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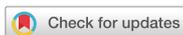
Pharmacokinetic profiles of levofloxacin after intravenous, intramuscular and subcutaneous administration to rabbits (*Oryctolagus cuniculus*)

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ABSTRACT

Levofloxacin pharmacokinetic profiles were evaluated in 6 healthy female rabbits after intravenous (I/V), intramuscular (I/M), or subcutaneous (S/C) administration routes at a single dose of 5 mg/kg in a 3 × 3 cross-over study. Plasma levofloxacin concentrations were detected using a validated Ultra Performance Liquid Chromatography method with a fluorescence detector. Levofloxacin was quantifiable up to 10 h post-drug administration. Mean AUC_{0-last} values of 9.03 ± 2.66, 9.07 ± 1.80, and 9.28 ± 1.56 mg/h*L were obtained via I/V, I/M, and S/C, respectively. Plasma clearance was 0.6 mL/g*h after I/V administration. Peak plasma concentrations using the I/M and S/C routes were 3.33 ± 0.39 and 2.91 ± 0.56 μg/mL. Bioavailability values, after extravascular administration were complete, - 105% ± 27% (I/M) and 118% ± 40% (S/C). Average extraction ratio of levofloxacin after I/V administration was 7%. Additionally, levofloxacin administration effects on tear production and osmolarity were evaluated. Tear osmolarity decreased within 48 h post-drug administration. All 3 levofloxacin administration routes produced similar pharmacokinetic profiles. The studied dose is unlikely to be effective in rabbits; however, it was calculated that a daily dose of 29 mg/kg appears effective for I/V administration for pathogens with MIC < 0.5 μg/mL.

Keywords: Levofloxacin; rabbits; pharmacokinetics; tears; osmolar concentration

INTRODUCTION

Rabbits have a small role as food-producing veterinary species [1]; however, they are frequently kept as companion animals. Like other small mammals, rabbits are susceptible to a variety of microbial infections, with the most common infective organisms identified as *Pasteurella* spp., *Enterobacteriaceae* spp., *Streptococcus* spp., and *Staphylococcus* spp. [2,3].

Fluoroquinolones, among the most important antimicrobial drugs in veterinary medicine [4], are known for their bactericidal action against a broad spectrum of microorganisms and for their high penetration to tissues and intercellular fluid after systemic administration [5,6].

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The developing threat of antimicrobial resistance due to over- or misuse of antimicrobials [5] can limit the use of existing antimicrobial agents, especially fluoroquinolones in veterinary medicine. Fluoroquinolones are used in veterinary medicine based on strong evidence of their efficacy and the lack of alternative treatment options. Therefore, understanding of drug kinetics and efficacy from experimental modeling of as-yet unapproved drugs for animal use can contribute to the potential use of these drugs in the future. At present, levofloxacin is approved for veterinary use in some countries [7] and might be used in other countries where antimicrobial agent use is not regulated/controlled by local laws. Regardless, the authors do not endorse the extra-label use of levofloxacin; instead, we undertook this study to investigate the potential use of levofloxacin in rabbits as a basis for further research.

Levofloxacin, a third-generation fluoroquinolone, is active against a wide range of Gram-positive and Gram-negative microorganisms and has improved activity, compared to older fluoroquinolones, against streptococci and anaerobes [6,8,9]. The pharmacokinetics (PKs) of levofloxacin has already been established in several domesticated mammalian pets [7,10,11], non-pets [12-14], and birds [15-18]. Moreover, there are several research papers published in recent years that show increased interest in levofloxacin having potential application as an off-label drug for some pet animals (dogs). Pharmacokinetic/pharmacodynamic (PK/PD) indices of fluoroquinolones indicate the effectiveness of this class of drugs [19,20], and they imply that levofloxacin has promise in the treatment of infections in animals [7,11].

In rabbits, the PKs of levofloxacin have been studied only after intravenous (I/V) administration, with limited samples taken following drug administration [21]. Further, the animals in that study were infected with *Streptococcus pneumoniae* for use as a model for meningitis; thus, the kinetics obtained may have been altered due to infective processes. Regardless, the full PK profile of levofloxacin in healthy rabbits has not been established.

I/V administration requires specific administration skills and is unlikely to become routinely used in rabbits as prey species are less tolerant of handling than predator species [9]. In contrast, intramuscular (I/M) and subcutaneous (S/C) routes of administration are suitable for use in rabbits [22] as those methods are easily performed, minimizing handling of and stress to the animal. Thus, I/M or S/C administration in rabbits is more convenient and faster for veterinary practitioners, and, in exceptional cases, the drug could even be administered by the owner. Despite all 3 routes of administration being parenteral, the PKs of each route could differ, affecting the onset and duration of action and bioavailability.

Rabbits have been used as a model to test the effects of eye drops containing fluoroquinolones [23,24]. However, there is no data on the effect on tear production and quality after parenteral administration of levofloxacin or any other fluoroquinolone approved for systemic use in rabbits. The ocular surface requires a tear film to cover the eye surface in order to maintain eye health and function. Dry eye syndrome (DES) occurs as a result of decreased tear production or increased tear film evaporation. DES in humans and animals can lead not only to discomfort but also corneal and conjunctival damage. There are reports in humans and animals showing that systemic use of drugs such as beta-blockers, angiotensin-converting enzyme inhibitors, diuretics, and antimicrobials have ocular side effects, and most of those drugs have been reported to cause DES [25-27]. In addition, there is evidence that systemic administration of other antimicrobial agents—sulphonamides—can decrease tear production in rabbits [26].

The aims of this study were to establish and compare the PK profiles of levofloxacin after single administration via I/V, I/M, and S/C routes in healthy rabbits. Subsequently, the antimicrobial efficacy of levofloxacin was predicted based on the area under the concentration-time curve to the minimal inhibitory concentration (AUC/MIC) ratio obtained, and additionally, the effects on tear quantitative and qualitative parameters were assessed.

MATERIALS AND METHODS

Animals

Six cross-bred female rabbits (*Oryctolagus cuniculus*) (body mass 4.21 ± 0.74 kg), 6 months of age at the beginning of the study, were obtained from the animal facility of the Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies. Animals were determined to be healthy based on physical examination, complete blood analysis, and complete ocular examination including biomicroscopy, indirect ophthalmoscopy, and tonometry. Animals received no drug treatment before the study and were allowed to acclimate in their cages for 7 days before the beginning of the study. Rabbits were housed individually in cages under 12-h light/12-h dark cycle with *ad libitum* access to drinking water and hay. Animals were fed standard pelleted food once daily (Purina Professional Rabbit Feed, Purina, USA). The room temperature was maintained at 20°C. Before the study, animals were randomly divided into 3 groups of 2 using research randomizer software. Identifying numbers were placed on each of the animal cages. Animals were weighed immediately before the beginning of the study and before every drug administration period.

Chemicals and reagents

Analytical standard (purity > 98%) levofloxacin and enrofloxacin (used as the internal standard) and tetraethylammonium chloride were purchased from Sigma-Aldrich (USA). Acetonitrile, methanol, sodium dihydrogen phosphate, sodium hydrogen phosphate, chloroform, and isopropanol were of high-performance liquid chromatography grade. A levofloxacin solution (Levoflox 500 mg/100 mL; Claris, India) was used for administration to the animals.

Experimental design and sample collection

A 3-phase, 3-treatment cross-over study design was applied. The experimental protocol was approved by the Animal Ethics Committee of the Republic of Latvia Food and Veterinary Service (Permission 025564). The study was performed according to the guideline for the care and use of laboratory animals. The levofloxacin solution was administered as a single dose of 5 mg/kg body weight. In each phase, doses were administered as follows: I/V route—as a 1 min bolus into the marginal ear vein; I/M route—half of the dose was administered to each of the musculus biceps femoris consecutively (half dose used to avoid muscle damage due to large volume of solution to be administered); S/C route—administered as an injection in the back of the neck region. A fourteen-day washout period was applied, allowing animals to fully clear the drug and to recover from stress related to the experimental procedures. Animal groups for levofloxacin administration were rotated until all 3 phases of the study were completed.

For each phase, a sterile 24G catheter was placed in the central ear artery (for blood collection) and a second one into the marginal ear vein (for I/V drug administration) prior to drug administration on the day of commencement of the experiment. The venous catheter was removed immediately after I/V drug administration while the arterial one remained until

blood collection at 10 h post-administration. Catheters were flushed with heparin containing saline after blood collection, and before any blood collection, the first 0.3 mL of blood were discharged. Blood samples (approximately 0.5 mL) were collected immediately before levofloxacin administration and at 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 24, and 48 h post-administration. Blood samples at 24 and 48 h were collected by syringe from the jugular vein. Collected blood was immediately transferred to lithium-heparin containing test tubes, centrifuged at 1000 × g for 10 min, and the plasma harvested and stored at −20°C until analysis.

Tear fluid evaluations included tests of tear production and tear film osmolarity. All evaluations were conducted the day before levofloxacin administration to obtain baseline values, and then at 1, 4, 8, 10, 24, and 48 h after each levofloxacin administration.

Baseline Schirmer Tear Test values for tear production were obtained with standardized sterile Schirmer Tear Test I (Eickemeyer, Germany) tips that were inserted under the lower lateral eyelid margin for 1 min. The length of the wet section of the STT tip was immediately measured in millimeters (mm/min). Immediately after the STT result was obtained, STT strips were placed into 1.5 mL polypropylene vials and held at −20°C for further quantification of levofloxacin in the lacrimal fluid. Tear production was also evaluated by applying I-TEAR TEST strips (I-MED Pharma Inc., Canada) into both eyes at the same period post levofloxacin solution administration as that for the STT-based evaluations. A strip was applied to the central lower lid tear meniscus without touching the cornea or conjunctiva in accordance with the manufacturer's instructions. The number of millimeters on the strip reached in 5 sec was obtained (unit: mm/5 sec). Tear film osmolarity was assessed by applying the I-PEN VET device (I-MED Pharma Inc.) immediately after the tear production tests were performed. The I-PEN VET sensor was applied to the palpebral conjunctiva until a sound signal, indicating the end of the measurement, was heard (unit: mOsms/L).

Plasma chromatographic analysis

Levofloxacin concentrations in plasma samples were assessed using a Waters Acquity H Class Ultra Performance Liquid Chromatography system equipped with a fluorescence detector (Waters Corporation, USA). The chromatographic analytical method and the sample extraction procedure were based on those previously described by Lee et al. [16]. Briefly, to 200 μL of plasma, 100 μL of 10 μg/mL internal standard solution in methanol, 800 μL of phosphate buffer solution (pH = 7.0), and 4 mL of chloroform:isopropanol (5:1 v/v) were added. The mixture was shaken by a vertical rotating device (Biosan Bio-RS 24, Latvia) at 30 rotations per minute for 20 min, and then centrifuged at 3,000 × g for 10 min at 4°C. Three milliliters of the lower organic layer was transferred into a clean polypropylene tube and evaporated to dryness under a nitrogen stream at 40°C. The dry residue was reconstituted with 200 μL of the mobile phase. One microliter of the resultant solution was injected into the chromatographic system. The chromatographic column used was a Waters Acquity C18 BEH 2.1 × 75 mm with a 1.7 μm particle size (Waters Corporation). The column temperature was maintained at 35°C. The mobile phase was 83% 0.02 M potassium dihydrogen phosphate solution with 0.012 M tetraethylammonium chloride (pH = 2.5) and 17% acetonitrile. The isocratic flow rate was 0.3 mL/min. The fluorescence detector wavelengths were set to 295 nm excitation and 420 nm emission. The sample run time was 5 min.

Chromatographic method validation

Drug-free rabbit plasma was used for both standard curve construction and quality control method validation in accordance with the Guideline on Bioanalytical Method Validation

(EMEA/CHMP/EWP/192217/2009). Drug-free pooled plasma was harvested from all 6 experimental rabbits (2 mL of blood collected) immediately before the beginning of the first phase of the experiment but after the catheters had been placed.

The calibration curve was linear from 0.01 to 10 µg/mL ($R^2 > 0.999$). The levofloxacin recovery from plasma was 96% ± 3.5%. The lower limit of quantification was 0.01 µg/mL. Five level standards of levofloxacin quality controls of 0.01, 0.025, 0.05, 0.5, and 5 µg/mL were used. The between-run accuracy of the method was 1.0%–13.9% and the within-run accuracy was within 15%. The inter- and intra-day precision coefficients of variation were below 5.73% and 5.84%, respectively.

PK analysis

Individual PK parameters were estimated for every animal after treatment using all 3 administration routes. Estimation was performed using non-compartmental analysis and based on visual inspection of the obtained graph (ThothPro Version 1.6.66, Poland). The linear trapezoidal interpolation method was used to calculate the area under the plasma vs. time curve (AUC) after I/V administration, whereas the linear up/log down method was used for the I/M and S/C routes of administration. At least 3 of the last points of the elimination phase of the plasma vs. time curve were used to calculate the elimination constant. The peak plasma concentration (C_{max}), and time to reach peak plasma concentration (t_{max}) were obtained from the data. The bioavailability (F%) was calculated for every single subject as F% = ($AUC_{I/M\ or\ S/C}/AUC_{I/V}$) × 100, and the mean absorption time (MAT) as MAT = $MRT_{I/M\ or\ S/C}$ − $MRT_{I/V}$. Numerical differences of individual $AUC_{0–last}$ values were lower than 20% of $AUC_{0–inf}$, and the R^2 of the terminal phase regression line was > 0.85. Extraction ratio (E%) after I/V administration was calculated using the clearance value after I/V administration and the cardiac output value (i.e., E% = clearance/cardiac output ×100), where cardiac output = 180 × body weight$^{-0.19}$ [28].

PK/PD index

Because the levofloxacin concentrations were below the LOQ at 24 h, in order to predict the $AUC_{0–24h}$ and to calculate the PK/PD surrogates, a dose 5 times that administered was modeled. The levofloxacin concentration values for all sampled times from 0.083 h to 10 h post-administration were multiplied by 5. Applying the superposition principle and assuming the same first-order kinetics [29], approximate values of the concentration at 24 h post-administration were calculated for each rabbit for all 3 routes of administration. The non-compartmental PK analysis was re-run to obtain an $AUC_{0–24h}$ value from this adjusted data, and the PK/PD surrogate $AUC_{0–24h}$/MIC was calculated. Since fluoroquinolones produce a concentration-dependent antimicrobial effect over time [5], a target $AUC_{0–24h}$/MIC ratio for fluoroquinolones of 72 was used [11].

Drug accumulation prediction

A prediction based on a single administration was used to evaluate the possible accumulation ratio (R) at 12 h dosing intervals (τ). The following formula was used [30]:

$$R = \frac{1}{\left[1 - (0.5)^{\tau/t_{1/2}}\right]}$$

where τ is the dosing interval and $t_{1/2}$ is the half-life of elimination.

MIC breakpoints prediction

Based on the equation AUC_{0-24h}/MIC > 72, the antimicrobial activity breakpoint for the theoretically computed dose of 25 mg/kg for rabbits, a MIC < AUC_{0-24h}/72 was assumed to be effective [11]. The AUC was expressed in terms of the unbound drug; levofloxacin was previously reported to be 25% bound to plasma proteins in rabbits [21].

Theoretical effective daily dose calculation

As fluoroquinolones are antimicrobials that possess concentration/time-dependent effects, a theoretical optimal daily dosage was calculated for all 3 routes of administration based on the following formula [19]:

$$Dose\ per\ day\ =\ \frac{\frac{AUC}{MIC}\ \times\ MIC\ \times\ Cl}{fu\ \times\ F}\ /24\ \text{h}$$

where AUC_{0-24h}/MIC is the ratio for optimal efficacy (= 72), Cl = clearance, fu = free fraction of drug in plasma (= 0.75) and F = bioavailability (considered 1 if complete).

Statistical analyses

Statistical analysis was performed using SPSS (version 21.0; IBM Corporation, USA). Most statistical parameters are reported as mean ± standard deviation (SD) values. The exceptions are for plasma half-lives (harmonic means were calculated) and t_{max} (median values are reported). The normality of the data was assessed using the Shapiro-Wilk test. Paired t-tests were used to compare the statistical differences for PK parameters with normal data distributions in different administration groups. Where data did not have a normal distribution (e.g., V_{area}/F after I/M or S/C administration), the Wilcoxon test was applied. The p values lower than 0.05 were considered to indicate statistical significance.

RESULTS

For all 3 administration routes, the drug was quantifiable in plasma for up to 10 h post-administration of 5 mg/kg.

Animals

All 6 animals received levofloxacin via I/V or I/M routes; however, only 4 completed the S/C administration. In the third phase of the cross-over study, 2 animals were excluded—one animal was excluded because of the inability to fix the catheter in either ear artery. The other animal suffered cramps post I/V administration of levofloxacin and died within 48 h post-administration. Post-mortem examination of this animal showed no respiratory tract, kidney, gastrointestinal tract, or liver abnormalities.

The semilogarithmic plots of mean levofloxacin plasma concentrations (± SD) after the 5 mg/kg single dose via all 3 routes of administration are presented in **Fig. 1**. The mean values of PK parameters obtained (± SD) are reported in **Table 1**. The average AUC_{0-last} values were 9.03 (± 2.66), 9.07 (± 1.80) and 9.28 (± 1.56) mg*h/L after I/V, I/M, and S/C administration, respectively. Maximum plasma concentration reached 3.33 (± 0.39) and 2.91 (± 0.56) µg/mL after I/M and S/C administrations, respectively. The mean extraction rate after 5 mg/kg I/V administration was 7.2% ± 2.1%.
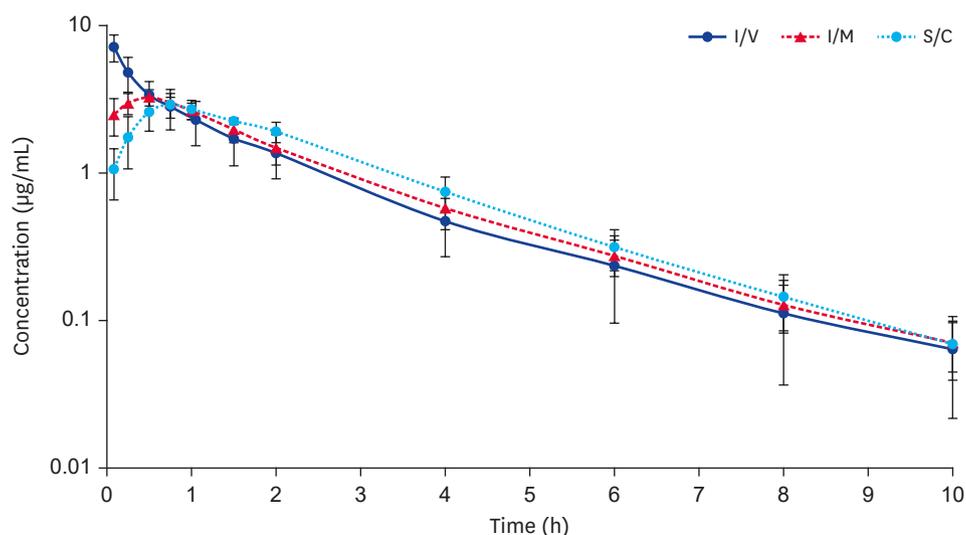
Fig. 1. Semilogarithmic plots of average levofloxacin plasma concentrations in rabbits (error bars represent standard deviations) after I/V (n = 6), I/M (n = 6), and S/C (n = 4) levofloxacin administration of 5 mg/kg bodyweight. I/V, intravenous; I/M, intramuscular; S/C, subcutaneous.

Table 1. Mean (± SD) pharmacokinetic parameters of levofloxacin in plasma following I/V, I/M or S/C administration to rabbits at a dose of 5 mg/kg bodyweight

PK parameters	Units	I/V (n = 6)	I/M (n = 6)	S/C (n = 4)
AUC_{0-last}	mg*h/L	9.03 ± 2.66	9.07 ± 1.80	9.28 ± 1.56
AUC_{0-inf}	mg*h/L	9.08 ± 2.64	9.07 ± 1.80	9.31 ± 1.50
$AUMC_{0-last}$	mg*h*h/L	22.93 ± 12.46	37.87 ± 18.35*	36.62 ± 17.35
$AUMC_{0-inf}$	mg*h*h/L	23.64 ± 12.17	37.89 ± 18.34*	36.98 ± 16.82
C_{max}	µg/mL	N/A	3.33 ± 0.39	2.91 ± 0.56
C_{first}	µg/mL	7.13 ± 1.47	N/A	N/A
t_{max} MEDIAN	h	N/A	0.50 (0.08–0.75)[†]	0.75
$t_{1/2\lambda z}$ HM	h	2.06 ± 0.18	2.01 ± 0.24	1.80 ± 0.14
λ_z	1/h	0.34 ± 0.03	0.34 ± 0.04	0.39 ± 0.03
MRT_{0-last} HM	h	2.19 ± 0.83	3.75 ± 1.16*	3.44 ± 1.31
MRT_{0-inf} HM	h	2.27 ± 0.80	3.75 ± 1.16*	3.52 ± 1.25
MAT HM	h	N/A	1.29 ± 0.61	0.45 ± 1.47
Cl	mL/g*h	0.60 ± 0.18	N/A	N/A
Cl/F	mL/g*h	N/A	0.57 ± 0.11	0.55 ± 0.10
V_{ss}	mL/g	1.37 ± 0.39	N/A	N/A
V_{area}/F	mL/g	N/A	1.66 ± 0.34	1.42 ± 0.18
F	%	N/A	105.69 ± 27.50	118.93 ± 40.51

PK, pharmacokinetic; AUC_{0-last}, area under the plasma-concentration time curve from zero to the last quantified sampling point time; AUC_{0-inf}, area under the plasma-concentration time curve from zero extrapolated to infinity; $AUMC_{0-last}$, area under the first moment curve from zero to the last quantified sampling point time; $AUMC_{0-inf}$, area under the first moment curve from zero extrapolated to infinity; C_{max}, maximum plasma drug concentration; C_{first}, concentration at first sample collection point; t_{max}, time of the maximum plasma concentration; $t_{1/2\lambda z}$, half-life of the elimination part of the curve; λz, slope of the elimination part of the curve; MRT_{0-last}, mean residence time from zero to the last quantified sampling point time; MRT_{0-inf}, mean residence time from zero extrapolated to infinity; MAT, mean absorption time; Cl, total plasma clearance; Cl/F, plasma clearance corrected to the bioavailability; V_{ss}, volume of distribution at steady-state; V_{area}/F, volume of distribution corrected to the bioavailability; n, number of experimental animals receiving levofloxacin via the corresponding route of administration; I/V, intravenous; I/M, intramuscular; S/C, subcutaneous; N/A, not applicable; HM, harmonic mean.
*Significantly different from I/V administration ($p < 0.05$); [†]Range reported.

PK/PD index

The *in silico* obtained AUC_{0-24h} values for the theoretical dose of 25 mg/kg were 44.98 (± 12.54) mg*h/L for I/V administration, 43.11 (± 6.85) mg*h/L for I/M administration, and 43.62 (± 13.65) mg*h/L for S/C administration. The levofloxacin accumulation ratio when administered twice daily (τ =12 h) was predicted to be 1.019 (± 0.006).

To obtain the AUC/MIC of 72, considering that levofloxacin is 25% bound to plasma proteins, it was calculated that 25 mg/kg of levofloxacin by I/V administration would be effective against pathogens with a MIC < 0.47 µg/mL. In the case of I/M and S/C routes of administration, this dose would be effective against pathogens with a MIC < 0.45 µg/mL. Thus, an effective daily dose against pathogens with a MIC of 0.5 µg/mL was calculated for the I/V administration to be 29 (± 8) mg/kg body weight.

## Effects on tear quality

Average tear production observed with STT was 6.4 (± 3.1) mm/min and 7.0 (± 3.1) mm/min, for left and right eyes, respectively (no significant difference, $p = 0.536$). Absolute values varied from 2 to 14 mm/min. No significant changes in tear production were observed among all routes of drug administration within 48 h (data not shown).

Strip meniscometry values, obtained by following the manufacturer's instructions, of 5 mm and higher are considered to indicate normal tear production while smaller values suggest decreased tear production. The average SM measurement results were normal, 6.9 (± 1.3) mm/5 sec and 6.3 (± 1.9) mm/5 sec, for the left and right eyes, respectively (no significant difference, $p = 0.145$). No significant changes in baseline tear production after levofloxacin I/V, I/M, and S/C administration were observed (data not shown).

Tear osmolarity was 324 (± 21) mOsms/L and 331 (± 22) mOsms/L for the 2 eyes prior to drug administration, and the difference was not significant ($p = 0.255$). Mean tear osmolarity decreased in all 3 routes of administration within 48 h after treatment. Changes in tear osmolarity up to 48 h after levofloxacin administration are summarized in **Fig. 2**.

Another area of interest was the quantification of the levofloxacin level in tear fluid in order to evaluate the rationale of ocular infection treatment (conjunctival and corneal infection treatments may be affected by drug distribution in tears). However, the small volume of tear fluid harvested, and the limited sensitivity of the detection method used did not allow quantification of levofloxacin in rabbit tear fluid.
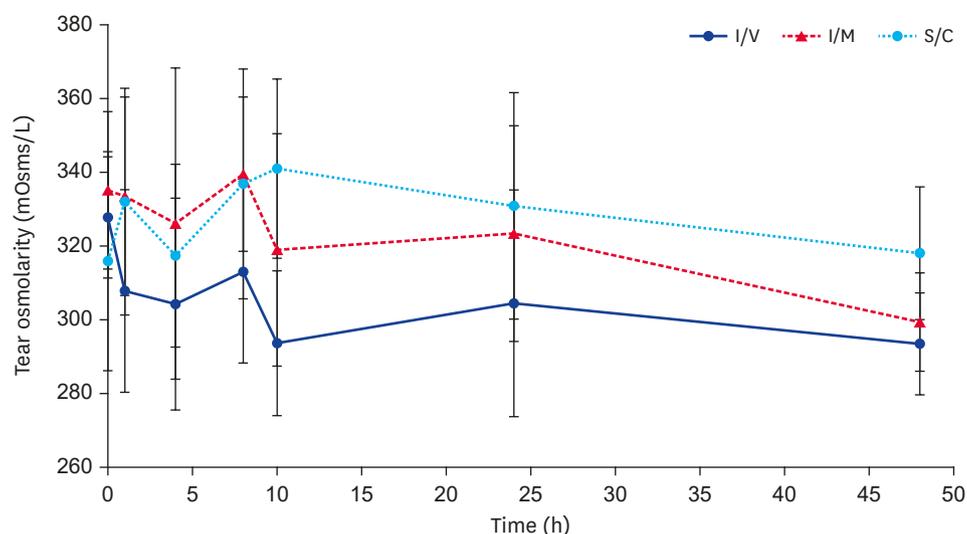
Fig. 2. Changes in tear osmolarity in rabbits after a single 5 mg/kg levofloxacin dose administered via I/V (n = 6), I/M (n = 6), or S/C (n = 4) routes (mean values indicated; error bars represent standard deviation). I/V, intravenous; I/M, intramuscular; S/C, subcutaneous.

DISCUSSION

To the authors' best knowledge, this is the first time levofloxacin PK profiles after I/M and S/C administration in healthy rabbits were evaluated, although I/V administration had been examined previously in rabbits infected by *S. pneumoniae*.

The 5 mg/kg dose used in this study was based on the dose used previously in a levofloxacin study involving broiler chicken [16]. This dose is within the range of doses previously used in other mammalian and bird species [10,15,17,18,31]; a dose associated with reduced risks of side effects. Fluoroquinolones are reported to cause tendon damage, seizures, diarrhea in humans, and blindness in cats [4], and Madsen et al. [11] reported side effects, including vomiting, soft feces, and depression, after I/V administration of 15 mg/kg of levofloxacin in dogs. One rabbit died during the current experiment, and the death may be attributed to the stress of the sampling procedures. While necropsy showed no noticeable organ changes in the rabbit, a single I/V dose of levofloxacin in humans has been reported to produce cardiovascular side effects—increased heart rate and QT interval prolongation [32]; thus, cardiovascular effects may also be involved in the lethal outcome in this individual.

All 3 routes of administration (I/V, I/M, and S/C) used in this study produced very similar results for key PK parameters. This could be explained by the fast absorption and rapid distribution of the drug after the extravascular administration routes mimicking the PK profile of the I/V administration. In this study, the AUC values for all 3 routes of administration were similar, and there was complete (calculated over 100%) systemic bioavailability of levofloxacin reported following both I/M and S/C administration. Maximal plasma concentrations for both extravascular routes were reached at around the same time (30–45 min post-administration) and were of similar value (around 3 μg/mL). Similar parallel results were observed for S/C and I/M mean residence times, clearances, and volumes of distribution compared to those for I/V administration. These similarities in PKs suggest that the same drug efficacy should be expected for all 3 routes of administration when levofloxacin is given at a dose of 5 mg/kg. Moreover, previous studies of other fluoroquinolones in rabbits [33,34] and of levofloxacin in other animal species [11,16,35] showed very similar PK profiles after different routes of administration. The levofloxacin terminal plasma half-life appeared to be one of the shortest among the species tested (1.8–2.06 h, depending on the route of parenteral administration).

The volume of drug distribution at a steady-state after I/V administration of 1.37 L/kg suggests moderate penetration of the drug through the biological membranes of the body. This value is within the range reported in avian and mammalian species, 0.92 L/kg in sheep and 2.88–3.25 L/kg in broiler chickens [15,16,35].

Complete bioavailability of levofloxacin after extravascular administration has also been reported in other species [11,13,16]. Interestingly, other fluoroquinolones studied in rabbits after I/M and S/C administration have also shown complete bioavailability, with actual values exceeding 100% [33,36,37]. This may be due to various factors that have already described in the literature [5,6,38], e.g., non-linear clearance. The I/M administration of orbifloxacin, norfloxacin, danofloxacin, and marbofloxacin have all been reported to exceed the 100% bioavailability level [33,34,36,39]. Moreover, S/C ofloxacin, orbifloxacin, and danofloxacin administration also showed complete bioavailability [33,37,40]. These observations indicate that, in general, fluoroquinolones are well absorbed and widely distributed to plasma after I/M or S/C administration in rabbits.

Compared to the study in rabbits infected with *S. pneumoniae* [21], the AUC values of levofloxacin were much lower (at least twice corrected to the dose administered) in the present study. The plasma terminal half-lives of the drug were at least 3 times longer than that observed in our study. These differences might be due to differences in rabbit breed (New Zealand white vs. cross-bred in this study), size of the animals in the 2 studies (2–3 kg vs. 4.2 kg in this study) and the provision of other drugs (e.g., anesthetic administration in [21]). Additionally, the presence of infection may have slowed the elimination of the drug from the body in a manner similar to that observed in a PK study of marbofloxacin in infected rabbits [39].

The AUC values reported for rabbits appear to be the lowest among the other species studied, taking into account the administered dose differences. This might be related to the rapid elimination of the drug from the rabbit body. The average plasma clearance of levofloxacin was 0.6 mL/g*h with some variability among the experimental animals. This is the highest clearance rate thus far reported in all previous mammalian and avian species studied, except sheep, which had similar reported clearance (0.55 mL/g*h [35] vs. 0.6 mL/g*h in rabbits) and half-life of elimination (2.38 h vs. 2.06 h in rabbits) values. However, another study in sheep [14] showed a lower clearance of 0.2 mL/g*h and a longer elimination half-life (3.3 h), but that study was performed using sheep with a body mass almost twice as large, possibly, resulting in slower drug elimination. The high rate of elimination in rabbits may be due to their high cardiac output and heart rate [41]. Higher clearance in rabbits is observed after administration of other fluoroquinolones; orbifloxacin, norfloxacin, danofloxacin, and moxifloxacin are cleared even faster than levofloxacin with clearance values of 0.9, 0.8, 0.8, and 0.8 mL*g/h, respectively [33,34,36,37]. These results indicate that parenteral fluoroquinolone administration in rabbits will require frequent dosing. Alternatively, the route of administration could be changed to consider practitioners' convenience and/or reduction of the handling stress of the infected animal.

A low extraction ratio (around 7%) may indicate that levofloxacin is not fully metabolized and may be excreted unchanged by the kidney [5,6]. This suggests the use of orally administered dosage forms [28]. Although extraction ratio values were not computed in other species in which levofloxacin PKs were established, we calculated approximate extraction ratios for the above-mentioned studies. Low levofloxacin extraction ratios were predicted in cats, dogs, and rabbits (around 2%) based on the clearance and mean animal body weights reported by Albarellos et al. [10], Landoni and Albarellos [7], Madsen et al. [11] and Destache et al. [21]. In food-producing animals, the levofloxacin extraction rate is also low. Based on data provided by Goudah and Abo-El-Sooud [13], Goudah and Hasabelnaby [14], and Patel et al. [35], the authors have calculated average extraction levels for goats, sheep, and camels of 3.2%, 3.9%, and 9.5%. The estimated extraction ratio values in all of the animal species investigated indicate similar drug elimination abilities among the species.

As the elimination half-life of levofloxacin for all 3 routes of administration was short, frequent administration, which is potentially stressful to the animal, would be required. The authors, therefore, do not suggest than any of these parenteral routes are suitable for regular clinical use of levofloxacin in the studied dosage form. While the therapeutic efficacy of fluoroquinolones may be inferred through PK/PD assessment and the use of the AUC/MIC ratio, the low AUC value and the inability to quantify levofloxacin in rabbit plasma at 24 h post drug administration resulted in the inability to perform these surrogate calculations based on our experimental data. Based on our results, a dose of 5 mg/kg of levofloxacin is unlikely to produce a therapeutic effect in rabbits. Our calculated effective daily dose for

levofloxacin, based on an *Enterobacteriaceae* MIC value of 0.5 µg/mL reported in dogs [11], was 29 (± 8) mg/kg, based on a plasma protein binding value of 25% [21]. The estimate is in agreement with the oral dose of 25 mg/kg in dogs to attain similar PK/PD therapeutic targets. In rabbit management, the oral route for drug administration (in medicated feed or water) is the most common one used. Levofloxacin is reported to have complete oral bioavailability in 2 other mammalian species; dog (104 [± 30]%) [11] and cat (86 [± 43]%) [10]. If this oral trend in oral bioavailability is similar in rabbits, the effective daily dose of levofloxacin reported in the current study could be added to pelleted rabbit food or drinking water. However, as infected animals may lose their appetite while maintaining water intake, we suggest the daily dose could be prepared in 50–100 mL of drinking water (i.e., the average daily water intake of rabbits) [42].

This study is the first to investigate the effect of systemic administration of levofloxacin on ocular parameters. The high variability in the qualitative parameters of tears between individual animals before and after treatment with levofloxacin made identification of trends difficult. The authors suggest that the dose may have been too small or a single administration insufficient to produce any discernible effects on tear production. The basal level of the tear production assessed with STT method (7 [± 3] mm/min) was slightly higher than those reported for English angora rabbits and Dutch rabbits (5.4 and 4.6 mm/min, respectively) [43]. Regardless, tear osmolarity appeared to decrease slightly but significantly ($p = 0.002$) at 48 h after drug administration. The authors, therefore, suggest that levofloxacin administration at 5 mg/kg is unlikely to cause major changes in the qualitative and quantitative properties of tears. However, studies with multiple-dose administration and a larger number of animals are warranted.

In conclusion, a levofloxacin dose of 5 mg/kg is unlikely to be effective in rabbits. Moreover, a single administration of that dose is unlikely to have any effect on tear parameters. Based on our calculations, a daily dose of 29 mg/kg may be effective for I/V administration of levofloxacin, but further PK/PD assessments are required to determine its effects.

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