



Pharmacokinetics of Azithromycin, Levofloxacin, and Ofloxacin in Rabbit Extraocular Tissues After Ophthalmic Administration

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ABSTRACT

Introduction: Azithromycin demonstrates high tissue distribution and prolonged elimination half-life. In this study, we monitored the pharmacokinetics of a single ophthalmic administration of 1% azithromycin ophthalmic solution containing polycarbophil in the extraocular tissues, including the eyelid, and compared it with that of two commercial ophthalmic products, 1.5% levofloxacin ophthalmic solution and 0.3% ofloxacin ophthalmic ointment.

Methods: Rabbits were treated with either a single topical administration of 1% azithromycin ophthalmic solution, 1.5% levofloxacin ophthalmic solution, or 0.3% ofloxacin ophthalmic ointment. The eyelid, conjunctiva, and cornea were collected at 0.25, 0.5, 1, 2, 4, 8, and 24 h post-administration. Tissue samples were pretreated for drug concentration measurements by ultra-performance liquid chromatography-tandem mass spectrometry. Pharmacokinetic parameters were determined by non-compartmental analysis.

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Results: Azithromycin was rapidly absorbed, and its levels remained near the observed maximum concentrations for up to 24 h post-administration in all tissue. In contrast, extraocular tissue concentrations of levofloxacin and ofloxacin decreased with time. The maximum concentrations of azithromycin, levofloxacin, and ofloxacin were 35.6, 34.1, and 55.1 $\mu\text{g/g}$ in the eyelid, 44.2, 46.8, and 20.4 $\mu\text{g/g}$ in the conjunctiva, and 79.9, 18.0, and 2.21 $\mu\text{g/g}$ in the cornea, respectively. The values of the area under the curve from 0 to 24 h after administration of azithromycin, levofloxacin, and ofloxacin were 602, 58.5, and 267 $\mu\text{g}\cdot\text{h/g}$ in the eyelid, 837, 43.2, and 51.9 $\mu\text{g}\cdot\text{h/g}$ in the conjunctiva, and 1250, 26.4, and 5.46 $\mu\text{g}\cdot\text{h/g}$ in the cornea, respectively.

Conclusion: The drug concentrations of azithromycin and levofloxacin were relatively comparable among the extraocular tissues following topical administration of the respective ophthalmic solutions, whereas the concentrations of ofloxacin varied following dosing of its ophthalmic ointment. The slow elimination profile in any extraocular tissue of rabbits was unique to azithromycin, and led to the demonstration of high exposures of azithromycin in all extraocular tissues after ophthalmic administration.

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Keywords: Azithromycin; Conjunctiva; Cornea; Drug concentration profile; Eyelid; Levofloxacin; Ofloxacin; Rabbit

INTRODUCTION

Extraocular tissues such as the eyelid, conjunctiva, and cornea are exposed to a variety of exogenous materials and microorganisms, including pathogenic bacteria and viruses. Infectious diseases including blepharitis, conjunctivitis, and keratitis are caused by pathogenic bacteria [1–5], which are typically treated with topical formulations of antimicrobial agents [6, 7].

Azithromycin is a broad-spectrum macrolide antibiotic with anti-inflammatory properties. A formulation of 1% azithromycin ophthalmic solution (AzaSite[®] 1% sterile topical ophthalmic drops) approved by the Food and Drug Administration is commercially available in the United States for the treatment of conjunctivitis. In addition, a 2009 US survey on blepharitis found that azithromycin was most frequently prescribed as treatment for this ophthalmologic condition by both ophthalmologists and optometrists [8]. In Japan, a formulation of 1% azithromycin ophthalmic solution (Azimycin[®] ophthalmic solution 1%) containing the same ingredients as AzaSite[®] was recently approved for treatment of extraocular infectious diseases including conjunctivitis and blepharitis.

The 1% azithromycin ophthalmic solution includes a polymeric ocular drug delivery system based on polycarbophil (DuraSite[®]). Topical administration of 1% azithromycin ophthalmic solution with polycarbophil led to a higher azithromycin concentration in the ocular tissues, including the eyelid, compared with that observed after administering a formulation without polycarbophil [9]. Moreover, azithromycin has shown prolonged elimination half-life in ocular tissues [9, 10].

In Japan, 1.5% levofloxacin ophthalmic solution (Cravit[®] ophthalmic solution 1.5%) and 0.3% ofloxacin ophthalmic ointment (Tarivid[®] ophthalmic ointment 0.3%) are

commercially available. Although these formulations are indicated for treatment of blepharitis, their concentration profiles in the eyelid after topical administration have not been reported.

In this report, we assess the pharmacokinetics of azithromycin in the extraocular tissues, including the eyelid, after a single topical dose of 1% azithromycin ophthalmic solution containing polycarbophil to rabbits, which we compare with that of levofloxacin and ofloxacin in these tissues after a single topical administration of the respective ophthalmic formulations.

METHODS

Animals

A total of 42 male Japanese White Rabbits (2.0–2.5 kg) were obtained from Kitayama Labes (Ina, Japan) and housed individually in cages under controlled environmental conditions with ad libitum access to water and fed 100 g dry pellet once daily. All animals were managed in accordance with the local standard operating procedures in a facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All institutional and national guidelines for the care and use of laboratory animals were followed. The study protocol, including all experimental procedures, was approved by the Institutional Animal Care and Use Committee of Senju Pharmaceutical Co.

Drugs

The 1% azithromycin ophthalmic solution containing polycarbophil and other ingredients was prepared according to the formulation approved in Japan. The 1.5% levofloxacin ophthalmic solution (Cravit[®] ophthalmic solution 1.5%; Santen Pharmaceutical Co., Osaka, Japan) and the 0.3% ofloxacin ophthalmic ointment (Tarivid[®] ophthalmic ointment 0.3%; Santen Pharmaceutical Co., Osaka, Japan) were commercially available.

Drug Administration and Ocular Sample Collection

Ophthalmic dosing of any two of the following were applied to the eye of each animal ($n = 4$ eyes per sampling time point): a single dose of 30 μL of 1% azithromycin ophthalmic solution, 30 μL of 1.5% levofloxacin ophthalmic solution, or 46 μL (approximately 40 mg) of 0.3% ofloxacin ophthalmic ointment. At predetermined time points of 0.25, 0.5, 1, 2, 4, 8, and 24 h after dosing, animals were euthanized with more than 100 mg/kg of pentobarbital (intravenous administration), and ocular samples were surgically collected and separated into the eyelid, conjunctiva, and cornea.

Analysis of Drug Concentration

Ocular tissues were pretreated for quantification of each drug using an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) system. Each tissue was homogenized with zirconia balls in water using a beads homogenizer (Precellys[®] Evolution; Bertin Technologies, France). The homogenates were recovered and further pretreated by solid-phase extraction using an Oasis[®] HLB $\mu\text{Elution}$ plate (Waters Corporation, Milford, MA, USA). The UPLC-MS/MS system consisted of a UPLC instrument (Waters Corporation, Milford, MA, USA) and an API 4000 triple quadrupole mass spectrometer (AB SCIEX, Framingham, MA, USA). Phenytoin (FUJIFILM, Wako Pure Chemical Corporation, Osaka, Japan) was used as an internal standard for all analytes, and the analytes and the internal standard were detected in positive ion mode. Lower limits of quantification for azithromycin were 0.0220, 0.0825, and 0.0275 $\mu\text{g/g}$ for the eyelid, conjunctiva, and cornea, respectively, and those for levofloxacin and ofloxacin were 0.0132, 0.0165, and 0.0132 $\mu\text{g/g}$ for the eyelid, conjunctiva, and cornea, respectively. Assay performance was monitored by the quality control samples.

Pharmacokinetic Analysis

The maximum concentration (C_{max}), the time to reach the C_{max} (T_{max}), and the values of the

area under the curve from 0 to 24 h after administration (AUC_{0-24}) were calculated with Phoenix WinNonlin Build 8.1.0 software (Certara L.P., Princeton, NJ, USA) using non-compartmental analysis as the sparse sampling method.

RESULTS

Azithromycin was rapidly absorbed into the extraocular tissues after administration (Table 1; Fig. 1). The azithromycin concentrations in the examined tissues were sustained at nearly C_{max} levels for 24 h after dose administration; i.e., at 24 h post-administration, the concentrations in the eyelid, conjunctiva, and cornea were 50.6%, 84.8%, and 42.2% of their respective C_{max} value. Levofloxacin was rapidly absorbed into the extraocular tissues, but its concentration decreased with time after reaching the C_{max} . At 24 h post-administration, its concentrations in the eyelid, conjunctiva, and cornea were 5.0%, 0.5%, and 0.1% of their respective C_{max} value. Ofloxacin was also rapidly absorbed into the extraocular tissues, and its concentrations decreased with time after the C_{max} . At 24 h after the ofloxacin administration, its concentrations in the eyelid, conjunctiva, and cornea were 16.6%, 2.7%, and 1.7% of their respective C_{max} value.

DISCUSSION

Azithromycin has a long elimination half-life in the ocular tissues after ophthalmic instillation [9, 10]. Similar to previous reports, our study showed that azithromycin concentrations rapidly reached the C_{max} in the extraocular tissues, which were sustained at high levels until 24 h after instilling 1% azithromycin ophthalmic solution. In contrast, we found that after instilling the ophthalmic formulations of levofloxacin or ofloxacin, their concentrations rapidly reached the C_{max} in the conjunctiva and cornea, and then decreased with time. In addition, we report for the first time that the concentrations of these drugs in the eyelid also rapidly reached the C_{max} and decreased with

Table 1 Pharmacokinetic parameters of azithromycin, levofloxacin, and ofloxacin after a single topical administration to rabbits

Tissue	Drug	C_{\max} ($\mu\text{g/g}$)	T_{\max} (h)	AUC_{0-24} ($\mu\text{g}\cdot\text{h/g}$)
Eyelid	Azithromycin	35.6 ± 12.2	0.5	602 ± 77
	Levofloxacin	34.1 ± 9.8	0.25	58.5 ± 12.6
	Ofloxacin	55.1 ± 29.1	0.5	267 ± 24
Conjunctiva	Azithromycin	44.2 ± 2.8	0.5	837 ± 195
	Levofloxacin	46.8 ± 34.6	0.25	43.2 ± 6.9
	Ofloxacin	20.4 ± 3.8	0.25	51.9 ± 6.6
Cornea	Azithromycin	79.9 ± 45.7	4	1250 ± 214
	Levofloxacin	18.0 ± 4.4	0.25	26.4 ± 2.1
	Ofloxacin	2.21 ± 0.57	1	5.46 ± 0.37

C_{\max} represents the mean \pm standard deviation ($n = 4$). T_{\max} represents the mean. AUC_{0-24} represents the mean \pm standard error ($n = 4$ eyes at each time point)

C_{\max} maximum concentration, T_{\max} time to reach C_{\max} , AUC_{0-24} value of area under the curve from 0 to 24 h after administration

time. Thus, our results indicate that after ocular administration, the elimination profile of azithromycin in any extraocular tissue differs from that of levofloxacin or ofloxacin. Azithromycin showed high retention in cells due to its weakly basic nature, which likely led to its prolonged terminal half-life in the body [11]. These characteristics of azithromycin may lead to its slow elimination in the ocular tissues, compared with that of the other two drugs.

Adding polycarbophil to an ophthalmic formulation ensures a high bioavailability of the active pharmaceutical ingredient in the ocular tissues by retaining it on the ocular surface after ophthalmic instillation [9]. We found that the C_{\max} value of azithromycin in any tissue was comparable to or higher than that of levofloxacin and ofloxacin. Moreover, the AUC_{0-24} values of azithromycin in all tissues were higher, and the differences in the AUC_{0-24} values were larger than those in the C_{\max} values. These findings indicate that the high drug exposure in the extraocular tissues observed after the topical instillation of 1% azithromycin ophthalmic solution containing polycarbophil was dependent on both the characteristic azithromycin elimination profile and polycarbophil.

The structural properties of ofloxacin and levofloxacin are extremely similar, as ofloxacin is the racemic mixture of levofloxacin [12–14]. In rabbits, instilling ophthalmic solutions of levofloxacin and ofloxacin resulted in similar drug concentration values and comparable profiles in the cornea [15]. Interestingly, in our study, the pharmacokinetic properties of the 1.5% levofloxacin ophthalmic solution and the 0.3% ofloxacin ophthalmic ointment varied notably in the extraocular tissues. Our data showed that the C_{\max} of ofloxacin in the cornea was eightfold lower than that of levofloxacin. In the conjunctiva, however, the C_{\max} of ofloxacin was twofold lower than that of levofloxacin, although there was an approximately fourfold difference in the dosing amount of active pharmaceutical ingredient between these drugs. Furthermore, the ofloxacin C_{\max} value in the eyelid was higher than that of levofloxacin. These results imply that an ophthalmic ointment is a dose formulation for effectively delivering drugs to the eyelid and conjunctiva with limited delivery to the cornea. Certain ointment characteristics might have contributed to the observed tissue-specific differences in the drug distributions: (1) low

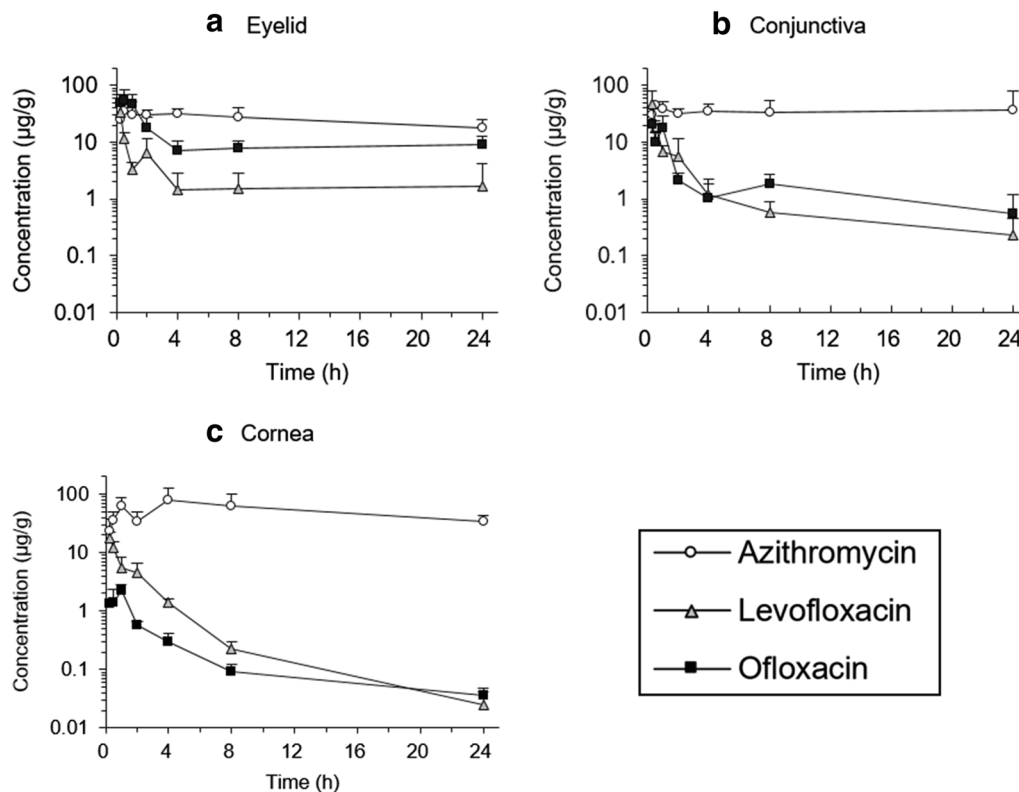


Fig. 1 Time-dependent concentration profiles of azithromycin, levofloxacin, and ofloxacin in rabbit eyelid (a), conjunctiva (b), and cornea (c) after a single topical

administration. The concentration data represent the mean + standard deviation ($n = 4$)

dispersibility from the conjunctival sac contributed to the low accessibility to the cornea and the high distribution to the eyelid and conjunctiva; (2) long-term sustainability at the ocular surface resulted in high penetration of the tissues; and (3) the hydrophobic base affected the tissue permeability. A previous study found that drug concentrations in the eyelid margin after instilling an ophthalmic solution were higher than in other parts of the eyelid [16]. We observed that a portion of ointment leaking from the conjunctival sac was frequently localized to the eyelid margin. The remarkably high C_{max} in the eyelid after administering a dose of ofloxacin ophthalmic ointment might have been caused by high absorption through the eyelid margin such as through the eyelash follicles.

The antimicrobial activity of azithromycin is not as potent as that of levofloxacin or ofloxacin against a large proportion of bacteria

isolated from the ocular surface [17, 18]. In our study, the topical instillation of 1% azithromycin ophthalmic solution containing polycarbophil attained the highest drug exposure in all extraocular tissue among the three drugs, including the ointment formulation. Our data might suggest that the high exposure after instillation of 1% azithromycin ophthalmic solution with polycarbophil could compensate for its moderate antimicrobial activity against the specific bacteria. Furthermore, earlier reports indicated that azithromycin accumulates in polymorphonuclear leukocytes [19, 20], and is distributed to infected tissues after oral dosing [21]. Thus, these specific pharmacokinetic behaviors might cause additional exposure of azithromycin in the infected ocular tissues and improve the antimicrobial efficacy after ophthalmic instillation. In clinical practice, the approved 1% azithromycin ophthalmic solution is indicated for treating conjunctivitis

and can be used in blepharitis therapy in the United States [8].

Our study showed that the decline in levofloxacin and ofloxacin concentrations from the C_{\max} to the levels at 24 h post-administration was slower in the eyelid than in the conjunctiva and cornea. Unlike the conjunctiva and cornea, the eyelid has a complex structure with multiple compartments, including the muscle, skin, meibomian gland, and eyelash. We measured the drug concentrations for the whole eyelid in this study. However, a previous study observed sustained drug concentrations in the eyelid margin but not in the conjunctival epithelium or meibomian gland [16]. Thus, studying intra-tissue concentrations of levofloxacin and ofloxacin in the eyelid may help reveal the reasons for differences in inter-tissue concentrations of the antimicrobial agents observed in our study.

The current study was based on single-dose pharmacokinetic data in the extraocular tissues of rabbits. However, the respective approved indications for 1% azithromycin ophthalmic solution, 1.5% levofloxacin ophthalmic solution, and 0.3% ofloxacin ophthalmic ointment are multiple-dose and different for each drug. Further multiple-dose pharmacokinetic studies with the respective clinical usage would be needed to more precisely estimate the antimicrobial potential of these drugs.

CONCLUSION

Our study showed that there were differences in the drug concentration profiles in the eyelid, conjunctiva, and cornea of rabbits following ophthalmic dosing of azithromycin ophthalmic solution, levofloxacin ophthalmic solution, and ofloxacin ophthalmic ointment. Concentrations of azithromycin or levofloxacin were comparable among these extraocular tissues, whereas ofloxacin concentrations were markedly different. Azithromycin had a slow elimination profile in every extraocular tissue, which differed from the other two drugs. This specific behavior of azithromycin ensured higher drug exposures in these tissues following its

ophthalmic administration, compared with those of the two ophthalmic comparator drugs.

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Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the entire work, and have given their approval for this version to be published.

Disclosures. Tatsuya Sakai is an employee of Senju Pharmaceutical Co. Keisuke Shinno is an employee of Senju Pharmaceutical Co. Masaaki Kurata is an employee of Senju Pharmaceutical Co. Akio Kawamura is an employee of Senju Pharmaceutical Co.

Compliance with Ethics Guidelines. All institutional and national guidelines for the care and use of laboratory animals were followed. The study protocol, including all experimental procedures, was approved by the Institutional Animal Care and Use Committee of Senju Pharmaceutical Co.

Data Availability. The data sets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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