Characterization of the pharmacokinetic disposition of levofloxacin in stallions after intravenous and intramuscular administration

A. GOUDAH*,† 1 
K. ABO EL-SOOUDE 
J.-H. SHIM† 
H.-C. SHIN‡ & 
A. M. ABD EL-ATY*,† 1

*Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt; †Natural Products Chemistry Laboratory, Institute of Agricultural Science and Technology, College of Agriculture and Life Science, Chonnam National University, Buk-gu, Gwangju, Republic of Korea; ‡Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Konkuk University, Kwangjin-gu, Seoul, Republic of Korea


The target of the present study was to investigate the plasma disposition kinetics of levofloxacin in stallions (n = 6) following a single intravenous (i.v.) bolus or intramuscular (i.m.) injection at a dose rate of 4 mg/kg bwt, using a two-phase crossover design with 15 days as an interval period. Plasma samples were collected at appropriate times during a 48-h administration interval, and were analyzed using a microbiological assay method. The plasma levofloxacin disposition was best fitted to a two-compartment open model after i.v. dosing. The half-lives of distribution and elimination were 0.21 ± 0.13 and 2.58 ± 0.51 h, respectively. The volume of distribution at steady-state was 0.81 ± 0.26 L/kg, the total body clearance (Cl tot) was 0.21 ± 0.18 L/h/kg, and the areas under the concentration–time curves (AUCs) were 18.79 ± 4.57 μg.h/mL. Following i.m. administration, the mean t1/2α and AUC values were 2.94 ± 0.78 h and 17.21 ± 4.36 μg.h/mL. The bioavailability was high (91.76% ± 12.68%), with a peak plasma mean concentration (C max) of 2.85 ± 0.89 μg/mL attained at 1.56 ± 0.71 h (T max). The in vitro protein binding percentage was 27.84%. Calculation of efficacy predictors showed that levofloxacin might have a good therapeutic profile against Gram-negative and Gram-positive bacteria, with an MIC ≤ 0.1 μg/mL.

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Dr A. M. Abd El-Aty, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Konkuk University, 1 Hwayang-dong, Kwangjin-gu, Seoul 143-701, Republic of Korea. E-mail: abdelaty44@hotmail.com

†The two authors contributed equally to this paper.

INTRODUCTION

Fluoroquinolones have experienced enormous clinical success in the past 20 years (Cock et al., 2006), both in humans and in veterinary medicine. Levofloxacin [(S)-(−)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid], the active l-isomer of the racemate ofloxacin, is a third-generation fluoroquinolone (Fish, 1997; Martin et al., 1998; Norrby et al., 1998; North et al., 1998). Levofloxacin bactericidal effects are caused by the inhibition of both bacterial DNA gyrase (type-II topoisomerase and topoisomerase IV) (Wolfson & Hooper, 1989; Drlica & Zhao, 1997; Albarellos et al., 2005).

In humans, levofloxacin exhibits a rapid and wide tissue distribution including lung, skin, urinary tract, prostate and other soft tissues and body fluids (Langtry & Lamb, 1998). Several studies have presented levofloxacin as a safe and effective treatment for community-acquired pneumonia, and have indicated it to be at least equivalent to cephalosporins like ceftriaxone and cefuroxime (Shah & Members of the International Study Group, 1997; Norrby et al., 1998). The pharmacokinetic profile of levofloxacin supports once-daily administration and, because of its high tissue distribution, levofloxacin may also be suitable in the treatment of bone diseases (Djabarouti et al., 2004). The drug undergoes a limited metabolic process and is primarily excreted by kidneys, mainly as active drug. Inactive
metabolites (N-oxide and demethyl metabolites) represent <5% of the total dose (Langtry & Lamb, 1998; Hurst et al., 2002). Based on its pharmacological profile, levofloxacin could be a promising therapeutic tool for several soft tissue infections in equines.

Fluoroquinolones are characterized by concentration-dependent bactericidal activities and the abilities to induce a postantibiotic effect against both Gram-positive and Gram-negative bacteria (Spreng et al., 1995). They have some additional characteristics, including low plasma protein binding, and a relatively low minimal inhibitory concentration (MIC) against target micro-organisms (Spreng et al., 1995; Brown, 1996). In recent years, it has been suggested that the optimal dosage should be set in terms of pharmacokinetic–pharmacodynamic (PK/PD) relationships (Schentag, 1999). For this class of antimicrobials, drug exposure, as measured by the area under the plasma concentration vs. 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 (Hyatt et al., 1995; Meinen et al., 1995; Craig, 1998). Thus, variations of drug exposure can be associated with variations in the probability of a successful outcome with a specific dosage regime.

Throughout the literature, there have been multiple reports on the pharmacokinetics of fluoroquinolones in horses and foals (Giguère et al., 1996; Kaartinen et al., 1997; Bermingham et al., 2000; Rebuelto et al., 2000; Haines et al., 2001; Bousquet-Melou et al., 2002; Carretero et al., 2002; Papich et al., 2002; Epstein et al., 2004; Gardner et al., 2004; Davis et al., 2006; Peyrou et al., 2006). Additionally, the pharmacokinetics of levofloxacin has been investigated in a limited number of animal species including rabbits (Mochizuki et al., 1994; Destache et al., 2001), rats (Ito et al., 1999; Cheng et al., 2002), guinea pigs (Edelstein et al., 1996) and calves (Dumka & Srivastava, 2006, 2007). However, there is no available information on the kinetics of levofloxacin in the horses. Therefore, the present study was undertaken to determine the pharmacokinetics and bioavailability of levofloxacin in stallions following a single intravenous (i.v.) or intramuscular (i.m.) administration of 4 mg/kg bwt.

MATERIALS AND METHODS

Drugs and chemicals
Tavanic® (100 mL vial of solution of levofloxacin hemihydrate equivalent to 500 mg (5 mg/mL) levofloxacin was obtained from Aventis (Frankfurt, Germany). Mueller–Hinton agar was supplied by Mast Group Ltd. (Merseyside, UK).

Experimental animals
Six healthy adult stallions aged 5–7 years old and weighing 300–350 kg were enrolled in this study. The animals were housed in a box stall the night before and during the experimental periods. Before the beginning of each trial, the animals were determined to be clinically healthy based on their previous histories and physical examinations. Stallions were fed on commercially-prepared concentrates with alfalfa hay and wheat straw, and were given free access to water. The study was approved by the Bioethics Committee of the Faculty of Veterinary Medicine, Cairo University.

Drug administration
This study was performed in two phases, using a crossover design (3 × 3) with a 15-day washout period. Three animals were given a single i.v. injection of levofloxacin into the left jugular vein at a dose of 4 mg/kg bwt and the other three were injected intramuscularly into the gluteal muscles with the same dose of the drug. Blood samples of 5 mL quantity 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, 0.166, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12, 18, 24, 36 and 48 h postinjection. All of the blood samples were centrifuged at 3000 g for 15 min and the plasma was separated. The plasma samples were frozen at −20 °C until the assay was performed. After a washout period, the animals that had been injected intravenously were injected intramuscularly and vice versa. Blood was collected and processed as indicated above.

Analytical method
Quantitation of levofloxacin in plasma samples was accomplished by a modified agar diffusion bioassay method reported previously by Bennett et al. (1966), Child et al. (1995), Böttcher et al. (2001) and Albarellos et al. (2005) using Escherichia coli (ATCC 10536) as the reference organism. The medium was prepared by dissolving 9.5 g Mueller–Hinton agar in 250 mL distilled water in a 0.5 L flat-bottomed flask, which was autoclaved for 20 min. After cooling to 50 °C in a water bath, 0.4 mL of the diluted suspension of reference organism was added to the media. After the medium was poured (25 mL) and solidified, six wells were cut at equal distances in the solidified bioassay plates. Triplicate plasma samples and a known standard concentration of the drug in plasma were placed directly into the wells without any clean-up step. The standard (in plasma) was included in each assay plate in order to compensate for any plate-to-plate variations. The plates were kept at room temperature for 2 h before being incubated at 37 °C for 18 h. Zones of inhibition were measured using micrometers, and the results from the standards were used to calculate the concentration in each sample. A linear relationship existed between the zone of inhibition and the logarithm of levofloxacin concentrations (0.039–10 μg/mL) in plasma, with a correlation coefficient of 0.995. The limit of quantification was 0.039 μg/mL. The mean percent recovery of levofloxacin (measured by comparing zones of inhibition of the spiked samples with external standards in phosphate-buffered saline) from plasma was 89%. The within-day variation coefficient was <4.3.
Protein binding

The extent of protein binding of various levofloxacin concentrations (0.32, 0.625, 1.25, 2.5 and 5 µg/mL) in triplicate was determined in vitro according to the method described previously by Craig and Suh (1991). This method was based on the diffusion of free antibiotic into the agar medium. The differences in the diameters of the inhibition zones between the solutions of the drugs in the buffer and plasma samples were then estimated.

Pharmacokinetic analysis

A computerized curve-stripping program (R Strip; Micromath Scientific Software, Salt Lake City, UT, USA) was used to analyze the concentration–time curves for each individual animal following the administration of levofloxacin. For i.v. injection, the appropriate pharmacokinetic model was determined by the visual examination of individual concentration–time curves and by application of Akaike’s Information Criterion (AIC) (Yamaoka et al., 1978). The plasma concentration–time relationship was best described as a two-compartment open model with the following equation:

\[ C_p = Ae^{-\alpha t} + Be^{-\beta t} \]

where \( C_p \) is the concentration of drug in the plasma at time \( t \); \( A \) is the intercept of the distribution phase with the concentration axis expressed as µg/mL; \( B \) is the intercept of the elimination phase with the concentration axis expressed as µg/mL; \( \alpha \) is the distribution rate constant expressed in units of reciprocal time (h\(^{-1}\)); \( \beta \) is the elimination rate constant 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 \( t_{1/2b} \), respectively) and the volume of distribution at steady-state (Vdss) were calculated using standard equations (Gibaldi & Perrier, 1982).

Levofloxacin plasma disposition curves after i.m. administration were analyzed following the same procedure as used for i.v. analysis. Each individual curve of levofloxacin over time was analyzed in order to determine the peak concentration (\( C_{\text{max}} \)) and the time to peak concentration (\( T_{\text{max}} \)). The program also calculated the noncompartmental parameters using the statistical moment theory (Yamaoka et al., 1978). The terminal elimination half-life (\( t_{1/2\beta} \)) and absorption half-life (\( t_{1/2a} \)) were calculated as ln2/\( K_{\beta} \) or ln2/\( K_{a} \), respectively. The areas under the concentration–time curves (AUCs) were calculated by the trapezoidal rule, and were 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 (Gibaldi & Perrier, 1982). The systemic clearance was calculated as \( C_l = \text{Dose}/AUC \). The absolute bioavailability (F) was calculated as \( \frac{AUC_{\text{i.m.}}}{AUC_{\text{i.v.}}} \times 100 \).

The pharmacodynamic efficacy of levofloxacin was determined by calculating the \( C_{\text{max}} \) (\( C_p^0 \)) in case of i.v.)/MIC\(_{90}\) and AUC\(_{0-24}/MIC_{90}\) ratios following i.v. and i.m. administration of the drug. There have been no studies reporting the MIC values of levofloxacin from bacteria isolated from horses. Therefore, in order to calculate the PK/PD efficacy predictors, hypothetical MIC values were used. These values were derived from those determined in the studies involving antibacterial activity of levofloxacin against strains isolated from human beings; 0.1 µg/mL for Klebsiella spp., Proteus spp., Enterobacter spp., Salmonella spp. and Staphylococcus spp. (Marshall & Jones, 1993).

Statistical analysis

The mean plasma pharmacokinetic variables of levofloxacin were statistically compared by nonparametric analysis using the Mann–Whitney test and Instat version 3.00 (GraphPad Software, San Diego, CA, USA). Means were considered significantly different at \( P < 0.05 \).

RESULTS

Clinical examination of all animals before and after each trial did not reveal any abnormalities. No toxicity was detectable via physical examination.

Intravenous injection

The concentrations of the drug in the plasma decreased in a biphasic manner, as characterized by a two-compartment open model. A semilogarithmic plot of the mean concentration of levofloxacin in the plasma following i.v. injection of 4 mg/kg bwt is shown in Fig. 1. A summary of pharmacokinetic parameters following i.v. administration is listed in Table 1. In stallions, levofloxacin has a Vdss of 0.81 ± 0.26 L/kg and \( C_l \) of 0.21 ± 0.18 L/h/kg. Its distribution half-life, \( t_{1/2a} \), was 0.21± 0.13 h and the elimination half-life, \( t_{1/2b} \), was 2.58 ± 0.51 h.

![Fig. 1. Mean ± SD plasma concentrations of levofloxacin in stallions after intravenous (■) and intramuscular (●) injection of 4 mg/kg bwt (n = 6).](image-url)
Table 1. Mean ± SD plasma pharmacokinetic parameters of levofloxacin in stallions following i.v. and i.m. administration at a dose rate of 4 mg/kg bwt (n = 6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>i.v.</th>
<th>i.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$ ($k_{ad}$)</td>
<td>h$^{-1}$</td>
<td>3.49 ± 0.62</td>
<td>1.93 ± 0.74*</td>
</tr>
<tr>
<td>$t_{1/2a}$ ($t_{1/2ab}$)</td>
<td>h</td>
<td>0.21 ± 0.13</td>
<td>0.49 ± 0.26*</td>
</tr>
<tr>
<td>$\beta$ ($k_{el}$)</td>
<td>h$^{-1}$</td>
<td>0.28 ± 0.21</td>
<td>0.25 ± 0.34</td>
</tr>
<tr>
<td>$t_{1/2b}$ ($t_{1/2d}$)</td>
<td>h</td>
<td>2.58 ± 0.51</td>
<td>2.94 ± 0.78</td>
</tr>
<tr>
<td>$K_{12}$</td>
<td>h$^{-1}$</td>
<td>1.44 ± 0.82</td>
<td>–</td>
</tr>
<tr>
<td>$V_{dss}$</td>
<td>L/kg</td>
<td>0.81 ± 0.26</td>
<td>–</td>
</tr>
<tr>
<td>$C_{tot}$</td>
<td>L/h/kg</td>
<td>0.21 ± 0.18</td>
<td>–</td>
</tr>
<tr>
<td>$AUC$</td>
<td>µg h/mL</td>
<td>18.79 ± 4.57</td>
<td>17.21 ± 4.36</td>
</tr>
<tr>
<td>$MRT$</td>
<td>h</td>
<td>3.94 ± 0.61</td>
<td>4.72 ± 0.54*</td>
</tr>
<tr>
<td>$C_{max}$</td>
<td>µg/mL</td>
<td>–</td>
<td>2.85 ± 0.89</td>
</tr>
<tr>
<td>$T_{max}$</td>
<td>h</td>
<td>–</td>
<td>1.56 ± 0.71</td>
</tr>
<tr>
<td>$F$</td>
<td>%</td>
<td>–</td>
<td>91.76 ± 12.68</td>
</tr>
</tbody>
</table>

$\alpha$, distribution rate constant; $\beta$, elimination rate constant; $t_{1/2a}$, distribution half-life; $t_{1/2b}$, absorption half-life; $t_{1/2d}$, elimination half-life; $K_{12}$ and $K_{21}$, first-order rate constants for drug distribution between the central and peripheral compartments; $V_{dss}$, volume of distribution at steady-state; $C_{tot}$, total body clearance; $AUC$, area under the curve from zero to infinity by the trapezoidal integral; $MRT$, mean residence time; $MAT$, mean absorption time; $C_{max}$, maximum plasma concentration; $T_{max}$, time to peak concentration; $F$, bioavailability.

*P < 0.05; **P < 0.01.

Intramuscular injection

The dispositions of i.m.-administered levofloxacin in stallions were best fitted to a two-compartment open model with first-order absorption (Fig. 1). Levofloxacin was absorbed rapidly after i.m. administration and the maximum plasma concentration ($C_{max}$) was attained at 1.56 ± 0.71 h ($T_{max}$) after injection. The area under the curve ($AUC$) was 17.21 ± 4.36 µg h/mL, the mean residence time ($MRT$) was 4.72 ± 0.54 h, and the half-life ($t_{1/2d}$) was 2.94 ± 0.78 h. The systemic bioavailability was 91.76% ± 12.68%.

Statistical analysis

Statistical analysis of the plasma pharmacokinetic parameters revealed significant differences in the distribution (absorption) rate constant, distribution (absorption) half-life, and $MRT$ between i.v. and i.m. administration.

Protein binding

The in vitro protein binding percent of levofloxacin in stallion plasma ranged from 20% to 29% with average of 27.84%.

Efficacy predictors

Following i.v. and i.m. administration of the drug, the $C_{max}$/MIC ratios were 102.1- and 28.5-fold, and the $AUC_{0-24}$/MIC ratios were 182.9 and 167.3 h, respectively.

DISCUSSION

In the current investigation, we measured the residual concentrations of levofloxacin in the plasma of stallions using the microbial inhibition test. The reasons that we selected the bioassay are: (a) the bioassay measures the total activity, which could be more useful for pharmacodynamic evaluations than HPLC (McKellar et al., 1999); (b) congruent results between the data determined by the microbiological assay and those determined by HPLC (Auten et al., 1991; Böttcher et al., 2001); (c) the bioassay method is precise, reproducible, and does not require specialized equipment or toxic solvents (Ev Lda & Schapoval, 2002); and (d) because there is no report on the clinically relevant active metabolites in animal species or human beings. For these reasons, the application of the microbiological assay for measuring levofloxacin concentration is suitable (Albarellos et al., 2005).

Intravenous administration

Plasma levofloxacin disposition curves after i.v. injection were best fit to an open two-compartmental model in all stallions, which is in accordance with the results reported for rabbits (Destache et al., 2001), cats (Albarellos et al., 2005) and calves (Dumka & Srivastava, 2007). The Vdss is a clearance-independent volume of distribution that is used to calculate the drug amount in the body under equilibrium conditions (Toutan & Bousquet-Melou, 2004). Fluoroquinolones are lipid-soluble drugs that have a large volume of distribution (Brown, 1996). In the current study, however, the Vdss for levofloxacin in stallions was relatively small 0.81 L/kg (less than unity). The present finding was lower than that reported for enrofloxacin, orbifloxacin and marbofloxacin in horses (Papich et al., 2002; Davis et al., 2006; Peyrou et al., 2006); respectively. Such differences are relatively common and are frequently related to inter-species variation, assay methods used, the amount of time between blood samplings, and/or the health status and age of the animal (Haddad et al., 1985).

The high value of $AUC$ 18.79 µg h/mL is consistent with that recorded for enrofloxacin (Peyrou et al., 2006) and stands in contrast to that reported for marbofloxacin (Peyrou et al., 2006) in horses.

The clearance of Levofloxacin in stallions (0.21 L/h/kg) was similar to the value reported for enrofloxacin (0.19 L/h/kg, Papich et al., 2002) and was somewhat slower than those of marbofloxacin and orbifloxacin in horses (0.28 L/h/kg, Peyrou et al., 2006; Davis et al., 2006, respectively).

Intramuscular administration

The rapid appearance of levofloxacin in plasma following i.m. administration suggested that the drug could reach the central compartment rapidly. Average plasma concentration of 0.032–0.5 µg/mL has been reported to be the median minimum inhibitory concentration (MIC$_{50}$) of levofloxacin against most...
Gram-positive, Gram-negative and atypical bacteria (Marshall & Jones, 1993; Chulavatnatol et al., 1999). Keeping in mind the synergistic effects of the immune system and other in vivo factors, and to cover most susceptible organisms, the 0.1 μg/mL MIC of levofloxacin has been taken into consideration in this discussion. The peak plasma level of levofloxacin attained in this study was approximately 28.5-fold higher than the target MIC of levofloxacin, and the drug was detected above the minimum therapeutic plasma level 24 h after administration. Similar to our findings, a peak plasma concentration of 3.07 μg/mL was attained after a single i.m. injection of levofloxacin in calves (Dumka & Srivastava, 2006). The high value of AUC 17.21 μg.h/mL in this study is inconsistent with 4.69 μg.h/mL of norfloxacin-glycine acetate in horses (Park & Yun, 2003). The time required to reach the maximum concentration of levofloxacin in stallions (T_{max} = 1.56 h) was longer than that recorded for norfloxacin-glycine acetate in horses (0.86 h) (Park & Yun, 2003). The MRT of levofloxacin (4.72 h) was lower than that recorded for norfloxacin-glycine acetate (29.51 h) (Park & Yun, 2003). The mean terminal half-life of levofloxacin (2.94 h) was lower than that recorded for marbofloxacin, norfloxacin-glycine acetate and danofloxacin in horses (5.47, 9.47 and 5.36 h, respectively) (Carretero et al., 2002; Park & Yun, 2003; Fernández-Varón et al., 2006, respectively).

The systemic bioavailability of levofloxacin in stallions after i.m. administration was 91.76%, which indicates good absorption of the drug from the site of injection. This value was similar to those reported for marbofloxacin and danofloxacin in horses (5.7, 9.47 and 5.36 h, respectively) (Carretero et al., 2002; Fernández-Varón et al., 2006; respectively), whereas it was higher than that reported following i.m. administration of norfloxacin-glycine acetate (55%; Park & Yun, 2003).

**Protein binding**

The mean binding of levofloxacin to the plasma proteins of stallions (27.84%) was in accordance with the corresponding values of 24% in humans (Langtry & Lamb, 1998) and similar to 17–24% for orbifloxacin in horses (Davis et al., 2006). This low degree of protein binding will not inhibit the distribution of the drug to the interstitial fluid (the site of action for most antibacterial drugs), and interstitial fluid drug concentrations are expected to equal the plasma concentrations (Davis et al., 2006).

**Efficacy predictors**

It has been established that for concentration-dependant fluoroquinolones, the AUC_{0–24}/MIC\_90 ratio is the most important predictor of efficacy with a clinical cure rate greater than 80% when this ratio is higher than 100–125 (Lode et al., 1998). A second predictor of efficacy for concentration-dependent antibiotics is the C_{max}/MIC\_90 ratio, considering that values ≥10 lead to better clinical results (Toutain et al., 2002). It is now accepted that high C_{max}/MIC\_90 values are necessary in order to avoid the emergence of bacterial resistance (Walker, 2000). It is suggested that the critical breakpoints that determine the efficacy of fluoroquinolones are C_{max}/MIC\_90 ≥ 10 and AUC_{0–24}/MIC\_90 ≥ 100 (Walker, 2000; Toutain et al., 2002). Considering the AUC_{0–24}/MIC\_90 and C_{max}/MIC\_90 ratios determined in this study, the C_{max}/MIC\_90 ratios were 102.1- and 28.5-fold and the AUC_{0–24}/MIC\_90 ratios were 182.9 and 167.3 h, following i.v. and i.m. administration of levofloxacin, respectively.

It can be concluded that levofloxacin administered intravenously or intramuscularly in the applied dosing schedule is efficacious against bacteria with MIC ≤ 0.1 μg/mL. Consequently, levofloxacin could be useful in the treatment of systemic infections in stallions after specific assessment of susceptible micro-organisms. However, further studies are needed to establish an appropriate multiple dosage regime and clinical efficacy.

**REFERENCES**


