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Pharmacokinetics, pharmacodynamics and therapeutics of pradofloxacin in the dog and cat

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Pradofloxacin is a third-generation fluoroquinolone, licensed in the EU for use in a range of indications in the dog and cat and authorized more recently in the USA for one therapeutic indication (skin infections) in the cat. This review summarizes and appraises current knowledge on the physico-chemical, pharmacological [pharmacokinetics (PK) and pharmacodynamics (PD)], safety and therapeutic properties of pradofloxacin in the target species. Pradofloxacin contains two centres of asymmetry and is the pure SS enantiomer. After oral dosing of tablets (dog) or tablets and oral suspension (cat), maximum plasma concentrations (C_{max}) are achieved in less than 3.0 h, and terminal half-life is of the order of 5–10 h. Accumulation is slight or absent with once daily oral dosing. Free drug concentrations in plasma are in the range of 63–71% of total concentration. As for other fluoroquinolones, antibacterial activity is attributable to inhibition of bacterial replication at two sites, subunit A of topoisomerase II and topoisomerase IV. The antimicrobial spectrum includes gram-negative and gram-positive organisms, anaerobes, *Mycoplasma* spp. and some intracellular organisms (*Rickettsia* spp. and *Mycobacterium* spp.). The killing action is of the concentration-dependent type. Pradofloxacin has high potency (low *MIC* values) in comparison with first- and second-generation fluoroquinolones. Integration of *in vivo* PK and *in vitro* PD data provides values of C_{max}/MIC and area under plasma concentration–time curve ($AUC_{24\ h}$)/*MIC* ratios predictive of good clinical efficacy against sensitive organisms, when administered at recommended dose rates. Clinical trial evaluation of pradofloxacin, in comparison with other authorized antimicrobial drugs, has demonstrated either noninferiority or superiority of pradofloxacin. Data indicating clinical and, in some instances, bacteriological cure have been reported: (i) in cats, for wound infections, abscesses, upper respiratory tract infections, conjunctivitis, feline infectious anaemia and lower urinary tract infections and (ii) in dogs, for wound infections, superficial and deep pyoderma, acute urinary tract infections and adjunctive treatment of infections of gingival and periodontal tissues. At clinical dose rates pradofloxacin was well tolerated in preclinical studies and in clinical trials. Among the advantages of pradofloxacin are (i) successful treatment of infections caused by strains resistant to some other fluoroquinolones, as predicted by PK/PD data, but depending on the specific *MIC* of the target strain and (ii) a reduced propensity for resistance development based on MPC measurements. The preclinical and clinical data on pradofloxacin suggest that this drug should commonly be the fluoroquinolone of choice when a drug of this class is indicated. However, the PK/PD data on pradofloxacin, in comparison with other fluoroquinolones, are not a factor that leads automatically to greater clinical efficacy.

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INTRODUCTION

Sixteen years have elapsed since publication of an excellent review on fluoroquinolones in animal health (Brown, 1996). The reader is referred to this early review for a description of the chemistry, pharmacological properties and therapeutic uses of earlier licensed drugs of this class.

Pradofloxacin is a third-generation fluoroquinolone, recently licensed in the EU and USA. It is recommended for use in the treatment of a range of microbial infections in the dog and cat. Pradofloxacin is available commercially in two oral formulations: flavoured tablets (Veraflox®, Bayer Animal Health GmbH, Leverkusen, Germany, 15 mg Tablet for cats and small dogs, Veraflox® 60 mg and 120 mg Tablet for dogs) and a 2.5% oral suspension (Veraflox® 25 mg/mL Oral Suspension) for feline use. In the latter formulation, pradofloxacin is bound to an ion exchange resin to avoid its bitter taste and to ensure good palatability. Within the upper gastrointestinal tract, at low pH values, pradofloxacin is released from the resin within a few minutes. The recommended dose rates are 3 mg/kg (tablet formulation) and 5 mg/kg (oral suspension) once daily in the EU and 7.5 mg/kg (cat only) in the USA. The relative bioavailability of the tablet formulation is higher than that of the suspension.

References cited in this review include peer-reviewed articles and abstracts of presentations to meetings of scientific societies. It is recognized that the latter, while providing useful data, have not, in most instances, undergone full peer review.

CHEMICAL STRUCTURE AND PHYSICO-CHEMICAL PROPERTIES

Pradofloxacin is a brownish yellow crystalline compound of molecular mass 396.42. Its melting point is 242 °C. pKa values for the molecule's two dissociation constants at 21 °C are 5.5 and 8.8, respectively. The solubility in water at 21 °C is equal to 33.5 g/L, and the pH of an aqueous solution is 7.3. Percentage hydrolysis in a 0.1% w/v solution at 90 °C after 7 days was 83% in 0.1N NaOH and negligible in 0.1N HCL. Among fluoroquinolones, lipid solubility varies. For example, enrofloxacin and pradofloxacin are more lipophilic than ciprofloxacin.

The synthetic pathway has been described by Himmler *et al.* (2002). Pradofloxacin is an 8-cyanofluoroquinolone containing two centres of asymmetry (Fig. 1). The chemical name is 8-cyano-1-cyclopropyl-7-([S,S]-2,8-diazabicyclo(4,3,0)non-8-y1)-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid. Pradofloxacin differs from enrofloxacin in the presence of an electron withdrawing cyano group at position C-8, in place of hydrogen and an S,S-pyrrolidinopiperidine replacement of an ethylpiperazine moiety at C-7 (Fig. 1). Enantiomerically, pradofloxacin is the pure SS isomer. Comparative studies have demonstrated its greater potency than the other three isomers (*vide infra*). Enhanced potency has been achieved by the substitutions in C-7 and C-8 positions in the molecule (Himmler *et al.*, 2002; Wetzstein & Hallenbach, 2004, 2011).

PHARMACOKINETICS

An analytical method for quantitation of pradofloxacin in serum and urine, based on turbulent flow chromatography coupled with tandem mass spectrometry and using sarafloxacin as internal standard, has been described by Krebber (2003). The method has also been used to determine pradofloxacin in canine skin biopsies (Krebber & Hoffend, 2004).

Cat

Fraatz (2006) described the pharmacokinetic profile of pradofloxacin after intravenous administration at a dose rate of

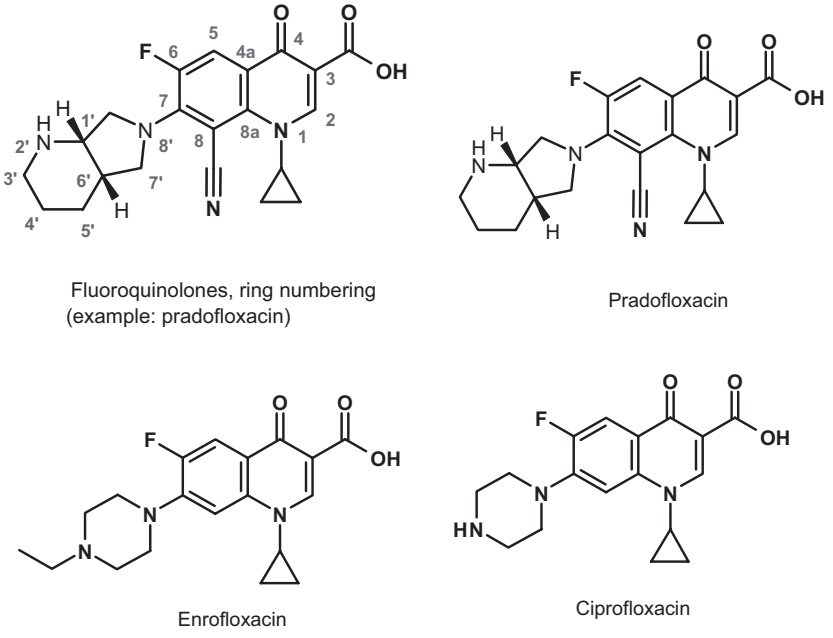

Fig. 1. Structures of enrofloxacin, ciprofloxacin and pradofloxacin.

3 mg/kg; mean values for the pivotal pharmacokinetic parameters were 0.28 L/h/kg (whole body clearance), 0.06 L/h/kg (renal clearance), 4.5 L/kg (volume of distribution) and 10 h (terminal half-life). Renal clearance thus comprised 21% of whole body clearance. In the same study, oral dosing of pradofloxacin in a tablet formulation provided a C_{max} of 1.19 μg/mL, T_{max} of 0.5–1.0 h, *AUC* of 4.96 mg·h/L and bioavailability of 70%. In a second study, repeat dosing of pradofloxacin tablets over 5 days at 24-h intervals indicated no accumulation (accumulation index = 1.01:1), and C_{max} and *AUC* values after the fifth dose were 1.33 μg/mL and 5.71 mg·h/L (Fraatz, 2006).The low accumulation index is somewhat surprising, in the light of the terminal half-life. The explanation for low accumulation is not known.

After oral dosing of pradofloxacin at 24-h intervals, formulated as an oral suspension, at three dose rates (2.5, 5.0 and 10.0 mg/kg) for a total of five administrations, dose-dependent C_{max} values of 0.9–3.2 μg/mL were achieved rapidly within 0.5–1.0 h. Terminal half-life (7.2–9.8 h) and mean residence time (8.8 h) were of medium duration (Daube *et al.*, 2006). The pharmacokinetic profile of the suspension was similar to the tablet formulation and also independent of administered dose, that is, the profile was linear. The accumulation index was slightly higher, in the range of 1.13:1–1.26:1, for the suspension, compared to the tablet formulation.

In a third feline report, Hartmann *et al.* (2008a) compared the distribution of pradofloxacin and doxycycline in serum, saliva and tear fluid. Doxycycline penetration into both nonvascular fluids was slight, whereas the ready penetration of pradofloxacin was indicated by the pharmacokinetic variables reported in Table 1. Terminal half-life was longer, and C_{max} concentrations of pradofloxacin were higher in both fluids compared to serum. The authors proposed that the high peak concentrations in both fluids are probably attributable to an active transport mechanism. Although active transport has not been reported for pradofloxacin, other investigators have demonstrated active secretion of ciprofloxacin across human intestinal (Caco-2) cells (Griffiths *et al.*, 1993, 1994; Cavet *et al.*, 1997). Moreover, several groups have shown that fluoroquinolones are substrates of the ATP-binding ABC transporters, including the multidrug resistance protein 1 (MDR1), a P-glycoprotein (P-gp) and the multidrug resistance-associated proteins 1 and 2 (MRP1 and 2) (Lowes & Simmons, 2002; Sasabe *et al.*, 2004; Schrickx & Fink-Gremmels, 2007). Hartmann *et al.* (2008a) proposed that P-gp/MDR1, which is expressed in the respiratory tract, may be responsible for secretion of pradofloxacin into saliva and tear fluid. However, not all fluoroquinolones are substrates for this transporter so that its role in relation to pradofloxacin transport, if any, remains to be elucidated. Irrespective of the transport mechanism, and whether active or passive, Hartmann *et al.* (2008a) indicated that the distribution of pradofloxacin was favourable for the treatment of upper respiratory tract and conjunctival infections in cats caused by organisms such as *Chlamydophila felis* (Greene, 2006).

Table 1. Pharmacokinetic variables for pradofloxacin in biological fluids of the cat after oral dosing of an oral suspension at a dose rate of 5 mg/kg

	Fluid		
Variable (units)	Serum	Saliva	Tear fluid
C_{max} (μg/mL)	1.09 ± 0.52	6.33 ± 6.97	13.41 ± 20.85
T_{max} (h)	1.75 ± 1.26	0.50 ± 0	0.75 ± 0.27
$t_{½}$ (h)	2.95 ± 1.08	18.03 ± 10.21	16.36 ± 33.45
MRT (h)	5.12 ± 1.54	16.77 ± 13.23	3.30 ± 4.34
$AUC_{0–24}$ (μg·h/mL)	5.32 ± 1.03	6.77 ± 4.92	7.23 ± 8.40

Values are mean ± SD (n = 6). C_{max}, maximum concentration; T_{max}, time of maximum concentration; $t_{½}$, terminal half-life; *MRT*, mean residence time; *AUC*, area under concentration–time curve. Data from Hartmann *et al.* (2008a).

Dog

Fraatz *et al.* (2003a) described the pharmacokinetic profile of pradofloxacin in fasted Beagle dogs after intravenous and oral dosing, the latter with the commercially available tablets, at dose rates of 3 and 6 mg/kg. Pivotal pharmacokinetic parameters after intravenous dosing of 3 and 6 mg/kg pradofloxacin were 0.24 L/h·kg (total body clearance, both doses), 2.22 and 2.56 L/kg (volume of distribution) and 6.60 and 7.63 h (elimination half-life), respectively. Elimination half-life was similar after oral dosing, C_{max} values were 1.2 and 2.5 μg/mL, and bioavailabilities were 1.05 and 1.06, respectively. T_{max} values of 2.1 and 2.8 h were determined after 3 and 6 mg/kg dose rates, respectively. Dose normalization of *AUC* values indicated linear pharmacokinetics. Of the administered doses, 45% and 40% were recovered in urine after intravenous and oral dosing, respectively, with 85% of urinary excretion occurring within 24 h.

Fraatz *et al.* (2002, 2003b) compared serum and skin concentrations of pradofloxacin in Beagle dogs after daily oral dosing with tablets over 28 days. Four dose rates were compared, namely 1, 3, 6 and 9 mg/kg. Skin biopsies taken 4 h after dosing on days 1, 7, 14, 21 and 28 were homogenized prior to analysis. The data established dose linearity by linear regression for the variables C_{max} and $AUC_{0–24\ h}$. The study confirmed the findings of Fraatz *et al.* (2003a) regarding: (i) mean serum T_{max} values ranging from 1.1 to 2.1 h, (ii) a low mean accumulation index of 1.1:1 for all dose groups and (iii) mean terminal half-lives in the range of 5.6–7.2 h. Skin concentrations of pradofloxacin on all dosing days were of the order of 30–60% higher than serum concentrations 4 h after dosing. Day 1 and steady-state concentrations are presented in Table 2.

Restrepo *et al.* (2008) assessed the penetration of pradofloxacin into canine skin of animals with pyoderma and in healthy animals. Higher concentrations were determined in lesional skin. The disposition of pradofloxacin into skin is clearly relevant to its use in the treatment of superficial and deep pyoderma, as previously identified for enrofloxacin (DeManuelle

Table 2. Mean (±SD) skin and serum pradofloxacin concentrations (μg/g or μg/mL) 4 h post-treatment on days 1 and 28 of dosing

	Day 1		Day 28	
Dose (mg/kg)	Skin	Serum	Skin	Serum
1	0.4 ± 0.1	0.3 ± 0.0	0.5 ± 0.2	0.3 ± 0.1
3	1.1 ± 0.3	1.1 ± 0.3	1.3 ± 0.4	0.9 ± 0.6
6	1.5 ± 0.7	2.1 ± 0.3	2.9 ± 1.1	1.8 ± 0.3
9	2.2 ± 0.6	2.7 ± 0.4	3.8 ± 0.8	3.0 ± 0.5

Data from Fraatz *et al.* (2003b).

et al., 1998). However, interpretation of 'whole tissue concentration' data for skin (as for any tissue) must be interpreted with caution (Mouton *et al.*, 2008). The skin is not the biophase in pyoderma, for which the important skin pathogenic organism is *Staphylococcus pseudintermedius*. Based on possibly high intracellular relative to extracellular concentrations, the total skin concentration of pradofloxacin may overestimate concentration in the biophase.

Plasma protein binding

In vitro studies have shown that pradofloxacin binding to plasma proteins is independent of total concentration over the concentration range 150–1500 ng/mL. Mean values for free drug concentration ranged from 63.4 to 64.2% (dog) and 68.6 to 71.2 (cat) (Bregante *et al.*, 2003). The therapeutic significance of this degree of plasma protein binding is that it is generally accepted that it is only 'free' drug that is microbiologically active (Zeitlinger *et al.*, 2004).

Metabolism and Excretion

The major excretion products are unchanged drug (dog and cat) and glucuronide conjugate (dog). As a percentage of administered dose, 40% and 10% are excreted in urine as parent drug plus glucuronide in the dog and cat, respectively (European Public Assessment Report, EMA/142130/2011). There are no published data on excretion of pradofloxacin in faeces, but as less than 50% of the administered dose is excreted in urine, it is likely that most of the parent drug and metabolites are excreted in faeces. The antimicrobial activity of pradofloxacin glucuronide, if any, is unknown, but it is likely that only parent drug possesses antimicrobial activity, as glucuronides of most drugs are polar and poorly lipid-soluble molecules, which do not readily penetrate cell membranes, including cell walls and cell membranes of bacterial cells.

PHARMACODYNAMICS

Molecular mechanism of action

The antibacterial activity of pradofloxacin is attributable to inhibition of replication at two enzyme sites, subunit A of topoisomerase II (also termed DNA gyrase) and topoisomerase IV (Körber *et al.*, 2002). DNA gyrase introduces negative superhelical twists in the bacterial DNA double helix ahead of the replication fork, thereby catalysing the separation of daughter chromosomes, essential for initiation of DNA replication. Topoisomerase IV is primarily involved in decatenation, the unlinking of replicated daughter chromosomes. Older fluoroquinolones in veterinary use can also act at both sites, but the enzyme primarily targeted depends on bacterial species (gram positive or gram negative), one enzyme generally being targeted preferentially in a given species. DNA gyrase and topoisomerase IV are the primary and secondary targets, respectively, of gram-negative bacteria, and target preference is reversed in gram-positive bacteria (Peterson, 2001; Drlica & Malik, 2003). Compared to earlier generation veterinary fluoroquinolones, pradofloxacin targets both enzymes with increased affinity (Wetzstein *et al.*, 2005a,b). Wetzstein *et al.* (2005a,b) identified topoisomerase IV as the primary and DNA gyrase as the secondary target for pradofloxacin in *Staphylococcus aureus*, a gram-positive organism. However, compared to ciprofloxacin, pradofloxacin had a 16-fold higher affinity for the secondary target. The end response of inhibition of topoisomerases II and IV is stabilization of DNA double-strand breaks in covalent enzyme-DNA complexes, resulting in inhibition of DNA replication and chromosome regeneration, respectively.

For fluoroquinolones, Lewin *et al.* (1991) defined bactericidal mechanisms A, B, B_1 and C, as follows: mechanism A requires cell division and protein synthesis; B requires neither of these; B_1 requires cell division and C requires protein synthesis. Körber *et al.* (2002) investigated the mechanism of the killing actions of pradofloxacin, enrofloxacin, marbofloxacin and ciprofloxacin against susceptible strains of *E. coli*, *S. aureus* and *S. pseudintermedius,* as well as single–step- and double-step-resistant mutants of the former two species. All four drugs displayed bactericidal mechanism A against all strains, but only pradofloxacin was effective in killing wild-type strains, through mechanism B, indicating high activity even in the absence of both protein synthesis and cell division and therefore exerting activity *in vitro* under conditions that may occur in infected tissues *in vivo*. In contrast, enrofloxacin and marbofloxacin exhibited reduced activity against *Staphylococcus* spp. under these conditions.

When fluoroquinolones trap bacterial type II topoisomerases in ternary drug–enzyme–DNA covalently linked complexes, this triggers induction of the SOS regulon. (The SOS system is a regulon; it controls expression of several genes distributed throughout the genome simultaneously. The SOS response is a state of high-activity DNA repair, activated by bacteria that have been exposed to DNA-damaging agents. In *E. coli,* more than 40 genes are induced in response to DNA damage.) Körber-Irrgang *et al.* (2004) quantified SOS-dependent expression of the *recA* gene, which encodes RecA, a central regulator of the SOS system. Using an SOS induction assay, they determined SOS-inducing concentrations (MISCs) of three fluoroquinolones for wild-type and three isogenic mutant-resistant strains of *E. coli*. Pradofloxacin had lower MISCs than enrofloxacin and ciprofloxacin for all four strains; for pradofloxacin,

the MISCs were 0.031-fold (three strains) and 0.063-fold (one strain) the corresponding *MIC*s. The assay thus detects and quantifies a biological action of pradofloxacin, underlying its bactericidal effect, at concentrations up to 31 times less than *MIC*s. These data further indicated that SOS induction is unaffected by the primary target, be it topoisomerase II in Wt and WT-4 strains or topoisomerase IV in WT-3-1 or WT-K strains. It is, however, argued by some workers that activation of the SOS response may have adverse effects (increased mutations conferring resistance, induction of horizontal gene transfer and persistence to fluoroquinolones and overexpression of resistance genes) (Beaber *et al.*, 2004; Dörr *et al.*, 2009; López & Blázquez, 2009; Briales *et al.*, 2012; Hocquet *et al.*, 2012). However, such studies have not been reported for pradofloxacin.

Spectrum of activity and potency

Pradofloxacin retains the broad spectrum of activity against gram-negative bacteria (bacilli and cocci) of first- and second-generation fluoroquinolones. Additionally, it possesses an extended spectrum of activity against gram-positive and anaerobic bacteria, as well as *Mycoplasma* species and intracellular organisms (*Rickettsia* spp. and *Mycobacterium* spp.) (Abraham *et al.*, 2002; Wetzstein & Ochtrop, 2002; de Jong & Bleckmann, 2003; Stephan *et al.*, 2003a, 2005, 2008; de Jong *et al.*, 2004; Silley *et al.*, 2007). MIC_{90} values for pradofloxacin against feline and canine pathogens are presented for EU, German and USA isolates in Table 3. There are some differences, depending on geographical locations. For three of nine bacterial species, MIC_{90} was higher for German isolates.

The high potency of pradofloxacin against a wide range of organisms isolated from clinical cases of wound, pyoderma and urinary tract infections in dogs and upper respiratory tract, wound infections and abscesses in cats was further illustrated by MIC_{50}, MIC_{90} and geometric mean *MIC* (GMIC) values (Pridmore *et al.*, 2005). Examples of GMIC values (μg/mL) for at least 66 strains per species are as follows: *S. pseudintermedius* 0.04; *Staphylococcus* spp. 0.07; *E. coli* 0.03; *Pasteurella* spp. 0.02; *Streptococcus canis* 0.07.

Table 3. MIC_{90} values (μg/mL) for pradofloxacin against feline and canine pathogen isolates from the EU*, Germany† and USA‡

Species	EU	Germany	USA
Bordetella bronchiseptica	0.25§	0.25	0.25
Escherichia coli	0.125	2.0	0.03
Klebsiella pneumoniae	0.062¶	0.25	0.06
Pasteurella spp.	≤ 0.016	0.015**	0.015
Proteus spp.	0.5	4.0††	0.25
Pseudomonas aeruginosa	0.5	2.0	>2.0
Salmonella spp.	Not tested	0.015	0.03
Staphylococcus pseudintermedius	0.125	0.06	0.06
Staphylococcus spp.	0.25	0.5‡‡	0.12
Streptococcus canis	0.125	Not tested	0.12§§

All determinations used the same methodology, which was undertaken using CLSI guidelines. *Data from Pridmore *et al.* (2005), †Data from de Jong *et al.* (2004); ‡Data from Abraham *et al.* (2002); §*Bordetella* spp.; ¶*Klebsiella* spp.; **Pasteurella multocida*; ††*Proteus mirabilis*; ‡‡*Staphylococcus aureus;* §§*Streptococcus* spp.

Silley *et al.* (2005) established MBC and *MIC* values for five strains of each of ten bacterial species, known to be important pathogens in canine and feline diseases. Of the 50 strains studied, it was shown that *MIC* and MBC were equal for 38%, and MBC was one dilution (18%), two dilutions (22%), three dilutions (16%) and four dilutions (6%) greater than *MIC*. Based on time-kill studies, pradofloxacin was shown to exert a concentration-dependent killing action against all organisms and strains tested, both aerobes and anaerobes, by the rapid killing effect and reduction in bacterial count of 5 $\log_{10}$ cfu/mL or greater. There was also absence of re-growth at 48 h with concentrations as low as 0.125 μg/mL (Silley *et al.*, 2012).

Wetzstein (2003) reported on the *in vitro* postantibiotic effect (PAE) of pradofloxacin, determined as the time for bacterial count to increase by 1 $\log_{10}$ cfu/mL, after short-term exposure to a high concentration. PAE values for clinical strains of *E. coli*, *S. aureus* and *S .pseudintermedius* were 2.3, 2.4 and 2.8 h, respectively, after previous exposure for 2 h to concentrations similar to the mutant prevention concentration (MPC). MPC describes the growth-inhibiting potential of an antibacterial drug for a large inoculum size; MPC or higher concentrations prevent the clonal expansion of quinolone-resistant subpopulations (Drlica & Zhao, 2007). When the previously listed three species were exposed to 0.5 *MIC*s of pradofloxacin, after exposure to the high concentrations used to determine PAE (that is sub-*MIC* PAE), the periods of growth inhibition relative to controls were 7.2, 9.0 and 6.1 h. Moreover, exposure to pradofloxacin at sub-*MIC* concentrations without initial exposure to a higher concentration also partially inhibited bacterial growth. The implication for clinical use is that when biophase concentrations initially exceed *MIC*s for target pathogens before decreasing to levels less than *MIC*, these lower concentrations can be expected to extend the period of growth inhibition. Furthermore, Wetzstein (2008) confirmed a pronounced postantibiotic sub-*MIC* effect of pradofloxacin in high-density bacterial populations. Relatively long PAE and sub-*MIC* PAE effects are typical of drugs with a concentration-dependent killing action. Wetzstein (2005) determined MPCs of several veterinary fluoroquinolones, including pradofloxacin, for strains of *E. coli* and *S. aureus*. Pradofloxacin had the lowest MPCs among the fluoroquinolones evaluated. However, it should be noted that it is the MPC/*MIC* ratio that is of importance in minimizing opportunities for the emergence of resistance. MPCs were achieved in plasma for target pathogens with therapeutic dose rates of pradofloxacin. Similarly, a low MPC of 0.3 μg/mL was determined for a single *Porphyromonas gingivalis* strain (Stephan *et al.*, 2007).

pH dependency of antimicrobial activity

Körber-Irrgang *et al.* (2009) investigated the pH dependency of the action of pradofloxacin against *E. coli* and *S. aureus* (2 reference strains and 12 clinical isolates of each species). For *E. coli*, pradofloxacin had highest potency (lowest *MIC*) at

alkaline pHs; pH8 = 7.3 > 6 > 5. For *S. aureus*, the potency order was pH 7.3 > 8 = 6 > 5 (Table 4). As the pHs in dog and cat skin are 7.4 and 6.4, respectively, and lower in both species in soft tissue infections, these *in vitro* data are relevant to prediction of *in vivo* activity in skin infections. The effects of pH on potency are of interest, yet somewhat surprising, in that the molecule contains an acid dissociation constant. Hence, the degree of ionization of the carboxylic acid group will be greater under alkaline conditions, and it would normally be expected that this would reduce potency.

Other studies have been conducted on the effect of pH on the activity of pradofloxacin against various bacteria. The *MIC*s are only moderately affected by acidic pH, and therefore, it is not anticipated that there will be reduced efficacy of pradofloxacin in treatment of urinary tract infections in dogs and cats (Stephan, personal communication). Bacteria that are released into the urine should be killed by the high urine concentrations of pradofloxacin, and for the bacteria in the tissues of the urinary tract, the pH is less relevant. Furthermore, clinical field studies have demonstrated the high clinical and bacteriological efficacy of pradofloxacin (Stephan *et al.*, 2006b). There is no necessity to alkalinise the urine when treating urinary tract infections.

The potency of pradofloxacin under differing pH conditions was compared to four analogs with substituents of H, Cl, F and OCH_3 in place of the CN grouping in position 8 of the structure (Körber-Irrgang *et al.*, 2009). The *MIC*s for the H and OCH_3 analogs established that the CN grouping was essential for high potency at neutral and slightly acidic pH values against *E. coli*. For *S. aureus*, the halogenated (Cl or F) substituents provided compounds with greater potency than pradofloxacin at some pH values. However, at slightly acidic pHs, pradofloxacin was the second most active of the five compounds.

Comparative potency studies and structure activity relationships

Detailed consideration of the potency of pradofloxacin relative to other fluoroquinolones used in cats and dogs is outside the scope of this review. However, it may be noted that Ganiere *et al.* (2005) determined MIC_{50} and MIC_{90} values of 18 antimicrobial drugs against 50 strains of *S. pseudintermedius* isolated in 2002 from canine pyoderma cases. Pradofloxacin was the most potent; MIC_{50} and MIC_{90} values were 0.032 and 0.063 μg/mL, respectively. Corresponding values were for enrofloxacin 0.125 and 0.5 μg/mL and for marbofloxacin 0.25 and 0.5 μg/mL.

Table 4. Range of *MIC* values for pradofloxacin against clinical isolates of *Escherichia coli* and *Staphylococcus aureus* at four pHs*

	MIC (μg/mL)			
Organism	pH5	pH6	pH7.3	pH8
Escherichia coli†	0.125–0.5	0.032–0.25	0.008–0.032	0.008–0.032
Staphylococcus aureus†	0.125–0.5	0.063–0.125	0.031–0.063	0.063–0.125

*Data from Körber-Irrgang *et al.* (2009); †12 clinical isolates of each organism.

Himmler *et al.* (2002) compared *MIC* values of pradofloxacin against six fluoroquinolones licensed for veterinary use (danofloxacin, difloxacin, enrofloxacin, marbofloxacin, orbifloxacin and sarafloxacin) and two references drugs, ciprofloxacin and moxifloxacin. For four *E. coli* strains, two *S. aureus* strains and two *S. pseudintermedius* strains, pradofloxacin had lower *MIC* values against all veterinary strains and equal or greater potency to the two reference drugs. Greater potency of pradofloxacin than other fluoroquinolones against feline and canine pathogens was also reported by Abraham *et al.* (2002), de Jong and Bleckmann (2003) de Jong *et al.* (2004) and Silley *et al.* (2007).

Structure activity relationships of pradofloxacin and related compounds were evaluated by Wetzstein and Hallenbach (2004) for eight strains of *S. aureus* and six strains of *E. coli*, with marbofloxacin and ciprofloxacin used as reference drugs. The strains selected included wild-type and fluoroquinolone-resistant mutant strains with differing enzyme structures for gyrase A or topoisomerase IVA or both. The SS isomer of pradofloxacin was more potent (lower *MIC*s) by a factor of 2–8 than the RR isomer for all strains. The SS enantiomer was also more potent than enrofloxacin and 8-cyano-enrofloxacin against all strains and more potent than decyano-8-H-pradofloxacin against several strains. The findings indicated that the potency of pradofloxacin was dependent on two groups, the amino (SS-pyrrolidinopiperidine) and cyano moieties.

Studies of structure activity relationships were extended by Wetzstein and Hallenbach (2006) for single strains of *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538). *MIC*s and MPCs were determined for pradofloxacin compared to eight derivatives with various chemical substitutions and also for enrofloxacin and ciprofloxacin as controls. Based on MPCs, measured after 14 days of incubation, activities relative to pradofloxacin (100%) were, respectively, 69, 141, 53 and 12 for enrofloxacin, ciprofloxacin, descyano-8-H-pradofloxacin and the RR-pyrrolidinopiperidine enantiomer for *E. coli*. Corresponding values for *S. aureus* were 17, 9, 15 and 29. Absolute MPC values are important in relation to drug pharmacokinetics, and MPC/*MIC* ratios are important in regard to mutant-selection window; the smaller the ratio, the less time in the window. These data indicate that the pradofloxacin structure is optimized for both MPC values and MPC/*MIC* ratios.

Stephan *et al.* (2003a) compared the potencies of pradofloxacin and metronidazole against 178 obligate anaerobe species isolated from both feline and canine clinical cases and healthy carriers. Geometric mean *MIC* values (μg/mL), pradofloxacin first and metronidazole second, were 0.48, 1.38 (*Clostridium*), 0.37, 0.70 (*Bacteroides*), 0.18, 0.42 (*Prevotella*), 0.46, 0.49 (*Fusobacterium*) and 0.062, 0.09 (*Porphyromas*). Silley *et al.* (2007) compared the activity of pradofloxacin, marbofloxacin, enrofloxacin, ibafloxacin and difloxacin against 141 strains of anaerobic bacteria. Pradofloxacin demonstrated the lowest *MIC*s of the fluoroquinolones tested, and, based on the GMIC, was at least threefold more potent than the other veterinary fluoroquinolones.

Biswas *et al.* (2010a) reported 4-fold or greater potency of pradofloxacin than enrofloxacin for 8 of 9 feline strains of *Bartonella henselae*, the causative agent of cat-scratch disease (CSD), which is also implicated in other feline presentations including endocarditis. For pradofloxacin, the *MIC* range was 0.004–0.125 μg/mL. Both fluoroquinolones (in contrast to azithromycin) retained activity for five or more passages, and the fluoroquinolones were considered more likely to achieve treatment success than azithromycin in clinical use (Biswas *et al.*, 2010b).

Emergence of resistance and clinical breakpoints

No claim can be made that pradofloxacin is 'resistance breaking', as the mechanisms of resistance are the same as for other fluoroquinolones, and cross-resistance is complete within the class. However, pradofloxacin exhibits lower *MIC*s than other fluoroquinolones against wild-type strains and first-step fluoroquinolone-resistant variants of *E. coli* and wild-type *S. aureus*. Because of the rapid killing action and low MPC concentrations of pradofloxacin, it is likely that its use in clinical subjects will not readily lead to clonal expansion of resistant mutants. For some earlier fluoroquinolones, epidemiological cut-off values are available for the respective pathogens and in some cases can be ascertained from published susceptibility distributions. A CLSI clinical breakpoint for pradofloxacin has not yet been set. In the course of EU registration, a tentative resistance breakpoint of ≥ 2 μg/mL was used.

PK–PD integration

As discussed by Toutain *et al.* (2002), Frimoldt-Möller (2002) and others, the integration of pharmacokinetic and pharmacodynamic data represents, in most instances, the most appropriate approach to determining dosing regimens of antimicrobial drugs for subsequent evaluation in disease models and clinical trials. PK–PD integration gives no guarantee of clinical and bacteriological efficacy, but it is considered to be preferable, in most instances, to dose titration studies (Toutain *et al.*, 2002). As fluoroquinolones, against most if not all susceptible pathogens, kill bacteria by a concentration-dependent killing action, the PK–PD indices widely used to predict effective doses are C_{max}/MIC and AUC/MIC ratios, where C_{max} and AUC refer to plasma or serum free drug concentrations. The required ratios are known to be drug and bacterial species specific, and the scientific literature suggests that numerical targets for fluoroquinolones are $C_{max}/MIC \geq 10$ and $AUC_{0-24}/MIC \geq 125$ h for gram-negative bacteria (Drusano *et al.*, 1998), $C_{max}/MIC \geq 10$ and $AUC_{0-24}/MIC \geq 40$ h for gram-positive bacteria (Andes & Craig, 2002) and $AUC_{0-24}/MIC \geq 7.5$ h for anaerobes (Noel *et al.*, 2005). It must be emphasized that these values provide general guidance, and lower or higher numerical values may apply against organisms of all classes.

The *in vivo* pharmacokinetic and *in vitro MIC* data summarized in this review indicate that pradofloxacin, at clinically recommended dose rates, meets most of these targets for species against which activity is claimed. Table 5 presents data for C_{max}/MIC_{90} and AUC/MIC_{90} ratios for pradofloxacin for large numbers (n = 135 to 1097) of field isolates of several bacterial species, after administration of therapeutic dose rates to dogs and cats,calculated from data provided by P. Silley (personal communication). For administration of pradofloxacin tablets (3 mg/kg) to dogs, C_{max}/MIC_{90} (no units) and AUC/MIC_{90} (h) ratios were, respectively, 16.4 and 132 h (*S. pseudintermedius* and *E. coli*) and 3.78 and 32.8 h (*Prevotella* species and all anaerobes). For cats dosed with pradofloxacin suspension (5 mg/kg), corresponding values were 11.7 and 49.7 h (*S. pseudintermedius*), 46.9 and 200 h (*E. coli*) and 90.4 and 388 h (*Pasteurella multocida*). For cats, lower PK-/PD-integrated values were determined for pradofloxacin tablets administered at a dose rate of 3 mg/kg.

In a recent study, Körber-Irrgang *et al.* (2012) compared the activity of pradofloxacin and marbofloxacin against three

Table 5. PK/PD ratios for target pathogens

Pathogen	Number	MIC_{90s} (μg/mL)	C_{max}/MIC_{90s}*	AUC_{0-24}/MIC_{90s}* (h)
In dogs following administration of pradofloxacin tablets at the dose rate of 3 mg/kg (C_{max} = 1.01 μg/mL and AUC_{0-24} = 13 8.19 μg.h/mL)				
Staphylococcus pseudintermedius	1097	0.062	16.4	132
Escherichia coli	173	0.062	16.4	132
Porphyromonas spp.	310	0.125	8.19	65.5
Prevotella spp.	320	0.25	3.78	32.8
All anaerobes	630	0.25	3.78	32.8
In cats following administration of pradofloxacin tablets at the dose rate of 3 mg/kg (C_{max} = 0.83 μg/mL and AUC_{0-24} = 4.14 μg.h/mL)				
Staphylococcus pseudintermedius	184	0.125	6.90	33.1
Escherichia coli	135	0.031	26.9	134
Pasteurella multocida	323	0.016	51.8	259
In cats following administration of pradofloxacin suspension at the dose rate of 5 mg/kg (C_{max} = 1.45 μg/mL and AUC_{0-24} = 6.21 μg·h/mL)				
Staphylococcus pseudintermedius	184	0.125	11.7	49.7
Escherichia coli	135	0.031	46.9	200
Pasteurella multocida	323	0.016	90.4	388

MIC_{90s} = MIC_{90} for the susceptible part of the population only, where the distributions are bimodal or multimodal;* all C_{max} and AUC values and ratios based on free drug concentrations, comprising 0.63 and 0.69 fraction of total concentrations of pradofloxacin in the dog and cat, respectively.

strains of *S. pseudintermedius* and one strain of *S. aureus* in an *in vitro* PK–PD model based on the pharmacokinetics of each drug in the dog. The model simulated free drug concentrations in dogs provided by single oral doses of pradofloxacin (3 mg/kg) and marbofloxacin (2 mg/kg). For *S. pseudintermedius,* values of C_{max}/MIC (pradofloxacin first, marbofloxacin second) were 13.3 and 2.7, and $AUC_{0–24\ h}/MIC$ values were 142 h and 35 h, respectively. Corresponding C_{max}/MPC ratios were 5.9 and 0.48, and $AUC_{0–24\ h}$/MPC ratios were 63.2 h and 6.1 h, respectively. Similar differences between the two drugs were determined for *S. aureus.* For example, $AUC_{0–24\ h}$/MPC ratios were 32.2 h (pradofloxacin) and 5.4 h (marbofloxacin). The killing action of pradofloxacin was more rapid and more sustained than that of marbofloxacin. Subpopulations with reduced susceptibility to either fluoroquinolone did not emerge.

EFFICACY IN DISEASE MODELS AND CLINICAL TRIALS

In the EU, a requirement for licensing approval of novel antimicrobial drugs by regulatory authorities is demonstration of noninferiority in respect of efficacy in clinical trials. Clinical trials are thus designed and analysed statistically accordingly. The publications reviewed in this section should be considered with this requirement in mind. In fact, in some instances, only noninferiority was demonstrated, but in others, pradofloxacin was shown to be superior to other licensed products. In the latter circumstance, pradofloxacin might be considered to be the drug of choice. *However, the clinician has to carefully balance this advantage against the consideration that national and/or international guidelines on the use of antimicrobial drugs recommend that all fluoroquinolones should be reserved in some circumstances as second-line treatments.*

As the studies reviewed below compared pradofloxacin with control antimicrobial products, the contribution of self-cure to overall treatment efficacy cannot be assessed. However, it may be noted that only bacteriologically positive dogs and cats were included in most studies, and many animals showed systemic signs of illness, and these were sometimes severe.

Cats

The indications and manufacturer's recommended dosages for pradofloxacin in the EU are presented in Table 6.

Comparison of pradofloxacin oral suspension (5 mg/kg once daily) and amoxicillin/clavulanic acid (12.5 mg/kg twice daily) treatments, each for 7 days, was made by Stephan *et al.* (2006a) for the treatment of wound infections and abscesses in a blinded, randomized trial. Clinical cure rates were virtually complete with both treatments: 97.3% for pradofloxacin and 98.8% for amoxicillin/clavulanic acid.

Stephan *et al.* (2005) also compared the efficacy of pradofloxacin (5 mg/kg once daily) against amoxicillin/clavulanic acid (12.5 mg/kg twice daily) in cats with upper respiratory tract infection with treatment over 5 days. Total clinical score decreases were virtually identical (84.3% pradofloxacin and 84.6% amoxicillin/clavulanic acid), but bacteriological cure rate was significantly higher for pradofloxacin (97.9%) compared to amoxicillin/clavulanic acid (81.3%).

Table 6. Pradofloxacin indications for dogs and cats (as authorized in the EU)

Species	Indication	Duration of treatment	Target organisms – susceptible strains of:
Dog*	Wound infections	7 days	*Staphylococcus pseudintermedius*
	Superficial pyoderma	14–21 days	*Staphylococcus pseudintermedius*
	Deep pyoderma	14–35 days	*Staphylococcus pseudintermedius*
	Acute urinary tract infections	7–21 days	*Escherichia coli* *Staphylococcus pseudintermedius*
	Adjunctive treatment of severe infections of the gingival and periodontal tissues	7 days	*Porphyromonas* spp. *Prevotella* spp.
Cat	Acute infections of the upper respiratory tract†	5 days	*Pasteurella multocida* *Escherichia coli* *Staphylococcus intermedius*
	Wound infections and abscesses‡	7 days	*Pasteurella multocida* *Staphylococcus intermedius* group

*Dosage = 3 mg/kg tablets once daily; †Dosage = 3 mg/kg tablets or 5 mg/kg oral suspension, both once daily; ‡Dosage = 5 mg/kg oral suspension once daily.

Hartmann *et al.* (2008b) evaluated pradofloxacin oral suspension (5 mg/kg once daily) plus placebo against doxycycline tablets (5 mg/kg twice daily) plus placebo, administered for 42 consecutive days in cats with upper respiratory tract disease or conjunctivitis attributable to *Chlamydophila felis* and/or *Mycoplasma* spp. Clinical signs of conjunctivitis, ocular and nasal discharge, sneezing, breathing pattern and lung sounds were each assessed using semiquantitative scores. In both groups, there was rapid improvement of clinical signs with no differences between the two treatments. Minor clinical signs, comprising conjunctivitis and ocular discharge, persisted in some cats in both groups at the end of the dosing period and hence 100% clinical cure was not achieved. However, this persistence might have been related to co-infection with feline herpes virus (FHV). In addition, the presence of *C. felis* (59% of cats) and *Mycoplasma* spp. (46% of cats) was detected by qPCR and nested PCR, respectively, on predosing conjunctival swabs. Eradication of *Mycoplasma* spp. was achieved in all cats with both treatments, while *C. felis* was eradicated in 22/22 cats treated with doxycycline and 13/17 cats receiving pradofloxacin.

A blinding by function study comparing the efficacy of pradofloxacin and amoxicillin in the treatment of upper respiratory tract infections was undertaken in 40 cats in a humane society colony (Spindel *et al.*, 2008). Potential primary or secondary pathogens isolated (and percentages) prior to therapy were FHV-1 (75%), *Mycoplasma* spp. (62.5%), *Bordetella* spp. (47.5%), *Staphylococcus* spp. (12.5%) and *Streptococcus* spp.

(10.0%). Single organisms were isolated in only 2/40 cats. *C. felis* was not detected, either by isolation or PCR assay. A range of clinical signs, including fever, nasal discharge, ocular discharge and depression, were scored semiquantitatively prior to and after treatments. The latter comprised amoxicillin (22 mg/kg every 12 h for 7 days), pradofloxacin oral suspension (5 mg/kg every 24 h for 7 days) and pradofloxacin oral suspension (10 mg/kg every 24 h for 7 days). A positive response comprised a reduction in total clinical score to ≤ 3, with mean pretreatment scores of approximately 6.0. Positive responses were determined in 10/15 cats (amoxicillin), 11/13 cats (pradofloxacin 5 mg/kg) and 11/12 cats (pradofloxacin 10 mg/kg). Group differences were nonsignificant.

Dowers *et al.* (2009) evaluated the efficacy of pradofloxacin against *Mycoplasma haemofelis* in a disease model. *M. haemofelis* is a virulent, gram-negative epicellular parasite of erythrocytes. It is a major causative agent of feline infectious anaemia, a disease characterized by anaemia, splenomegaly, fever, icterus and death with a worldwide prevalence rate of 4–23% in asymptomatic cats (Van Steenhouse *et al.*, 1993). Vector-driven transmission of the disease is through fleas. Previous studies with drugs of various classes have suggested that fluoroquinolones and tetracyclines are effective treatments during dosing. However, organism clearance, as indicated by PCR assays for *M. haemofelis* DNA, had not occurred with doxycycline days to weeks after cessation of therapy (Berent *et al.*, 1998).

The four treatment groups in the Dowers *et al.* (2009) study comprised: doxycycline (5 mg/kg every 12 h), pradofloxacin (5 mg/kg every 24 h), pradofloxacin (10 mg/kg every 24 h) and an infected, untreated control group. Drugs were administered orally for 14 days. *M. haemofelis* infection was then detected by PCR assay, and clinical signs of haemoplasmosis were assessed. Doxycycline and both pradofloxacin groups demonstrated significant improvement (total clinical scores) and reduced indicators of anaemia, for example, higher p.c.v. and more rapid resolution of anaemia. However, there were no major differences between the three treatments. Based on real-time qPCR assays to detect *M. haemofelis* DNA, copy numbers in blood were significantly lower in all three drug-treated groups compared to control from days 21 to 42. Copy numbers were also significantly lower than the doxycycline group in cats receiving low-dose pradofloxacin on days 28, 32, 35 and 42 and also in the high-dose pradofloxacin group on days 25 and 28. In addition, 6/12 pradofloxacin-treated and 0/5 doxycycline-treated cats yielded negative results in a cPCR assay. The authors suggested that pradofloxacin may be more effective than doxycycline and also older fluoroquinolones in eradicating *M. haemofelis*, as opposed to merely suppressing clinical signs.

Litster *et al.* (2007) investigated the efficacy of pradofloxacin (oral suspension, 5 mg/kg) administered every 24 h in 27 cats against two positive control treatments for the therapy of lower urinary tract infections. The controls were amoxicillin–clavulanate oral suspension (62.5 mg/cat every 12 h) in 28 cats and doxycycline oral paste (5 mg/kg loading dose, followed by 2.5 mg/kg every 12 h for two doses, then 2.5 mg/kg every 24 h) in 23 cats. All treatments were administered for a minimum of 10 days. Allocation to groups was based on prior bacterial susceptibility tests. The pretreatment percentages, respectively, of gram-negative bacilli and gram-positive cocci were 62 and 39 (pradofloxacin), 50 and 50 (doxycycline) and 41 and 59 (amoxicillin-clavulanate). Efficacy assessment was based on pre- and post-treatment urine bacterial culture and sensitivity tests. Post-treatment urine samples were negative for culture in all cats receiving pradofloxacin, whereas there were three treatment failures in each of the positive control groups, despite the allocation to groups based on the initial susceptibility tests. Differences between groups were nonsignificant, possibly as a consequence of the relatively small group sizes. Median therapy durations were 18 days (pradofloxacin), 21 days (amoxicillin-clavulanate) and 28 days (doxycycline) ($P < 0.05$ for pradofloxacin compared to each of the other treatments). Palatability scores indicated no significant differences between treatments, and all were classed as excellent.

Dog

The indications and dosages for pradofloxacin are presented in Table 6.

Mueller and Stephan (2007) compared two treatments, pradofloxacin tablets (3 mg/kg once daily) in 50 dogs and amoxicillin + clavulanic acid tablets (12.5 mg/kg twice daily) in 51 dogs, with deep pyoderma in a blinded, randomized trial. Deep pyoderma is associated with follicular rupture, inflammatory response and frequently cellulitis and recurrence of infection. Lesion, pruritus and general condition semiquantitative scores were evaluated weekly for 3 weeks and then every 2 weeks until 2 weeks after clinical remission with a maximum treatment duration of 9 weeks. A total of 231 bacterial isolates were determined pretreatment; the most frequent (in either pure or mixed culture) was *S. pseudintermedius* (36), and other *Staphylococcus* spp. (23), *Pseudomonas* spp. (10) and *E. coli* (7). Treatment outcomes (per protocol analysis) are indicated in Table 7; compared to baseline, both treatments provided highly significant responses within 7 days of commencing treatment, but with no significant differences between the groups for general condition score, lesion score, pruritus score and median time to cure. However, recurrence of signs and percentage of diseased

Table 7. Treatment outcomes expressed as percentage of total number of dogs treated for deep pyoderma in the per protocol analysis*

	Treatment	
Response	Pradofloxacin	Amoxicillin/Clavulanic acid
Remission†	85.8	72.5
Improvement‡	7.1	5.9
Poor§	7.1	9.8
Recurrence¶,**	0	11.8

*Data from Mueller and Stephan (2007); †Total disappearance of lesions; ‡Decrease in infected lesion score of >50% after 63 days therapy; §Decrease in infected lesion score of <50% after 63 days therapy; ¶Reappearance of skin lesions within 14 days after dog pronounced lesion free; **$P = 0.0082$.

skin surface were significantly less in the pradofloxacin-treated dogs. Some additional differences in favour of pradofloxacin were noted on specific assessment days, based on intention to treat analysis.

In an earlier pyoderma field study in 158 dogs, comparing pradofloxacin and amoxicillin/clavulanic acid, administered at the same dose rates as in the Mueller and Stephan (2007) investigation, Stephan *et al.* (2003b) reported 83.3 and 81.6% clinical cure rates on day 63 for pradofloxacin and amoxicillin/clavulanate, respectively, with mean treatment durations of 27 and 32 days. These cases were not classified into deep and superficial pyoderma.

Using the same treatment regimens over 7 days in 137 dogs with infected wounds, Stephan *et al.* (2003b) reported 52.2 and 100% clinical cures on days 7 and 14 (7 days post-treatment) in response to pradofloxacin and 47.1 and 94.0% cures on days 7 and 14 for amoxicillin/clavulanic acid.

In an uncontrolled trial, Restrepo *et al.* (2010) evaluated the clinical efficacy of pradofloxacin in 20 dogs with superficial (12) or deep (8) pyoderma, administered at a dosage of 3 mg/kg orally once daily. Diagnosis was based on histopathology and bacterial cytology assessments. Clinical resolution was defined as disappearance of all lesions and further scoring of excellent for >75% resolution of lesions, good for 50–75% resolution and failure for < 50% resolution of lesions. Responses at 42 days were resolution (one dog), excellent (three dogs), good (three dogs) and failure (one dog) in the deep pyoderma group. For dogs with superficial pyoderma, responses at 28 days were resolution (two dogs), excellent (nine dogs) and good (one dog), while up to 28 days additional dosing provided resolution in all dogs.

Stephan *et al.* (2004, 2006b) compared pradofloxacin tablets (85 dogs receiving 3 mg/kg once daily) with amoxicillin/clavulanic acid tablets (77 dogs receiving 12.5 mg/kg twice daily) for periods of 7–21 days. The treated conditions were cystitis (77% of dogs in both groups) and prostatitis (23% of dogs in both groups). The main organisms isolated were *E. coli* ($n = 139$), *S. pseudintermedius* ($n = 28$), *Pseudomonas* spp. ($n = 24$) and *Proteus mirabilis* ($n = 22$). Differences between treatments were significant for bacteriological but not for clinical cure rates (Table 8).These data are consistent with the fact that the activity of amoxicillin/clavulanic acid is weak or absent against some causative organisms, for example, *Pseudomonas* spp.

In a multi-centre, blinded, randomized European trial, pradofloxacin tablets (3 mg/kg once daily) were compared to clindamycin capsules (5.5 mg/kg twice daily) in the treatment of periodontal disease (Stephan *et al.*, 2006c). To establish a worst-case scenario, the teeth were not mechanically cleaned prior to treatment and both drugs were administered for 7 days. Efficacy data assessed 7 days after the end of treatment are summarized in Table 9; the veterinary and clinical assessments were similar for the two treatments, while reduction in total anaerobic count was superior for pradofloxacin. In a dose confirmation study, pradofloxacin produced significantly greater reductions in gram-negative bacteria in periodontal pockets than a control treatment, comprising metronidazole plus spiramycin, indicating a longer beneficial stabilizing effect on the oral flora than the control product (European Public Assessment Report, EMA/142130/2011).

Table 8. Efficacy of pradofloxacin and amoxicillin/clavulanic acid in the treatment of canine cystitis and prostatitis*

Response (%)	Pradofloxacin	Amoxicillin/clavulanic acid	Significance between treatments
Reduction in total clinical score	96.8	93.4	NS
Clinical cure rate	89.3	83.9	NS
Bacteriological cure rate	85.3	48.0	$P = 0.002$

*Data from Stephan *et al.* (2006b); NS, nonsignificant.

Table 9. Efficacy of pradofloxacin and clindamycin in the treatment of canine periodontal disease*

	Result	
Parameter	Pradofloxacin	Clindamycin
Reduction of pocket depth	0.41 mm $P < 0.00001$	0.27 mm $P < 0.00001$
Reduction of bleeding on probing	64.5% $P = 0.000003$	62.9% $P = 0.000011$
Reduction of total sub-gingival anaerobic count	80% $P < 0.05$	7.7% NS
Investigators' assessment of efficacy†	78%	75%

*Data from Stephan *et al.* (2006c); †Percentage of cases classified as very good or good; *P* values refer to changes from baseline measurements; NS, not significant.

TARGET SPECIES SAFETY

In the target species, dog and cat, pradofloxacin has been shown to be well tolerated at clinical dose rates in preclinical studies and in clinical trials. Data on adverse events harvested from clinical trials are presented in Table 10 (dog) and Table 11 (cat). The majority of events reported were limited to mild and transient gastrointestinal symptoms, with a similar incidence to control products.

A target animal safety study in dogs was performed up to and including five times the therapeutic dose recommended by the manufacturer (15 mg/kg bodyweight) over 91 days. No treatment related effects were observed at dose concentrations up to five times the therapeutic dose for a treatment duration three times longer than that recommended for clinical use.

At a daily dosage of 4 mg/kg for 90 days in young growing dogs (aged less than 6 months), pradofloxacin induced articular

Table 10. Adverse events reported in clinical studies with pradofloxacin tablets in dogs

Adverse event	No. of dogs (n = 395)	%
Diarrhoea	17	4.3
Vomiting	13	3.3
Tiredness/Sleepiness	5	1.3
Polydipsia	5	1.3
Salivation	3	0.75
Polyuria	3	0.75
Decreased appetite	1	0.25
Anorexia	1	0.25
Constipation	1	0.25
Weakness	1	0.25
Blood in faeces	1	0.25
Tremor*	1	0.25
Leakage of urine†	1	0.25

*After 3-fold overdose; †Symptom of urinary tract infection reported as adverse event.

Table 11. Adverse events in clinical studies with pradofloxacin tablets and 25 mg/mL oral suspension in cats

	Pradofloxacin Tablets, n = 70		Pradofloxacin 25 mg/mL Oral Suspension, n = 404		All Cases, n = 474	
Adverse event	No. of cats	%	No of. cats	%	No. of cats	%
Diarrhoea	6	8.6	6	1.5	12	2.5
Vomiting	2	2.9	4	1.0	6	1.3
Salivation	2	2.9	0	0	2	0.4
Anorexia	1	1.4	1	0.25	2	0.4
Polydipsia	0	0	1	0.25	1	0.2
Apathy	1	1.4	0	0	1	0.2

lesions of the type well recognized for other fluoroquinolones, but with no other manifestations of toxicity. The question of pradofloxacin-induced chondropathy is addressed in the product literature.

In cats, daily doses of pradofloxacin tablets up to five times the manufacturer's recommended dose (15 mg/kg bodyweight) were well tolerated over 21 consecutive days. The only sign, which might have been treatment related, was occasional emesis. The suspension formulation was evaluated in a similar 21-day study, assessing safety at up to five times the therapeutic dose (25 mg/kg bodyweight). The only abnormal clinical findings were occasional vomiting, soft faeces and salivation postdosing. Vomiting and salivation may have resulted from the high volumes of product administered.

The potential for oculotoxicity of pradofloxacin, at daily dose rates of 30 and 50 mg/kg, was investigated in cats. Specific ocular investigation involving ophthalmological examination, optical coherence tomography (OCT), electroretinography (ERG) and histological examination, including light and electron microscopy, as well as immunohistopathology, revealed no signs of retinal pathology (Messias *et al.,* 2008). No oculotoxicity occurred with pradofloxacin in either dosage group or a negative control group, in contrast to the retinal degeneration induced by enrofloxacin at high dosage (30 mg/kg) as a positive control.

Safety under conditions of clinical use was evaluated in pivotal field efficacy and safety studies in Europe. Tables 10 and 11 summarize adverse events reported in dogs and cats, respectively.

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CONFLICT OF INTEREST

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