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Ocular pharmacokinetics of atenolol, timolol and betaxolol cocktail: Tissue exposures in the rabbit eye

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ABSTRACT

Quantitative understanding of pharmacokinetics of topically applied ocular drugs requires more research to further understanding and to eventually allow predictive *in silico* models to be developed. To this end, a topical cocktail of betaxolol, timolol and atenolol was instilled on albino rabbit eyes. Tear fluid, corneal epithelium, corneal stroma with endothelium, bulbar conjunctiva, anterior sclera, iris-ciliary body, lens and vitreous samples were collected and analysed using LC-MS/MS. Iris-ciliary body was also analysed after intracameral cocktail injection. Non-compartmental analysis was utilized to estimate the pharmacokinetics parameters. The most lipophilic drug, betaxolol, presented the highest exposure in all tissues except for tear fluid after topical administration, followed by timolol and atenolol. For all drugs, iris-ciliary body concentrations were higher than that of the aqueous humor. After topical instillation the most hydrophilic drug, atenolol, had 3.7 times higher AUC_{iris-ciliary body} than AUC_{aqueous humor}, whereas the difference was 1.4 and 1.6 times for timolol and betaxolol, respectively. This suggests that the non-corneal route (conjunctival-scleral) was dominating the absorption of atenolol, while the corneal route was more important for timolol and betaxolol. The presented data increase understanding of ocular pharmacokinetics of a cocktail of drugs and provide data that can be used for quantitative modeling and simulation.

1. Introduction

Topical application is the most common route of ocular drug administration. Bioavailability of drugs after topical administration is less than 4% [1–3], since anatomical barriers restrict drug permeation into the inner eye (Fig. 1). Moreover, most of the instilled dose is rapidly removed from the ocular surface by tear turnover, induced lacrimation, and solution drainage into the nasolacrimal duct. During and after the

solution drainage, the drug is absorbed into systemic circulation across the conjunctiva, nasal mucosa and gastrointestinal tract [4–6].

Drug absorption into the inner eye takes place through corneal or non-corneal pathways. For the corneal route of absorption, drug penetrates through the corneal epithelium that constitutes the major barrier for drug absorption. Thereafter, the drug reaches the corneal stroma, endothelium, and aqueous humor. The corneal route is considered to be the most common pathway of topical ocular drug absorption [7–9]. On

Abbreviations: AUC_{inf}, area under the curve from time zero to infinity; AUC_{last}, area under the curve from the time of dosing to the time of the last measurable concentration; C_{max}, maximum observed concentration; internal standard, ISTD; t_{max}, time of maximum observed concentration; t_{1/2}, elimination half-life; MRT_{inf}, mean residence time; NCA, non-compartmental analysis.

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the other hand, drugs may also be absorbed across the conjunctiva and sclera (non-corneal route) reaching the ciliary body and iris with limited access to the aqueous humor [10–14]. However, the conjunctiva is a highly vascularized tissue and a major part of the drug dose may enter the blood circulation instead of diffusing into the sclera [14–18].

From aqueous humor, the drug may be cleared by aqueous humor outflow via trabecular meshwork into Schlemm's canal [19] or it may enter iris-ciliary body blood vessels and then systemic blood circulation [20–22]. From the aqueous humor drugs also distribute to the lens and vitreous humor, but the concentrations in these tissues are low [13,23].

The corneal epithelium is the main anterior barrier for ocular drug absorption due to the intercellular tight-junctions, particularly limiting permeation of hydrophilic drugs. The acellular corneal stroma allows relatively free diffusion of drugs but partitioning of lipophilic drugs from the lipoidal epithelium to the hydrophilic stroma may be restricted [7–9,24].

In fact, the conjunctiva and sclera are more permeable than the cornea, being sclera less permeable than conjunctiva [14,24–26]. For hydrophilic compounds conjunctiva is ~ 15–25 times more permeable than cornea, and sclera is half as permeable as conjunctiva [25]. Although drug diffusion across the conjunctiva and sclera is easier than through the cornea [11,14,26], the presence of blood and lymphatic flows in the conjunctiva and episclera results in drug elimination from the eye into the systemic circulation [27–29].

Additionally, iris and ciliary body have a vascular bed with blood flows of 62 $\mu\text{l}/\text{min}$ and 82 $\mu\text{l}/\text{min}$ in rabbits, respectively [21,30]. Iridial vessels and ciliary muscle vessels with tight junctions are parts of the blood-aqueous barrier. Moreover, the epithelial blood-aqueous barrier (posterior iris epithelium and non-pigmented ciliary epithelium) may limit drug diffusion further into the vitreous [29,31]. Clearance via the iris and ciliary body vessels seems to be faster for lipophilic drugs than hydrophilic compounds [32–34].

The present study was performed to quantitatively understand the ocular pharmacokinetics of the three model drugs: betaxolol, timolol, and atenolol cited in decreasing order of lipophilicity (Table 1). The compounds were administered as eye drops and intracameral injection. Pharmacokinetic parameters were estimated in nine ocular tissues. This study provides a comprehensive description of the drug distribution in the eyes after topical administration, also including the analysis of the corneal vs non-corneal absorption processes.

2. Material and method

2.1. Animal experiments

Animals. Thirty-two albino New Zealand rabbits, age 3–6 months and weight 2.8–3.2 kg were used in the experiment sixteen for the topical study and sixteen for the intracameral one. The animals were housed in a temperature and humidity-controlled environment with a 12/12 light/dark cycle. The animals were individually housed and fed a normal diet. All rabbits underwent an ocular examination before the experiments. The study complies with ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and EU Directive 2010/63/EU for animal experiments.

Topical administration. The cocktail containing 20 mM atenolol (USP reference standard, Sigma), 10 mM betaxolol hydrochloride (USP reference standard, Sigma) and 10 mM timolol maleate (USP reference standard, Sigma) in phosphate-buffered saline (PBS, ThermoFisher Scientific) (pH adjusted to 7.4; 322 mOsm/kg) was administered onto the upper cornea-scleral limbus of both eyes (25 $\mu\text{l}/\text{eye}$) in each rabbit as previously reported [1]. The tear fluid samples of 1 μl were withdrawn from each eye with disposable microcapillaries (Microcaps, Drummond Scientific). Corneal epithelium, corneal stroma with endothelium, bulbar conjunctiva, anterior sclera, aqueous humor, iris-ciliary body, lens, and vitreous humor were collected and weighted at 5, 10, 20, 30, 60, 120, 180 and 240 min, and the number of eyes at each time point was four ($n = 4$).

Intracameral administration. A volume of 5 μl of the cocktail solution with atenolol, timolol and betaxolol (each at 1 mM in PBS) was injected into the anterior chamber (aqueous humor) of the rabbit eye [32]. The animals were sacrificed by injecting into the marginal ear vein a lethal dose of pentobarbital (Mebunat vet 60 mg/ml; Orion Pharma, Finland). In these experiments, aqueous humor and iris-ciliary body samples were collected and weighted at time points 10, 20, 30, 60, 120, 180 and 240 min with number of eye per each time point 4 ($n = 4$) as previously described [32]. All samples were cooled on ice following storage at $-80\text{ }^{\circ}\text{C}$ until analyses.

Dissection of ocular tissues. The excised eyeballs were dipped to PBS. Firstly, the corneal epithelium was collected from the corneal surface by gently scraping with a scalpel blade. Then, a piece of approximately 5 mm \times 5 mm size of bulbar conjunctiva was collected with small scissors by first cutting a small hole through conjunctiva from the limbus, detaching the conjunctiva from the surface of the eye and

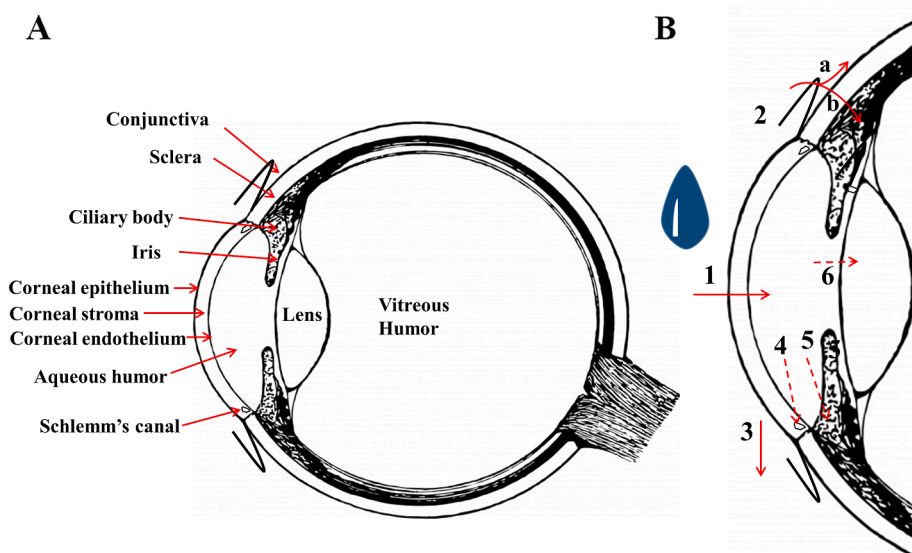
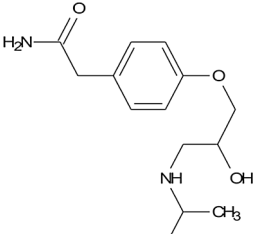
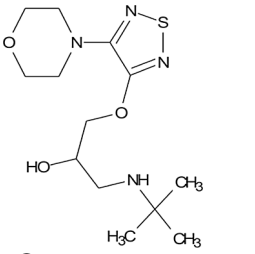
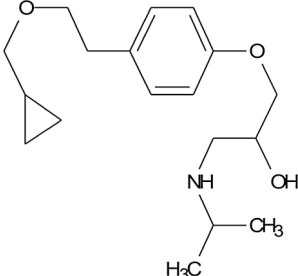


Fig. 1. Ocular absorption, distribution and elimination routes after topical administration. **A:** Anatomy of the eye. **B:** Absorption, distribution and clearance pathways after topical administration. 1: corneal absorption; 2: a) conjunctival absorption into conjunctival blood vessels reaching the systemic circulation and b) conjunctiva-scleral absorption into ciliary body and iris; 3: tear flow and extra volume drainage by the nasolacrimal system; 4: clearance from trabecular meshwork into Schlemm's canal; 5: distribution into the ciliary body and iris and the blood circulation therein; 6: distribution to the lens. (<https://www.publicdomainpictures.net/en/view-image.php?image=130389&picture=medical-eye>).

Table 1

Physicochemical properties of atenolol, timolol and betaxolol (calculated with ACD/Percepta, version 2254, Advanced Chemistry Development, Inc. Toronto, Canada).

Drugs	Molecular structure	Molecular weight	Log D _{7.4}	Polar surface area	Hydrogen bond donor
Atenolol		266.34	-1.85	84.5	4
Timolol		316.42	-0.35	107.9	2
Betaxolol		307.43	0.77	50.7	2

finally removing the conjunctival piece. The sclera was cut open from the same spot as the conjunctiva was collected, and a ~ 5 mm × 5 mm piece of anterior sclera was removed. From the scleral piece, remaining extraocular tissues and retina were removed with scissors. Then, the anterior part (corneal stroma with endothelium, iris-ciliary body and lens) was detached from the posterior tissues (vitreous, retina, choroid, sclera) by cutting with scissors 2 mm posterior from the limbus. Cutting was started from a pre-made hole in the sclera, enabling removal of the iris-ciliary body and lens with the cornea. Then, lens and iris-ciliary body were collected with tweezers, and the sclera was cut off from the corneal stroma and endothelium with scissors to obtain the corneal sample. From the posterior part, vitreous was dissected on a separate dish by gently pulling the vitreous from the eyecup with tweezers. When necessary, the vitreous sample was cleaned from retinal contamination with tweezers and scissors to obtain a clean vitreous sample.

2.2. LC-MS/MS analyses

The corneal epithelial samples were lysed by adding 38-fold volume of 0.1 N NaOH (based on the sample weight) and then pipetted and vortexed until homogenous tissue lysate was obtained. Corneal stroma-endothelium, bulbar conjunctiva and anterior sclera samples were cut into smaller pieces with scalpel before homogenization. The samples were transferred to the 2 ml or 7 ml tubes containing 2.8 mm ceramic beads (Omni International, USA), PBS buffer was added and the tissues were homogenized at 4–6 m/s speed in total for 0.5–12 min using Bead Ruptor Elite (Omni International, USA). For corneal stroma-endothelium, bulbar conjunctiva, anterior sclera, iris ciliary body and lens the homogenization was performed in two steps and PBS buffer was added between steps. Homogenization conditions were optimized for each tissue to obtain smooth tissue homogenate, suitable for pipetting (Supplementary Table S1). Some samples of early time points (corneal

epithelium, corneal stroma-endothelium, bulbar conjunctiva, iris-ciliary body) were diluted with similarly prepared blank tissue homogenate.

One part of each sample was mixed with 3 parts of internal standard solution (e.g. 30 µl of sample and 90 µl) by vortexing for 10 sec. Internal standard (ISTD) solution contained 50 ng/ml atenolol-d7 (Toronto Research Chemicals, Canada), 5 ng/ml betaxolol-d5 (Toronto Research Chemicals, Canada), 5 ng/ml rac timolol-d5 maleate (Toronto Research Chemicals, Canada), 1% formic acid in acetonitrile. In the case of the vitreous samples, one part of the vitreous homogenate was mixed with one part of ISTD solution. All the samples were incubated at RT for 10 min and thereafter centrifuged (10 min, +4°C, 13 000 rpm). The supernatant was collected for the LC-MS/MS analysis. Standards (0.5–4000 nM, in duplicates) and quality controls (in triplicates, 4–6 levels) were prepared from the drug cocktail (containing 20 mM atenolol, 10 mM timolol maleate and 10 mM betaxolol hydrochloride in PBS) into blank tissue homogenates in the same way as the samples were treated.

Tear fluid samples were prepared in the same way as aqueous humor samples in our previous article [32] with the exception that ISTD solution was 50 ng/ml atenolol-d7, 5 ng/ml betaxolol-d5, 5 ng/ml rac timolol-d5 maleate, 1% formic acid in 50% acetonitrile. Tear fluid samples were first diluted with ISTD solution (dilution factors were 20–4000) to achieve final concentrations in the range of calibration curve (0.1 – 5000 nM). Standards and quality controls were prepared by diluting the beta-blocker cocktail in PBS by ISTD solution. Additional quality controls (250 nM, n = 2), which were prepared in similar manner as tear fluid samples (dilution of tear fluid 1:20 and 1:2000 with ISTD solution), were included into LC-MS/MS analysis.

All samples, standards and quality controls were analysed by LC-MS/MS (Agilent 1290 liquid chromatograph and Agilent 6495 triple quadrupole mass spectrometer, Agilent Technologies Inc., USA) as described earlier [32]. The calibration curves were generated using 7–13

concentration levels. Mean accuracy of the standards of most standard curve points ($\geq 78\%$) was 80–120%. The accuracy of QC samples was 80–120% for $\geq 67\%$ of quality controls. For additional tear fluid QC samples the accuracy was 86–110%.

2.3. Pharmacokinetic analysis

Mean concentration–time profiles of drugs in the tissues were analysed using non-compartmental analysis (NCA) with Phoenix WinNonlin (build 8.1, Certara L.P.) using linear–linear trapezoidal interpolation. The following pharmacokinetic parameters were estimated: area under the curve (AUC) from time zero until last sampling point (AUC_{last}), total AUC until infinity (AUC_{inf}), mean residence time (MRT_{inf}), peak concentration (C_{max}), time to peak concentration (t_{max}) and elimination half-life ($t_{1/2}$). The AUC_{inf} and MRT_{inf} were reported for the tissues with the terminal phase of the concentration–time profiles longer than two half-lives.

3. Results

The dose-normalized (to 250 nmol) concentration–time profiles for the topically applied drugs in eight ocular tissues (this study) and the aqueous humor (from our previous study) [1] are shown in Fig. 2. The

aqueous humor [32] and iris-ciliary body (present study) concentration–time profiles after intracameral administration of 5 nmol of atenolol, timolol and betaxolol are presented in Supplementary Fig. S1.

Betaxolol concentrations were higher than the levels of timolol and atenolol in most tissues, but in the tear fluid atenolol concentrations were the highest (Fig. 2, Supplementary Fig. S2). Drug concentrations in various tissues show a wide range as the concentrations in the corneal epithelium are at least 1000 times higher than in the vitreous.

The pharmacokinetic parameters for the topical and intracameral beta-blockers are listed in Table 2 (see in Supplementary Table S2 the original data without dose normalization). As expected, the MRT values are shorter in the tear fluid than in the other tissues. In some tissues, the MRT values are relatively constant regardless of the drug (e.g. corneal epithelium), while in other tissues (e.g. aqueous humor, corneal stroma-endothelium, vitreous, iris-ciliary body) hydrophilic atenolol has longer retention times than the more lipophilic timolol and betaxolol (Supplementary Fig. S3).

The AUC_{inf} values of the drugs are shown in Fig. 3. The data shows that AUC_{inf} values span over the range of four orders of magnitude from tear fluid to lens and vitreous. Atenolol has the highest AUC_{inf} in tear fluid whereas betaxolol has the highest AUC values in the corneal epithelium and other tissues. The AUC_{inf} values of three drugs are higher in the iris-ciliary body than in the aqueous humor. AUC_{inf} values of

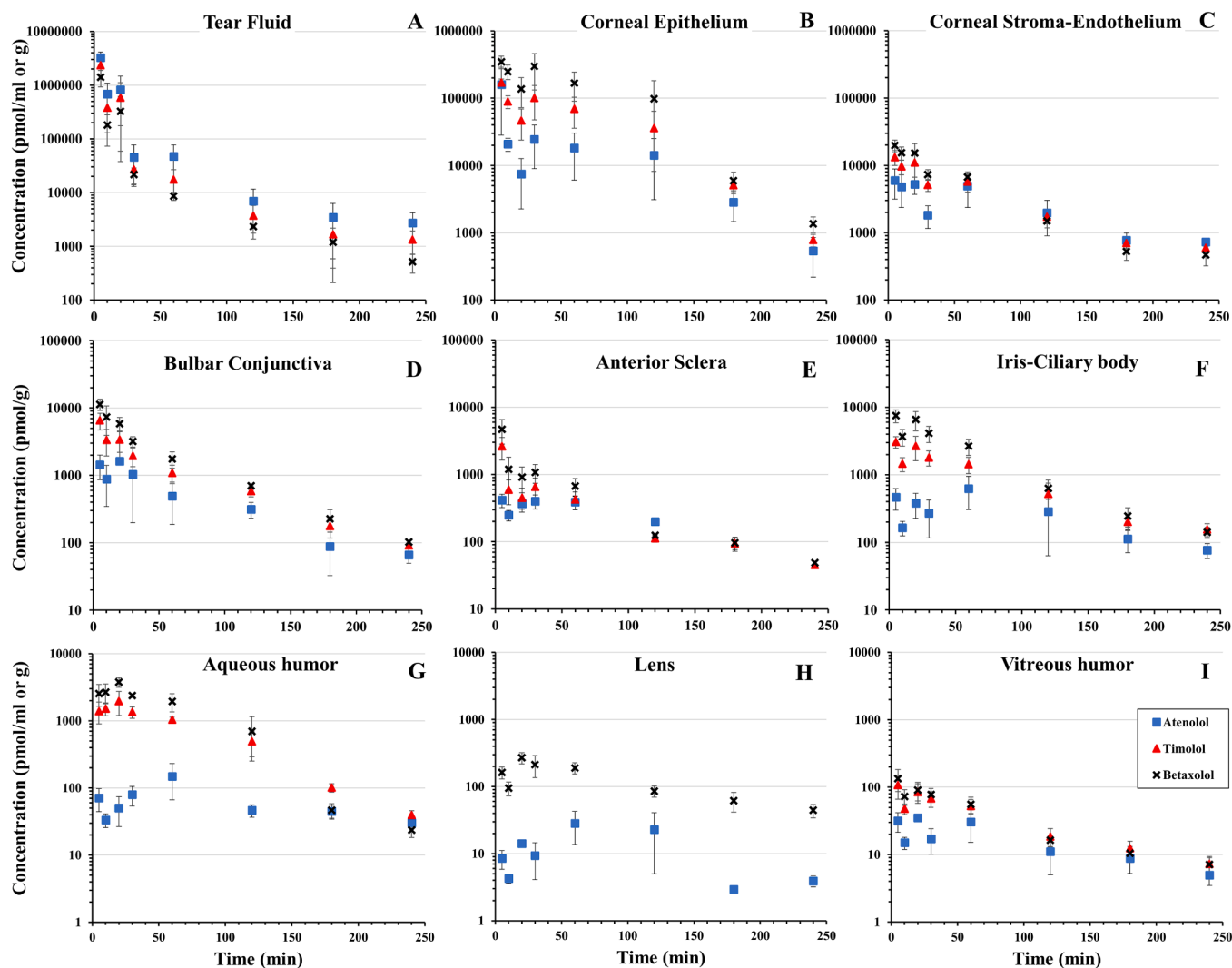


Fig. 2. Concentration–time profiles of drugs in nine ocular matrices (A–I) of rabbits after topical administration of atenolol (250 nmol dose-normalized), timolol (250 nmol dose), and betaxolol (250 nmol dose). Mean drug concentrations \pm standard errors of the mean ($n = 3–4$) are presented. Timolol concentration could not be quantified in the lens due to the lack of a reliable control sample.

Table 2

Pharmacokinetic parameters estimated with NCA analysis of the three beta-blockers concentrations in nine ocular tissues after topical administration including the aqueous humor [1], and iris-ciliary body, and aqueous humor [2] after intracameral administration with a dose-normalization to 250 nmol.

Topical	Atenolol						Timolol						Betaxolol					
	AUC _{inf} (min*nmol/ml or g)	AUC _{last}	C _{max} (nmol/ml or g)	t _{max}	t _{1/2} (min)	MRT	AUC _{inf} (min*nmol/ml or g)	AUC _{last}	C _{max} (nmol/ml or g)	t _{max}	t _{1/2} (min)	MRT	AUC _{inf} (min*nmol/ml or g)	AUC _{last}	C _{max} (nmol/ml or g)	t _{max}	t _{1/2} (min)	MRT
Tear fluid	71720	71542	3240	5	45	9	58398	58311	2345	5	45	6	39799	39769	1398	5	40	5
Corneal epithelium	3387	3361	159	5	34	63	9642	9611	171	5	27	62	24760	24712	348	5	24	60
Corneal stroma -endothelium	627	562	5.98	5	62	104	819	774	13.2	5	53	74	978	949	19.6	5	43	57
Bulbar conjunctiva	208	198 164*	3.24	20	52	74	234	228 197*	6.55	5	47	59	378	372 334*	11.3	5	42	50
Anterior sclera	-	39.1*	0.41	5	84	-	71.5	67.7 57.3*	2.60	5	59	72	111	108 96.7*	4.70	5	47	55
Iris-ciliary body	72.4	66.2 49.5*	0.63	60	56	105	213	201 169*	3.07	5	52	77	398	390 352*	7.52	5	39	55
Aqueous humor	19.6	15.8	0.15	60	87	148	151	149	1.97	20	37	64	252	251	3.76	20	29	54
Lens	3.65	3.35	0.03	60	53	112	**	**	**	**	**	**	32.8	27.2	0.27	20	88	133
Vitreous humor	4.22	3.69	0.04	20	74	118	8.13	7.52	0.11	5	61	89	8.53	7.97	0.13	5	56	80
Intracameral																		
Iris-ciliary body	9939	7624	85.8	60	85	149	4615	4456	106	10	47	60	5013	4907	145	10	41	51
Aqueous humor	28711	26397	365	20	59	84	10895	10819	204	20	31	45	6520	6486	142	20	31	39
TOP: ICB/AH	3.69						1.41						1.58					
IC: ICB/AH	0.35						0.42						0.77					

AUC_{inf}: Area under the curve from 0 to infinity.

AUC_{last}: Area under the curve from 0 to last sampling time (240 min) unless otherwise indicated.

C_{max}: Maximum concentration.

t_{max}: Time to peak concentration.

t_{1/2}: Elimination half-life.

MRT: Mean residence time to infinity.

* AUC_{last} until 120 min.

** timolol could not be quantified due to the lack of a reliable control sample.

-: unreliable estimate because the concentration-time profile was shorter than two half-lives.

ICB/AH: AUC_{inf} ratio between iris-ciliary body and aqueous humor after topical (TOP) and intracameral (IC) administrations.

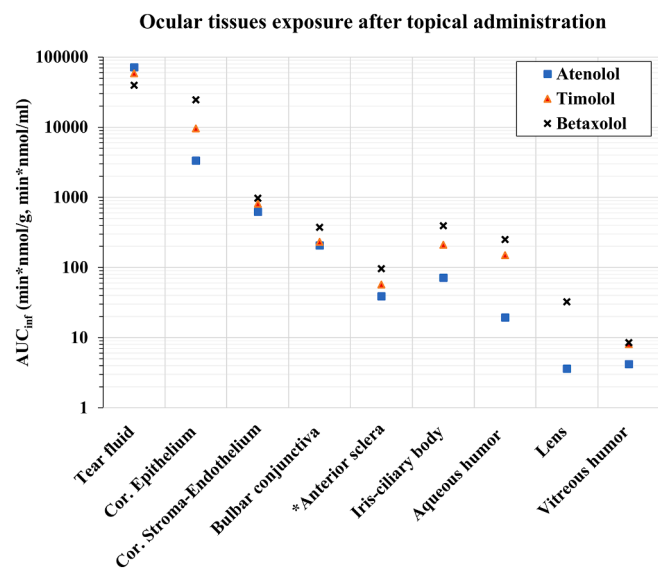


Fig. 3. The AUC_{inf} values of betaxolol, timolol and atenolol in the ocular tissues after topical eye drop instillation to the rabbit eyes with a dose-normalization to 250 nmol. For anterior sclera $AUC_{last} = 120$ min values are shown (*). Abbreviation: Cor = cornea.

betaxolol in aqueous humor, corneal epithelium, and iris-ciliary body were 12, 7, and 5.5 times higher than the values of atenolol, while betaxolol had 2, 2.5, and 1.9 higher values than timolol.

The AUC_{inf} ratios between the adjacent tissues are presented in Fig. 4. Lipophilic betaxolol shows the highest partitioning from tear fluid to the corneal epithelium and bulbar conjunctiva. Likewise, it shows the highest partitioning from sclera to iris-ciliary body. Interestingly, atenolol shows the highest ratios of iris-ciliary body/aqueous humor, corneal stroma-endothelium/epithelium and vitreous/aqueous humor ratios. Overall, concentration ratios between neighboring tissues show wide range of values from ≈ 0.001 to less than 10.

Iris-ciliary body concentration–time profiles after topical and intracameral administration of the drugs are presented in Supplementary Fig. S4. Atenolol shows a bigger difference than timolol and betaxolol between the iris-ciliary body concentrations after topical and intracameral administrations. Differences are also seen among AUC_{inf} ratios between

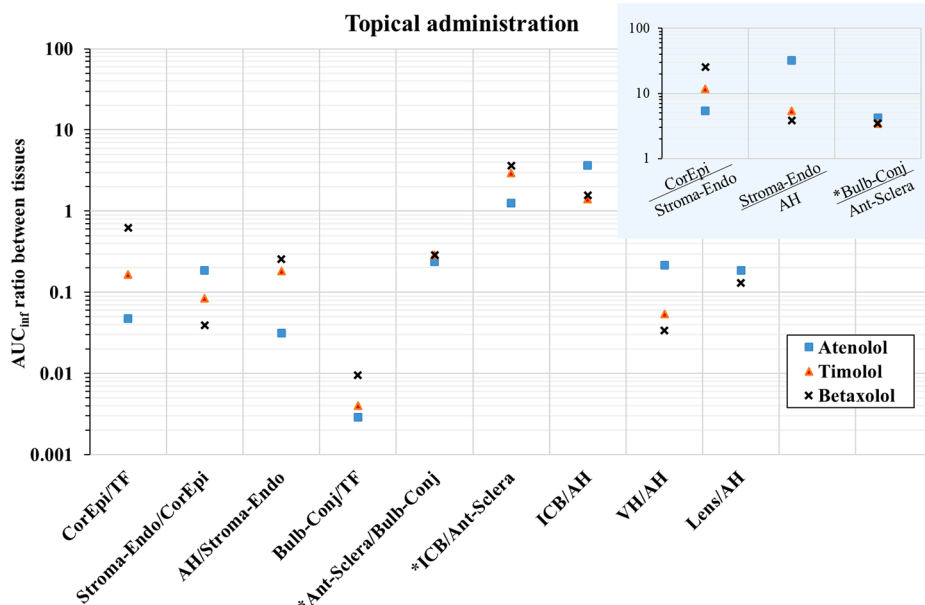


Fig. 4. The AUC_{inf} ratios among neighboring tissues after topical eye drop instillation of betaxolol, timolol and atenolol to the rabbit eyes. The inset shows AUC_{inf} ratios in the direction of drug accumulation into corneal epithelium, corneal stroma-endothelium and bulbar conjunctiva. The AUC_{inf} (Table 2) was used except for anterior sclera-related ratios when $AUC_{last=120min}$ was used (*). Abbreviations: CorEpi = corneal epithelium, TF = tear fluid, Stroma-Endo = corneal stroma-endothelium, AH = aqueous humor, Bulb-Conj = bulbar conjunctiva, Ant-Sclera = anterior sclera, ICB = iris-ciliary body, and VH = vitreous humor.

iris-ciliary body and aqueous humor (Table 2).

4. Discussion

A cocktail approach was utilized to estimate the pharmacokinetic behavior of betaxolol, timolol and atenolol in the ocular tissues of the rabbit eye. The current study provides pharmacokinetic data and parameters to better understand tissue exposure, partitioning and elimination of the drugs.

4.1. Corneal drug absorption

In the tear fluid, atenolol shows higher levels than the more lipophilic timolol and betaxolol (Table 2, Fig. 2A and 3, Supplementary Fig. S2A). Flow patterns of the instilled solution is identical for all drugs in this study as a cocktail approach was used. Thus, the differences among three drugs can be explained by their different rates of elimination from lacrimal fluid across the membranes (cornea, conjunctiva); with the more lipophilic compounds betaxolol and timolol having higher permeability [14,35]. Since the short initial half-life in the tear fluid cannot be obtained using the NCA analysis, the reported half-lives in Table 2 correspond to later phases in the disposition process that may involve drug equilibration to the tissues, and back-diffusion to the lacrimal fluid [11,24,36].

In the corneal epithelium, betaxolol and timolol showed higher concentrations and corneal partitioning than atenolol, which is expected due to their increased lipophilicity. Corneal stroma is not posing an anatomical barrier for diffusion of small molecules [7]. Interestingly, it seems that hydrophilic atenolol has higher corneal stroma-endothelium / aqueous humor ratio and longer MRT in the corneal stroma-endothelium (inset of Fig. 4, Table 2). This may reflect lower permeability of atenolol in corneal endothelium as compared to timolol and betaxolol.

Betaxolol had the highest AUC_{inf} value in the aqueous humor (Fig. 3, Table 2), probably due to its lipophilicity and higher corneal permeation. Concentration profiles of betaxolol and timolol had similar first-order decline in aqueous humor and in corneal-epithelium (Supplementary Fig. S2A). This supports the notion that corneal absorption is the main route of drug absorption to the aqueous humor and corneal epithelium forms a drug depot after eye drops instillation [1,9,12]. On the contrary, the concentration profile of atenolol is flatter, reflecting

the first-order decline rate in the corneal stroma-endothelium (Supplementary Fig. S2A). This suggests that corneal stroma, not epithelium, is a depot site for atenolol following topical administration.

As expected [7,9] lipophilicity plays a positive impact on the corneal drug absorption to the aqueous humor. From the aqueous humor, the drugs distribute into the iris-ciliary body, lens, and vitreous. It is known that hydrophilic atenolol has lower clearance from the aqueous humor than timolol and betaxolol [32], this is in line with the longer MRT in the aqueous humor.

4.2. Non-corneal drug absorption

Drugs permeate from tear fluid to the conjunctiva and in this case also betaxolol showed the highest conjunctival concentrations, followed by timolol and atenolol (Fig. 2, Table 2, Supplementary Fig. S2B). Lipophilic drugs permeate faster into cellular conjunctiva, but they are also eliminated faster into the systemic circulation from the conjunctiva [11,14,26]. For this reason, the conjunctival AUC_{inf} values are much lower than the non-vascular corneal ones and the AUC_{inf} differences among the drugs are also smaller in conjunctiva than in cornea (Fig. 2, Fig. 3, Table 2).

Scleral kinetics of betaxolol and timolol showed similar decline as in the bulbar conjunctiva, and no differences among drugs were seen in the $AUC_{last=120\text{ min}}$ ratios between these two tissues (Fig. 4). From sclera the drugs may reach iris-ciliary body. Atenolol concentrations were slightly higher in the iris-ciliary body than the sclera, for the more lipophilic compounds there was a bigger difference in the iris-ciliary body compared to scleral concentrations (Table 2, Fig. 3), possibly due to entry of some drug from the aqueous humor to the iris-ciliary body [20,22,37]. Higher AUC_{inf} ratio of hydrophilic atenolol between iris-ciliary body and aqueous humor may be partly due to its higher non-corneal entry into iris-ciliary body as compared to timolol and betaxolol. The slower clearance of hydrophilic atenolol than timolol and betaxolol via iris blood vessels could also contribute in the cited higher AUC_{inf} ratio of atenolol [32].

After intracameral administration, the concentration time profiles of timolol and betaxolol in aqueous humor and iris-ciliary body are quite similar, but atenolol shows different kinetic behaviour: slower distribution and elimination in the iris-ciliary body (Supplementary Fig. S1). This behavior reflects the slower permeation of atenolol into the blood-aqueous barrier. As intracameral administration results in iridial drug distribution only from the aqueous humor, it is interesting to compare these data with the topical eye drop data that involves also non-corneal drug delivery. Comparison of the AUC_{inf} ICB/AH ratios of hydrophilic atenolol after topical (3.69) and intracameral (0.35) administrations reveals ten times higher ratio for topical administration, suggesting the importance of non-corneal atenolol absorption. The difference was only 2–3-fold for more lipophilic timolol and betaxolol. Therefore, atenolol has the lowest AUC_{inf} in the iris-ciliary body target tissue after topical administration, but the highest contribution from non-corneal route of absorption. Non-corneal absorption was earlier seen also for topical suspension of brinzolamide [2], and timolol insert placed in the conjunctival sac [34]. Comparisons of these ratios inform about the relative importance of the non-corneal route, but these values do not give absolute non-corneal contributions, because *trans*-corneal delivery to the iris-ciliary body may differ from the iris-ciliary body distribution after intracameral injection.

4.3. Lens and vitreous

Atenolol and betaxolol distributed to a lower extent from aqueous humor into the lens (Fig. 3). Poor lenticular distribution is due to its tight structure that resulted in low lens/buffer partition coefficients for various drugs *in vitro* [23]. Thus, the lens acts as a barrier between the anterior chamber and vitreous, but it does not seem to act as a drug reservoir.

Drugs may distribute also from the aqueous humor into the vitreous humor, and the expected route is via permeation across the iris-ciliary body, since lens is quite impermeable and aqueous humor flow from posterior to anterior direction prevents drug transfer to the vitreous. The drug concentrations and AUC_{inf} values in the vitreous humor are 1–2 orders of magnitude lower than in the aqueous humor (Figs. 2–3) as it has been observed earlier [34,38,39]. Ratio of AUC_{inf} values between vitreous and aqueous humor is several times higher for atenolol than for timolol and betaxolol (Fig. 4). Atenolol has much lower clearance to the blood flow in the iris-ciliary body than timolol and betaxolol [32], since atenolol does not permeate across the walls of the uveal blood vessels that represent the blood-aqueous barrier. Therefore, atenolol may reach the vitreous at a higher level than timolol and betaxolol. However, the concentrations of all three drugs in the vitreous humor are four orders of magnitude lower than in the tear fluid at early times and bioavailability of topically applied drugs to the vitreous humor is negligible (Fig. 2).

5. Conclusion

The present study shows the influence of lipophilicity on ocular pharmacokinetic processes after topical administration and gives insights on the relative contributions of the corneal and non-corneal routes of drug absorption. Detailed information on the disposition of three drugs to nine different ocular tissues over time following topical administration can be used to build physiological models of drug distribution in the eye with the ultimate aim of developing predictive *in silico* models that would allow the number of preclinical studies in animals to be reduced or replaced. Ultimately speeding up ocular drug development.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejpb.2021.06.003>.

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