MEMBERS' PAPERS

COLON CONTENTS CONCENTRATION AND AREA UNDER THE CURVE/ MINIMUM INHIBITORY CONCENTRATION RELATIONSHIPS FOR VALNEMULIN – PREVENTION, TREATMENT AND ELIMINATION OF SWINE DYSENTERY

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Summary

Following the introduction of the European Guideline EMEA/CVMP/627/01-FINAL – ('Guidelines for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances'), using pharmacokinetic/pharmacodynamic (PK/PD) methods of analysis are recommended for evaluating medicines. The guidelines were set with bactericidal antimicrobial models in mind, with bacterial elimination as an endpoint to try to reduce resistance development; but many products used in pig medicine are primarily bacteriostatic. Valnemulin for the prevention and treatment of swine dysentery was applied to a large intestine infection PK/PD model. This demonstrated that the minimum bactericidal concentration (MBC) was more relevant than minimum inhibitory concentration (MIC). Prevention of swine dysentery is a valid and definable claim with a good antibacterial endpoint. Treatment is more complex due to the nature of the disease and the mode of action of the antibiotic. Significantly higher concentrations of valnemulin (times 10) are required to eliminate infection and therefore there is a higher risk of incomplete treatment and potential resistance development. Fortunately, Brachyspira hyodysenteriae develops resistance slowly to valnemulin.

Introduction

The pharmacokinetic (PK)/pharmacodynamic (PD) relationships of antimicrobials and their efficacy in the control of gut infections in the pig have been infrequently reported (Guyonnet *et al*, 2003). It was the purpose of this paper to examine the PK/PD relationships of valnemulin (Econor premix – Novartis), a pleuromutilin antibiotic, approved for administration via the feed, for the prevention, treatment and possible elimination of swine dysentery caused by *Brachyspira hyodysenteriae*, with the results from artificial challenge studies.

Materials and Methods

a. Pharmacokinetics

The colon contents concentration (CCC) for valnemulin, after giving feed containing 75ppm valnemulin (treatment level), achieved a level of 1.68µg/ml in the large intestine (ref. product information). As the CCC is a relatively steady concentration due to its slow passage (Clemens *et al*, 1975), the area under the curve (AUC) 24 hours was calculated at 40.3µg hr/ml (CCC x 24) and extrapolations of CCC and AUC at different in-feed inclusion levels were based on these figures.

b. Pharmacodynamics - Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of valnemulin against B. hyodysenteriae

Valnemulin is primarily a bacteriostatic-acting antibiotic, which is highly active against *B. hyodysenteriae* and the MBC has not been reported previously. Originally the MIC for the strain of *B. hyodysenteriae* used in the challenge studies was reported at 0.025µg/ml based on an agar plate test, using doubling dilutions of valnemulin. It was reported that it was very difficult to perform MBC tests from plate tests (Burrows, personal communication). It was therefore decided to do the MIC test using broth dilution methods and also sub-culturing into broth with no antibiotic, to determine the MBC. Growth/lack of growth was determined by the presence/absence of turbidity in the liquid medium after 5 days of culture.

c. Artificial challenge studies – dose titration for prevention and treatment

In the prevention dose-titration study (Burrows *et al*, 1996a), valnemulin was administered in the feed at 0, 5, 10 and 20ppm for 21 days, starting the day after the second *B. hyodysenteriae* challenge was given. Faecal swabs were taken

and cultured twice weekly. Necropsy was performed 21 days after infection and the colons were examined for the presence of lesions and scraped in four places and cultured for *B. hyodysenteriae.*

In the treatment study (Burrows *et al*, 1996b), valnemulin was given in feed at 0, 50, 75 and 100ppm for 10 days following the outbreak of disease in the pigs. All of the valnemulin-treated pigs responded clinically and were negative for *B. hyodysenteriae* culture at the end of treatment. After a further 14-day observation, the pigs were necropsied and their colons examined for lesions and cultured as before.

Results and Calculations

a. Pharmacokinetics

For valnemulin at 75ppm, the CCC = 1.68µg/ml; the AUC 24hours = 40.3µg hr/ml (CCC x 24).

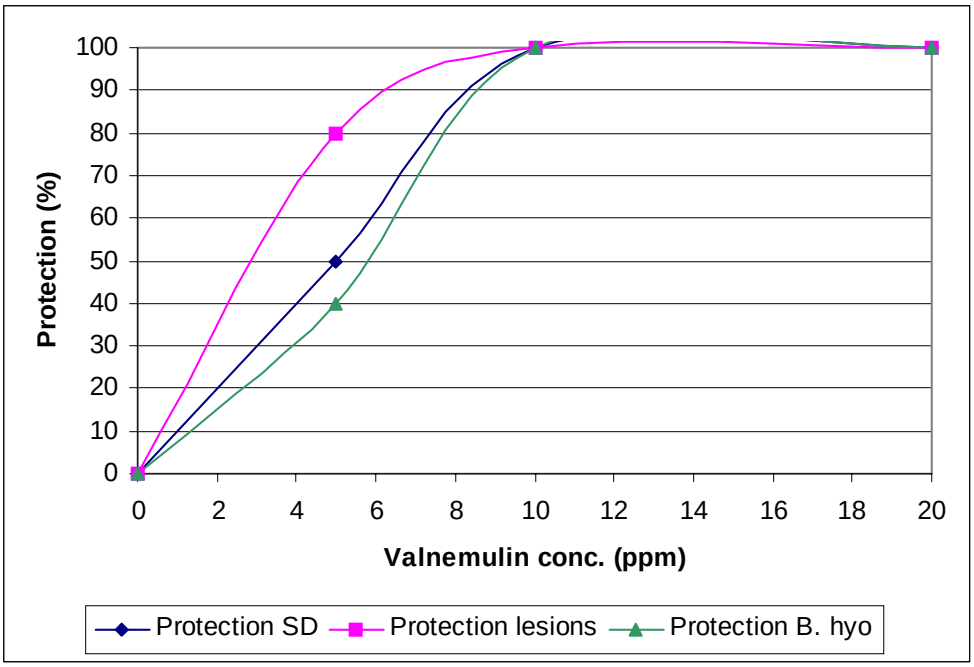
b. Pharmacodynamics

MIC of valnemulin = 0.003µg/ml; MBC of valnemulin = 0.0125µg/ml in broth cultures.

c. Artificial challenge studies

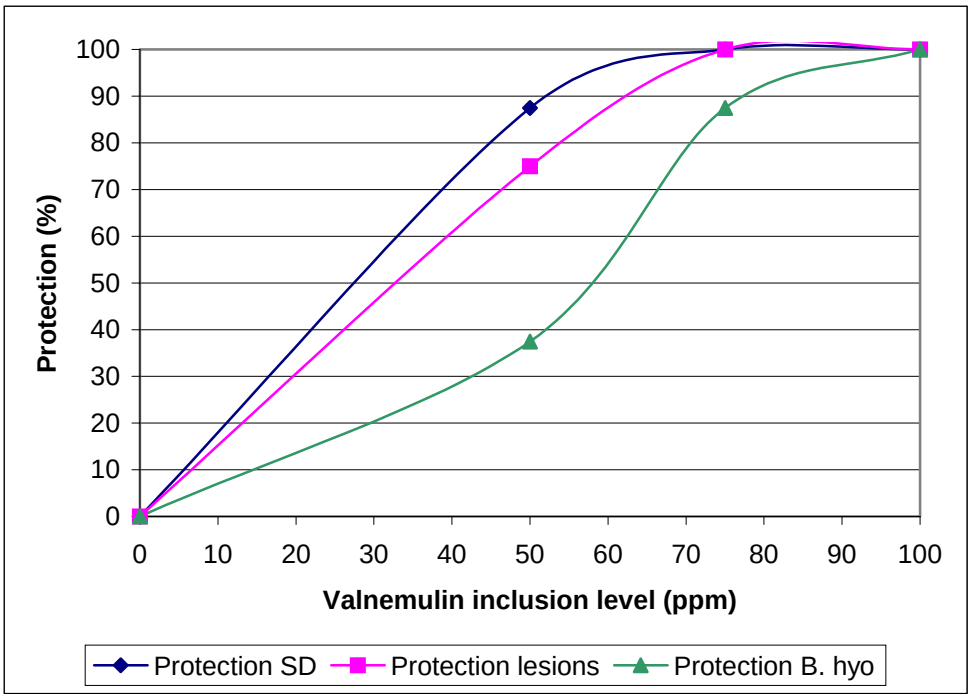
Prevention study - levels of 10ppm and above completely prevented the development of swine dysentery, gross lesions and the growth and re-isolation of the organism at autopsy (see Graph 1); but there was no unmedicated observation period.

Graph 1 - Prevention of swine dysentery with valnemulin – disease, lesions and isolation of *B. hyodysenteriae*



Treatment study - At the end of the 14-day observation period following treatment in the 50ppm valnemulin group, 12.5% had relapsed and 25% of the pigs had gross lesions. *B. hyodysenteriae* was isolated from 62.5% of the 50ppm treated pigs, 12.5% from the 75ppm group and 0% from the 100ppm group (see Graph 2).

Graph 2 - Treatment of swine dysentery with valnemulin – disease, lesions and isolation of *B. hyodysenteriae*



Calculations

Inclusion (ppm)	**10**	**50**	**100**
Effect	bacteriostatic	bactericidal	elimination
CCC (µg/ml)	0.22	1.13	2.24
CCC/MIC	*73*	*377*	*747*
CCC/MBC	**18**	**90**	**180**
AUC 24hr (µg hr/ml)	5.4	27	54
AUIC	*1800*	*9000*	*18000*
AUC/MBC	**432**	**2160**	**4320**

Discussion and Conclusions

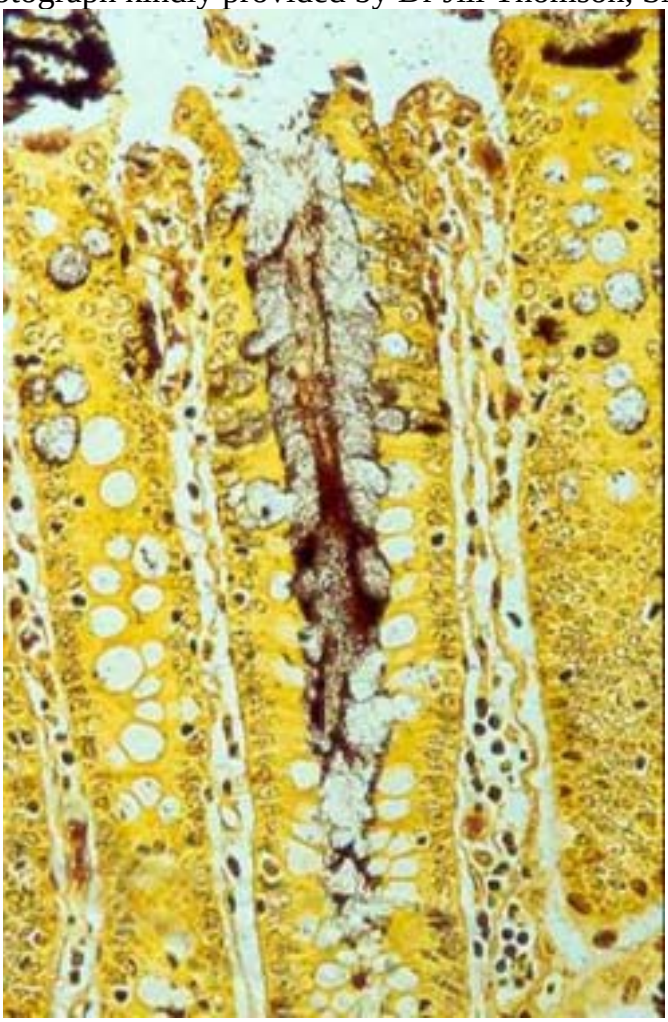
Commonly, the plasma parameters used for successful treatment with bactericidal compounds, such as the aminoglycosides, are Cmax/MIC = **8-10** and the fluoroquinolones AUIC = **100 - 125** (Toutain, 2003). These figures are substantially exceeded by the ratios for valnemulin in the prevention and treatment of a colonic infection, swine dysentery. One hundred and eighty times (**180**) the CCC/MBC and **4320** the AUC/MBC was required to eliminate *B. hyodysenteriae.*

The MIC is not the best parameter to use if a bactericidal activity is to be compared, especially with primarily bacteriostatic compounds. The MBC gives a more comparable result, as has been demonstrated for enzootic pneumonia (mycoplasmal) infections in pigs (Burch, 2004). In the case of swine dysentery treatment, other factors must come into play such as faecal binding, which may affect the bioavailability and diffusion of valnemulin from the colon, although precise information on this is not available.

The difference in MIC against *B. hyodysenteriae*, using different methods such as broth dilution and agar plate, is quite dramatic with an eight-fold difference (four dilutions), highlighting the need to standardise methods when trying to make assessments. Interestingly, the MBC by broth is similar to the MIC by agar plate (two-fold difference or one dilution). Rohde *et al* (2004) reported that there was normally an average of one dilution difference between agar plate and broth dilution methods for *B. hyodysenteriae*.

The artificial challenge studies highlight that prevention of swine dysentery is a valid claim, as a dose-titration effect can be observed and bacterial inhibition is achieved. The likelihood of bacterial resistance may be reduced as few, if any, bacteria are left to mutate (Ripley, personal communication). The treatment study demonstrates that a clinical cure may be achieved without a complete bacterial cure and at least 5-10 times the amount of valnemulin is required to penetrate the lesion and destroy the organisms in comparison with prevention. The bacteria colonise deep into the colonic crypts (see photograph) and a greater concentration gradient needs to be achieved to counter mucus, exudate, even haemorrhage and other inflammatory debris (Toutain *et al*, 2002) flowing in the opposite direction. Large numbers of organisms, in excess of 10^5 per gram, may increase the MIC for pleuromutilins substantially (Drews *et al*, 1975) requiring higher concentrations of antimicrobial to be used. Incomplete kill, in the case of treatment, may actually encourage the development of resistance; but, fortunately this is relatively slow (83 passages) with regard to valnemulin (Karlsson *et al*, 2001).

Photograph – Dark stained spirochaetes penetrating colonic crypts and goblet cells.
(Photograph kindly provided by Dr Jill Thomson, SAC)



In conclusion, the classical PK/PD parameters used for systemic infections such as Cmax/MIC and AUIC, when modified to CCC/MIC and AUIC or even CCC/MBC and AUC/MBC ratios, cannot be simply applied to valnemulin and *B. hyodysenteriae* for the treatment and elimination of swine dysentery as other factors come into play. A different model needs to be developed.

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