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Azithromycin pharmacokinetics in the serum and its distribution to the skin in healthy dogs and dogs with pyoderma

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ABSTRACT

Serum and skin tissue azithromycin (AZM) concentrations were analysed in healthy and pyoderma affected dogs to determine AZM pharmacokinetics and to establish the effect of disease on AZM skin disposition. AZM was administered orally to two groups of healthy dogs: (1) at 7.02 mg/kg ($n = 7$) and (2) at 11.2 mg/kg ($n = 9$). A crossover design was used on five of them. Seven dogs with pyoderma were treated with AZM at 10.7 mg/kg. The two groups of healthy dogs received AZM once daily over three consecutive days and dogs with pyoderma received the same treatment repeated twice with an interval of 1 week. AZM concentrations were determined by liquid chromatography–tandem mass spectrometry.

AZM was rapidly absorbed and slowly excreted. In healthy dogs, maximum serum concentrations appeared 2 h after administration and were (mean ± standard deviation) 0.60 ± 0.25 μg/mL and 1.03 ± 0.43 μg/mL, and the half-lives were 49.9 ± 5.10 and 51.9 ± 6.69 h for doses of 7.02 and 11.2 mg/kg, respectively. Clearance (CL_{0-24}/F) was similar in both dosing groups (1.24 ± 0.24 and 1.29 ± 0.24 L/h/kg) and the respective mean residence time (MRT_{0-24}) was 11.1 ± 0.8 and 8.4 ± 2.2 h. The skin concentration in healthy dogs was 3.5–6.5 and 5.0–12.0 times higher than the corresponding serum concentration after the two doses and increased after the cessation of AZM administration. The ratio increased significantly in inflamed tissue (9.5–26.2).

Introduction

Azithromycin (AZM) is the first semi-synthetic macrolide derivative classified as an azalide (Girard et al., 1987). AZM differs from erythromycin (ERY) in the methyl-substituted nitrogen at position 9a in the lactone ring creating a 15-membered macrolide (Mutak, 2007), which improves the potency against Gram-negative bacteria (Retsema et al., 1987; Dunkin et al., 1988). Furthermore, AZM is more stable than ERY in an acidic environment (Fiese and Steffen, 1990), improving AZM bioavailability. AZM free base suspension bioavailability in dogs was 97% whereas ERY was 52% (Shepard and Falkner, 1990). Bioavailability in other species was lower: 57% in cats (Hunter et al., 1995), 46% in rats (Shepard and Falkner, 1990) and 37% in human beings (Foulds et al., 1990).

AZM tissue concentrations in various organs of mice, rats, gerbils, dogs and humans were significantly above the minimum inhibition concentration (MIC) of the relevant pathogens, while AZM serum concentrations were below the MIC (Girard et al., 1987; Foulds et al., 1990; Retsema et al., 1990; Foulds and Johnson, 1993). AZM skin concentrations have not been investigated, although it has been found to be effective in therapy of skin and subcutaneous infections, including those caused by *Staphylococcus* spp. (Daniel, 1991; Mallory, 1991; Amaya-Tapia et al., 1993; Rodriguez-Solares et al., 1993; Noli and Boothe, 1999).

Staphylococcus pseudintermedius is the primary pathogen in canine pyoderma, followed by other coagulase negative *Staphylococcus* spp., coagulase positive *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Pseudomonas* spp. and *Proteus* spp. (Miller et al., 2012). Treatment of canine pyoderma involves long term oral administration of antibiotics relying strongly on dog owner-dependent administration compliance (Stegemann et al., 2007). Once daily administration of AZM is potentially beneficial in achieving owner compliance (Norrby, 1991; Noli and Boothe, 1999). The aim of the present study was to determine the pharmacokinetics (PK) of AZM in healthy dogs and to investigate AZM distribution to normal and pyoderma affected skin.

Materials and methods

Experimental design

Two groups of healthy dogs and one group of pyoderma affected dogs received AZM tablets (Zeto 250 mg, Unipharm) according to the dosing regimen described below. Plasma and skin biopsies were collected according to the design below

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and in Table 1. The AZM concentrations in plasma and skin were analysed using a liquid chromatography–tandem mass spectrometry (LC/MS/MS) method and AZM PK were determined for each group of dogs.

Ethical approval

The experiments and the suggested protocols were approved by the Ethics Committee of Faculty of the Agricultural, Food and Environmental Quality Sciences of the Hebrew University of Jerusalem for Clinical Trials of the Koret School of Veterinary Medicine (KSVM-VTH/6_2006). All owners signed an informed consent form.

Study animals

Eleven mixed breed, healthy dogs, 9 months to 3 years of age and weighing 22.7 ± 11.7 kg (mean ± standard deviation; range 8.0–40.5 kg) were included in the study. All dogs were healthy based on physical examination, complete blood count (CBC) and serum chemistry panel results prior to the study. The target AZM doses were 5 mg/kg (seven dogs) and 10 mg/kg (nine dogs), resulting in exact doses of 7.02 ± 0.50 mg/kg and 11.2 ± 1.66 mg/kg; five dogs received both doses (crossover), with a period of 2.5 weeks in between the treatments.

Seven dogs with clinical pyoderma, 9 months to 12 years of age and weighing 33.0 ± 6.47 kg (range 22–40 kg), admitted to the Dermatology Department of the Veterinary Teaching Hospital, Koret School of Veterinary Medicine, were enrolled in the study. Three dogs were male, one female and three were castrated males. There were two mixed breed dogs, two Golden Retrievers and one each of Great Dane, Boxer and Staffordshire bull terrier. Prior to inclusion in the study, all dogs exhibited clinical superficial pyoderma due to various causes (atopic dermatitis, flea allergy, short coated breed dermatitis) without apparent accompanying systemic disease based on physical examination, CBC and serum chemistry panel results. One dog had epilepsy that was medically controlled (phenobarbital and potassium bromide). Pyoderma was diagnosed according to clinical signs (Miller et al., 2012). Samples of skin tissue were examined histologically for the presence of bacteria and neutrophil infiltration. All seven dogs received AZM at 10.7 mg/kg actual dose once daily for three consecutive days. This protocol was repeated twice with weekly intervals.

Blood and tissue samples

The timeline of blood and skin sample collection relative to drug administration is shown in Table 1. Blood was collected from a peripheral vein (cephalic, saphenous or metatarsal veins), allowed to clot and centrifuged at 2000 *g* for 5 min. The serum samples were stored at −80 °C until analysis.

For the determination of AZM concentrations in skin, biopsies were performed in the trunk area of the dogs using an aseptic technique, including clipping the hair and applying a topical analgesic ointment containing 5% lidocaine (Emla, AstraZeneca) with a 6–8 mm diameter punch biopsy device followed by 2–0 nylon sutures. The sutures were inspected daily and removed after 10 days. Biopsies were taken according to the protocol described in Table 1 and stored at −80 °C until analysis.

Blood and skin tissue sample collection, as well as the drug administration protocol, from dogs with pyoderma was slightly different from that used for the healthy dogs (Table 1). Sample collection coinciding with drug administration was performed immediately before AZM administration. The sample preparation was identical to that described above.

Azithromycin analysis

Serum samples were prepared by the addition of 0.5 mL acetonitrile (J.T. Baker) to 0.2 mL serum. The tube was mix-vortexed, centrifuged at 16,000 *g* for 10 min; 100 μL of the supernatant solution were diluted with 100 μL 0.01 M ammonium formate, pH 3.5 (Sigma–Aldrich). The resulting mixture was used for injection to the LC/MS/MS instrument. AZM (Sigma–Aldrich) standard solution was prepared in methanol at 1 mg/mL and working standards (0.01–100 μg/mL) were kept at −30 °C. Standard curves for serum analysis were prepared by fortifying drug free dog serum with AZM to produce a concentration range from 0.0025 to 5 μg/mL.

AZM concentrations were determined using an LC/MS/MS method comprising a Hewlett–Packard H-P 1100 (Agilent Technologies) high performance liquid chromatography (HPLC) system (binary pump, degasser, thermostat column compartment and autosampler) combined with a tandem mass spectrometer (MS/MS) ABI 3200 QTrap (ABSciex). Liquid chromatography separation was performed on a C18 Hypersil Gold column (3 μm, 100 mm × 2.1 mm; Thermo Electron Corporation). The mobile phase consisted of 0.01 M ammonium formate at pH 3.5 and acetonitrile. A linear gradient of acetonitrile concentration from 5% to 70% within 1 min was applied at a flow rate of 0.45 mL/min. The column compartment was maintained at 30 °C and the injection volume was 5 μL. Turbo ion electrospray (ESI/MS/MS) in positive ion mode was operated at a temperature of 500 °C. Multiple reaction monitoring (MRM) was applied. AZM was detected by identifying the precursor ion 734 *m/z* and the product ion 576 *m/z*. The lower limit of quantification (LLOQ) and the higher limit of quantification (HLOQ) were 0.005 μg/mL and 5 μg/mL, respectively. The calibration curve was linear within the range 0.005–5 μg/mL (coefficient of correlation 0.9972). Recovery was 86–93%. The per cent coefficient of variation (%CV) within and between days was 8–14%.

For preparation of skin samples, each biopsy sample was weighed and acetonitrile (10:1 V/W) was added. The samples were placed overnight in a shaker bath at 37 °C. The tube was centrifuged at 3000 *g* for 10 min. The acetonitrile layer was collected, evaporated to dryness and the residue was re-dissolved in 0.01 M ammonium formate (pH 3.5):methanol (1:1 V/V). The volume was proportional to biopsy weight (2:1 V/W). The solution was then used for injection to the LC/MS/MS as described above. Standard curves for skin analysis were prepared by fortifying blank dog skin with AZM. The LLOQ and the HLOQ were 0.005 μg/g and 5 μg/g, respectively. The calibration curve was linear within the range 0.005–5 μg/g (coefficient of correlation 0.9972). Recovery was 65–71% and the %CV within and between days was 12–19%. Samples for which the AZM concentration exceeded the HLOQ were diluted and re-analysed.

Pharmacokinetic analysis

The PK parameters of AZM were determined by use of the non-compartmental approach based on the statistical moment theory (Yamaoka et al., 1978) using a computer programme (Yamaoka, 1986). The linear terminal slope λ_z after the third daily administration was calculated by linear, least squares regression analysis,

Table 1
Timeline of administration of azithromycin to healthy dogs and pyoderma affected dogs.

Time (h)	Healthy dogs 5 mg/kg			Healthy dogs 10 mg/kg			Dogs with pyoderma 10 mg/kg		
	Drug administration	Serum	Skin	Drug administration	Serum	Skin	Drug administration	Serum	Skin
0	x	x	x	x	x	x	x	x	x
2	–	x	–	–	x	–	–	x	–
4	–	x	–	–	x	–	–	–	–
6	–	x	–	–	x	–	–	–	–
10	–	x	–	–	x	–	–	–	–
16	–	x	–	–	x	–	–	–	–
24	x	x	x	x	x	x	x	x	x
48	x	x	–	x	x	–	x	x	x
60	–	x	–	–	–	–	–	–	–
72	–	x	x	–	x	x	–	–	–
96	–	x	–	–	x	–	–	–	–
120	–	x	–	–	x	–	–	–	–
144	–	x	–	–	x	–	–	–	–
168	–	x	x	–	x	x	x	x	x
192	–	–	–	–	–	–	x	–	–
216	–	–	–	–	–	–	x	–	–
336	–	–	–	–	–	–	x	–	–
360	–	–	–	–	–	–	x	–	–
384	–	–	–	–	–	–	x	–	–
504	–	–	–	–	–	–	–	x	x

Serum and tissue sample collection times are indicated with 'x' and no sample with '–'.