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

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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 (*AUC*s) were 18.79 ± 4.57 μg.h/mL. Following i.m. administration, the mean $t_{1/2el}$ 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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INTRODUCTION

Fluoroquinolones have experienced enormous clinical success in the past 20 years (Czock *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, 2003; Swoboda *et al.*, 2003; Martinez *et al.*, 2006) with a wide spectrum of bactericidal activity *in vitro* (Davis & Bryson, 1994; Martin et al., 1998). The spectrum of activity includes Gram-positive aerobic organisms such as *Streptococcus pneumoniae* and *Staphylococcus aureus*, Gram-negative bacteria such as *Escherichia coli*, *Moraxella catharralis*, *Haemophilus influenzae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, and intracellular pathogens responsible for atypical pneumonia, including *Legionella*, *Mycoplasma*, *Chlamydia* and *Mycobacterium* spp. (Davis & Bryson, 1994; Klesel *et al.*, 1995; Eliopoulos *et al.*, 1996; Fish & Chow, 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; α is the distribution rate constant expressed in units of reciprocal time (h^{-1}); β 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 ($t_{1/2\alpha}$ and $t_{1/2\beta}$, 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_{max}) and the time to peak concentration (T_{max}). The program also calculated the noncompartmental parameters using the statistical moment theory (Yamaoka *et al.*, 1978). The terminal elimination half-life ($t_{½el}$) and absorption half-life ($t_{½ab}$) were calculated as $\ln 2/K_{el}$ or $\ln 2/K_{ab}$, respectively. The areas under the concentration–time curves (*AUC*s) were calculated by the trapezoidal rule, and were further extrapolated to infinity by dividing the last experimental plasma concentration by the terminal slope (β). 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 Cl = Dose/*AUC*. The absolute bioavailability (F) was calculated as $AUC_{i.m.}/AUC_{i.v.} \times 100$.

The pharmacodynamic efficacy of levofloxacin was determined by calculating the C_{max} (Cp^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 Cl_{tot} of 0.21 ± 0.18 L/h/kg. Its distribution half-life, $t_{1/2\alpha}$, was 0.21± 0.13 h and the elimination half-life, $t_{1/2\beta}$, was 2.58 ± 0.51 h.

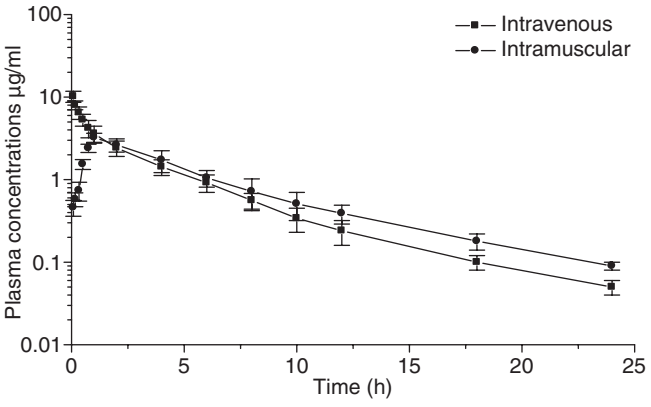

Fig. 1. Mean ± SD plasma concentrations of levofloxacin in stallions after intravenous (■) and intramuscular (•) injection of 4 mg/kg bwt (n = 6).

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)

Parameters	Unit	i.v.	i.m.
α (k_{ab})	h^{-1}	3.49 ± 0.62	1.93 ± 0.74**
$t_{1/2\alpha}$ ($t_{1/2ab}$)	h	0.21 ± 0.13	0.49 ± 0.26*
β (k_{el})	h^{-1}	0.28 ± 0.21	0.25 ± 0.14
$t_{1/2\beta}$ ($t_{1/2el}$)	h	2.58 ± 0.51	2.94 ± 0.78
K_{21}	h^{-1}	1.44 ± 0.82	–
K_{12}	h^{-1}	1.67 ± 0.86	–
Vdss	L/kg	0.81 ± 0.26	–
Cl_{tot}	L/h/kg	0.21 ± 0.18	–
AUC	μg·h/mL	18.79 ± 4.57	17.21 ± 4.36
MRT	h	3.94 ± 0.61	4.72 ± 0.54*
MAT	h	–	1.26 ± 0.64
C_{max}	μg/mL	–	2.85 ± 0.89
T_{max}	h	–	1.56 ± 0.71
F	%	–	91.76 ± 12.68

α, distribution rate constant; β, elimination rate constant; $t_{1/2\alpha}$, distribution half-life; $t_{½ab}$, absorption half-life; $t_{1/2\beta}$ ($t_{½el}$), elimination half-life, K_{12} and K_{21} first-order rate constants for drug distribution between the central and peripheral compartments, Vdss, volume of distribution at steady-state; Cl_{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 2.85 ± 0.89 μg/mL (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, $t_{1/2el}$, 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_{90}) 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_{90} 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 (87.9 and 88.48%, 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 $C_{max}/MIC_{90} \geq 10$ and $AUC_{0-24}/MIC_{90} \geq 100$ (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 $MIC \leq 0.1$ 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.

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