THE *IN-VITRO* BACTERICIDAL ACTIVITY OF DANOFLOXACIN AND CEFTIOFUR AGAINST RESPIRATORY PATHOGENS IN CATTLE

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Introduction

The fluorinated quinolones and the third generation cephalosporins are highly efficacious against gram-negative bacteria. Danofloxacin* and ceftiofur** are newly developed antimicrobials belonging to the fluoroquinolones and third generation cephalosporins, respectively. Danofloxacin and ceftiofur are very active against major bacterial pathogens in farm animals[1,2]. The objective of the present study was to investigate the bactericidal effect and time-kill kinetics exerted by danofloxacin and ceftiofur on bovine respiratory pathogens.

Materials and Methods

Strains: Three Pasteurella hemolytica and 3 Pasteurella multocida strains isolated from pneumonic lung tissue of calves at the National Veterinary Institute, Uppsala were used.

Antimicrobials: Analytical grade danofloxacin was provided by Pfizer, and analytical grade ceftiofur was provided by Upjohn.

MIC and MBC determinations: Minimum inhibitory concentration (MIC) was determined by broth dilution (100μl) in microtiter panels. Each antibacterial was incorporated in the panels at appropriate concentrations, using doubling dilution patterns. Mueller Hinton broth (MHB) supplemented with yeast extract (1%) was used as growth medium. After incubation in the panels for 18 h at 37°C, subcultures (10 μl) were seeded on solid agar and incubated for 48 h at 37°C. Minimum bactericidal concentration (MBC) was defined as at least 99.9% kill of the initial bacterial number.

Time-kill kinetics: The kill rate over time was determined in 5 ml MHB supplemented as described above. Each broth tube was inoculated with 10^5 to 10^6 bacteria in log phase. Antimicrobials were added at concentrations corresponding to 1x, 2x or 4x their established MBC. Counts for viable organisms were performed at 0, 2, 4, 6, 8, 10, 12 and 24 hours after exposure to the antimicrobials.

Results

The P. hemolytica strains were inhibited by 0.03 mg l^{-1} or lower concentrations, whereas the P. multocida strains were inhibited by 0.008 mg l^{-1} or lower concentrations of the antimicrobials tested. MBC was equal to, or one doubling dilution above MIC for both danofloxacin and ceftiofur, except for P. multocida and ceftiofur (4 x MIC) (Table 1).

* Advocin, trademark of Pfizer, Inc
**Naxcel, Excenel, trademarks of The Upjohn Company

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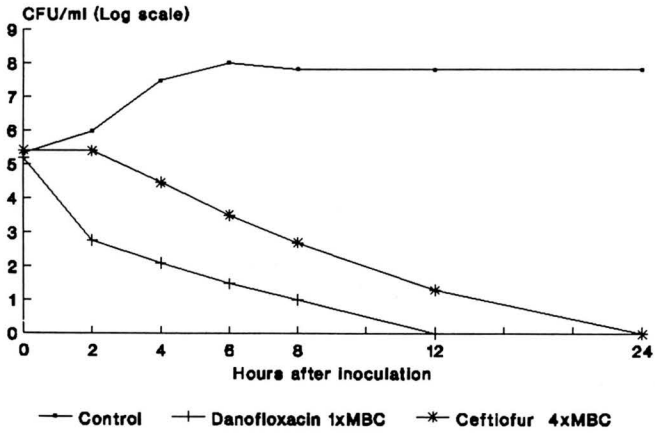
Table 1: MIC and MBC (mcg/ml of danofloxacin and ceftiofur)

	P. HEMOLYTICA			
	Danofloxacin		Ceftiofur	
Strain No.	MIC	MBC	MIC	MBC
1228/89	.03	.06	.015	.015
1262/89	.03	.03	.008	.015
1274/89	.03	.03	.015	.015
	P. MULTOCIDA			
	Danofloxacin		Ceftiofur	
Strain No.	MIC	MBC	MIC	MBC
1202/89	.008	.016	.004	.016
1261/89	.008	.016	.004	.016
1271/89	.008	0.16	.004	.016

Time-kill kinetic results of both antibacterials against one strain each of P. hemolytica and P. multocida are presented in Figures 1 and 2.

Fig.1: Killing curves for danofloxacin and ceftiofur against P.hemolytica



strain 1228/89

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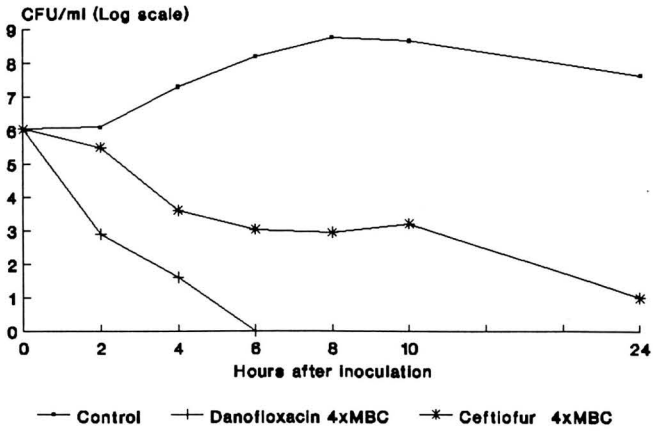
Fig. 2: Killing curves for danofloxacin and ceftiofur against P.multocida



strain 1202-88

A 99.9% reduction of the initial P. hemolytica culture was obtained within 4 hours after exposure to danofloxacin at 1 x MBC. There were no surviving bacteria 12 h post inoculation. A 99.9% reduction of the initial P. multocida culture was obtained within two hours after exposure to danofloxacin at 4 x MBC. There were no surviving bacteria 6 h post inoculation. For all other strains tested, a 99.9% reduction of the initial bacterial inoculum was obtained within 4 hours; when exposed to danofloxacin at 2x or 4x MBC. For ceftiofur at 4 x MBC, a 99.9% reduction of the initial inoculum was obtained within 6 to 12 h for all strains. There were always survivors of P. multocida but not of P. hemolytica 24 h post inoculation. The kill rates at the various concentrations tested did not differ essentially from each other for either of the antimicrobials.

Summary

Danofloxacin and ceftiofur exerted bactericidal effect on P. hemolytica and P. multocida at concentrations equal to or at most one doubling dilution above MIC. After exposure to danofloxacin at 1, 2 or 4 x MBC for up to 12 h there were no P. hemolytica or P. multocida survivors. At 4 x MBC of ceftiofur there were still survivors of P. multocida but not of P. hemolytica 24 h post inoculation.

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Resumen

Danofloxacin y ceftiofur mostraron un efecto bactericida contra P. haemolytica y P. multocida generalmente a concentraciones iguales a la concentracion minima inhibitoria (CMI), o en algunos casos a concentraciones tan solo una dilucion arriba de la correspondiente a la CMI. Despues de una exposicion a danofloxacin en concentraciones de 1, 2 o 4 veces la concentracion minima bactericida (CMB) por periodos de 12 horas, no hubo unidades formadoras de colonias sobrevivientes de P. haemolytica o P. multocida.

A concentraciones 4 veces mayores a la CMB de ceftiofur, todavia quedaron unidades formadoras de colonias de P. multocida, pero no de P. haemolytica, 24 horas despues de la exposicion.

Résumé

L'auteur montre que la danofloxacine et le ceftiofur possèdent un effet bactéricide sur P. haemolytica et P. multocida à des concentrations égales, ou au maximum deux fois supérieures, à leurs concentrations minimales inhibitrices (CMI). Aucune souche de P.haemolytica ou P.multocida n'a pu survivre à des concentrations de danofloxacine égales à 1, 2, ou 4 fois la concentration bactéricide minimale (CBM) pendant un maximum de 12 heures.

Après avoir été soumises à une concentration égale à 4 fois la CBM du ceftiofur pendant 24 heures, seule P. multocida était encore présente.

References

1. McGuirk PR, Jefson MR, Shyrock TR and Schaaf TK. The synthesis and antibacterial activity of danofloxacin (CP-76.136): A new quinolone for veterinary medicine. Proceedings of Twenty-ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, 17-20 September 1989, p. 303. 2. Brown, S.A., Yancey, Jr R.J. and Steffan, J. ceftiofur sodium: Antimicrobial activity of parent Ceftiofur and metabolites. Proceedings of the fifth Congress of the European Association for Veterinary Pharmacology and Toxicology, 18-22. 8. 1991. Acta Vet. Scand. 1991, Suppl. 87, 95.

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