Update on the pharmacokinetic profile of marbofloxacin in loggerhead sea turtles *(Caretta caretta)* after intravenous and intramuscular injection

Antibiotic therapy is an essential part of the treatment of sick or injured loggerhead sea turtles (*Caretta caretta*). Marbofloxacin is commonly used in loggerhead sea turtles at a dose of 2 mg/kg, as in other species. The aim of this study was to increase our understanding of marbofloxacin pharmacokinetics in *Caretta caretta*. Six healthy turtles were divided in two groups: the intravenous (IV) group (subjects A, B and D) and the intramuscular (IM) group (subjects C, E and F). Each animal received a single dose of Marbocyl® 2%, corresponding to 2 mg/kg of marbofloxacin. The drug was administered by the IV or IM route. After its administration, 13 blood samples were collected at different times: for the IV group at 0, 10, 20, 30 and 45 minutes, 1, 2, 4, 8 and 12 hours and 1, 2 and 2.5 days; for the IM group at 0 and 30 minutes, 1, 1.5, 2, 3, 4, 6, 8 and 12 hours and 1, 2 and 2.5 days. A high performance liquid chromatography ultraviolet light (λ 295 nm) analytical method was used to measure marbofloxacin concentrations in blood. The pharmacokinetic behaviour of the drug was best described by a non-compartmental model. The plasma concentration profiles of marbofloxacin were similar for the two routes of administration and the residual concentrations at 1 day were almost identical. The molecule was detected up to 2.5 days. The apparent volume of distribution was >1 L/kg. The IM route resulted in rapid and complete marbofloxacin absorption (F = 131, 43±12.06 %).

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INTRODUCTION

The loggerhead sea turtle (*Caretta caretta*) is the most widespread species of marine turtle in the Mediterranean Sea[1,2] and it is, therefore, found relatively frequently in Italian Recovery Centres. The most common clinical presentations of this species are infectious diseases (bacterial and viral), trauma, and nutritional or parasitic disorders[3,4]. Numerous bacteria are responsible for infections in these turtles. The bacteria most frequently isolated from beached examples of this species

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are: *Pseudomonas* spp., *Aeromonas* spp., *Escherichia coli, Pasteurella* spp., *Proteus* spp. and *Vibrio*[5,6,7,8]. Antibiotic treatment is, therefore, often necessary during the clinical management of beached turtles.

There are clinically relevant differences in the distribution and elimination of antibiotics between reptiles and mammals[9,10]. It is, therefore, necessary to know the specific pharmacokinetics in different animal species in order to provide appropriate antibiotic treatment. Fluoroquinolones are among the widely used antibiotics in reptiles and in *Caretta caretta* because of their broad spectrum of action. Furthermore, they have low toxicity, excellent pharmacokinetic characteristics (e.g., high volume of distribution, low percentage of binding to serum proteins), low values of minimal inhibitory concentration (MIC) for sensitive bacteria and have a predominantly concentration-dependent bactericidal activity[11,12,13,14].

Jacobson and colleagues evaluated the plasma concentrations of enrofloxacin in *Caretta caretta* after oral administration[14], confirming the low toxicity of this drug and the good pharmacokinetics, although showing that the absorption following oral administration is strongly influenced by the presence of food.

Reptiles often show clinically relevant differences from mammals with regards to the distribution and elimination of antibiotics.

Marbofloxacin is a fluoroquinolone used exclusively in veterinary medicine which, compared to enrofloxacin, has a comparable spectrum of antibacterial activity, but better pharmacokinetic characteristics (e.g., higher bioavailability and longer half-life). Although marbofloxacin has shown different pharmacokinetic profiles in the various animal species in which it has been studied, a dose of 2 mg/kg seems to have good clinical efficacy and a valid kinetic profile. There are only two published studies on the pharmacokinetics of marbofloxacin in *Caretta caretta,* only one of which described the kinetic profiles following the intravenous and intramuscular routes of administration, that are commonly used in this species[15,16].

The limited number of subjects that can usually be recruited into studies on *Caretta caretta* means that such studies normally lack the power necessary for an adequate statistical analysis. On this background, the aim of the present study, also carried out on a small number of animals, is to provide further information on the pharmacokinetics of marbofloxacin in *Caretta caretta*, integrating the data already available in literature.

MATERIALS AND METHODS

In vivo study

Six clinically healthy examples of *Caretta caretta*, housed at the "Turtle Point" of "Anton Dohrn" Zoological Station (Naples, Italy), were used for this research.

The turtles were kept singly in tanks (1.5 x 1.0 x 0.6 m) with a capacity of 1000 L, connected directly with the water treatment system of the Zoological Station. The water in the tanks was drawn at a distance of about 1 km away from the coast and at a depth of 3 m. Each tank was aerated and the suspended waste matter removed mechanically by appropriate systems (respectively: SCL V5, Effepizeta Spa, Concorrezzo, Italy; SHS 50-125/22/A, Lowara Srl, Montecchio Maggiore, Italy and LSC 48, Lacron-Waterco, Sidney, Australia). The control of bacterial, viral and fungal loads was ensured by an UV irradiation system (REX M-2PE140-C, WEDECO Ag, Herford, Germany), while the temperature of the water was maintained at about 21°C by a heater (RE/U 21, ZPCE, Saint Barthélemy d'Anjou Cedex, France).

Throughout the whole period of the investigation the animals were kept in standard environmental conditions (temperature and light-dark cycle) and given the diet, based on various shellfish and oily fish, usually provided in the Centre.

The turtles, which had a fasting body weight ranging from 10 to 42.3 kg, were divided into two groups of three animals each, trying to keep body weight as similar as possible within each group (Table 1). Each animal was given a single dose of injectable Marbocyl® 2% (Azienda Terapeutica Italiana A.T.I. s.r.l. Gruppo Fatro, Ozzano Emilia-Bologna, Italy) equivalent to 2 mg/kg of body weight. Animals in the intravenous (IV) group (turtles A, B and D) were administered by injection into the jugular vein, while animals in the intramuscular (IM) group (turtles C, E and F) were given the drug into the pectoral muscle (Table 1). The choice of dose of marbofloxacin (2 mg/kg) was based on published data indicating that this is a therapeutic dose in most animal species (mammals and not) for all routes of administration.

Table 1 - Distribution of treatment with marbofloxacin (a single dose of 2 mg/kg) in the animals included in this study

Animal	Fasting weight (kg)	Marbocyl 2% (2 mg/kg)	Route of administration
A	29,45	2,95 ml	IV
B	27,00	2,70 ml	IV
C	42,28	4,20 ml	IM
D	10,00	1,00 ml	IV
E	31,00	3,00 ml	IM
F	39,00	3,90 ml	IM

In order to determine the pharmacokinetic profile of marbofloxacin, 13 blood samples were taken from each animal at the following times: IV group 0, 10, 20, 30 and 45 min, 1, 2, 4, 8 and 12 h and 1, 2 and 2.5 days; IM group 0 and 30 min, 1, 1.5, 2, 3, 4, 6, 8 and 12 h and 1, 2 and 2.5 days. The blood was taken from the contralateral jugular vein using vacuum plastic test-tubes (Vacutest KIMA Arzergrande, Italy, 4 mL, lithium-heparin). The amount of blood taken at each sampling was sufficient to guarantee 1 mL of plasma. The blood samples were centrifuged to separate the plasma which was then stored in a freezer at -20°C until analysis.
The investigation was conducted in compliance with the legislation on the welfare of animals undergoing experiments and following authorisation from the Ministry of Health (Legislative Decree n. 116/1992 – Ministerial Authorisation - Decree n. 10/2004-C issued on 11/02/2004 by the Ministry of Health).

Each animal was given a single dose (2 mg/kg of body weight) of injectable Marbocyl® 2% (Azienda Terapeutica Italiana A.T.I. s.r.l. Gruppo Fatro, Ozzano Emilia-Bologna, Italy), administered intravenously (via the jugular vein) in the IV group (turtles A, B and D) and intramuscularly (into the pectoral muscle) in the IM group (turtles C, E and F).

Marbofloxacin analysis

Marbofloxacin was extracted and purified from the plasma samples using the method described by Schneider and colleagues[17], with slight modifications to the aqueous extraction phase and the mobile chromatographic phase. The analysis of the test samples and the validation of the analytic method, in terms of specificity, sensitivity and linearity, were performed using a HPLC-UV with a Beckman Gold System (Gold Programmable Solvent Module 126 pump system, Gold Autosampler system 507, Beckman Programmable Detector Module 168 as diode UV detector and a control station IBM Thinkcenter – Software 32 Karat). A Phenomenex Gemini C18 5 µm column (250 x 4.6 mm), kept at room temperature, was used. The mobile phase (flow 0.7 mL/min) consisted of a H_3PO_4 : CH_3CN solution (85:15, *v/v*). The injection volume was 50 µL. The analyses were performed using UV spectrophotometry at a wavelength of 295 nm.
Under the chromatographic conditions used, marbofloxacin has a retention time of about 9.5 min, while for ofloxacin (Internal Standard) it was about 11.5 min, demonstrating the good specificity of the method. The "blank" samples, consisting of plasma taken immediately before the treatment (time 0), did not show interferences at the afore-mentioned retention times. The method is highly sensitive, with limits of detection (LOD) and quantification (LOQ) of 3 ppb and 5 ppb, respectively. The correlation between the concentration of marbofloxacin and the relationship between the area of the peak and the area of the relative internal standard was linear ($R^2 \geq 0.998$) in the wide range of concentrations used (5-10000 ppb). The samples were analysed in conformity with ISO 9001 standards, for which the Department of Veterinary Medical Sciences is certified.

Pharmacokinetic evaluation

The main pharmacokinetic parameters of marbofloxacin were determined on the basis of the blood concentration/time curve, starting from the plasma concentrations found in each animal and using WinNonlin Prof 6.1 software (Pharsight Corp, St Louis, MO, USA). A non-compartmental analysis was performed on the plasma concentrations of marbofloxacin. The following parameters were calculated for each animal: the maximum concentration (C_{max}), time to reach the peak concentration (T_{max}), terminal phase half-life ($t_{1/2\lambda z}$), area under the curve from time 0 until the last measurable concentration (AUC_{last}), apparent volume of distribution (Vd), volume of distribution corrected by the fraction of dose absorbed (Vd/F), apparent volume of distribution at the steady state (V_{ss}), body clearance (Cl), body clearance corrected by the fraction of dose absorbed (Cl/F), and bioavailability (F).

The good distribution of marbofloxacin, for both routes of administration, was demonstrated by the high volumes of distribution: Vd, Vd/F and Vss were above or only just below 1 L/kg (Table 2).

RESULTS AND DISCUSSION

No adverse reactions (systemic or at the injection site) to the antibiotic occurred throughout the study in any of the turtles treated with either route of administration. Food consumption and general activity remained unaltered and no particular behavioural abnormalities were noticed. These observations are concordant with those reported by various Authors who conducted similar investigations in *Caretta caretta*[15,16], as well as in other animal species[11,12], and, more generally, with the use of fluoroquinolones[13,14]. Figure 1 shows the trend of marbofloxacin mean concentrations (±SD) as a function of time for the two administration routes.
Following both routes of administration, marbofloxacin was still be detected at the last assay time-point (2.5 days); furthermore, the blood levels were very similar

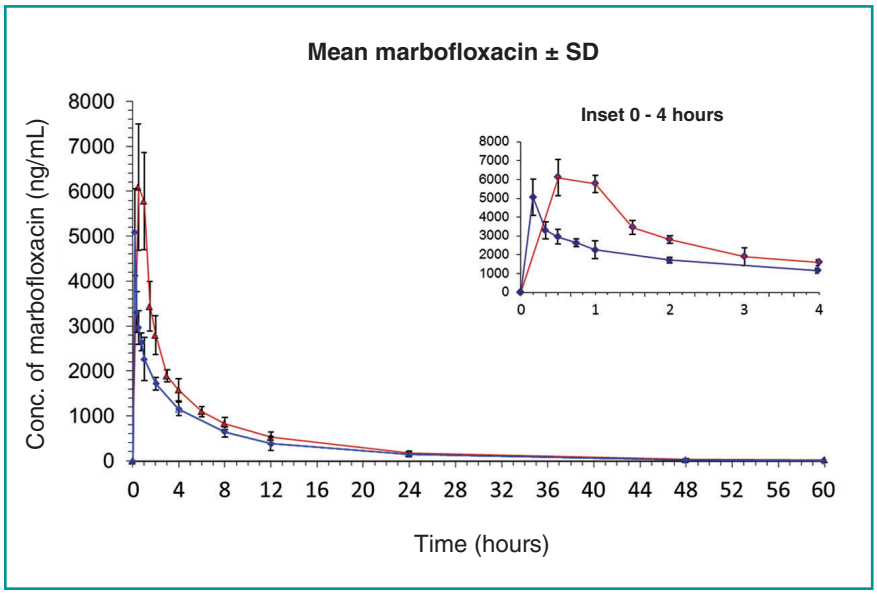

Figure 1 - Plasma concentrations profile of marbofloxacin in Caretta caretta following intravenous or intramuscular administration of a single 2 mg/kg dose. The blue circles represent mean values for intravenous administration. The pink squares represent the mean values for intramuscular administration.

Table 2 - Pharmacokinetic parameters (mean values ± SD) of marbofloxacin in *Caretta caretta* administered as a single dose of 2 mg/kg intravenously or intramuscularly

Parameters		IV	IM
C_{max}	µg/mL	5,09±0,96	6,65±1,11
T_{max}	h	0,17±0,00	0,67±0,29
$t_{1/2\lambda z}$	h	11,76±1,32[a]	12,78±1,14[a]
AUC_{last}	µg·h/mL	20,11±3,49	26,42±4,21
Vd	L/kg	1,71±0,36	
Vd/F	L/kg		1,41±0,27
V_{ss}	L/kg	0,94±0,05	
Cl	mL·h/kg	100,45±18,79	
Cl/F	mL·h/kg		76,20±12,32
F	%		131,43±12,06

[a] Harmonic mean.

C_{max}, maximum concentration; T_{max}, time to peak concentration; $t_{1/2\lambda z}$, terminal phase half-life; AUC_{last}, area under the curve from time 0 until last measurable concentration; Vd, apparent volume of distribution; Vd/F, volume of distribution corrected for the fraction of dose absorbed; V_{ss}, apparent volume of distribution at steady state; Cl, body clearance; Cl/F body clearance corrected for the fraction of dose absorbed; F, bioavailability.

(Figure 1), especially after 2 hours (IV) and 4 hours (IM), becoming almost identical at 1 day, as reported by Lai and colleagues[15], although the sampling times were slightly different. IM administration is characterised by high bioavailability (Table 2). The good distribution of marbofloxacin, by both routes of administration, is demonstrated by its volumes of distribution: Vd, Vd/F and V_{ss} were above or just below 1 L/kg (Table 2), contrasting with the findings of Lai and colleagues[15]. The antibacterial activity of marbofloxacin in many systemic infections (e.g., trauma and pneumonia) is therefore supported by the high values of these pharmacokinetic parameters, due to the ability of fluoroquinolones to penetrate into tissues[17,18]. As far as regards the elimination of the drug, although statistical analyses were not performed, the elimination half-lives seemes similar for the IV and IM routes, with clearance values (Cl and Cl/F) much higher than those found by Lai and colleagues[15], but well correlated with other data in the literature[16,17,18].

CONCLUSIONS

Marbofloxacin showed good tolerability in *Caretta caretta,* without clinically relevant secondary effects. The therapeutic protocols for antibiotics (doses and intervals between doses) are related to the PK/PD ratio; for fluoroquinolones, which are mainly concentration-dependent, the AUC/MIC and C_{max}/MIC ratios of the sensitive and/or more resistant bacterial populations are those that provide the best indications[19]. A PK/PD extrapolation was not planned in this study, in part because the MIC of marbofloxacin for the main bacterial species isolated from *Caretta caretta* are still unknown[6]. The general lack of undesired effects in the turtles studied and the favourable pharmacokinetic parameters of marbofloxacin administered at a dose of 2 mg/kg IV or IM suggest that this fluoroquinolone can be used for the treatment of bacterial infections in *Caretta caretta*, although, as emphasised in previous studies[15,16], further research is necessary, particularly for repeated doses and to establish the PK/PD ratio. Furthermore, considering that enrofloxacin can cause necrotic lesions at the injection site[20], long-term assessments of the clinical use of marbofloxacin will be useful.

KEY POINTS

- There are only two published studies on the pharmacokinetics of marbofloxacin in *Caretta caretta,* only one of which describes the pharmacokinetic profiles following intravenous and intramuscular administration, routes that are commonly used to administer antibiotic therapy in this species.
- The animals were divided into two groups of three turtles each, maintained in standard environmental conditions (temperature and light-dark cycle) and given a normal diet.
- Marbofloxacin was extracted and purified from plasma samples using ofloxacin as internal standard and following analyte extraction through an organic phase.
- Following both routes of administration, marbofloxacin levels could still be assayed at the end of the experimental protocol (2.5 days).
- The good distribution of marbofloxacin, following both routes of administration, is demonstrated by the large volumes of distribution: Vd, Vd/F and Vss were above or only just below 1 L/kg, contrasting with previous studies, but in accordance with pharmacokinetic studies in other animal species (mammals, birds and reptiles).

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