

Pharmacokinetics of marbofloxacin in horses

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Summary

Marbofloxacin is a fluoroquinolone antibiotic expected to be effective in the treatment of infections involving Gram-negative and some Gram-positive bacteria in horses. In order to design a rational dosage regimen for the substance in horses, the pharmacokinetic properties of marbofloxacin were investigated in 6 horses after i.v., subcutaneous and oral administration of a single dose of 2 mg/kg bwt and the minimal inhibitory concentrations (MIC) assessed for bacteria isolated from equine infectious pathologies. The clearance of marbofloxacin was mean \pm s.d. 0.25 ± 0.05 l/kg/h and the terminal half-life 7.56 ± 1.99 h. The marbofloxacin absolute bioavailabilities after subcutaneous and oral administration were $98 \pm 11\%$ and $62 \pm 8\%$, respectively. The MIC required to inhibit 90% of isolates (MIC₉₀) was 0.027 μ g/ml for enterobacteriaceae and 0.21 μ g/ml for *Staphylococcus aureus*.

The values of surrogate markers of antimicrobial efficacy (AUC, C_{max}/MIC ratio, time above MIC₉₀) were calculated and the marbofloxacin concentration profiles simulated for repeated administrations. These data were used to determine rational dosage regimens for target bacteria. Considering the breakpoint values of efficacy indices for fluoroquinolones, a marbofloxacin dosage regimen of 2 mg/kg bwt/24 h by i.v., subcutaneous or oral routes was more appropriate for enterobacteriaceae than for *S. aureus*.

Introduction

Marbofloxacin is a fluoroquinolone antibiotic expected to be effective in the treatment of infections involving Gram-negative and some Gram-positive bacteria in horses. Fluoroquinolones act as concentration-dependent antibiotics for Gram-negative bacteria, whereas their action against certain Gram-positive bacteria is generally considered to be time-dependent.

Several surrogate efficacy indices have been proposed to rationalise antimicrobial therapy in human and veterinary medicine. These are hybrid indices in that they rely on both pharmacokinetic (PK) and pharmacodynamic (PD) properties of drugs.

The clinical outcome, when antibiotics exhibiting a concentration-dependent killing mechanism are used, is correlated with 2 main indices: (i) the area under the inhibitory concentration-time curve (AUC), which combines data from *in vitro* susceptibility testing such as minimal inhibitory concentrations

(MIC) or MIC₉₀ (MIC for which 90% of the isolates of the same genus and species are inhibited) and the drug exposure level of patients (as measured by AUC, i.e. the area under the plasma concentration vs. time curve); (ii) the peak concentration/MIC ratio, which is also linked with a lower incidence of development of bacterial resistance (Duddley 1991; Hyatt *et al.* 1995). In contrast, the activity of time-dependent antibiotics relies on the maintenance of a minimal plasma concentration throughout the dosage interval, represented by T_{>MIC}, the time that plasma concentration exceeds the MIC (Garraffo *et al.* 1990).

The objective of the present study was to evaluate the main pharmacokinetic parameters of marbofloxacin in the horse after i.v., subcutaneous (SC) or oral administration, to report the susceptibility of germs of equine origin and to compute different surrogate efficacy indices, in order to design a rational dosage regimen for marbofloxacin in horses.

Materials and methods

Horses and material design

Six saddle-bred horses (3 geldings, 3 females) weighing 497–639 kg were used. They received 3 meals of a commercial food per day (5–6 kg/day). They were placed in straw-bedded boxes and given water *ad libitum*.

The horses (1 female, 1 gelding) were assigned randomly to 3 groups in a 3 period crossover study with respect to the route of administration. A 15 day washout period was observed between administrations. The horses were weighed the day before each drug administration to determine the required marbofloxacin dose. The nominal dose was 2 mg/kg bwt whatever the administration route.

A 10% w/v marbofloxacin solution (V1205)¹ was used for the i.v. and SC injections. The i.v. injection was made in the right jugular vein via a catheter. The SC administration was performed in the right side of the neck.

For the oral administration, a 5% w/v solution (V6203)¹ was mixed with 250 g bran and given before the morning meal. The horses had no access to straw during the trial.

Blood samples (5 ml) were collected into heparinised tubes by direct venipuncture of the left jugular vein and centrifuged within 1 h (1400 g, 10 min, 10–12°C). The plasma was stored at -20°C until assayed.

Blood samples were taken just before and 1, 2, 4, 8, 15, 30 min, 1, 2, 4, 8, 24, 36, 48 and 72 h after i.v. administration, and just before and 10, 20, 30 min, 1, 2, 3, 4, 6, 8, 24, 36, 48 and 72 h after SC and oral administrations.

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Marbofloxacin assay

Plasma marbofloxacin concentrations were measured using a validated HPLC method (Schneider *et al.* 1996). The limit of quantification was 0.01 µg/ml. The extraction recoveries exceeded 70%. The repeatability and reproducibility, based on the coefficients of variation, were below 7%.

Pharmacokinetic analysis

Both compartmental and statistical moment approaches were used for the pharmacokinetic analysis of marbofloxacin concentrations, using a programme adapted from Multi (Yamaoka *et al.* 1981).

The plasma concentration vs. time curves were fitted with polyexponential equations and the number of exponents determined by application of the Akaike's Information Criterion (AIC; Yamaoka *et al.* 1978). The parameters were estimated by nonlinear regression. The data points were weighted with the inverse of the squared fitted value.

Intravenous administration: A tri-exponential equation was selected for the i.v. administration:

$$C(t) = Y_1 e^{-\lambda_1 t} + Y_2 e^{-\lambda_2 t} + Y_3 e^{-\lambda_3 t} \quad \text{Equation 1}$$

where $C(t)$ represents the marbofloxacin plasma concentration at time t ; Y_1 , Y_2 and Y_3 are the plasma concentrations extrapolated to time 0 of the first, second and third phases of marbofloxacin plasma disposition and λ_1 , λ_2 and λ_3 are the slopes of the first, second and third phases of marbofloxacin plasma disposition.

The elimination half-life and the volumes of distribution were calculated from the classical equations associated with compartmental analysis (Gibaldi and Perrier 1982). The area under the curve ($AUC_{(0-\text{inf})}$) was calculated using the linear trapezoidal rule with extrapolation to infinity. The extrapolated part of the curve ($AUC_{(C_{\text{last}} - \text{inf})}$) was estimated using equation 2 :

$$AUC_{(C_{\text{last}} - \text{inf})} = C_{\text{last}}/\lambda_z \quad \text{Equation 2}$$

where C_{last} is the last observed concentration and λ_z is the slope of the last phase obtained from the compartmental analysis. The extrapolated part of the AUC was always <10% of the total area.

The plasma clearance (Cl) was calculated using equation 3:

$$Cl = \text{Dose}/AUC_{(0 - \text{inf})} \quad \text{Equation 3}$$

where Dose is the actual administered dose. The mean residence time (MRT) was calculated using the linear trapezoidal rule with extrapolation to infinity.

Extravascular administrations: A tri-exponential equation describing a bicompartamental open model with first-order absorption was selected for the SC administration:

$$C(t) = -(Y_1 + Y_2)e^{-k_a t} + Y_1 e^{-\lambda_1 t} + Y_2 e^{-\lambda_2 t} \quad \text{Equation 4}$$

Equation 4 incorporating a lag time (t_{lag}) was selected for the oral route :

$$C(t) = -(Y_1 + Y_2)e^{-k_a(t - t_{\text{lag}})} + Y_1 e^{-\lambda_1(t - t_{\text{lag}})} + Y_2 e^{-\lambda_2(t - t_{\text{lag}})} \quad \text{Equation 5}$$

In equations 4 and 5, Y_1 and Y_2 are the plasma concentrations of the first and second phases of plasma concentration decrease extrapolated to time 0; λ_1 and λ_2 are the first-order rate constants of the first and second phases of plasma concentration decrease and k_a is the apparent absorption rate constant.

The observed maximum concentration ($C_{\text{max,obs}}$) and its occurring time ($T_{\text{max,obs}}$) were obtained from the observed concentrations. C_{max} and T_{max} were also calculated by solving equations 4 and 5. The absolute bioavailability (F%) of marbofloxacin after SC and oral administrations was calculated using the ratio of the $AUC_{(0-\text{inf})}$ obtained after extravascular ($AUC_{\text{SC,oral}}$) and i.v. ($AUC_{\text{i.v.}}$) administrations of the same marbofloxacin dose (equation 6):

$$F(\%) = \frac{AUC_{\text{SC,oral}}}{AUC_{\text{i.v.}}} \times 100 \quad \text{Equation 6}$$

Pharmacodynamics

In vitro susceptibility tests: Strains of enterobacteriaceae, *S. aureus*, *Streptococci* and *Pseudomonas aeruginosa* collected in France and the UK were tested using a microdilution technique. Enterobacteriaceae were isolated from cutaneous, digestive and uterine pathologies. *S. aureus* strains were isolated from cutaneous diseases. *Pseudomonas aeruginosa* and *Streptococcus* spp. were isolated from cutaneous and respiratory pathologies. The concentrations of marbofloxacin which inhibited the visible growth of 50% and 90% of the strains tested in each species were designated MIC_{50} and MIC_{90} .

Pharmacodynamic surrogate markers: Three efficacy indices were computed, using pharmacokinetic parameters and selected MIC_{90} values. AUC_{24} was defined as the area under the curve for the period of time ($T_1 - T_2$) that the concentrations were above the MIC, divided by the MIC (Sanchez-Navarro and Sanchez Recio 1999). AUC_{24} is generally calculated over a 24 h period. In this case, AUC_{24} (h) was calculated using equation 7:

$$AUC_{24} = AUC_{[T_1 - T_2]}/MIC_{90} \quad \text{Equation 7}$$

The C_{max}/MIC_{90} ratio was calculated using fitted C_{max} values.

The mean pharmacokinetic parameters obtained for each administration route were used to predict plasma marbofloxacin concentrations up to 48 h after a single marbofloxacin administration (1 or 2 mg/kg bwt), up to 168 h after daily administration for 5 days (1, 2 or 3 mg/kg bwt) or after twice daily administration for 5 days (1 or 2 mg/kg bwt). These mean curves were used to compute the times above selected MIC_{90} ($T_{>MIC}$).

Statistical analysis

The statistical analyses were performed using Statgraphics Plus Software package (version 5.0)². The arithmetic means and s.d. of the different parameters were calculated. Harmonic means were calculated for the half-lives of elimination and absorption and their s.d. computed using a Jackknife technique (Lam *et al.* 1985). A classical additive linear model was used for statistical analysis of the different parameters (AUC, MRT and half-life). The factors tested were administration route, period, sequence and animals

TABLE 1: Pharmacokinetic parameters describing the plasma disposition of marbofloxacin after a single i.v., subcutaneous (SC) and oral administration of marbofloxacin at a dose level of 2 mg/kg bwt in 6 horses. Data are presented as mean \pm s.d.

Parameters (units)	Route of administration		
	i.v.	SC	Oral
Cl (l/kg bwt/h)	0.249 \pm 0.045	--	--
V _{ss} (l/kg bwt)	1.48 \pm 0.30	--	--
V _{area} (l/kg bwt)	2.83 \pm 0.75	--	--
MRT (h)	5.96 \pm 0.95	8.66 \pm 1.41*	8.97 \pm 2.23*
t _{1/2 λz} (h)	7.56 \pm 1.99	10.41 \pm 4.27*	8.78 \pm 2.70
AUC _(0-inf) (μg.h/ml)	8.26 \pm 1.67	7.940 \pm 1.025	5.11 \pm 1.00*
T _{max_{obs}} (h)	--	0.72 \pm 0.31	0.58 \pm 0.20
C _{max_{obs}} (μg/ml)	--	1.07 \pm 0.30	0.89 \pm 0.14
t _{1/2 ka} (h)	--	0.16 \pm 0.20	0.055 \pm 0.102
t _{lag} (h)	--	--	0.14 \pm 0.03
F (%)	--	97.6 \pm 11.3	62.4 \pm 8.12

Cl = plasma clearance; V_{ss} = steady-state volume of distribution; V_{area} = volume of distribution of the terminal phase; MRT = mean residence time; t_{1/2 λz} = half-life of the terminal phase; AUC = area under the plasma concentration-time curve; T_{max_{obs}} = occurring time of the maximum plasma concentration (C_{max_{obs}}); t_{1/2 ka} = half-life of absorption; t_{lag} = lag time for absorption; F = absolute bioavailability. T_{max_{obs}} and C_{max_{obs}} were obtained from the raw data for each horse. AUC, V_{ss}, V_{area}, Cl and MRT were calculated using the linear trapezoidal rule with extrapolation to infinity. t_{1/2 λz}, t_{1/2 ka} and t_{lag} were obtained by data fitting. *Significantly different (P<0.05) from i.v.

nested in sequence. In the first step, special attention was paid to the sequence effect using the Grizzle approach (Grizzle 1965), which consisted of first inspecting for a differential carry-over effect using a level of significance of P = 0.1.

Results

Pharmacokinetics

The mean plasma marbofloxacin concentrations vs. time after i.v., SC and oral administrations are presented in Figure 1. Figure 2 shows the observed and fitted data for a representative horse.

Table 1 gives the mean values of the marbofloxacin pharmacokinetic parameters. The plasma marbofloxacin clearance for the i.v. route was mean \pm s.d. 0.25 \pm 0.05 l/kg bwt/h. The steady-state volume of distribution (V_{ss}) was 1.48 \pm 0.30 l/kg bwt. The terminal half-life (t_{1/2 λz}) was 7.56 \pm 1.99 h and the MRT was 5.96 \pm 0.95 h.

After SC administration, the absolute bioavailability was 98 \pm 11% and the terminal half-life 10.4 \pm 4.3 h. The C_{max_{obs}} was 1.07 \pm 0.30 μg/ml and occurred 0.72 \pm 0.31 h after the marbofloxacin administration. In all horses, SC administration of marbofloxacin triggered an oedema (of approximately 5 cm diameter) at the injection site. This local reaction disappeared within 2 or 3 weeks.

After oral administration, the absolute bioavailability was mean \pm s.d. 62 \pm 8% and the terminal half-life 8.8 \pm 2.7 h. The C_{max_{obs}} was 0.89 \pm 0.14 μg/ml and occurred 0.58 \pm 0.20 h after marbofloxacin administration.

The statistical analysis did not reveal any sequence effect, but

TABLE 2: The *in vitro* activity of marbofloxacin against pathogenic equine strains collected in France and the UK

Tested strains	No.	Range of MIC (μg/ml)		MIC ₅₀ (μg/ml)	MIC ₉₀ (μg/ml)
Enterobacteriaceae	26	0.008	0.03	0.017	0.027
<i>S. aureus</i>	15	0.12	0.25	0.125	0.210
Streptococcaceae	21	0.5	2.0	0.854	1.667
<i>Pseudomonas aeruginosa</i>	7	0.12	2.0	NC	NC

Enterobacteriaceae = *Salmonella* sp. (11), *E. coli* (10), *Klebsiella pneumoniae* (5). Streptococcaceae = *Streptococcus zooepidemicus* (14), *Streptococcus equi* (7). NC = not computed. MIC₅₀ and MIC₉₀ were not reported when the number of isolates was less than 10.

a significant (P<0.05) effect of the administration routes was observed for MRT, AUC and terminal half-life (see Table 1).

Pharmacodynamics and surrogate markers of efficacy

Table 2 gives the *in vitro* activities of marbofloxacin against pathogenic equine bacteria collected in France and the UK. The MIC₉₀ values were 0.027 μg/ml for enterobacteriaceae and 0.21 μg/ml for *Staphylococcus aureus*. The MIC₉₀ for the *Streptococci* spp. exceeded 1.6 μg/ml. The range of MIC for *Pseudomonas aeruginosa* was 0.12–2.0 μg/ml, but the MIC₅₀ and MIC₉₀ were not calculated due to the low number of isolates.

The efficacy parameters AUC₂₄ and C_{max}/MIC₉₀ are presented in Table 3. These were calculated for all 3 administration routes, taking into account the MIC₉₀ values for enterobacteriaceae and *S. aureus*.

The plasma marbofloxacin concentrations were predicted for different dosage regimens and routes of administration, and the T_{>MIC} calculated (Tables 4, 5). For a single administration, the T_{>MIC} for enterobacteriaceae (0.027 μg/ml) was 27–32 h after 2 mg/kg bwt depending on the route of administration. The T_{>MIC} for *S. aureus* (0.21 μg/ml) was 6–8 h after 2 mg/kg bwt (Table 4).

The simulated marbofloxacin concentrations for daily administration for 5 consecutive days were higher than the MIC₉₀ for enterobacteriaceae for more than 120 h whatever the dosage regimen tested. In contrast, the simulated marbofloxacin concentrations for dosage regimens from 1–3 mg/kg bwt/24 h exceeded the MIC₉₀ for *S. aureus* for only 16–50% of the 5 day period (Table 5).

Discussion

Any rational dosage regimen, especially in the case of antibacterial drugs, should take into account both the pharmacokinetics (PK) and pharmacodynamics (PD) of the drug in question. Several efficacy indices based on both microbiological and pharmacokinetic parameters (AUC₂₄, C_{max}/MIC, T_{>MIC}) have been proposed to predict the favourable outcome of fluoroquinolones in the treatment of infectious diseases (Hyatt *et al.* 1995; Sanchez-Navarro and Sanchez Recio 1999). The present study was designed to determine the basic pharmacokinetic parameters of marbofloxacin and then to compute the surrogate parameters of efficacy in order to determine possible dosage regimens for this new quinolone when administered by i.v., SC or oral routes in horses.

The PK/PD nature of the dosage regimen is illustrated by the

TABLE 3: Values of the efficacy indices AUC₂₄ (h) and C_{max}/MIC calculated from the pharmacokinetic parameters obtained after the administration of 2 mg/kg bwt marbofloxacin in 6 horses. Data are presented as mean ± s.d. (range)

	Route of administration		
	i.v.	SC	Oral
MIC ₉₀ = 0.027 µg/ml - Enterobacteriaceae			
AUC ₂₄	297 ± 90 (229–462)	238 ± 30 (207–293)	155 ± 21 (137–193)
C _{max} /MIC	632 ± 450 (239–1702)	36 ± 7.0 (30–49)	31 ± 4.5 (27–37)
MIC ₉₀ = 0.21 µg/ml - <i>Staphylococcus aureus</i>			
AUC ₂₄	31 ± 11 (23–52)	24 ± 4 (20–31)	12.0 ± 2.4 (10–17)
C _{max} /MIC	81 ± 69 (31–219)	4.70 ± 0.89 (3.9–6.3)	3.90 ± 0.58 (3.10–4.70)

TABLE 4: Time (h) above selected MIC₉₀ (T_{>MIC}) for different single doses of marbofloxacin administered by i.v., subcutaneous (SC) or oral routes in the horse. The data were obtained by simulating curves with the mean kinetic parameters of 6 horses. T_{>MIC} are expressed in hours

Route of administration (mg/kg bwt)	Dose	MIC ₉₀ (µg/ml)	
		0.027 (Enterobacteriaceae)	0.21 (<i>S. aureus</i>)
i.v.	1	20	4
	2	28	7
SC	1	22	5
	2	32	8
Oral	1	19	3
	2	27	6

following equation, which describes the relation between a dose and a target plasma concentration:

$$\text{Dose}_{\text{per unit time}} = \frac{Cl \times C_{\text{target}}}{F} \quad \text{Equation 8}$$

where C_{target} corresponds to the mean concentration over the dosing interval. The major pharmacokinetic parameters involved in this relationship are plasma clearance and absolute bioavailability, both of which control drug exposure.

The plasma marbofloxacin clearance estimated from the i.v. study was about 4 ml/kg bwt/min, which represents 10% of the blood flow in horses (Holt *et al.* 1968). This is a relatively low clearance value, indicating that, in horses, the capacity of the clearing organs to eliminate marbofloxacin is rather poor.

The second pharmacokinetic parameter in equation 8 is the absolute bioavailability of the formulation. For the SC route, the marbofloxacin absolute bioavailability was close to 100%, indicating the favourable kinetic properties of this route of administration. The plasma half-life of marbofloxacin after SC administration was about 10 h, i.e. longer than the i.v. plasma half-life, indicating that the absorption rate was a limiting factor in drug elimination (flip-flop situation). Kaartinen *et al.* (1997) published a very variable absolute bioavailability (range 30–170%; coefficient of variation 26%) for enrofloxacin after i.m.

TABLE 5: Time above selected MIC₉₀ (T_{>MIC}) for different doses and dosage intervals (12 or 24 h) of marbofloxacin administration by i.v., subcutaneous (SC) and oral routes during 5 consecutive days in horses. T_{>MIC} are expressed in percent of the total 120 h dosing period (100% = 120 h)

Route of administration	Dose (mg/kg bwt)	Interval (h)	MIC ₉₀ (µg/ml)	
			0.027 (Enterobacteriaceae)	0.210 (<i>S. aureus</i>)
i.v.	1	12	110%	20.4%
		24	104%	32.5%
	2	12	116%	40.0%
		24	108%	49.2%
SC	1	12	116%	27.5%
		24	110%	36.6%
	2	12	125%	36.6%
		24	115%	52.5%
Oral	1	12	111%	16.6%
		24	104%	20.0%
	2	12	118%	34.2%
		24	108%	40.0%

administration in horses. In the present experiment, the interindividual variability of the absolute bioavailability of marbofloxacin by SC route was lower (range 79–110%; coefficient of variation 12%).

The absolute bioavailability of marbofloxacin by the oral route was about 62%, which is much higher than that reported by Dowling *et al.* (1995) for ciprofloxacin in ponies (6.8%). The absolute bioavailability of enrofloxacin administered by intragastric route was about 60% (Guiguère *et al.* 1996). However, this figure was obtained from a microbiological assay which may have overestimated the actual bioavailability because of transformation, by hepatic first-pass effect, of a fraction of enrofloxacin into ciprofloxacin which is microbiologically more potent than enrofloxacin (Cester *et al.* 1996).

Based on the hypothesis that marbofloxacin is only cleared by the liver, i.e. plasma clearance equals hepatic clearance, the maximum bioavailability for the oral route (F_{max}) can be calculated using equation 9:

$$F_{\text{max}} = 1 - Cl_{\text{TOT}}/\dot{Q}_h \quad \text{Equation 9}$$

where Cl_{TOT} is the total clearance and \dot{Q}_h the hepatic flow, which represents 30% of the cardiac output, i.e. about 15 ml/kg bwt/min (Holt *et al.* 1968). Using this approach, the theoretical marbofloxacin F_{max} is about 73%. This value is close to the absolute bioavailability actually found after oral administration (mean ± s.d. 62 ± 8%) suggesting that under this hypothesis the hepatic first-pass effect is sufficient to explain the incomplete availability of marbofloxacin and, concurrently, that absorption is almost complete. This conclusion, based on the assumption that marbofloxacin is cleared only by the liver, can be checked easily by measuring the renal plasma clearance of marbofloxacin.

Langston *et al.* (1996) reported very variable AUC after oral administration of enrofloxacin in the horse, illustrated by a coefficient of variation of 76%. The coefficient of variation of AUC for the oral administration of marbofloxacin was 20%, suggesting a lower variability in exposure to marbofloxacin compared with enrofloxacin after oral administration.

Variability in the absolute bioavailability is of major concern

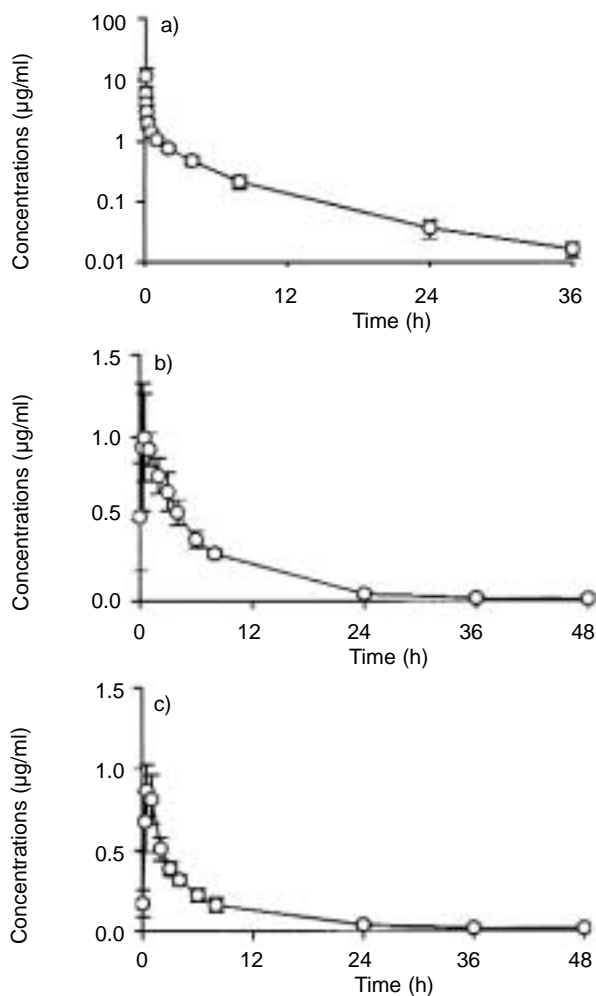


Fig 1: Mean \pm s.d. plasma concentration-time curves for marbofloxacin in 6 horses. a) Semilogarithmic plot after i.v. marbofloxacin administration (2 mg/kg bwt). b) Arithmetic plot after subcutaneous marbofloxacin administration (2 mg/kg bwt). c) Arithmetic plot after oral marbofloxacin administration (2 mg/kg bwt).

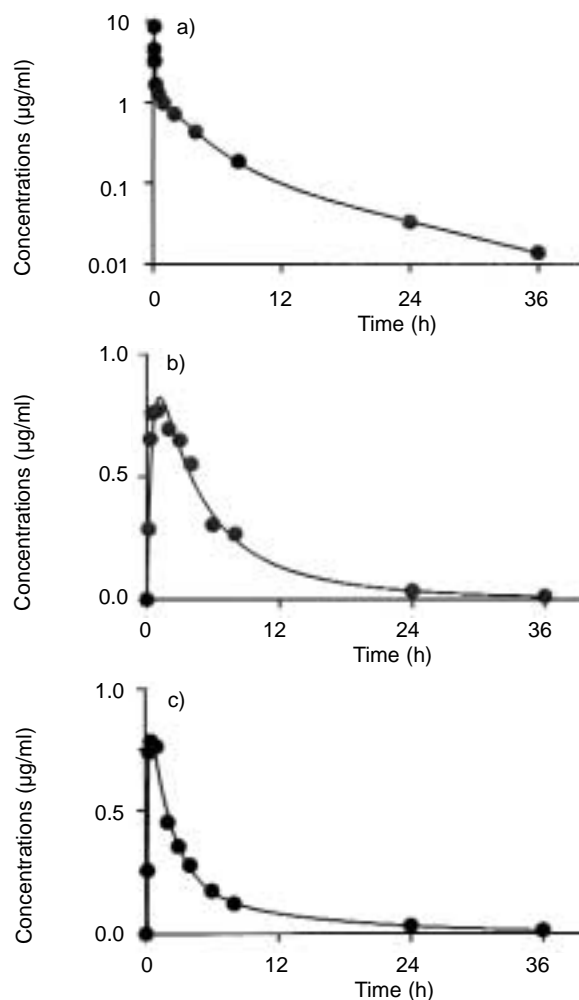


Fig 2: Observed (●) and fitted (—) plasma marbofloxacin concentrations in a representative horse. a) Semilogarithmic plot after i.v. marbofloxacin administration (2 mg/kg bwt). b) Arithmetic plot after subcutaneous marbofloxacin administration (2 mg/kg bwt). c) Arithmetic plot after oral marbofloxacin administration (2 mg/kg bwt).

because it is associated with 2 major risks: (i) underexposure of animals with low bioavailability which favours the emergence of resistance; and (ii) overexposure of animals with high bioavailability, with a risk of side effects. Erratic enrofloxacin bioavailability led Langston *et al.* (1996) to suggest the therapeutic monitoring of this drug after oral administration. It is well known that the feeding pattern may greatly influence oral bioavailability in horses (Maittho *et al.* 1986). It should be pointed out that, in our experiment, marbofloxacin was given before a regular meal and further experiments will be necessary to assess the characteristics of oral marbofloxacin bioavailability under different feeding conditions.

Finally, both extravascular routes tested in this experiment exhibited a low interindividual variability in the absolute bioavailability of marbofloxacin, which constitutes an advantageous property for the two marbofloxacin formulations. Moreover, there was no evidence of major local intolerance with SC administration of marbofloxacin, but final recommendations for this route of administration would require additional tolerance study.

Equation 8 shows how the dosing rate can be calculated to obtain a target concentration (the mean plasma concentration over

the dosage interval) and AUC_{24} can be used to assess the value of this concentration. When AUC_{24} is close to the AUC_{0-24h}/MIC ratio ($AUC_{[T1-T2]} = AUC_{0-24h}$) a target concentration can be calculated using equation 10:

$$C_{\text{target}} = \frac{AUC_{24} \times MIC}{24 \text{ h}} \quad \text{Equation 10}$$

which can be substituted into equation 8 to calculate the corresponding dosing rate:

$$\text{Dose}_{\text{per unit time}} = \frac{Cl}{F} \times \frac{AUC_{24} \times MIC}{24 \text{ h}} \quad \text{Equation 11}$$

The AUC_{24} is generally calculated at steady-state for repeated administrations. The AUC_{24} calculated for marbofloxacin in horses after a single administration was close to that expected at steady-state, simulations indicating that for a 24 h dosing interval, the steady-state accumulation of marbofloxacin did not exceed 25%. Moreover, it should be noted that plasma protein binding was not taken into account in equation 11: the MIC (which corresponds to a free drug concentration *in vitro*) was assimilated to a total plasma concentration. This assumption is

reasonable assuming that marbofloxacin binds weakly to plasma albumin in the horse, as in other animal species.

The pivotal point for the rational design of dosage regimens in veterinary medicine is the availability of breakpoint values for the different surrogate markers considered relevant for antimicrobial efficacy in the targeted animal species and infectious pathologies. At present, the available breakpoint values for these efficacy indices concern human antimicrobial therapy, and their correlations with clinical responses have not been determined in horses.

In human medicine, an $AUC_{24} \geq 125$ has been proposed as a target value to guarantee the clinical efficacy of fluoroquinolones (Forrest *et al.* 1993), which corresponds to a mean plasma concentration over 24 h equal to 5.2 times (125/24) the MIC_{90} of the target bacteria. When this value is used for marbofloxacin in horses, the target plasma concentration for enterobacteriaceae ($MIC_{90} = 0.027 \mu\text{g/ml}$) is $0.14 \mu\text{g/ml}$ (equation 10) and the corresponding i.v. dosing rate is $0.8 \text{ mg/kg bwt/24 h}$ (equation 11). Conversely, for a dosage regimen of 2 mg/kg bwt/24 h , an $AUC_{24} \geq 125$ can be obtained with $MIC_{90} \leq 0.067, 0.063$ and $0.04 \mu\text{g/ml}$ for i.v., SC and oral routes, respectively. The AUC_{24} for the MIC_{90} of enterobacteriaceae was largely above the 125 h value whatever the route of administration (Table 3). According to some authors (Madaras-Kelly *et al.* 1996; Papich 2000), AUC_{24} values of 50–75 are likely to be effective, especially in immunocompetent patients. This illustrates the complexity of clinical situations and confirms that an AUC breakpoint of 125 should not be accepted as universally applicable (Dalla Costa and Derendorf 1996).

Concerning the C_{max}/MIC ratio, evaluation of the efficacy of ciprofloxacin against *Streptococcus pneumoniae*, using a mouse protection model, indicated that 100% protection was obtained for a C_{max}/MIC above 10.6 (Sullivan *et al.* 1993). Moreover, Blaser *et al.* (1987) showed that peak concentrations higher than 8–10 times the MIC could prevent the emergence of organisms resistant to fluoroquinolones, even when plasma and tissue concentrations fell below the MIC. Values for marbofloxacin C_{max}/MIC higher than 10 were obtained with all 3 administration routes for the MIC_{90} of enterobacteriaceae and with the i.v. route for the MIC_{90} of *Staphylococcus aureus*.

The third pharmacokinetic parameter of interest when determining a suitable dosage regimen is the terminal half-life, which controls both drug accumulation and the time required to attain equilibrium. The plasma half-life is also a major factor influencing the $T_{>MIC}$. The i.v. plasma half-life of marbofloxacin was mean \pm s.d. $7.56 \pm 1.99 \text{ h}$, i.e. relatively long compared to that of enrofloxacin (Kaartinen *et al.* 1997). Moreover, this half-life was significantly increased after SC administration due to the flip-flop phenomenon.

For some Gram-positive germs, quinolones can act as time-dependent antibiotics and $T_{>MIC}$ is, therefore, a good predictor of their efficacy. Generalisation should nevertheless be avoided in view of the study by Meinen *et al.* (1995), which indicated that the killing of *staphylococci* by enrofloxacin was concentration-dependent in neutropenic mice.

We calculated $T_{>MIC}$ for selected MIC_{90} during multiple dosing, assuming that the disposition of marbofloxacin was linear (Table 5). After 5 repeated administrations at 2 mg/kg bwt/24 h , marbofloxacin was able to maintain plasma concentrations above the MIC_{90} of enterobacteriaceae throughout the entire dosing period (120 h), whatever the route of administration. In contrast, none of the dosage regimens tested were able to maintain plasma

concentrations above the MIC_{90} of *S. aureus* for more than 50% of the dosing period.

In this report, we have presented an approach for the rational design of dosage regimen which combines pharmacokinetics and surrogate markers of antimicrobial efficacy. It can be concluded from breakpoint values of the efficacy indices for fluoroquinolones proposed for human antimicrobial therapy that a marbofloxacin dosage regimen of 2 mg/kg bwt/24 h administered by i.v., SC or oral routes should be appropriate to treat infections due to bacteria with MIC_{90} of about $0.03 \mu\text{g/ml}$ and possibly efficacious for bacteria with MIC_{90} of up to $0.2 \mu\text{g/ml}$.

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Manufacturers' addresses

¹Vétoquinol, Magny-Vernois, Lure, France.

²STSC Inc., Rockville, Maryland, USA.

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