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Comparative quantification of the *in vitro* activity of veterinary fluoroquinolones

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Abstract

The aim of this study was to compare the *in vitro* antimicrobial activity of the veterinary fluoroquinolones against a panel of recently isolated porcine and bovine bacterial pathogens. The study used enrofloxacin as a benchmark against which other agents were compared, being the most common fluoroquinolone used in treatment of bovine and porcine infections. The activity of ciprofloxacin was also assessed as it is the main metabolite of enrofloxacin in cattle. Enrofloxacin and ciprofloxacin generally showed higher antibacterial activity, in terms of MIC_{50} values, for most pathogen species when compared with marbofloxacin, difloxacin, danofloxacin and norfloxacin. Ciprofloxacin showed significantly greater *in vitro* antibacterial activity than enrofloxacin against *M. haemolytica*, *P. multocida* and *E. coli,* whereas enrofloxacin showed greater activity than ciprofloxacin against *S. aureus*. Marbofloxacin was significantly more active than enrofloxacin against *M. haemolytica*, *E. coli* and *B. bronchiseptica* but less active against *P. multocida, S. aureus,* coagulase negative Staphylococci*, S. dysgalactiae, S. uberis, A. pleuropneumoniae* and *S. suis*. Danofloxacin was significantly less active than enrofloxacin against *P. multocida*, *E. coli*, *S. uberis*, *A. pleuropneumoniae* and *S. suis*. Enrofloxacin and its metabolite ciprofloxacin showed the highest *in vitro* activities against most bovine pathogens tested and the porcine pathogens also showed a high degree of sensitivity to enrofloxacin. These data facilitate further pharmacokinetic/pharmacodynamic comparison of fluoroquinolones currently used in veterinary medicine.

Keywords: Veterinary antibiotics; Fluoroquinolone activity; Enrofloxacin; Ciprofloxacin

1. Introduction

The use of pharmacokinetic/pharmacodynamic (PK/PD) analyses is of increasing importance during the development of new antimicrobial agents, to optimise treatment strategies (McKellar et al., 2004)

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and reduce the development of antibiotic resistant bacterial strains (Lathers, 2002). PK characterisation involves defining parameters such as the area under the concentration time-curve (AUC) and the maximum plasma concentration (C_{max}) (Coulet et al., 2002). The most commonly used PD parameter is minimum inhibitory concentration (MIC), which is key to the derivation of therapeutic AUC/MIC and C_{max}/MIC ratios. PK and PD components can be combined experimentally either by integration or by modelling (Kietzmann et al., 2004; McKellar et al., 2004; Toutain and Lees, 2004).

Fluoroquinolones are important therapeutic agents in veterinary medicine, having a broad range of antimicrobial activity (Brown, 1996). They act by inhibiting the action of the topoisomerase gene products thereby disrupting DNA replication (Hawkey, 2003). Many studies assess the MICs of fluoroquinolones against veterinary pathogen collections, to compare the efficacy of the different agents. However, such studies have a number of limitations: (1) most do not consider the full panel of veterinary fluoroquinolones (Cruz et al., 1997; De Oliveira et al., 2000; Yoshimura et al., 2001, 2002a,b; Zhao et al., 2005); (2) studies often use differing methodologies, introducing variability into the absolute MIC values attained (Gombert and Aulicino, 1985; Koeth et al., 2000; Wallmann et al., 2006) even when using procedures standardised according to the Clinical and Laboratory Standards Institute ([CLSI] NCCLS, 2002); (3) a number of fluoroquinolone resistance monitoring studies do not accurately determine true *in vitro* potencies, as antibiotic concentrations at the lower end of the sensitivity range were not used (Watts et al., 1997; Mevius and Hartman, 2000; Yoshimura et al., 2001); (4) MIC values should be determined for a large group of isolates for each type of organism, minimising the impact of any variation between isolates (Lees et al., 2004; Toutain and Lees, 2004). To overcome these limitations in the background data we assessed the *in vitro* antimicrobial activities of a range of fluoroquinolones relevant to veterinary practice, comprising enrofloxacin and the comparators ciprofloxacin, danofloxacin, difloxacin, marbofloxacin and norfloxacin. Though not licensed for use in veterinary medicine the inclusion of ciprofloxacin was considered important, as it is the main enrofloxacin metabolite in cattle.

2. Materials and methods

2.1. Minimum inhibitory concentration testing

MIC values were determined using the microbroth dilution method in accordance with the CLSI guidelines (NCCLS, 2002). Sensititre microtitre plates (MCS Diagnostics, Swalmen, Netherlands) were coated with 11 or 12 two-fold dilutions of the fluoroquinolones: enrofloxacin (0.002–4 μg/mL), ciprofloxacin (0.002–4 μg/mL), danofloxacin (0.002–4 μg/mL), difloxacin (0.002–4 μg/mL), norfloxacin (0.004–4 μg/mL) and marbofloxacin (0.002–4 μg/mL).

2.2. Bacterial strains

Ten bovine and porcine pathogen species, comprising 422 isolates were tested. The isolates were collected between 2001 and 2005 from sources in Germany, Belgium and France and comprised: 49 bovine respiratory *Mannheimia haemolytica* isolates; 60 bovine respiratory and 15 porcine respiratory *Pasteurella multocida* isolates; 50 bovine mastitis and 20 bovine intestinal *Escherichia coli* isolates; 47 bovine mastitis *Staphylococcus aureus* isolates; 24 bovine mastitis isolates of coagulase negative Staphylococci; 25 bovine mastitis *Streptococcus dysgalactiae* isolates; 24 bovine mastitis *Streptococcus uberis* isolates; 39 porcine respiratory *Actinobacillus pleuropneumoniae* isolates; 29 porcine respiratory *Bordetella bronchiseptica* isolates; 40 *Streptococcus suis* isolates from porcine septicaemia and associated conditions. As the study was not designed to assess the incidence of resistance to fluoroquinolones, any isolate that was not sensitive to an antibiotic in the concentration range tested was deemed resistant and excluded from the analyses. Control strains *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were tested concomitantly as part of each antibiotic sensitivity assay.

2.3. Data analysis

Statistical analyses were carried out using the SAS software (release 8.2). Pairwise comparisons of *in vitro* MIC activities were made for enrofloxacin versus the comparator fluoroquinolones. The mean differ-