Determination of Plasma and Skin Concentrations of Orbifloxacin in Dogs with Clinically Normal Skin and Dogs with Pyoderma*

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ABSTRACT

Plasma and skin concentrations of orbifloxacin (Orbax tablets, Schering-Plough Animal Health) were assessed in 14 clinically normal dogs and 14 dogs with pyoderma following oral administration of the drug at 7.5 mg/kg once daily for 5 to 7 days. Skin biopsies and whole blood samples were obtained before dosing and at the time of the expected maximum concentration in skin (3 hours after dosing) on the first and on the fifth to seventh day of dosing. Skin biopsies and plasma were analyzed for orbifloxacin concentrations by high-performance liquid chromatography.

Dogs with pyoderma had significantly higher mean skin concentrations of orbifloxacin within 3 hours of administration (Day 0: 7.80 ± 3.40 µg/g, Days 4 to 6: 9.47 ± 6.23 µg/g) than did dogs with normal skin (Day 0: 3.85 ± 1.08 µg/g, Days 4 to 6: 5.43 ± 1.02 µg/g). After dosing on Day 0 and after five to seven daily treatments, dogs with pyoderma had significantly higher mean orbifloxacin skin:plasma ratios (1.40 and 1.44, respectively) than did clinically normal dogs (0.81 and 0.96, respectively). The accumulation of orbifloxacin in diseased skin may contribute to the efficacy of this compound for the treatment of bacterial skin infections.

INTRODUCTION

Orbifloxacin (Orbax, Schering-Plough Animal Health) is a fluoroquinolone antibiotic that exhibits bactericidal activity against aerobic gram-negative and gram-positive bacteria. This drug exhibits a high degree of therapeutic efficacy, even when multiple pathogens
are present. Orbifloxacin is rapidly absorbed from the gastrointestinal tract in fasted animals, and the absolute bioavailability of an oral dose is approximately 100%. Peak plasma concentrations are usually attained within 1 hour of administration.\textsuperscript{1,3} In the United States, orbifloxacin is approved at a flexible dosage range of 2.5 to 7.5 mg/kg for the management of diseases in dogs and cats associated with bacteria susceptible to this drug.

Orbifloxacin may be used to treat dermal infections.\textsuperscript{1} In one clinical field trial, 88% of 56 dogs with bacterial skin infections (wounds and abscesses) were clinically healed within 11 days of multiple daily dosing; 43% of these dogs were considered completely healed within 5 days.\textsuperscript{4} In that trial, 95% of isolated pathogens (including \textit{Staphylococcus intermedius}, \textit{Pseudomonas} spp, \textit{Escherichia coli}, \textit{Pasteurella multocida}, \textit{Proteus mirabilis}, \textit{Klebsiella pneumoniae}, \textit{Streptococcus} \textit{β}-hemolytic group G, \textit{Enterobacter} spp, and \textit{Enterococcus} spp) were eliminated after 5 to 10 days of therapy with orbifloxacin.

A key factor for therapeutic success of an antimicrobial is the ability to achieve therapeutic concentrations at the site of infection. Pyoderma, the most common bacterial skin infection of dogs,\textsuperscript{5} was identified as an ideal naturally occurring canine model to better characterize the tissue penetration of orbifloxacin into diseased skin. Clinical manifestations of superficial pyoderma include papules, pustules, crusts, and collarettes. Although \textit{S. intermedius} is the most commonly isolated organism, multiple pathogens may be involved in cases that have progressed from superficial to the more severe and extensive deep form of pyoderma.\textsuperscript{6,7} The objective of the present study was to determine plasma and skin concentrations of orbifloxacin in dogs with healthy skin and in dogs with pyoderma following single and multiple oral doses of orbifloxacin.

### MATERIALS AND METHODS

#### Dogs and Treatments

Fourteen laboratory beagles with clinically healthy skin and 14 client-owned dogs with pyoderma were treated orally with orbifloxacin tablets at a target dosage of 7.5 mg/kg once daily for 5 to 7 days. To accommodate client schedules, owners were instructed to treat their dogs once daily until they could be returned to the clinic for follow-up sampling (5 to 7 days). Or-bax tablets (5.7, 22.7, and 68 mg orbifloxacin per tablet) were used; when necessary, the scored 22.7- and 68-mg tablets were bisected to provide more accurate dosing. Client-owned dogs were of various breeds and were from several clinics participating in the study. Dogs ranged in age from 1.4 to 8 years, with an equal distribution of males and females.

Pyoderma was diagnosed in client-owned dogs based on physical examination findings, including the presence of pustules, papules, collarettes, crusts, or erythema. The diagnosis was confirmed by histopathologic evaluation of a skin biopsy. Dogs were excluded from the study if an alternative disease process other than pyoderma was present histopathologically or if the dog had received an antimicrobial orally or topically within the previous week, antiinflammatory drugs or nutraceuticals within the previous 2 weeks, or an intermediate or long-acting corticosteroid within the previous 4 weeks. Additionally, German shepherds were excluded from the study based on reports that suggest certain cases of pyoderma in this breed represent a distinct clinical syndrome associated with a breed-related autosomal recessive trait and immunologic differences in T cell function.\textsuperscript{8,9}

#### Skin Biopsy and Blood Sampling

On either Day –1 or Day 0 (before initiation of treatment), a skin biopsy was taken from each dog for histopathologic evaluation. Additionally, on either Day –1 or Day 0, a whole
blood sample (approximately 3.5 ml collected by venipuncture into a tube containing sodium heparin) and another skin biopsy were obtained from each dog to serve as controls for analysis of orbifloxacin. A third skin biopsy and blood sample were collected on Day 0 approximately 3 hours after dosing, and all dogs were similarly sampled approximately 3 hours after the fifth, sixth, or seventh dose (Day 4, 5, or 6).

Skin biopsy samples were collected using separate 8-mm disposable dermal punches. The selected skin biopsy sites (most commonly the sites were abdomen, axilla, or inguinal area) were shaved, scrubbed gently with a 7.5% povidone–iodine skin cleanser, and wiped with 70% ethyl or isopropyl alcohol. Lidocaine (containing no epinephrine) was administered subcutaneously to infiltrate the appropriate area to facilitate sample collection. Biopsy sites were closed by staples or sutures.

Skin samples intended for histopathology were preserved in formalin and examined by a board-certified pathologist. In addition to routine histopathologic evaluation to confirm the clinical diagnosis of pyoderma, the number of inflammatory cells per field was determined by examining three nonreplicated (step) sections collected approximately 20 µm apart on each slide and evaluating two fields per section using the 10× objective. The dermoepidermal junction was fixed at the top of each field before commencing each cell count. The results were averaged to yield a mean leukocyte count per field for each skin biopsy.10

Skin biopsy samples intended for orbifloxacin analysis were trimmed of visually detectable subcutaneous tissue (i.e., fat). The specimens were stored in polypropylene tubes, shipped frozen on dry ice, and subsequently stored at –70°C.

Sample Analysis

Orbifloxacin concentrations in plasma and skin were assayed in the sponsor’s laboratory using validated high-performance liquid chromatography (HPLC). The extraction procedure involved deproteination using phosphate buffer and acetonitrile followed by centrifugation. The supernatant was partitioned with hexane. Water was added to the extract and acetonitrile was removed using a nitrogen evaporator. The extract was filtered and analyzed on a polymeric column using HPLC with fluorescence detection. Treated plasma samples were quantified using a linear calibration curve based on matrix standards. Calibration standards prepared at five concentrations responded in a linear fashion ($r^2 \geq 0.98$). Analysis of plasma samples collected from all dogs before dosing did not show any peaks that could interfere with the assay for orbifloxacin. The limit of quantitation for the method was established as 0.1 µg/ml and the method limit of detection was 0.025 µg/ml.

Statistical Analysis

Orbifloxacin concentrations were compared between groups (pyoderma vs normal skin) at each sampling time using a t-test and the Satterthwaite degrees of freedom when homogeneity of variances was not confirmed. Data for all client-owned dogs at different sites were pooled together for the statistical comparison versus laboratory dogs with normal skin. Skin:plasma ratios of orbifloxacin were calculated by dividing the concentration in skin (µg/g) by the concentration in plasma (µg/ml) for each animal, and the mean was calculated for each group. The relationship between the mean...
number of inflammatory cells per field during histopathologic examination of skin biopsies and the concentration of orbifloxacin in the skin at each sampling time was determined by linear regression. Statistical significance was declared when $P \leq .05$.

**RESULTS**

**Skin Orbifloxacin Concentrations**

Three hours after dosing on Day 0, the mean concentration of orbifloxacin in healthy canine skin was $3.85 \pm 1.08$ µg/g compared with $7.80 \pm 3.40$ µg/g for dogs with pyoderma ($P = .0008$) (Table 1, Figure 1). The range of orbifloxacin concentrations in skin on Day 0 was 3.17 to 15.79 µg/g in dogs with pyoderma compared with 1.45 to 5.29 µg/g in dogs with normal skin. By 3 hours after dosing on Days 4 to 6, the mean concentration of orbifloxacin in the skin of clinically normal dogs had increased to $5.43 \pm 1.02$ µg/g compared with $9.47 \pm 6.23$ µg/g for dogs with pyoderma ($P = .0316$) (Table 1, Figure 1). The range of orbifloxacin skin concentrations on Days 4 to 6 was 0.82 to 25.50 µg/g in dogs with pyoderma compared with 3.66 to 6.97 µg/g in dogs with normal skin. An aberrant result was noted for one dog with pyoderma (Case 9). For this dog, the skin concentration was 0.82 µg/g after the fifth dose compared with 5.68 µg/g on Day 0. The plasma concentration for this dog also was unexpectedly low after receiving orbifloxacin once daily for 5 to 7 days (0.62 µg/ml) compared with the value on Day 0 (4.42 µg/ml). It can be speculated that this dog may not have received a full dose of orbifloxacin on one or more days. Nevertheless, data for this dog were retained in all calculations and analyses.

**Plasma Orbifloxacin Concentrations**

There were no significant differences between the mean plasma orbifloxacin concentrations in clinically normal dogs and dogs with pyoderma following administration of single ($P = .1201$) or multiple ($P = .3260$) doses. Three hours after receiving the initial dose on Day 0, dogs with normal skin had a mean plasma orbifloxacin concentration of $4.99 \pm 0.90$ µg/ml and dogs with pyoderma had a mean concentration of $5.66 \pm 1.29$ µg/ml (Table 1). After 5 to 7 days of dosing, beagles with normal skin had a mean orbifloxacin plasma concentration of $5.76 \pm 0.74$ µg/ml and dogs with pyoderma had a mean concentration of $6.38 \pm 2.18$ µg/ml; the increase from Day 0 to Day 4, 5, or 6 was not significant.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Skin Concentration (µg/g)</th>
<th>Plasma Concentration (µg/ml)</th>
<th>Skin:Plasma Ratio (ml/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 5*</td>
<td>Day 0</td>
</tr>
<tr>
<td>Normal beagles (n = 14)</td>
<td>3.85 ± 1.08</td>
<td>5.43 ± 1.02</td>
<td>4.99 ± 0.90</td>
</tr>
<tr>
<td>Dogs with pyoderma (n = 14)</td>
<td>7.80 ± 3.40</td>
<td>9.47 ± 6.23</td>
<td>5.66 ± 1.29</td>
</tr>
</tbody>
</table>

*Actual sampling day was Day 4, 5, or 6.
Orbifloxacin Skin:Plasma Ratio

Three hours after dosing on Day 0, beagles with healthy skin had a mean orbifloxacin skin:plasma ratio of 0.81 ± 0.31 ml/g compared with a ratio of 1.40 ± 0.57 ml/g for dogs with pyoderma (P = .0029) (Table 1). At 3 hours after dosing on Day 4, 5, or 6, the mean orbifloxacin skin:plasma ratio for beagles with normal skin was 0.96 ± 0.24 ml/g compared with 1.44 ± 0.65 ml/g for dogs with pyoderma (P = .0193).

Histopathology

Based on histopathologic assessment of the inflammatory cell infiltrate from pretreatment skin biopsies, beagles with normal skin had a grand mean leukocyte count of 67.7 ± 26.1 cells per field versus 4,345.5 ± 2,584.9 cells per field for dogs with pyoderma. Although leukocyte and orbifloxacin concentrations were numerically higher in diseased skin than in normal skin, there was no statistical correlation between orbifloxacin skin concentrations and leukocyte count.

DISCUSSION

Mean orbifloxacin concentrations in the skin of dogs with pyoderma were approximately 1.4 times the concentrations in plasma following single and multiple dosing of orbifloxacin at 7.5 mg/kg. According to data from the National Committee on Clinical Laboratory Standards (NCCLS),11 the minimum inhibitory concentration (MIC) breakpoint of 1.0 µg orbifloxacin/ml is the point at which canine and feline dermal isolates (including *Staphylococcus* spp, gram-negative enteric bacilli, and other bacteria) would be considered susceptible and would be expected to respond well to the lowest dosage (2.5 mg/kg) of orbifloxacin. The flexible MIC breakpoint at which elevated doses of 5.0 to 7.5 mg/kg may be advisable is 2 to 4 µg/ml. As demonstrated in the present study, mean concentrations of orbifloxacin in infected skin (7.80 to 9.47 µg/g) following administration of 7.5 mg/kg (the high end of the flexible dose range) were nearly double the upper limit of the flexible MIC breakpoint of 4 µg/ml. Orbifloxacin plasma pharmacokinetics have been demonstrated to be linear; therefore, it is reasonable to assume that the skin pharmacokinetics would also be linear.3 Based on this assumption, mean skin concentrations following 5
consecutive days of oral administration of orbifloxacin at 2.5 mg/kg would be expected to be approximately 3.16 µg/g, still exceeding by threefold the susceptible breakpoint of 1.0 µg/ml or less. (Figure 2).

The organism that is recognized as most commonly associated with canine pyoderma is coagulase-positive *S. intermedius*, which has a reported orbifloxacin MIC$_{90}$ of 0.39 µg/ml. The mean skin concentration of orbifloxacin achieved in dogs with pyoderma following multiple daily dosing of 7.5 mg/kg is 24 times the MIC$_{90}$ of *S. intermedius*, and extrapolated mean concentrations achieved following a 2.5-mg/kg dose would still be eight times the MIC$_{90}$. It is also important to note that several dogs in this study had skin concentrations that were much higher than the mean (some as high as 25.50 µg/g); thus the ratio of the concentration of orbifloxacin to a pathogen’s MIC$_{90}$ is considerably higher in certain dogs (in this instance 65 times the MIC$_{90}$ for *S. intermedius*).

The scientific literature consistently describes the bactericidal activity of fluoroquinolones as concentration-dependent. As such, high maximum concentration (C$_{max}$):MIC and area under the curve (AUC):MIC ratios have been advocated to predict antimicrobial effect. Concentrations of orbifloxacin in nondiseased skin approximate those in plasma (thus legitimizing the use of plasma C$_{max}$ or AUC for pharmacokinetic/pharmacodynamic purposes); however, orbifloxacin achieves much higher concentrations in the diseased skin of dogs with pyoderma. This indicates that the use of plasma kinetic parameters as a determinant of efficacy of orbifloxacin in canine skin disease is rather conservative.

Results of this study demonstrated that plasma orbifloxacin concentrations are similar in both clinically normal dogs and dogs with pyoderma. However, orbifloxacin is preferentially distributed or targeted to areas of infection within skin. Although leukocyte and orbifloxacin concentrations were numerically higher in diseased skin than in normal skin, no statistical correlation between orbifloxacin skin concentrations and leukocyte count was evident. This finding differs from a similar study in which a statistical correlation between dermal inflammatory cell count and drug concentration in infected skin was noted in dogs with pyoderma that had received enrofloxacin. As noted in that paper, it is difficult to determine how much of the increased drug concentration in the skin of dogs with pyoderma is attributable to the presence of inflammatory mediators producing increased vascular permeability and enhanced blood flow.

**CONCLUSION**

Following both single and multiple administrations of orbifloxacin at 7.5 mg/kg, dogs with pyoderma had significantly higher mean skin concentrations of orbifloxacin than did clinically normal dogs with healthy skin. Additionally, dogs with pyoderma had significantly higher mean orbifloxacin skin:plasma ratios than did dogs with normal, healthy skin. There were no significant differences between the mean plasma orbifloxacin concentrations in dogs with normal skin and dogs with pyoderma on either Day 0 or following five to seven doses of orbifloxacin.

The high degree of clinical efficacy of orbifloxacin for the treatment of skin infections reported previously may be attributable to the propensity of the drug to accumulate in infected skin, its concentration-dependent bactericidal activity, and the postantibiotic effect that is attributed to fluoroquinolones.

**REFERENCES**

3. Heinen E: Comparative serum pharmacokinetics of the fluoroquinolones enrofloxacin, difloxacin, mar-


