

Comparative *in vitro* activities of enrofloxacin, ciprofloxacin and marbofloxacin against *Staphylococcus intermedius* isolated from dogs

Branka Šeol*

Department of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

ŠEOL, B.: Comparative *in vitro* activities of enrofloxacin, ciprofloxacin and marbofloxacin against *Staphylococcus intermedius* isolated from dogs. Vet. arhiv 75, 189-194, 2005.

ABSTRACT

Fifty strains of *Staphylococcus intermedius* were isolated from different pathological specimens originating from dogs during a six-month period. Antimicrobial susceptibility patterns against three fluoroquinolones were obtained on 50 *Staphylococcus intermedius* strains. *In vitro* antimicrobial susceptibility testing was performed employing the disk diffusion method (Kirby-Bauer). The sensitivity against enrofloxacin and marbofloxacin showed that 98.0% of *S. intermedius* strains were sensitive and 96% were sensitive to ciprofloxacin. One of the tested strains showed multiple resistance to all tested fluoroquinolones.

Key words: *Staphylococcus intermedius*, fluoroquinolones, dogs

Introduction

Staphylococcus intermedius is the predominant type of coagulase-positive staphylococci on normal canine skin (BERG et al., 1984), and is the causative agent of pyoderma and otitis externa in dogs (AMINE-KHODJA et al., 1983; BIBERSTEIN et al., 1984; MEDLEAU et al., 1986; IHRKE, 1987). Dermatoses account for a large percentage of cases in dogs in the veterinary practice. A primary or secondary bacterial skin infection by bacteria of the *Staphylococcus* genus is often a component of the skin diseases (PELLERIN et al., 1998; LLOYD et al., 1991; NOBLE and KENT, 1992). The fluoroquinolones have revolutionized treatments against bacterial pathogens resistant to traditional antibacterial agents (WALKER, 2000).

* Contact address:

Prof. Dr. Branka Šeol, Department of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, University of Zagreb, P.O. Box 190, Zagreb, Croatia, Phone: +385 1 2390 205; Fax: +385 01 244 1390; E-mail: seol@vef.hr

Enrofloxacin, marbofloxacin and ciprofloxacin are members of the fluoroquinolones, a class of synthetic antibiotics acting on bacterial DNA topoisomerases II and IV (WOLFSON and HOOPER, 1989; DRLICA and ZHAO, 1997). They exhibit high bactericidal activity against a broad spectrum of aerobic Gram-negative and Gram-positive bacteria and mycoplasmas (HANNAN et al., 1997; WATTS et al., 1997). Nearly complete oral bioavailability, excellent pharmacokinetic properties and low toxicity make them a first choice of drugs for the treatment of several bacterial infections in dogs and cats (VANCUTSEM et al., 1990; BROWN, 1996). Enrofloxacin is the first fluoroquinolone developed for veterinary application and is approved in dogs and cats for treating urinary tract, respiratory and skin infections (BOOTHE, 1994). Marbofloxacin, difloxacin and orbifloxacin were introduced in a number of countries for use in animals (SCHNEIDER et al., 1996; MATSUMOTO et al., 1997; VAN DEN HOVEN, 1997), but not in Croatia (ŠEOL et al., 2002). Ciprofloxacin, fluoroquinolone labelled for humans is also used for animal treatment. In our study we investigated a small collection consisting of 50 *S. intermedius* strains originating from dog and tested them against three fluoroquinolones: enrofloxacin, marbofloxacin and ciprofloxacin. The intention was to present a brief overview about possible resistance to those very extensively used antibiotics. Both enrofloxacin and ciprofloxacin have been used for animal treatment for several years, although marbofloxacin is not yet registered in Croatia.

Materials and methods

Over a short period, clinical specimens collected from dogs suffering from different syndromes suspected to have an infectious aetiology were submitted for bacteriological examination to the Department of Microbiology and Infectious Diseases by several clinics of the Faculty of Veterinary Medicine, University of Zagreb.

Identification of *S. intermedius* was carried out according to the procedure described by QUINN et al. (1994): Gram-positive cocci, catalase-positive, facultative anaerobe, β -hemolytic, coagulase-positive, DNase-positive. The precise species identification was made using the API Staph (BioMérieux, France). In the present study, 50 *S. intermedius* isolates were tested by the Kirby-Bauer's disk-diffusion method on Mueller-Hinton agar (PRESCOTT and BAGGOT, 1998) for antimicrobial susceptibility against three antimicrobial agents: enrofloxacin-ENO (5 μ g), ciprofloxacin-CIP (5 μ g) and marbofloxacin-MAR (5 μ g). A standard concentration of a pure culture of *S. intermedius* was placed on Mueller-Hinton agar (Becton Dickinson, Cockeysville, U.S.A.), and individual filter paper disk containing known concentrations of individual antibiotics were placed on the pathogen. The culture was incubated for 18-24 hours at 37 °C. The zone of inhibition around each disk was measured, and the measurement compared to a chart (presented in Table 1) which classifies the organism into three categories: sensitive, resistant and intermediate sensitive.

Results and discussion

The majority of all 50 tested strains were sensitive against three tested fluoroquinolones. Marbofloxacin and enrofloxacin were active against 49 (98%) *S. intermedius* isolates and ciprofloxacin against 48 (96%) tested strains. There were no intermediate sensitivity strains. One *S. intermedius* strain showed multiple resistance against all three tested fluoroquinolones.

Table 1. Zone diameter interpretative chart (National Committee for Clinical Laboratory Standards)

Antimicrobial agent	Zone diameter interpretative standards (mm)			
	Symbol	R (resistant) ≤	I (intermediate s.) =	S (sensitive) ≥
ciprofloxacin	CIP-5	15	16-20	21
enrofloxacin*	ENO-5	16	17-19	20
marbofloxacin**	MAR-5	14	14-18	19

* National Committee for Clinical Laboratory Standards. 1997. Tentative standard M31-T. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. NCCLS, Wayne, Pa.

** National Committee for Clinical Laboratory Standards. 1997. Tentative standard M31-A Vol. 19 N 11 (June 1999) Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard.

Fluoroquinolones are a group of chemotherapeutics that are a rapid-acting bactericidal against a wide variety of clinically important microorganisms. They have been used extensively during the past 15 years in veterinary dermatology, predominantly for the management of canine pyoderma (IHRKE et al., 1999). More recently, marbofloxacin has also proven successful in treating canine pyoderma (SPRENG et al., 1995; CARLOTTI et al., 1999; LLOYD et al., 1999; PARADIS et al., 2001). Our results showed no marked resistance to selected fluoroquinolone antibiotics. Only one strain exhibited resistance to marbofloxacin, but the same strain showed multiple resistance also to enrofloxacin and ciprofloxacin. The same sensitivity pattern was observed to enrofloxacin; 49 strains were sensitive (98%) and two strains (96%) were resistant to ciprofloxacin. PIRIZ et al. (1996) determined antimicrobial susceptibility of 91 methicillin-resistant and methicillin-susceptible *S. intermedius* strains to 15 different antimicrobial agents. Of the fluoroquinolones, ciprofloxacin and enrofloxacin proved to be a useful alternative for the treatment of methicillin-resistant *S. intermedius* strains. CARLOTTI et al. (1999) isolated 47 strains of *S. intermedius* from dogs with pyoderma

and found only one strain resistant to marbofloxacin, while LLOYD et al. (1999) found two *S. intermedius* strains, out of 247 tested, to be resistant to enrofloxacin, marbofloxacin and enrofloxacin. LLOYD et al. (1999) suggested that multiple resistance in *S. intermedius* detected as resistance to enrofloxacin, extended to marbofloxacin and ciprofloxacin probably results from the same, or very similar, mutations in the *gyrA* and/or *norA* genes. Recent studies (GANIÈRE et al., 2001) showed that MICs (minimal inhibitory concentrations) of enrofloxacin against *S. intermedius* ranged from 0.063 µg/mL in 1995 to 64 µg/mL in 1999. This suggests that inappropriate use might favour the development of resistant strains *in vivo*. Results of our study are very similar to those discussed above and confirmed the excellent activity of fluoroquinolones in treatment of *S. intermedius* infections.

Acknowledgements

The author would like to thank Charles-Eric Descotes, Vétérinaire Spécialités Pharmaceutiques Vétérinaires, Lure Cedex, France, for supplying us with marbofloxacin sensitivity discs.

References

- AMINE-KHODJA, C. A., A. PELLERIN, J. L. CHANTAL, J. MILON (1983): A. Antibiogram in pyoderma and suppurated otitis of the dog. Note 2: The results of bacterial examinations carried out in the National Veterinary School in Toulouse from 1975 to 1979. *Rev. Méd. Vét.* 10, 533-540.
- BERG, J. N., D. E. WENDELL, C. VOGELWEID, W. H. FALES (1984): Identification of the major coagulase-positive *Staphylococcus* spp. of dogs as *Staphylococcus intermedius*. *Am. J. Vet. Res.* 45, 1307-1309.
- BIBERSTEIN, E. L., S. S. JANG, D. C. HIRSH (1984): Species distribution of coagulase-positive staphylococci in animals. *J. Clin. Microbiol.* 19, 610-615.
- BOOTHE, D. M. (1994): Enrofloxacin revisited. *Vet. Med.* 89, 744-753.
- BROWN, S. A. (1996): Fluoroquinolones in animal health. *J. Vet. Pharmacol. Ther.* 19, 1-14.
- CARLOTTI, D. N., E. GUAGUERE, D. PIN, P. JASMIN, E. THOMAS, V. GUIRAL (1999): Therapy of difficult cases of canine pyoderma with marbofloxacin: a report of 39 dogs. *J. Small Anim. Pract.* 6, 265-270.
- DRLICA, K., X. ZHAO (1997): DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol. Molecul. Biol. Rev.* 61, 377-392.
- GANIÈRE, J. P., C. MÈDAILLE, A. LIMET, N. RUVOEN, G. A. FONTAINE (2001): Antimicrobial activity of enrofloxacin against *Staphylococcus intermedius* strains isolated from canine pyodermas. *Vet. Dermatol.* 12, 171-177.
- HANNAN, P. C. T., G. D. WINDSOR, A. DE JONG, N. SCHMEER, M. STEGEMANN (1997): Comparative susceptibilities of various animal-pathogenic mycoplasmas to fluoroquinolones. *Antimicrob. Agent. Chemother.* 41, 2037-2040.
- IHRKE, P. J. (1987): An overview of bacterial skin disease in the dog. *Br. Vet. J.* 143, 112-118.

B. Šeol: Comparative *in vitro* activities of enrofloxacin, ciprofloxacin and marbofloxacin against *Staphylococcus intermedius* isolated from dogs

- IHRKE, P. J., M. G. PAPICH, T. C. DEMANUELLE (1999): The use of fluoroquinolones in veterinary dermatology. *Vet. Dermatol.* 10, 193-204.
- LLOYD, D. H., R. P. ALLAKER, A. PATTISON (1991): Carriage of *Staphylococcus intermedius* on the ventral abdomen of clinically normal dogs and those with pyoderma. *Vet. Dermatol.* 2, 161-164.
- LLOYD, D. H., A. I. LAMPORT, W. C. NOBLE, S. A. HOWELL (1999): Fluoroquinolone resistance in *Staphylococcus intermedius*. *Vet. Dermatol.* 10, 249-251.
- MEDLEAU, L., R. E. LONG, J. BROWN, W. H. MILLER (1986): Frequency and antimicrobial susceptibility of *Staphylococcus* species isolated from canine pyoderma. *Am. J. Vet. Res.* 47, 229-231.
- MATSUMOTO, S., M. TAKAHASHI, M. YOSHIDA, T. KOMATSU, Y. KITADAI, Y. HORII, H. KATAE (1997): Absorption, distribution and excretion of orbifloxacin in dogs and cats. *J. Jap. Vet. Med. Assoc.* 50, 470-474.
- NOBLE, W. C., L. E. KENT (1992): Antibiotic resistance in *Staphylococcus intermedius* isolated from cases of pyoderma in the dog. *Vet. Dermatol.* 3, 71-74.
- PARADIS, M., L. ABBEY, B. BAKER, M. COYONE, M. HANNIGAN, D. JOFFE, B. PUKAY, A. TRETTIEN, S. WAISGLASS, J. WELLINGTON (2001): Evaluation of the clinical efficacy of marbofloxacin (Zenequin) tablets for the treatment of canine pyoderma: an open clinical trial. *Vet. Dermatol.* 12, 163-169.
- PELLERIN, J. L., P. BOURDEAU, H. SEBBAG, J. M. PERSON (1998) : Epidemiosurveillance of antimicrobial compound resistance of *Staphylococcus intermedius* clinical isolates from canine pyodermas. *Comp. Immun. Microbio. Infect. Dis.*, 21, 115-133.
- PIRIZ, S., J. WALLE, E. M. MATEOS, R. DE LA FUENTE, D. CID, J. A. RUIZ-SANTAQUITERIA, S. VADILLO (1996): *In vitro* activity of fifteen antimicrobial agents against methicillin-resistant and methicillin-susceptible *Staphylococcus intermedius*. *J. Vet. Pharmacol. Ther.* 19, 118-123.
- PRESCOTT, J. F., J. D. BAGGOT (1993): *Antimicrobial Therapy in Veterinary Medicine*, Iowa State University Press/Ames
- QUINN, P. J., M. E. CARTER, B. K. MARKEY, G. R. CARTER (1994): *Clinical Veterinary Microbiology*. Wolfe Publishing, London.
- SCHNEIDER, M., V. THOMAS, B. BOISRAME, J. DELEFORGE (1996): Pharmacokinetics of marbofloxacin in dogs after oral and parenteral administration. *J. Vet. Pharmacol. Ther.* 56-61.
- SPRENG, M., J. DELEFORGE, V. THOMAS, B. BOISRAME, H. DRUGEON (1995): Antibacterial activity of marbofloxacin. A new fluoroquinolone for veterinary use against canine and feline isolates. *J. Vet. Pharmacol. Ther.* 4, 284-289.
- ŠEOL, B., T. NAGLIĆ, J. MADIĆ, M. BEDEKOVIĆ (2002): *In vitro* antimicrobial susceptibility of 183 *Pseudomonas aeruginosa* strains isolated from dogs to selected antipseudomonal agents. *J. Vet. Med.* B 49, 188-192.

B. Šeol: Comparative *in vitro* activities of enrofloxacin, ciprofloxacin and marbofloxacin against *Staphylococcus intermedius* isolated from dogs

- VAN DEN HOVEN, R. (1997): A multi-centre observational study on the efficacy of Dicural Palatabs (difloxacin) for the treatment of canine cystitis. *J. Vet. Pharmacol. Therap.* 20 (Suppl. 1) 185-186.
- VANCUTSEM, P. M., J. G. BABISH, W. S. SCHWARK (1990): The fluoroquinolone antimicrobials: structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. *Cornell Vet.* 80, 173-186.
- WALKER, R. D. (2000): The use of fluoroquinolones for companion animal therapy. *Aust. Vet. J.* 2, 84-90.
- WATTS, J. L., S. A. SALMON, M. S. SHANCEZ, R. J. YANCEY (1997): *In vitro* activity of premafloxacin, a new extended-spectrum fluorowuinolone against pathogens of veterinary importance. *Antimicrob. Agent. Chemother.* 41, 1190-1192.
- WOLFSON, J. S., D. C. HOOPER (1989): Fluoroquinolone antimicrobial agents. *Clin. Microbiol. Rev.* 2, 378-424.

Received: 13 September 2004

Accepted: 5 May 2005

ŠEOL, B.: *In vitro* aktivnost enrofloksacina, ciprofloksacina i marbofloksacina na sojeve bakterije *Staphylococcus intermedius* izdvojene iz pasa. *Vet. arhiv* 75, 189-194, 2005.

SAŽETAK

U razdoblju od šest mjeseci iz pasa je bilo izdvojeno 50 sojeva vrste *S. intermedius* koji su identificirani uobičajenim bakteriološkim postupcima. Osjetljivost na enrofloksacin, ciprofloksacin i marbofloksacin istražena je disk-difuzijskim Kirby-Bauerovim postupkom. Od 50 pretraženih, 49 sojeva (98,0%) bilo je osjetljivo na enrofloksacin i marbofloksacin, a na ciprofloksacin 48 sojeva (96%). U jednog je soja uočena višestruka otpornost na sva tri fluorokinolona.

Ključne riječi: *Staphylococcus intermedius*, fluorokinoloni, psi
