of fully solubilized brinzolamide (4.5 μg/eye), aqueous humor concentration curve was well described with a two-compartment model and 1/Yhat$^2$ weighting (Fig. 3a). The estimated clearance after intracameral injection was 4.12 μL/min (CV % = 4.16), apparent volume of distribution at steady-state 673 μL (CV % = 6.48), and terminal half-life 3.4 h (CV% = 4.89). Brinzolamide concentration in iris-ciliary body declined only slowly after 8 h, and the concentration ratio iris-ciliary body/aqueous humor was 7.5 at the last sampling time (24 h), but less than 1.0 at earlier time points (Fig. 3a). PK parameters obtained with NCA are shown in Table 1.

After topical administration of 1% brinzolamide suspension (500 μg/eye), brinzolamide concentration in the iris-ciliary body was higher than its concentration in the aqueous humor at all time points (Fig. 3b). PK parameters are shown in Table 1. Absolute bioavailability of brinzolamide in aqueous humor was 0.10% (calculated based on the dose-normalized $AUC_{0\text{-}\infty}$ in aqueous humor after topical and intracameral administration reported in Table 1 and according to Equation (1)).

### *Intravenous Pharmacokinetics*

After intravenous administration of brinzolamide solution (0.75 mg/kg or 2250 μg/3 kg rabbit) with two-week sampling period, elimination half-life in plasma and whole blood appeared to be higher than two weeks (Fig. 4). Therefore, the PK parameters could not be estimated. Brinzolamide concentration in the whole blood was much higher than in plasma indicating drug accumulation into red blood cells. After 8 h, whole blood/plasma concentration ratio remained constant.

At the terminal time point of 14 days the concentrations of iris-ciliary body and aqueous humor were measured. The aqueous humor sample was below the limit of quantitation, but iris-ciliary body concentrations were reported for all three rabbits as 87.5, 84.6 and 64.1 ng/g respectively (Fig. 4).

The protein binding of brinzolamide in plasma based on measuring the pooled samples from each of the three intravenously administered animals was determined to be 47%–57%.

### *Brinzolamide Concentrations in Iris-Ciliary Body*

The concentration of brinzolamide in aqueous humor, iris-ciliary body, whole blood and plasma at the last sampling points

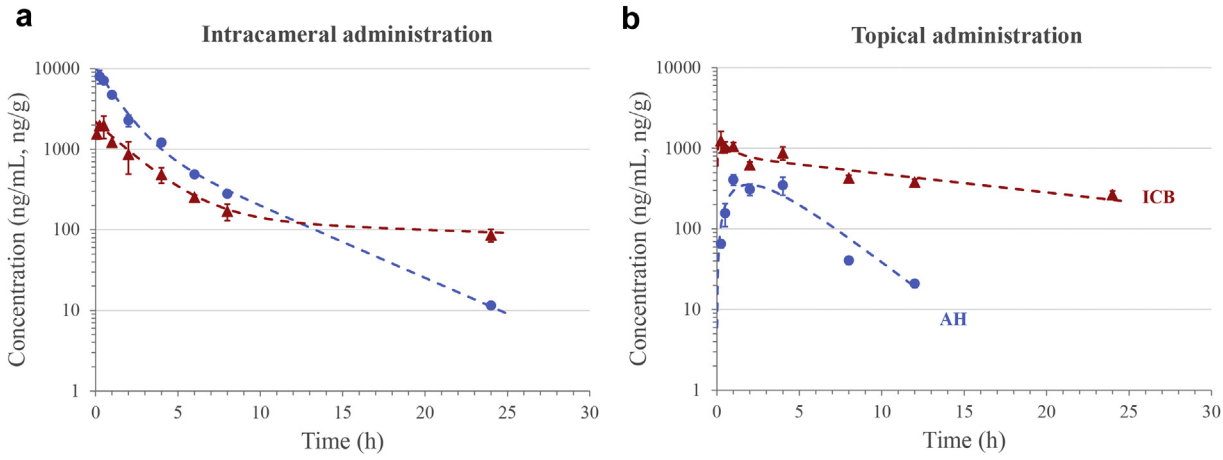



**Fig. 3.** Brinzolamide concentrations in aqueous humor (circles) and iris-ciliary body (triangles) following (a) intracameral solution (4.5 μg dose) and (b) topical suspension (500 μg dose) administrations. Each symbol represents the observed concentration ± standard error of the mean (n = 2 for intracameral administration and n = 4–6 for topical administration) while the dashed line represents the predicted profile.

**Table 1**
PK Parameters After Intracameral and Topical Administration with NCA.

| PK Parameters | Intracameral Administration (4.5 μg) | | Topical Administration (500 μg) | |
|---|---|---|---|---|
| | Aqueous Humor | Iris-Ciliary Body | Aqueous Humor | Iris-Ciliary Body |
| $C_{max}$ (ng/mL, ng/g) | 11,050 | 1964 | 408 | 1245 |
| $t_{max}$ (h) | 0.08 | 0.5 | 1 | 0.25 |
| Terminal $t_{1/2}$ (h) | 3.4 | 13 | 2 | 13.6 |
| $AUC_{0-24h}$ (h*ng/mL, h*ng/g) | 17,780 | 7225 | 1896[a] | 11,414 |
| $AUC_{0-\infty}$ (h*ng/mL, h*ng/g) | 17,836 | 8839 | 1955 | 16,628 |
| Dose-normalized $AUC_{0-\infty}$ (h*/mL, h*/g) | 4 | 2 | 0.004 | 0.03 |

[a] $AUC_{0-12h}$.

after the different administrations and the corresponding ratios with respect to the iris-ciliary body are reported in Table 2.

The iris-ciliary body/aqueous humor ratio at 12 h after topical administration was much higher (18.1) than the ratio obtained following intracameral administration at 24 h (7.47). The ratio obtained from the extrapolated topical 24-h data was even higher and estimated to be > 53.

Ratio obtained following intravenous administration was also high and estimated to be > 31. This ratio is based on comparing the concentrations in iris-ciliary body and aqueous humor at the terminal time point following IV administration. Since the aqueous humor concentrations at the last time point (336 h) were below the limit of quantitation, the LOQ (2.5 ng/mL) was used to determine the ratio as being >31.

## Discussion

In this study, the comprehensive ocular pharmacokinetics of brinzolamide in rabbit aqueous humor, iris-ciliary body, plasma, and whole blood was investigated following topical, intracameral, and intravenous administration. Compartmental analysis and NCA enabled the determination of critical pharmacokinetic parameters and the absolute bioavailability in aqueous humor for brinzolamide. The absolute bioavailability in aqueous humor of the topical brinzolamide suspension was determined to be 0.10% and is the first time this has been reported for any topical ophthalmic suspension in the literature.

Topical ocular drug delivery is an ideal route for treatment of ocular diseases due to the ease of administration. However, there are many challenges for ocular drug delivery due to barriers, such as tear turnover, nasolacrimal solution drainage, corneal epithelium, and systemic absorption. While there are many reports on the pharmacokinetics of topical eye drops, information on absolute drug bioavailability in the aqueous humor is sparse. Recently the aqueous humor bioavailability of topical ophthalmic solutions of atenolol, timolol and betaxolol has been reported with values from 0.07 to 4.31%[24] representing ã 60-fold, range of bioavailability.

Unlike these topical solutions however, AZOPT® (brinzolamide ophthalmic suspension, 1%) is a complex ophthalmic suspension comprised of drug crystals suspended in a crosslinked polymer Carbomer 974P.[6] Due to the partial solubility of brinzolamide in water, there is a dissolved drug fraction in the continuous phase. Formulating brinzolamide suspension with Carbomer 974P appears to prolong the retention of dissolved brinzolamide and suspended particles on the ocular surface thereby increasing ocular drug absorption. It is known that increasing eye drop viscosity increases ocular drug absorption several fold.[25]

However, after application in the viscous vehicle, the bioavailability of brinzolamide in the aqueous humor was only 0.10%, which is 15−43 times less than the bioavailability of timolol and betaxolol after instillation in simple non-viscous buffer solutions.[24] Porcine corneal permeability values of atenolol ($1.72 \pm 0.87 \times 10^{-7}$ cm/s) and brinzolamide ($1.36 \pm 0.35 \times 10^{-7}$ cm/s) are similar[9,10] and ocular bioavailability of these two drugs were in the same range (0.10% vs. 0.07%). Atenolol formulation is a simple solution, whereas brinzolamide is applied as a viscous suspension. It is possible therefore that only a fraction of the suspended brinzolamide dissolves in the tear fluid and partitions into ocular tissues before its removal from the ocular surface.

The concentration of brinzolamide in the aqueous humor is not only dependent on the extent of absorption across the corneal barrier, but also on the drug clearance from the aqueous humor. Moreover, the drug can permeate across the conjunctiva reaching systemic circulation or the iris-ciliary body via scleral absorption. In this study, compartmental analysis was performed to accurately describe the aqueous humor concentration curve, and to obtain brinzolamide clearance after intracameral injection. The number of eyes per time point in the intracameral study was two, kept

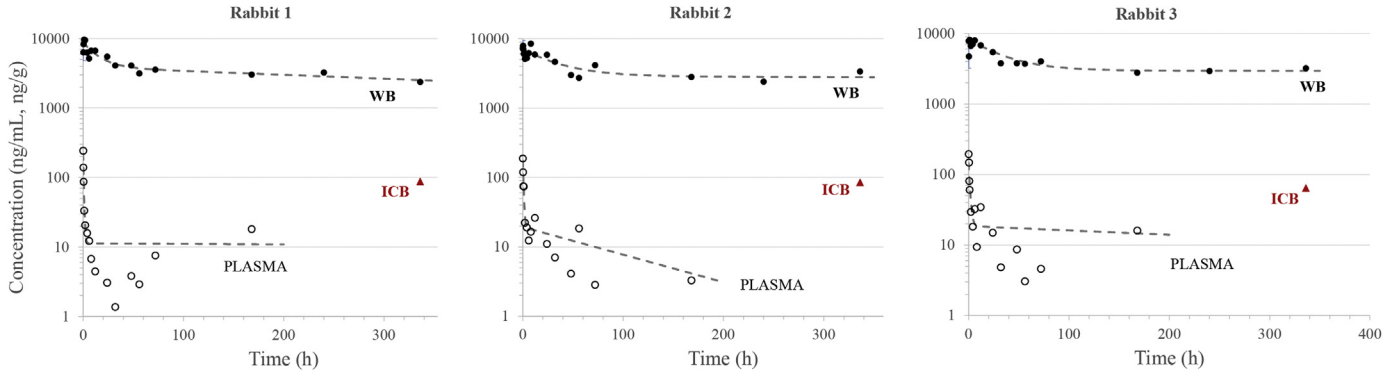



**Fig. 4.** Brinzolamide whole blood (black circles), plasma (open circles) and iris-ciliary body (ICB, triangles) concentrations following intravenous administration (0.75 mg/kg) in three rabbits.

**Table 2**
Mean Brinzolamide Concentration in Iris-Ciliary Body (ICB); Aqueous Humor (AH), Whole Blood (WB) and Plasma and Ratios of ICB Concentrations to Other Compartments.

| Administration Route And Dose | Time (h) | ICB Mean Conc. (ng/g) | AH Mean Conc. (ng/ml) | WB Mean Conc. (ng/ml) | Plasma Mean Conc. (ng/ml) | Ratio ICB/AH | Ratio ICB/WB | Ratio ICB/Plasma |
|---|---|---|---|---|---|---|---|---|
| Intracameral (4.5 μg/eye) | 24 | 85.9 | 11.5 | n.d. | n.d. | 7.47 | n.d. | n.d. |
| Topical (500 μg/eye) | 12 | 380 | 21 | n.d. | n.d. | 18.1 | n.d. | n.d. |
| | 24 | 266 | <5[a] | n.d. | n.d. | >53 | n.d. | n.d. |
| Intravenous (0.75 mg/kg) | 336 | 78.7 | <2.5[a] | 2990 | <2.5[a] | >31 | 0.026 | >31 |

[a] below LLOQ.

minimal for ethical reasons and being considered a reasonable number for a solution of brinzolamide administered directly into the anterior chamber, with minimal deviations as shown in Fig. 3A. Brinzolamide is not known to undergo any metabolism in the eye and the primary metabolites have been identified only in the blood and feces.[26] Therefore, aqueous humor turnover and drug elimination to iris-ciliary body blood flow are the primary routes for drug clearance from the anterior chamber. The intracameral clearance of brinzolamide was found to be 4.12 μL/min which is only slightly higher than the normal aqueous humor turnover of~3 μL/min in rabbits,[22] suggesting that the elimination to the iris-ciliary body blood flow is a minor contributor. Brinzolamide PK parameters after intracameral administration are similar to atenolol values with a volume of distribution of 687 μL and clearance of 6.44 μL/min.[27] The high volume of distribution of brinzolamide (0.673 mL) after intracameral administration is greater than the volume of the aqueous humor of ~0.25−0.3 mL in rabbits,[22] indicating high drug binding to the surrounding tissues, such as iris and ciliary body. The affinity of brinzolamide to the CA-II enzyme present in ciliary epithelial cells[28] seems apparent.

Slow elimination of brinzolamide from the iris-ciliary body after intracameral and topical administration is consistent with tight binding to carbonic anhydrase, that is important in determining the overall kinetics of brinzolamide. This matches previously cited elimination half-lives in intraocular tissues.[7,16] However no experimental data were included in these publications.[7,16] Brinzolamide concentrations in the iris-ciliary body were much higher than the levels in the aqueous humor at the last time points, both after topical and intracameral administrations. Furthermore, the ratio of the dose-normalized AUCs in iris-ciliary body and aqueous humor after topical administration (≈8) was greater than the ratio after intracameral administration (≈0.5), suggesting that major part of brinzolamide in the iris-ciliary body does not enter the tissue via aqueous humor. The brinzolamide concentrations were higher in the iris-ciliary body than in the aqueous humor even at the early time points following topical administration, further supporting the role of direct drug absorption across the conjunctiva and sclera to the iris-ciliary body. Two factors point to/may contribute to non-corneal absorption: 1) Dissolution of the suspended drug particles may take place in the conjunctival sac being in direct contact with the conjunctiva, but not with cornea; 2) Ex vivo permeability of brinzolamide in the rabbit and porcine conjunctiva is one order of magnitude greater than in the corneas of the same species.[9–11] Further analysis of the corneal and conjunctival tissue samples may help with estimating the relative contribution of these pathways to the accumulation of drug in the iris-ciliary body relative to the topical dose.

Based on studies in rabbits, monkeys and humans, brinzolamide is known to be absorbed into the systemic circulation following topical ocular administration, and after repeated dosing, high levels (several micrograms per mL in whole blood) have been achieved.[7] The intravenous elimination half-life of > two weeks seen from the present rabbit study is consistent with human data published in the prescribing information for AZOPT®, which indicates that after topical administration, brinzolamide is absorbed into the systemic circulation and distributes extensively into the red blood cells, exhibiting a long half-life in whole blood (approximately 111 days in humans). This is a well-known property of this drug class.[29] While the drug shows lower accumulation in iris-ciliary body compared to whole blood, based on iris-ciliary body/whole blood ratio at 14 days, the exposure in iris-ciliary body is 31 times more than in plasma and in aqueous humor (where the concentrations at the last measured time point were below the limit of quantitation).

## Conclusion

Pharmacokinetics of brinzolamide into anterior ocular tissues was investigated after topical, intracameral and intravenous administration to the eyes of rabbits. Brinzolamide bioavailability from topical suspension in the aqueous humor via corneal absorption pathway was low (0.10%), whereas significant brinzolamide absorption took place across conjunctiva and sclera, directly to the iris-ciliary body. Overall, brinzolamide levels in the iris-ciliary body were high and drug elimination from this tissue was slow. These findings improve our understanding of the pharmacokinetics of ocular suspensions and facilitate quantitative assessment and development of novel and generic ophthalmic suspension products. This can also further enable the development of pharmacokinetic and pharmacodynamic models that may be utilized for human predictions.

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