Research Article

Disposition Kinetics of Levofloxacin in Sheep after Intravenous and Intramuscular Administration

Ayman Goudah¹ and Sherifa Hasabelnaby²

¹ Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, Giza, P.O. Box 12211, Egypt
² College of Pharmacy, Ohio State University, Columbus, OH 43210, USA

Correspondence should be addressed to Ayman Goudah, aymangouda@yahoo.com

Received 31 May 2010; Revised 29 July 2010; Accepted 5 October 2010

Academic Editor: William Ravis

Copyright © 2010 A. Goudah and S. Hasabelnaby. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The present study was planned to investigate the disposition kinetics of levofloxacin in plasma of female native Barky breed sheep after single intravenous (IV) and intramuscular (IM) administration of 4 mg/kg body weight. The concentrations of levofloxacin in the plasma were measured using high-performance liquid chromatography (HPLC) with a UV detector on samples collected at 0, 0.08, 0.16, 0.33, 0.5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 32, and 48 h after treatment. Following intravenous injection, the decline in plasma drug concentration was biexponential with half-lives of $(t_{1/2\alpha}) 0.33 \pm 0.12$ h and $(t_{1/2\beta}) 3.29 \pm 0.23$ h for distribution and elimination phases, respectively. The volume of distribution at steady state $V_{(d(ss))}$ was $0.86 \pm 0.23$ l/kg. After intramuscular administration of levofloxacin at the same dose, the peak plasma concentration ($C_{max}$) was $3.1 \pm 0.35 \mu g/mL$ and was obtained at $1.64 \pm 0.29$ h ($T_{max}$), the elimination half-life ($T_{1/2\beta}$) was $3.58 \pm 0.30$ h, and AUC was $20.24 \pm 1.31 \mu g.h/mL$. The systemic bioavailability was $91.35 \pm 6.81\%$. In vitro plasma protein binding was $23.74\%$. When approved therapy fails, levofloxacin may be used in some countries for therapy of food animals, however, that is not true in the US.

1. Introduction

Levofloxacin is a recently introduced third-generation fluoroquinolones with high activity against a wide spectrum of Gram-positive and Gram-negative bacteria [1]. In human clinical trials, levofloxacin has been found to be very effective in the treatment of infections of upper and lower respiratory tract, genitourinary system, and skin and soft tissue [2]. Compared to other fluoroquinolones, ofloxacin and ciprofloxacin, levofloxacin has more pronounced bactericidal activity against organisms such as Pseudomonas and Enterobacteriaceae [3]. The bactericidal effect of levofloxacin is achieved through reversible binding to DNA gyrase and subsequent inhibition of bacterial DNA replication and transcription [4–6]. Fluoroquinolones act by a concentration-dependent killing mechanism, whereby the optimal effect is attained by the administration of high doses over a short period of time [7]. This concentration-dependent killing profile is associated with a relatively prolonged postantibiotic effect [8]. For this class of antimicrobials, drug exposure, as measured by the area under the plasma concentration versus time curve (AUC), has been used to calculate surrogate efficacy indices, such as the AUC/MIC ratio, where MIC stands for the in vitro minimal inhibiting concentration of the tested bacteria [9–11]. Thus, variations of drug exposure can be associated with variations in the probability of a successful outcome with a specific dosage regime.

The drug undergoes a limited metabolism in rats and human [12] and is primarily excreted by kidney mainly as active drug. Inactive metabolites (N-oxide and desmethyl metabolites) represent <5% of the total dose [13], as other fluoroquinolones are metabolised in chickens as reported in [14–16], as chickens metabolized marbofloxacin to N-desmethyl-marbofloxacin.

The pharmacokinetics of levofloxacin has been investigated in a limited number of animal species including rats [17], rabbits [18], calves [19, 20], goats [21], cats [22], male camels [23], and stallions [24]. However, there is no available information on the kinetics of levofloxacin in the sheep. Therefore, the present study was undertaken to determine
the disposition kinetics and bioavailability of levofloxacin in
sheep following a single intravenous (IV) or intramuscular
(IM) administration of 4 mg/kg bwt.

2. Material and Methods

2.1. Drugs and Chemicals. Levaquin (25 mg/mL of levoflo-
xacin solution) was obtained from Janssen Pharmaceutica N
V (Beerse, Belgium). Ciprofloxacin as internal standard was
purchased from Sigma, Chemical Company (St. Louis, MO,
USA). The solvents (Baker Inc., Phillipsburg, NJ, USA) used
during the chromatographic analysis of the drug were HPLC
grade.

2.2. Experimental Animals. The study was approved by the
Animal Care and Use Committee at the Faculty of Veterinary
Medicine, Cairo University. We used ten female native Barky
breed 2-3 years old, 45-55 kg body weight. The animals were
in optimal nutritional condition, fed on concentrated pellets,
hay, and alfalfa, and had free access to water ad libitum
daily. The health of all animals was monitored prior to and
throughout the experimental period.

2.3. Drug Administration. The study was performed in two
phases, following a crossover design (5 × 5). Animals were
randomly assigned into two groups, with each group con-
taining five animals. In phase one of the experiment, five animals
were given a single intravenous injection into the left jugular
vein at dose of 4 mg/kg bodyweight levofloxacin, and the
other five were injected intramuscularly into the lower third
region of the neck muscles with the drug at the same dose.
Three ml venous whole blood samples were taken by jugular
venepuncture into 10 mL heparinized Vacutainers (Becton
Dickinson vacutainer Systems, Rutherford, NJ, USA). The
sampling times were 0 (blank sample), 0.08, 0.16, 0.33, 0.5,
1, 2, 4, 6, 8, 10, 12, 18, 24, 32, and 48 h after treatment.
All the blood samples were centrifuged at 3000 g for 15 min
to separate the plasma. The plasma samples were frozen at
−20°C until analysed. After a washout period of 2 weeks, the
animals that had been injected intravenously with the drug
were injected intramuscularly and vice versa. Pilot studies
have shown that a 2-week period is enough to avoid carry
over effect. Blood was collected and processed as above.
The heparinized plasma samples were frozen at −20°C and
analyzed by high-performance liquid chromatography
(HPLC). All samples were analyzed within one week after
each experimental phase.

2.4. Analytical Method. Plasma concentrations of lev-
ofloxacin were measured using a modified HPLC method
[25]. Briefly, the HPLC system was performed on Shim-
adzu Liquid Chromatography System (Duisburg, Ger-
many) equipped with an LC9A pump, an automatic sampler
SIL6B, and a UV detector. Class LC 10 software version
1.6 (Shimadzu) was used for data analysis and processing.
Levofloxacin and ciprofloxacin (as internal standard, 99.2
pure, 1 μg/10 μL methanol) were isolated from plasma. The
plasma proteins were removed via methanol precipitation;
200 μL plasma were mixed with 400 μL methanol and vigor-
ously shaken. The precipitated proteins were removed via
centrifugation at 12000 × g for 5 min. Subsequently, 20 μL of
the supernatant were injected onto the column.

The HPLC separation was performed using a reversed-
phase C18 column (Discovery, Supelco, 5 μm, 4.6 mm ×
150 mm) with an injection volume of 20 μL. The mobile phase
consisted of water: acetonitrile (80 : 20, v/v) with 0.3% of
triethylamine and pH adjusted to 3.3 with phosphoric acid,
using an isocratic form with a flow rate of 1.0 mL/min.
The detector wavelength was set at 295 nm. The analytes were
identified from the retention times of 97–99% pure reference
standards.

The calibration curves of plasma were prepared with
seven different concentrations between 0.01 and 10 μg/mL
using blank sheep plasma. A calibration curve was obtained
by plotting the peak height ratio versus the nominal con-
centrations. The equation was calculated by the least-squares
method using linear regression. The limit of quantification
(LQO) based on a signal-to-noise ratio >5 was 0.04 μg/mL
of levofloxacin in supplemented sheep plasma. Under our
experimental conditions, the linearity of the method was
from 0.01 to 10 μg/mL of levofloxacin sheep plasma, and
the value of correlation coefficients (r) was >0.99. The peak
height ratios of an unknown specimen (peak height of lev-
ofloxacin/peak height of internal standard) were compared
with that of the standard.

The precision and accuracy of the method were evaluated
by repetitive analysis of the plasma samples (n = 12) spiked
with different known concentrations of levofloxacin. The
percentage recoveries were determined by comparing the
peak height of blank samples spiked with different amounts
of drug and treated as any sample, with the peak height of
the same standards prepared in phosphate buffer (n = 6).
Intra-assay variations were determined by measuring
six replicates (n = 6) of three standard samples used for
 calibration curves. The intra-assay variation coefficients
were <4.3%. Inters assay precisions were determined by assaying
the three standard samples on three separate days. The Inters assay
variation coefficients were <4.6%. Recovery of levofloxacin
from plasma was found to be 93%.

2.5. In Vitro Plasma Protein Binding. The extent of plasma
protein binding was determined in vitro using ultrafiltration
[26]; antimicrobial-free plasma from sheep fortified with
known concentrations of levofloxacin (0.01, 0.16, 0.32,
1.25, 5, and 10 μg/mL) was used. One mL of each sample
was placed on a conditioned semipermeable membrane
(Centriflow Cones CF-50, Amicon Corp., Lexington, MA,
USA) resting on porous conical polyethylene support on the
top of centrifuge tubes. The tubes were centrifuged at 1500 g
for 45–60 min. Plasma samples and their corresponding
ultrafiltrates were assayed by the same method (HPLC) as
described above. The percentage of plasma protein binding
was calculated according to the following equation:

\[
\text{Protein binding} \% = \frac{\text{Total concentration} - \text{Ultrafiltrate concentration}}{\text{Total concentration}} \times 100.
\]
2.6. Pharmacokinetic Analysis. Pharmacokinetic analysis of plasma levofloxacin concentration versus time data was conducted by noncompartmental analysis using WinNonLin Professional version 4.1 software package (Pharsight Corporation, Mountain View, California). For the intravenous data, the appropriate pharmacokinetic model was determined by visual examination of individual concentration-time curves and by application of Akaike’s Information Criterion (AIC) [27]. The plasma concentration-time relationship was best estimated as a two-compartment open model:

\[ C_p = A e^{-\alpha t} + B e^{-\beta t}, \]

where \( C_p \) is the concentration of drug in the plasma at time \( t \), \( A \) and \( B \) are the zero-time drug intercepts of the distribution and elimination phase expressed as \( \mu g/mL \), \( \alpha \) and \( \beta \) are the distribution and elimination rate constants expressed in units of reciprocal time (h\(^{-1}\)), and \( e \) is the natural logarithm base. The distribution and elimination half-lives \( (t_{1/2A} \) and \( t_{1/2B} \)) were calculated according to standard equations [28], while the volume of distribution at steady state \( (V_{d(ss)}) \) and the mean residence time \( (MRT) \) were calculated according to the following equations \( (V_{d(ss)} = \frac{C_{0}}{\alpha} \) and \( MRT = \frac{AUMC}{AUC} \), respectively.

Following IM administration of levofloxacin, plasma concentrations data were analyzed by both compartmental and noncompartmental methods based on the statistical moment theory [28]. The terminal elimination half-life \( (t_{1/2e}) \) and absorption half-life \( (t_{1/2a}) \) were calculated as \( \ln 2/k_d \) or \( \ln 2/k_a \), respectively, where \( k_d \) and \( k_a \) are the elimination and absorption rate constant, respectively. The areas under the concentration-time curves \( (AUC) \) were calculated by the trapezoidal rule and further extrapolated to infinity by dividing the last experimental plasma concentration by the terminal slope \( (\beta) \). The mean residence time \( (MRT) \) was calculated as \( AUMC/AUC \), where \( AUMC \) is the area under the first moment curve, each individual curve of levofloxacin over time was analyzed to determine the peak concentration \( C_{max} \) (extrapolated from the curve), and the time to peak concentration \( T_{max} \) was read from the data. The systemic clearance was calculated as \( Cl = Dose/AUC \). The absolute bioavailability (F\%) was calculated as \( (AUC_{IM}/AUC_{IV}) \times 100 \). In case of extravascular administration, the volume of distribution at steady state \( (V_{d(ss)}) \) and the systemic clearance \( (Cl_B) \) were calculated according to the following equations \( (V_{d(ss)} = \frac{V}{D/f}) \) and \( Cl_B = \frac{Cl_B}{F} \), respectively.

Pharmacodynamic efficacy of levofloxacin was determined by calculating the \( C_{max}/MIC \) and \( AUC_{24}/MIC \) ratios following IM administrations using the respective mean MIC value for susceptible \( Klebsiella \) spp. (0.06 \( \mu g/mL \)), \( Shigella \) spp. (0.06 \( \mu g/mL \)), \( Salmonella \) spp. (0.12 \( \mu g/mL \)), \( Proteus \) spp. (0.06 \( \mu g/mL \)), and \( Acinetobacter \) spp. (0.12 \( \mu g/mL \)) according to Marshall and Jones [29]; these values were derived from those determined in the studies involving antibacterial activity of levofloxacin against strains isolated from human beings.

3. Results

Clinical examination of all animals before and after each trial did not reveal any abnormalities. No adverse reactions were observed after the single-dose IV or IM administration of levofloxacin in the animals studied. Akaike’s Information Criterion test indicated that a two-compartment model best represented the plasma concentration versus time data after IV administration of levofloxacin in sheep.

The mean plasma concentration-time profiles of levofloxacin following single IV and IM administrations of 4 \( mg/kg \) b.wt are presented graphically in Figure 1. Mean ± SD values of pharmacokinetic parameters estimated from the curve fitting are shown in Table 1. \( In vitro \) plasma protein binding of levofloxacin was 23.74%. Following intramuscular administrations of levofloxacin using MIC \( \leq 0.12 \mu g/mL \), the \( C_{max}/MIC_{90} \) ratio was 25.83-fold, and the \( AUC_{0-24}/MIC_{90} \) ratio was 160.42 h.

4. Discussion

Plasma levofloxacin disposition curves after IV injection were best fit to an open bicompartamental model in all the animals, which is in accordance with the results reported for calves and lactating goats, respectively, [20, 21].

The \( V_{d(ss)} \) is a clearance-independent volume of distribution that is used to calculate the drug amount in the body under equilibrium conditions [30]. Fluoroquinolones are lipid-soluble drugs that have a large volume of distribution [31]. The \( V_{d(ss)} \) for levofloxacin was 0.86 l/kg in sheep indicating a relatively wide distribution after
IV administration and it was slightly differing from that reported for levofloxacin in lactating goats 0.73 L/kg [21] and moxifloxacin in lactating goats (0.79 L/kg) [32]. The discrepancies between values calculated for pharmacokinetic parameters may be attributed to the animal species, the drug formulation employed, the age, size or sex of the animals, to differences in fatty tissue deposits between animal species or breeds, or even to interindividual variations and also due to the method of analysis of the drug [33].

The clearance of levofloxacin in sheep was 0.2 L/h/kg similar to those values reported in calves and lactating goats 0.19 and 0.18 L/h/kg [20, 21], respectively.

The elimination half-life of levofloxacin following IV administration was 3.29 h. This value is close to that reported for levofloxacin in lactating goats 2.95 h [21] and longer than that reported in calves [20] 1.61 h, and shorter than that reported in rabbits 7.50 h [18].

Following intramuscular injection, the present data were best represented by a one-compartment model and the estimated $C_{\text{max}}$ (3.10 μg/mL) was similar to that data reported in calves and lactating goats (3.07 and 3.16 μg/mL) [19, 21], respectively.

The time to reach the maximum concentration of levofloxacin in sheep ($T_{\text{max}} = 1.64$ h) was longer than that recorded levofloxacin in calves (1.00 h) [19] and shorter than that reported in lactating goats 1.76 h [21]. The absorption process of levofloxacin was moderately fast as showed by the absorption rate constant ($k_a$) 1.39 h$^{-1}$, short absorption half-life ($t_{1/2a}$) 0.51 h, and confirmed by the short $T_{\text{max}}$ (1.64 h). The overall MRT was longer for IM administration compared with that for IV injection, with an estimated time of 5.33 h. This was expected as the MRT after IM injection depends on both the disposition and absorption rates. The MRT of levofloxacin was similar to that recorded for calves and lactating goats (5.57 and 5.24 h) [19, 21], respectively.

The elimination half-life value was 3.58 h after IM administration, similar to that recorded for calves and lactating goats (3.67 and 3.64 h) [19, 21], respectively.

The systemic bioavailability of levofloxacin in sheep after IM administration was 91.35%, and the absorption process was rapid with absorption half-life ($t_{1/2a}$) 0.51 h. This value indicates the excellent absorption of the drug from that injection site. This value was similar to values reported for levofloxacin in lactating goats [21] and moxifloxacin in sheep [34] and that reported value was effectively higher than that reported for calves 56.6% [19].

Protein binding has long been considered one of the most important physicochemical characteristics of drugs, playing a potential role in distribution, excretion, and therapeutic effectiveness as a low protein binding generally enables a rapid and extensive distribution into the intracellular and extracellular space [35]. In this study, the in vitro plasma protein binding experiment showed that levofloxacin displayed a low level of binding to plasma proteins (approximately 23.74%) to sheep plasma. The results of in vitro protein binding may differ substantially depending on the methodology and experimental conditions [36]. The low

### Table 1: Mean ± SD plasma pharmacokinetic parameters of levofloxacin (μg/mL) in sheep ($n = 10$) following IV and IM administration at a dose rate of 4 mg/kg b.w.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>IV</th>
<th>IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>h$^{-1}$</td>
<td>2.19 ± 0.17</td>
<td>—</td>
</tr>
<tr>
<td>$k_a$</td>
<td>h$^{-1}$</td>
<td>—</td>
<td>1.39 ± 0.15</td>
</tr>
<tr>
<td>$t_{1/2a}$</td>
<td>h</td>
<td>0.33 ± 0.12</td>
<td>—</td>
</tr>
<tr>
<td>$t_{1/2e}$</td>
<td>h</td>
<td>—</td>
<td>0.51 ± 0.11</td>
</tr>
<tr>
<td>$\beta$</td>
<td>h$^{-1}$</td>
<td>0.19 ± 0.09</td>
<td>—</td>
</tr>
<tr>
<td>$k_d$</td>
<td>h$^{-1}$</td>
<td>—</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>$t_{1/2d}$</td>
<td>h</td>
<td>3.29 ± 0.23</td>
<td>—</td>
</tr>
<tr>
<td>$t_{1/2\alpha}$</td>
<td>h</td>
<td>—</td>
<td>3.58 ± 0.30</td>
</tr>
<tr>
<td>$V_{d(\alpha)}$</td>
<td>L/kg</td>
<td>0.86 ± 0.23</td>
<td>1.02 ± 0.18</td>
</tr>
<tr>
<td>$C_{\text{Lag}}$</td>
<td>L/kg</td>
<td>0.20 ± 0.05</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>$\text{AUC}$</td>
<td>μg.h/mL</td>
<td>21.61 ± 1.24</td>
<td>20.24 ± 1.31*</td>
</tr>
<tr>
<td>$\text{MRT}$</td>
<td>h</td>
<td>4.26 ± 0.94</td>
<td>5.33 ± 1.05*</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>μg/mL</td>
<td>12.17 ± 1.73</td>
<td>3.10 ± 0.35</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>h</td>
<td>—</td>
<td>1.64 ± 0.29</td>
</tr>
<tr>
<td>$F$</td>
<td>%</td>
<td>—</td>
<td>91.35 ± 6.81</td>
</tr>
</tbody>
</table>

$\beta$ ($k_d$): elimination rate constant; $\alpha$ ($k_a$): distribution (absorption) rate constant; $t_{1/2a}$: distribution half-life; $t_{1/2e}$: absorption half-life; $t_{1/2d}$: elimination half-life; $V_{d(\alpha)}$: volume of distribution; $C_{\text{Lag}}$: total body clearance; AUC: area under the curve from zero to infinity by the trapezoidal integral; MRT: mean residence time; $C_{\text{max}}$: maximum plasma concentration; $T_{\text{max}}$: time to peak concentration; F(%): bioavailability; for IM, ($V_{d(\alpha)} = Vd/F$) and $C_{\text{Lag}} = C_{\text{Lag}}/F$

* $P < .05$
protein binding of levofloxacin in sheep plasma proteins is in agreement with previously reported value of 22% in lactating goats [21], 24% in human [12] and 25% in rabbits [18]. Nevertheless, it was relatively lower to that reported (17%) in calves [19].

It has been established that for concentration-dependant fluoroquinolones, the AUC0–24h/MIC90 ratio is the most important efficacy predictor, with the rate of clinical cure being greater than 80% when this ratio is higher than 100–125 [37]. A second predictor of efficacy for concentration-dependent antibiotic is the ratio Cmax/MIC90, considering that values ≥10 would lead to better clinical results [38]. High Cmax/MIC90 ratios have been associated with a lower incidence resistance development [39]. It is suggested that the critical break points determining the efficacy of fluoroquinolones are Cmax/MIC90 ≥8–10, and AUC0–24h/MIC90 ≥100 [38, 39]. The MIC of levofloxacin has not yet been determined for bacteria isolated from sheep. To cover most of the susceptible organisms, in this discussion, the MIC90 of 0.12 μg/mL of levofloxacin has been taken into consideration [29]. Based on this data, a dosage of 4 mg/kg levofloxacin IM in sheep would result in a Cmax/MIC90 ratio of 25.83-fold, which exceeds the recommended ratio of 10. The second surrogate marker AUC0–24h/MIC90 was 160.42 h. Based on the calculated Cmax/MIC90 and AUC0–24h/MIC90, a dosage of 4 mg/kg b.wt. is recommended to treat infections caused by bacteria with MIC ≤0.12 μg/mL.

It can be concluded that levofloxacin administered intravenously or intramuscularly in the applied dosing schedule is efficacious against bacteria with MIC≤0.12 μg/mL. Consequently, levofloxacin could be useful in the treatment of systemic infections in sheep after specific assessment of susceptible micro-organisms, also, when approved therapy fails, levofloxacin may be used in some countries for therapy of food animals, however, that is not true in the US. Only enrofloxacin is approved for beef cattle and cannot be used extralabel.

References


Submit your manuscripts at http://www.hindawi.com