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In Vitro Activity of Premafloxacin, a New Extended-Spectrum Fluoroquinolone, against Pathogens of Veterinary Importance

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The in vitro activity of premafloxacin against 673 veterinary pathogens was evaluated. Premafloxacin was equivalent to ciprofloxacin, enrofloxacin, and danofloxacin in activity against the gram-negative bacilli but was much more active (MIC for 90% of the strains tested [MIC_{90}], 0.015 to 0.25 μg/ml) than the comparison antimicrobial agents (MIC_{90}, 0.13 to 16.0 μg/ml) against the staphylococci, streptococci, and anaerobes tested.

Therapeutic treatment of animal diseases is necessary to reduce morbidity and mortality, as well as to decrease economic losses to farmers. The fluoroquinolones are a class of antimicrobial agents uniquely suited for use in treatment of animal diseases due to their activity against a broad range of veterinary pathogens, particularly the gram-negative bacilli (1, 3). Several fluoroquinolones are approved or under development for animal health use in the United States or Europe, including enrofloxacin, danofloxacin, sarafloxacin, and marbofloxacin (1–3, 7).

Premafloxacin (PD-140288, U-95376), chemically defined as 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-{3-[1-(methylamino)ethyl]-1-pyrrolidinyl}-4-oxo-3-quinolinecarboxylic acid, is a new fluoroquinolone antimicrobial agent under development for a variety of animal diseases (Fig. 1). The purpose of this study was to determine the in vitro activity of premafloxacin against pathogens of veterinary importance.

A total of 673 isolates of veterinary origin were used in the study. A list of the organisms tested and the source animals is presented in Table 1. All isolates were stored in Trypticase soy broth containing 10% glycerol at −70°C. Prior to testing, all isolates were subcultured on Trypticase soy agar (Becton-Dickinson Microbiology Systems, Cockeysville, Md.) containing 5% sheep blood and supplements as required. The following antimicrobial agents were tested: premafloxacin, enrofloxacin (Bayer Animal Health, Shawnee Mission, Kans.), danofloxacin (Pfizer Animal Health, Groton, Conn.), and ciprofloxacin (Sigma Chemical Co., St. Louis, Mo.). MICs for aerobic and facultative anaerobic organisms were determined by using a microdilution broth method that conforms to National Committee for Clinical Laboratory Standards recommendations (4, 6). MIC determinations for *Actinobacillus pleuropneumoniae* and *Haemophilus somnus* were performed as previously described (7, 9). MIC determinations for obligate anaerobic organisms were performed by using an agar dilution method conforming to National Committee for Clinical Laboratory Standards guidelines (5). The recommended quality control organisms for both aerobic and anaerobic MIC determinations were included on each day of testing (4–6).

The *Staphylococcus aureus* intracellular killing assay was performed as previously described (8). Briefly, bovine blood polymorphonuclear leukocytes (PMNs) and bacteria were mixed at a 1:1 ratio and incubated for 90 min, and extracellular *S. aureus*

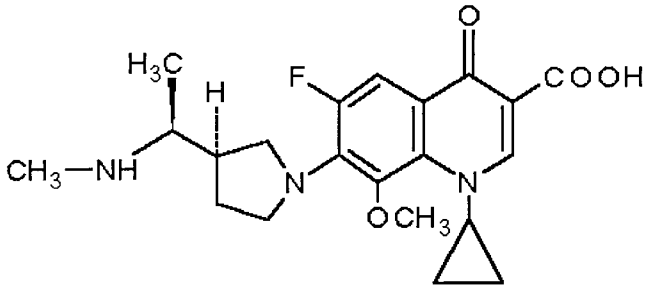

FIG. 1. Structure of premafloxacin (U-95376, PD-140288).

TABLE 1. Distribution of the isolates tested in this study

Organism	No. of isolates[a]	Source
Staphylococcus aureus	50	Bovine mastitis, poultry, canine
Staphylococcus intermedius	48	Bovine mastitis, canine, poultry
Staphylococcus hyicus	25	Swine, bovine mastitis
Staphylococcus sp.	46	Bovine mastitis
Streptococcus agalactiae	26	Bovine mastitis
Streptococcus dysgalactiae	27	Bovine mastitis
Streptococcus zooepidemicus	24	Horses
Streptococcus uberis	25	Bovine mastitis
Enterococcus faecalis	20	Bovine mastitis, poultry
Streptococcus suis	23	Swine
Actinobacillus pleuropneumoniae	28	Swine
Pasteurella multocida	50	Swine, cattle, sheep
Pasteurella haemolytica	26	Cattle
Haemophilus somnus	26	Cattle
Escherichia coli	45	Bovine mastitis, canine, swine, cattle, poultry
Salmonella sp.	82	Cattle, swine, poultry
Klebsiella pneumoniae	20	Canine
Proteus mirabilis	20	Canine, poultry
Clostridium perfringens	29	Cattle, swine, canine, poultry
Fusobacterium necrophorum	20	Cattle, horses
Bacteroides sp.	13	Cattle

[a] Total, 673.

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TABLE 2. Summary of MICs of premafloxacin and comparison quinolones against gram-negative bacteria of veterinary importance

Microorganism (no. of isolates tested)	Antimicrobial agent	MIC (μg/ml)		
		Range	For 50% of isolates	For 90% of isolates
Actinobacillus pleuropneumoniae (28)	Premafloxacin	NR[a]	≤0.015	≤0.015
	Enrofloxacin	≤0.015–0.06	0.03	0.03
	Danofloxacin	≤0.015–0.03	≤0.015	≤0.015
	Ciprofloxacin	NR	≤0.015	≤0.015
Pasteurella multocida (50)	Premafloxacin	NR	≤0.015	≤0.015
	Enrofloxacin	≤0.015–0.03	≤0.015	≤0.015
	Danofloxacin	≤0.015–0.03	≤0.015	≤0.015
	Ciprofloxacin	NR	≤0.015	≤0.015
Pasteurella haemolytica (26)	Premafloxacin	NR	≤0.015	≤0.015
	Enrofloxacin	≤0.015–0.06	≤0.015	0.03
	Danofloxacin	≤0.015–0.03	≤0.015	≤0.015
	Ciprofloxacin	≤0.015–0.03	≤0.015	≤0.015
Haemophilus somnus (26)	Premafloxacin	≤0.0039–0.015	≤0.0039	≤0.0039
	Enrofloxacin	0.0078–0.03	0.03	0.03
	Danofloxacin	0.03–0.06	0.03	0.06
	Ciprofloxacin	0.015–0.03	0.015	0.03
Escherichia coli (45)	Premafloxacin	≤0.015–0.06	≤0.015	0.03
	Enrofloxacin	≤0.015–0.03	≤0.015	0.03
	Danofloxacin	≤0.015–0.03	≤0.015	≤0.015
	Ciprofloxacin	NR	≤0.015	≤0.015
Salmonella sp. (82)	Premafloxacin	≤0.015–0.13	0.03	0.06
	Enrofloxacin	≤0.015–0.13	0.03	0.06
	Danofloxacin	≤0.015–0.06	0.03	0.03
	Ciprofloxacin	≤0.015–0.03	≤0.015	≤0.015
Klebsiella pneumoniae (20)	Premafloxacin	≤0.015–1.0	0.03	0.06
	Enrofloxacin	≤0.015–0.25	0.03	0.06
	Danofloxacin	≤0.015–0.5	0.03	0.06
	Ciprofloxacin	≤0.015–0.13	0.03	0.06
Proteus mirabilis (20)	Premafloxacin	0.03–0.5	0.13	0.25
	Enrofloxacin	0.06–0.5	0.13	0.25
	Danofloxacin	0.03–0.13	0.06	0.13
	Ciprofloxacin	≤0.015–0.03	≤0.015	0.03
Streptococcus agalactiae (26)	Premafloxacin	≤0.015–0.03	≤0.015	≤0.015
	Enrofloxacin	0.13–1.0	0.5	0.5
	Danofloxacin	0.06–2.0	0.5	1.0
	Ciprofloxacin	0.25–1.0	0.5	1.0
Streptococcus dysgalactiae (27)	Premafloxacin	NR	≤0.015	≤0.015
	Enrofloxacin	0.06–1.0	0.25	0.5
	Danofloxacin	0.13–1.0	0.5	0.5
	Ciprofloxacin	0.13–1.0	0.25	0.5
Streptococcus uberis (25)	Premafloxacin	≤0.015–0.06	≤0.015	0.06
	Enrofloxacin	0.13–0.5	0.25	0.5
	Danofloxacin	0.13–1.0	0.5	1.0
	Ciprofloxacin	0.13–2.0	0.5	1.0
Enterococcus faecalis (20)	Premafloxacin	≤0.015–0.06	≤0.015	0.03
	Enrofloxacin	0.25–2.0	0.5	1.0
	Danofloxacin	0.5–2.0	1.0	1.0
	Ciprofloxacin	0.25–2.0	1.0	1.0
Streptococcus suis (23)	Premafloxacin	≤0.015–0.03	≤0.015	≤0.015
	Enrofloxacin	0.25–1.0	0.5	0.5
	Danofloxacin	0.13–0.5	0.25	0.5
	Ciprofloxacin	0.13–0.5	0.25	0.5
Streptococcus zooepidemicus (24)[b]	Premafloxacin	NR	≤0.015	≤0.015
	Enrofloxacin	0.25–1.0	0.5	0.5
	Danofloxacin	0.03–2.0	0.5	0.5
	Ciprofloxacin	0.06–1.0	0.5	1.0
Staphylococcus aureus (50)	Premafloxacin	≤0.015–0.03	≤0.015	≤0.015
	Enrofloxacin	0.03–2.0	0.06	0.13
	Danofloxacin	0.03–1.0	0.06	0.13
	Ciprofloxacin	0.06–4.0	0.13	0.5
Staphylococcus hyicus (48)	Premafloxacin	≤0.015–0.03	≤0.015	≤0.015
	Enrofloxacin	≤0.015–0.13	0.06	0.13
	Danofloxacin	≤0.015–0.13	0.06	0.06
	Ciprofloxacin	0.03–0.25	0.06	0.25
Staphylococcus intermedius (25)	Premafloxacin	≤0.015–0.03	≤0.015	≤0.015
	Enrofloxacin	0.03–1.0	0.06	0.13
	Danofloxacin	≤0.015–1.0	0.06	0.13
	Ciprofloxacin	0.03–0.5	0.13	0.25
Staphylococcus sp. (46)	Premafloxacin	≤0.015–0.06	≤0.015	≤0.015
	Enrofloxacin	≤0.015–0.25	0.06	0.13
	Danofloxacin	0.03–0.25	0.06	0.13
	Ciprofloxacin	0.03–0.5	0.13	0.25
Clostridium perfringens (29)	Premafloxacin	≤0.06–0.13	≤0.06	0.13
	Enrofloxacin	0.13–0.25	0.25	0.25
	Danofloxacin	0.25–1.0	0.5	0.5
	Ciprofloxacin	0.13–0.5	0.5	0.5
Fusobacterium necrophorum (20)	Premafloxacin	≤0.06–0.25	≤0.06	0.13
	Enrofloxacin	≤0.06–4.0	0.5	0.5
	Danofloxacin	0.13–16.0	2.0	8.0
	Ciprofloxacin	0.13–4.0	0.5	2.0
Bacteroides sp. (13)	Premafloxacin	0.13–0.25	0.13	0.25
	Enrofloxacin	0.5–4.0	1.0	4.0
	Danofloxacin	2.0–8.0	2.0	4.0
	Ciprofloxacin	2.0–16.0	2.0	16.0

[a] NR, no range; all isolates yielded the same value.
[b] Includes 15 isolates of *S. equi* subsp. *zooepidemicus* and 9 isolates of *S. equi* subsp. *equi.*

bacteria were lysed by lysostaphin treatment for 15 min. The infected PMNs were washed three times and suspended in Hank's balanced salt solution, and 1 ml was pipetted into a six-well plastic tissue culture plate. The PMNs were allowed to attach for 10 min, the compounds were added to the individual wells (one six-well plate per drug), and then the plates were incubated overnight at 37°C. Lysostaphin was added to each well, and the monolayers were washed 15 min later. Cells were lysed by addition of water (1 ml), and the number of viable bacterial was determined by plate counting. The criterion for intracellular killing by an antimicrobial agent must be a significant reduction in the number of viable bacteria ($P < 0.05$) compared with untreated controls. All compounds were tested at 100 times the MIC for the *S. aureus* strain.

The MIC data for the organisms tested are presented in Table 2. The results for the quality control organisms fell within published ranges for all of the compounds tested. Premafloxacin demonstrated activity against the gram-negative veterinary pathogens equivalent to that of the comparison compounds, enrofloxacin, danofloxacin, and ciprofloxacin. With

the exception of strains of *Proteus mirabilis* and *Haemophilus somnus*, the overall MICs for 90% of the strains tested (MIC_{90}s) of all of the compounds were ≤0.06 μg/ml for these organisms. In particular, premafloxacin was much more active against *H. somnus* (MIC_{90} ≤0.0039 μg/ml) than were the comparison compounds (MIC_{90}, ≤0.06 μg/ml). Against the gram-positive cocci tested, premafloxacin was much more active than the comparison compounds. For example, the MIC_{90}s for the streptococcal and staphylococcal strains tested were ≤0.015 μg/ml for premafloxacin, compared with 0.13 to 1.0 μg/ml for the comparator compounds. Premafloxacin was only marginally more active than the comparison compounds against strains of *Clostridium perfringens* but was much more active against strains of *Fusobacterium necrophorum* and *Bacteroides* spp.

Premafloxacin also demonstrated the ability to penetrate PMNs and kill intracellular *S. aureus*. The percent reduction in viable *S. aureus*, compared to the untreated control, for premafloxacin, enrofloxacin, danofloxacin, and ciprofloxacin were 99.8, 78.6, 75.7, and 88.7%, respectively. The activities of the positive control (rifampin) and negative control (cloxacillin) were 94.1 and 7.7%, respectively. Thus, the activity of premafloxacin was greater than those of enrofloxacin, danofloxacin, and ciprofloxacin and equivalent to that of rifampin.

The results of this study indicate that premafloxacin has potent activity against a variety of veterinary pathogens. In particular, premafloxacin was more active against the staphylococci, streptococci, and anaerobes than were enrofloxacin, danofloxacin, and ciprofloxacin. Moreover, a recent study by Zerva et al. (10) demonstrated that premafloxacin is active against ciprofloxacin-resistant strains of methicillin-resistant *S. aureus* and vancomycin-resistant enterococci. In conclusion, premafloxacin demonstrated in vitro activity superior to that of currently available fluoroquinolones against gram-positive veterinary pathogens and equivalent activity against gram-negative veterinary pathogens.

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