Population Pharmacokinetics of 2% Topical Dorzolamide in the Aqueous Humor of Humans

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Purpose. To evaluate the concentration and kinetics of dorzolamide in the aqueous humor after its topical application.

Methods. Samples of aqueous humor were collected at the beginning of routine cataract surgery at defined intervals after topical application of a 2% solution of dorzolamide. After deep-frozen storage of the samples, drug extraction was achieved with a mixture of solvents. Quantification was carried out by high-performance liquid chromatography on a reversed-phase column.

Results. Peak concentrations of dorzolamide in aqueous humor were reached approximately 2 hours after application with 1000 ng/ml. Average values were approximately 1000 to 700 ng/ml after 4 to 6 hours and approximately 200 ng/ml after 12 hours. Mean half-life of absorption was 1.2 hours and for elimination 3.0 hours.

Conclusions. Pharmacokinetics of dorzolamide in the aqueous humor of humans are in comparable dimensions as previously reported in experimental trials in pigmented rabbits. There is a clear linear absorption and elimination kinetic, which is demonstrated using the Bateman function. A better knowledge of the distribution and kinetics of dorzolamide will help to explain its reported effects on intraocular hemodynamics, distinct from its intraocular pressure lowering effect. (*Invest Ophthalmol Vis Sci.* 1999;40:1621-1624)

The site of aqueous humor production is the nonpigmented ciliary epithelium of the eye. Fluid movement from the stroma of the ciliary processes to the posterior chamber is achieved by transepithelial movement of Na^+ and HCO_3^-. The hydration of CO_2 to HCO_3^- is catalyzed by carbonic anhydrase (CA). Inhibition of isoenzyme II of CA in the ciliary body slows down HCO_3^- production, thereby reducing aqueous humor secretion and lowering intraocular pressure (IOP).[1]

Dorzolamide, the first clinically available topical CA inhibitor, was approved by the FDA in 1995. Pharmacokinetic testing has been performed after topical administration of 2% dorzolamide in pigmented rabbits.[2] It revealed effective peak concentrations in the aqueous humor and in the ciliary body 2 hours after application, and it also showed reasonably high concentrations in the retina.

In healthy humans, only the whole-blood pharmacokinetics after topical application have been investigated, with the half-life being approximately 120 days.[3] The present study was undertaken to investigate the pharmacokinetic properties of a 2% solution of dorzolamide (Trusopt; Chibret, Haar, Germany) in the aqueous humor of humans after a single-dose topical administration.

Materials and Methods

Patient Selection and Sample Collection

Forty-three patients scheduled for routine cataract surgery who had no other ocular pathology than cataract were recruited for the study. They included 14 men and 29 women, with a mean age of 72.7 years (range, 18.5-89.9 years). Two of these patients served as controls, without any preoperative drug application. Each of the remaining 41 patients received one drop (average, 38 μl) of a 2% solution (=0.76 mg) of dorzolamide (Trusopt) into the conjunctival sac of the eye to be operated on at defined intervals before surgery (0.5, 1, 2, 4, 6, or 12 hours preoperatively). Other preoperative topical drugs (mydriatics, anti-inflammatory agents) were identical in dosage and application interval in all studied patients. Their application was delayed for a minimum of 10 minutes after the application of dorzolamide. None of the patients had been receiving topical therapy with dorzolamide or systemic therapy with other CA inhibitors preoperatively. After surface disinfection and draping, a paracentesis was performed before any other manipulation on the eye. A Sautter cannula mounted on a tuberculin syringe was introduced into the anterior chamber without touching the rest of the eye to prevent contamination with drugs on the outer eye. The withdrawn samples of aqueous humor were deep-frozen at −20°C immediately, while the anterior chamber volume was restored with BSS (Alcon, Freiburg, Germany). The exact interval (in minutes) from dorzolamide application to sample collection was recorded.

In nine samples, the collected aqueous humor volume was too small for analysis of dorzolamide concentrations. The volume among the remaining 32 evaluated patients (11 men, 21 women; mean, 72.6 years; range, 18.5-89.9 years) ranged from 25 to 180 μl. Informed consent for the procedures was obtained from all patients. The research protocol observed the tenets of the Declaration of Helsinki and was approved by members of the Ethics Committee of the medical faculty of the Otto-von-Guericke-University.

Analytical Methods

Concentrations of dorzolamide in aqueous humor were determined by a modification of high-performance liquid chromatography (HPLC) method with UV detection (252 nm) as described by Matuszewski et al. in 1994.[4] All analyses were performed with a HPLC pump (L 6000; Merck-Hitachi, Darmstadt, Germany) and a HPLC-UV detector (SPD 6A; Shimadzu, Berlin, Germany). The sample was separated on a reversed phase column (column 125 × 4 mm, Superspher 60 RP8; Merck, Darmstadt, Germany). Dorzolamide for calibration purposes was obtained from Merck Research Laboratories (Rahway, NJ).

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For preparation of the aqueous humor samples, water was replenished to 1 ml, and 1 ml of 0.2 M phosphate buffer, pH 8 (potassium phosphate monobasic; Laborchemie Apolda, Germany) was added. The samples were extracted with a mixture of 0.5 ml isopropanol and 9.5 ml dichlormethane (both Merck, Germany). For reextraction, the organic phase was transferred and 0.3 ml 0.015 M phosphoric acid (Laborchemie Apolda) were added. After phase separation, aliquots of the aqueous phase were injected in the HPLC system. Dorzolamide was eluted isocratically using a mobile phase consisting of a mixture of acetonitrile (Baker, Germany) and a solution containing 1.6 g of octanesulfonic acid (Serva, Germany) and 0.085% phosphoric acid in water (250:750, vol/vol). Under these conditions, retention time for dorzolamide was 13.5 minutes. The calibration function in the range of 100 to 1000 ng/ml aqueous humor was linear with $r = 0.9981$. The assay limit of detection was 50 ng/ml aqueous humor, and the limit of quantification was defined at 100 ng/ml aqueous humor with a 3.4% intra-day precision. This precision was accomplished without the use of an internal standard, which was not available at the time of our analyses.

Population Pharmacokinetics

As opposed to classical pharmacokinetic studies, there was only one sample from each patient available, which was collected at different time intervals from drug application. This represents a typical population pharmacokinetic trial, which also results in valuable pharmacokinetic information. For evaluation, the Bateman function was used, a mathematical model of a bisigmoidal curve fit:

$$C(t) = A(e^{-k_2 t} - e^{-k_1 t})$$

where C is concentration, t is time, A is the amount of drug, k_1 is the absorption rate constant, and k_2 is the elimination rate constant.

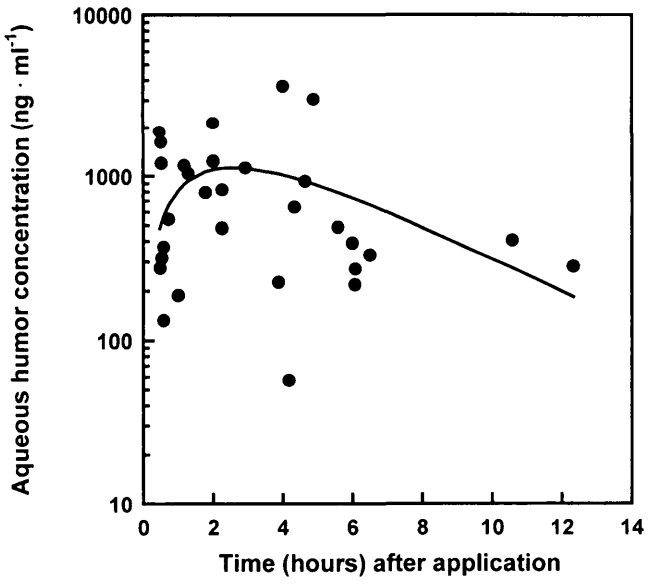

FIGURE 1. Dorzolamide concentration in aqueous humor versus time after topical instillation of one drop of a 2% ophthalmic solution (Trusopt) in 32 patients. $A = 2066$ ng, $k_1 = 0.596\ h^{-1}$, $k_2 = 0.237\ h^{-1}$, pseudo-$r^2 = 0.033$; $P = 0.811$.

TABLE 1. Pharmacokinetic Parameters of Dorzolamide in Aqueous Humor of Patients

Parameter	Mean	SE	95% Confidence Interval
k_1 (h^{-1})	0.596	1.235	−1.930, 3.123
k_2 (h^{-1})	0.237	0.540	−0.868, 1.341
A (ng)	2066	3392	−4872, 9004

$n = 32$ patients.

Data were fitted according to this equation with nonlinear regression by means of residual sums of square (SPSS software; SPSS Inc, Chicago, IL).

RESULTS

Aqueous humor samples of 32 patients were sufficient in amount to perform concentration measurements as described. In the two additional control samples of patients without prior application of dorzolamide, none of the substance was detected. The determined dorzolamide concentrations in the 32 treated patients showed large variability (Fig. 1). Maximum concentrations were reached 1 to 2 hours after application of the topical dose of 0.76 mg (1 drop). The average concentration was 1000 ng/ml after approximately 2 hours, approximately 1000 to 700 ng/ml after 4 to 6 hours, and approximately 200 ng/ml after 12 hours.

A coefficient of determination pseudo-$r^2 = 0.033$ was calculated. This is the proportion of the total variation of the dependent variable (dorzolamide concentration) around its mean at a distinct time that is explained by the model. The nonsignificant P value results from the small number of patients regarding the large variability.

The kinetic data are summarized in Table 1. Again, the ranges of values reflect the large variability. Nonetheless, the mean values are plausible. The half-lives of absorption and elimination as calculated from the constants k_1 and k_2 were 1.2 and 3.0 hours, respectively.

DISCUSSION

In 1954, acetazolamide was introduced for medical therapy of glaucoma.[5] This prototype of systemic CA inhibitor was soon shown to lower IOP by inhibiting aqueous humor production in the ciliary body.[6] Despite its very good ocular hypotensive effect after oral application, its use is limited by its systemic side effects. They are due to the high dose of acetazolamide needed to inhibit sufficiently the very active CA in order to achieve pressure-lowering effects. At least 99.7% of CA activity needs to be inhibited for a reduction in IOP.[7] Intolerable side effects are the reason for discontinuation of long-term therapy in up to 80% of patients.[8,9] For that reason, the topical application of CA inhibitors was investigated soon after the introduction of acetazolamide. Corneal penetration of acetazolamide is poor because of its very hydrophilic character, and the investigation the pressure-lowering effect of several of its derivatives after topical application failed in the 1950s.[10]

Around 1980, investigations were resumed, and in 1995, dorzolamide became the first approved and marketed topical CA inhibitor. The effect of dorzolamide on IOP has been shown preclinically and clinically,[11,12] and investigations on its effect on visual field and intraocular hemodynamics are ongoing.

Dorzolamide has a much greater ability than acetazolamide to penetrate the cornea because of its ampholytic characteristics. This property is achieved by the supplementation of the thienothiopyran-2-sulfonamide derivatives with an alkylamino side group that allows the substance to alternate between acidic and basic forms, depending on the pH level.[13,14] On the one hand, this increases the permeability through the lipophilic corneal epithelium and endothelium, whereas on the other hand, it was shown to be the most specific and most potent inhibitor of CA isoenzyme II of all investigated substances,[15] with an IC_{50} value of 0.18 ± 0.03 nM. A high level of inhibiting activity was shown against membrane-bound isoenzyme IV (IC_{50} = 6.9 ± 0.7 nM), which is currently under investigation for its suspected effect on IOP reduction,[16,17] but it showed a very low level of activity against isoenzyme I (IC_{50} = 600.0 ± 13.6 nM).

The pharmacokinetic properties of topically applied dorzolamide have been investigated in vivo in pigmented rabbits.[2] The instillation of 2% dorzolamide resulted in peak concentrations of 24.0 μg/g, 7.8 μg/ml, and 27.0 μg/g in the cornea, aqueous humor, and iris/ciliary body, respectively. The high concentrations in the cornea demonstrate the depot effect achieved by the ampholytic properties of the substance. This effect is estimated to be responsible for reaching and maintaining a sufficient concentration in the ciliary epithelium over a longer period.[18] Dorzolamide also was detected in retinal tissue, with a peak concentration of 5.29 μg/g, which may be one explanation for its effect on retinal blood flow after topical administration, as shown by Harris et al. in 1996.[19] Recently, activity of membrane-bound CA has also been demonstrated in endothelial cells of retinal capillaries.[20]

The results of our study clearly demonstrate that the curve of dorzolamide concentration in aqueous humor follows a Bateman function (Fig. 1), i.e., there is a distinct linear absorption and elimination kinetic. This is even more remarkable, because we are dealing with a population kinetic, in which every single value represents the concentration in the aqueous humor of one eye of one individual patient. The mean peak concentration is reached after approximately 2 hours with approximately 1000 ng/ml. This is approximately ⅛ of the corresponding value of 7800 ng/ml in pigmented rabbits,[2] but corresponds well with the relation of "one-third-or-less," which was established by Maren for other CA inhibitors (L-650, 719 and L-654, 230).[10]

In vitro studies have shown that there is no difference in corneal permeability between humans and rabbits. However, a whole series of parameters has been pointed out to explain the following in vivo differences: the blink rate is about four times greater in humans, the tear turnover rate is about twice as great, and the conjunctival/corneal surface is about half that of the rabbit.[21]

The great interindividual variability of the single values in our study may be explained with corresponding arguments: e.g., blink rates may vary extensively between individuals, especially in the preoperative setting with different degrees of anxiety. Anterior chamber volumes may vary significantly, especially in cataractous eyes because of increased lens mass, with the possibility of higher concentrations in smaller overall volumes. This variation in volume also made it impossible in some instances to collect enough aqueous humor for quantification. However, these variations do not seem to influence the clinical effect of dorzolamide because of its broad therapeutic range, as was shown in clinical trials.

Because peak concentration and kinetics in aqueous humor of humans are comparable to the experimental results in pigmented rabbits, as demonstrated by Sugrue,[2] we also expect comparable concentrations in other ocular tissues, both in the anterior and the posterior segment of the eye. Effective concentrations of dorzolamide in the posterior segment may help us to explain its effect on retinal and superficial optic disc blood flow and possibly on the retinal tissue itself. It seems interesting to find out, whether the substance has another antiglaucomatous effect apart from its IOP-lowering effect. Therefore, in future investigations we want to measure dorzolamide concentrations in other ocular compartments and tissues, which, unfortunately, are much more difficult to collect in humans.

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Suppression of Retinal Neovascularization by the NF-κB Inhibitor Pyrrolidine Dithiocarbamate in Mice

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Purpose. To evaluate the effect of pyrrolidine dithiocarbamate (PDTC), an inhibitor of nuclear factor κB (NF-κB), on retinal neovascularization in a murine model of ischemic retinopathy.

Methods. One-week-old C57BL/6N mice were exposed to 75% ± 2% oxygen for 5 days and then were returned to room air to induce retinal neovascularization. After the return to room air, the left and right eyes were injected intravitreally with PDTC or a vehicle, respectively. Retinal neovascularization was examined by injecting fluorescein dextran and angiography after 5 days in room air and was quantitated histologically with a masked protocol. The effects of PDTC on NF-κB activation were evaluated by immunohistochemistry. To examine the toxicity of PDTC, the histologic change in the retina was examined by light and electron microscopy.

Results. Retinal neovascularization in the eye injected with PDTC by intravitreal methods was reduced in 100% of animals compared with that apparent in the vehicle-treated eye. The inhibitory effect was dose-dependent, with a maximal inhibition of 39% ($P < 0.01$) at a dose of 1 nmole. The immunostaining intensity for NF-κB in the retina was reduced by PDTC injections. No side effects by PDTC in the retina were observed by light and electron microscopy.

Conclusions. NF-κB activation appears to be required for retinal angiogenesis, given that the administration of PDTC suppressed retinal neovascularization. PDTC may prove beneficial in the treatment of ischemic neovascular diseases such as diabetic retinopathy and retinal vein occlusion. (*Invest Ophthalmol Vis Sci.* 1999;40:1624-1629)

Angiogenesis, the formation of new capillary blood vessels, often accompanies wound healing, inflammation, and other pathologic conditions. Retinal neovascularization is a major cause of the blindness associated with such ischemic retinal disorders as diabetic retinopathy, retinopathy of prematurity, and retinal vein occlusion. Despite the prevalence of these diseases, an effective treatment for retinal neovascularization remains elusive. Neovascularization is induced by complex interactions among multiple cytokines and adhesion molecules. Several potential inhibitors of retinal neovascularization, including soluble vascular endothelial growth factor (VEGF) receptor and antagonists of both α_v-integrin and growth hormone, have been identified with the use of a highly reproducible model of ischemia-induced retinal neovascularization.[1-3] However, targeting pleiotropic regulators of multiple angiogenic factor and adhesion molecule genes may be required to inhibit neovascularization effectively.

With the animal model of ischemia-induced retinal neovascularization, we have previously shown that nuclear factor κB (NF-κB) activation may be important to induce retinal neovascularization in vivo.[4] In this model, exposure of neonatal animals to hyperoxic conditions results in extensive obliteration of retinal capillaries. When the animals are returned to room air, the inner retina presumably becomes relatively hypoxic, which results in activation of NF-κB, subsequent production of many cytokines and adhesion molecules, and retinal neovascularization.

We have also shown that the expression of interleukin-8 (IL-8), probably mediated by NF-κB activation, may contribute to the pathogenesis of intraocular neovascularization in individuals with diabetic retinopathy or retinal vein occlusion.[5] In addition to the IL-8 gene, NF-κB regulates many other angiogenesis-related genes, including those encoding tumor necrosis factor-α (TNF-α), vascular cell adhesion molecule-1, and intercellular adhesion molecule-1.[6] Pyrrolidine dithiocarbamate (PDTC), a specific inhibitor of NF-κB activation, and NF-κB antisense oligonucleotides inhibit angiogenesis in an in vitro model.[7,8] We also have shown that PDTC prevents hypoxia-induced NF-κB activation in retinal glial cells in vitro.[5] These observations suggest that NF-κB may represent a suitable target for therapeutic intervention in retinal neovascularization.

We have now examined the effects of PDTC on retinal neovascularization in the mouse model of proliferative retinop-

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