THE COMPARATIVE EFFICACY OF ANTIMICROBIALS FOR THE PREVENTION AND TREATMENT OF ENZOOTIC PNEUMONIA AND SOME OF THEIR PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIPS

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Summary

Enzootic pneumonia is a widespread respiratory disease of swine, caused by Mycoplasma hyopneumoniae. It has been a difficult disease to control with the use of medication. The purpose of this paper is to examine the activity of various antimicrobials, both in vitro and in vivo, and to attempt to correlate their pharmacokinetics in both blood and lung with their activity and efficacy. From this analysis, some basic pharmacodynamic relationships, applicable to both bactericidal and bacteriostatic antimicrobials, can be established.

Introduction

Enzootic pneumonia (EP) is usually a chronic respiratory disease of pigs caused by *Mycoplasma hyopneumoniae.* It is widespread in most pig populations of the world, infecting over 90% of herds and causing characteristic lung lesions in 40-50% of all pigs slaughtered. It causes a relatively mild disease on its own, with occasional coughing, depression in growth rate and feed conversion efficiency (FCE) and a low mortality rate. However, a field infection is commonly complicated by secondary bacterial invaders and viruses, which makes the disease situation much more severe and difficult to control. Antimicrobials were widely employed, prior to the introduction of *M. hyopneumoniae* vaccines, to try to prevent the disease developing. They still are commonly used but often more strategically at times when an increase in respiratory disease is expected, i.e. after moving and mixing, or for treatment when there is acute clinical disease present. The disease often involves a mixture of mycoplasmas, bacteria and viruses, causing the porcine respiratory disease complex (PRDC). *M. hyopneumoniae* is still considered of major significance in PRDC.

The purpose of this paper is to compare the *in vitro* activity of various antimicrobials against *M. hyopneumoniae* with their efficacy in infection models

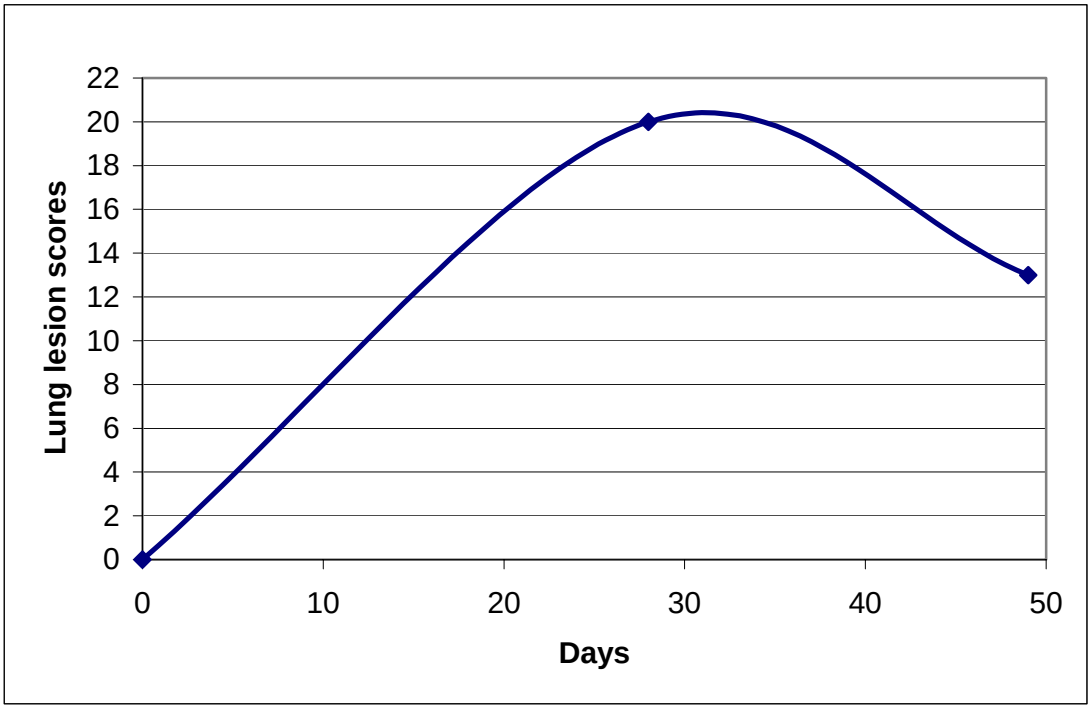
of EP and, where data is available, examine their pharmacokinetic/pharmacodynamic (PK/PD) relationships with their efficacy.

The disease – enzootic pneumonia

Whittlestone (1973) described the progressive development and resolution of enzootic pneumonia lesions induced either by infection with *M. hyopneumoniae* or ground lesion suspensions administered intra-nasally. Early gross lesions could be detected approximately 7-10 days after infection. These continued to develop and establish between 17-40 days and recovery and resolution of lesions took 69–262 days.

Goodwin (1979) also demonstrated this prolonged disease effect in an artificial challenge study, under non-intensive conditions, where pigs were killed at 28 and 49 days after infection and the lesions were scored and shown to be starting to regress at 7 weeks, although they were still described as active.

Graph 1 -EP lesion development and regression in an artificial challenge model



The lesions affect primarily the dependent parts of the lung lobes and may affect the cranial, middle and intermediate lobes and relatively small portions of the large caudal lobes. The organism progressively colonises the respiratory tract and can be identified mainly on the bronchiolar surfaces where there is depletion of cilia. *M. hyopneumoniae* is closely associated with the epithelial cell surface.

There is peribronchiolar and perivascular lymphoreticular hyperplasia and progressive development of alveolar-cell pneumonia. There appears to be a marked lymphoreticular response to the mycoplasmas but the clearance of the organism is very slow, suggesting immuno-defence interference, thought to be associated with reduced macrophage activity. As a result, resolution of the lesions is a prolonged process but, in the end, there is a solid immunity established and pigs are resistant to subsequent challenges by *M. hyopneumoniae*.

Secondary bacterial invaders are also very common, especially *Pasteurella multocida,* due to the reduced mechanical and immunological defence processes. It has also been shown that a simultaneous *P. multocida* infection can double the lung lesion size caused by *M. hyopneumoniae* alone (Ciprian *et al*, 1986). Enzootic pneumonia appears to enhance other bacterial infection such as *Actinobacillus pleuropneumoniae.* With the spread of new virus-associated diseases such as porcine reproductive respiratory syndrome (PRRS) virus and post-weaning multisystemic wasting syndrome (PMWS) associated with porcine circovirus type 2 (PCV-2), there has been an increase in other bacterial infections, especially *Haemophilus parasuis* and *Streptococcus suis* and almost all lobes of the lung can be affected.

Assessment and scoring of the gross lung lesions has been the basis for the evaluation of the efficacy of both antimicrobials and vaccines. A number of methods have been described, but the most common are by visual assessment and scoring, which is rapid and relatively consistent. Three methods are commonly employed and there are similarities in each.

Table 1 - Comparison of lung lesion scoring methods

Lung lobe	**Goodwin *et al* (1969)**	**Percentage**	**Thacker *et al,* (1988)**
L cranial	10	10	4 (100 x 0.04)
L caudal segment	10	10	9 (100 x 0.09)
L caudal	5	25	25 (100 x 0.25)
R intermediate	5	10	5 (100 x 0.05)
R cranial	10	10	7 (100 x 0.07)
R middle	10	10	15 (100 x 0.15)
R caudal	5	25	35 (100 x 0.35)
Total max.	55	100	100 (100 x 1)

The Goodwin *et al* (1969) method is adequate for EP, as it primarily affects the cranio-ventral aspects of the lobes, especially the cranial lobes and rarely goes further in uncomplicated situations. The maximum score of 55 is approximately

equivalent to 55% of the lung being involved. The percentage method is similar, very rapid to do, especially when working on a slaughter line, but takes into account the whole lung rather than just the normally EP-affected parts. This has become important with the upsurge of virus infections (PRRS and PMWS), where up to 80% of the total lung can be consolidated. The Thacker *et al* (1988) method is a more accurate approach to take into account the relative differences in the sizes of the lobes and gives them a weighting. but is more time-consuming.

In the efficacy trial comparisons, percentage lung lesion reductions in comparison with the untreated control will be used, so that it takes into account the different methods of assessment, but enables an overall comparison to be made.

Comparative antimicrobial activity

A variety of studies reported on the susceptibility of reference and field isolates of *M. hyopneumoniae* (Inamoto *et al*, 1994; Hannan *et al*, 1997; Aitken *et al*, 1999; Thongkamkoon *et al*, 2002) to differing antimicrobials, but all used similar methods of broth dilution to determine the minimum inhibitory concentrations (MIC) of the various substances. The determination of the MIC is the lowest concentration of antimicrobial, which prevents the colour change in the pH-dependent colour marker system (phenol red) associated with the fermentation of sugars in the broth. Culturing is normally for about 5 days as *M. hyopneumoniae* is a relatively slow growing organism. Some have used reference strains, usually the type 'J' strain, also referenced as NCTC10110, which are useful for direct comparative purposes of the investigator's methods and results. The references are summarised in Table 2 and the results are compared in Table 3.

Table 2 - References used and origin of isolates and number of isolates

Reference	Year	Country	Ref. strain	No. of isolates
1. Inamoto *et al*	1994	Japan	1	25
2. Hannan *et al*	1997	Worldwide	1	20
3. Aitken *et al*	1999	UK	-	10
4. Thongkamkoon *et al*	2002	Thailand	1 (not reported)	27

Table 3 - Comparison of MIC50, MIC90 and range (µg/ml) for the various antimicrobials against *M. hyopneumoniae* by reference

Antimicrobial	Reference	Ref. strain	MIC50	MIC90	Range
Chlortetracycline	1 4	12.5 -	12.5 0.39	≥100 1.56	0.78-≥100 <0.024-3.125
Oxytetracycline	1 2 3	0.78 0.25 -	0.78 0.25 0.078	6.25 1 0.31	0.1-12.5 0.025-1 0.039-0.63
Tiamulin	1 2 3 4	≤0.0125 0.025 - -	≤0.0125 0.05 0.039 0.006	0.05 0.05 0.078 0.048	≤0.0125-0.05 0.01-0.1 0.039-0.16 <0.006-0.097
Valnemulin	3 4	- -	0.0024 <0.006	0.0049 <0.006	0.0024-0.0098 <0.006
Tylosin	1 2 3	≤0.0125 0.025 -	0.025 0.1 0.16	0.05 0.25 0.31	≤0.0125-0.1 0.025-0.25 0.16-0.63
Tilmicosin	1 4	0.1 -	0.1 0.39	0.39 1.56	≤0.0125-0.39 <0.024-3.125
Acetylisovaleryltylosin	1	≤0.0125	≤0.0125	≤0.0125	≤0.0125-0.05
Josamycin	4	-	0.048	0.097	<0.006-0.195
Lincomycin	1 3 4	0.025 - -	0.025 0.31 0.048	0.1 0.63 0.097	≤0.0125-0.39 0.31-0.63 <0.006-0.39
Enrofloxacin	2	0.05	0.025	0.05	0.01-0.1
Danofloxacin	2	0.025	0.025	0.05	0.01-0.05

Most antimicrobials, including the pleuromutilins, macrolides and the fluoroquinolones, have relatively low MICs and presumably low levels of resistance have developed. In the case of the tetracyclines, the MICs tend to be higher, especially for chlortetracycline in Japan, but less so for oxytetracycline.

Variations in the MIC do occur depending on the media used and agar plates are normally higher than broth. High initial organism inoculum strength will also tend to increase the MIC. The pH can impact the activity of some antimicrobials; the initial pH is 7.8. A slightly alkaline condition benefits tiamulin, but may have a detrimental effect on chlortetracycline activity. Stability of the substance in the broth is also important, especially over prolonged culture. The endpoint, i.e. either at the first colour change of the control or after a further day or so of culture, can also have an impact on the MIC result.

Comparative antimicrobial efficacy

A number of challenge studies have been reported over the years as new antimicrobials were developed. However, there are relatively few comparative studies involving several products.

The majority of studies use EP lung lesion homogenate inoculated intra-nasally, (occasionally intra-tracheally), either on one or two occasions, to induce the infection. Occasionally, pigs are naturally infected and then removed for treatment. The EP infection is superimposed by a bacterial infection, either as part of the challenge or by a contaminant of the homogenate, or natural infection in the pig. In prevention studies, medication usually begins just before, or at the time of infection, and in treatment studies 10-28 days after infection when the lesions have started to develop.

The studies are summarised in Table 4 and the percentage lung lesion reductions in Table 5.

Table 4 - Summaries of EP model infections by reference

Trial reference	Antimicrobials	Route of admin.	Prevention days	Treatment	Infection route	Bacteria isolated
1.Thacker *et al* (2000)	Chlortetracycline	In feed	-3 to +10 Term 28	10 to 24 Term 28	I/T	-
2.Schuller *et al* (1977)	Tiamulin	In feed	-3 to +7 Term 35	-	I/N	Mh
3.Goodwin (1979)	Tiamulin	In feed	-	28 to 37 Term 49	I/N	-
4.Hannan *et al* (1982)	Tiamulin Tylosin	Gavage b.i.d.	-	14 to 24 Term 38	I/N	Mh, Ag, Ph, Pm
5.Simon *et al* (1990)	Tiamulin Enrofloxacin	In feed	-	0 to 10 Term 15 & 29	Natural	Yes - NR
6.Miller and Stipkovits, (1991)	Tiamulin + chlortetracycline Tylosin + sulphadimidine	In feed	0-28 Term 28	-	I/N	Pm, Bb
7.Stipkovits *et al* (2001)	Tiamulin + chlortetracycline Valnemulin + chlortetracycline Tilmicosin Lincomycin + chlortetracycline	In feed	-	9 to 21 Term 22-24	I/N aerosol Mh – 0 Pm – 8 App - 15	Pm, App

Table 4 contd. on next page

Table 4 (contd.) - Summaries of EP model infections by reference

8.Morgan *et al* (1996)	Valnemulin	In feed	0 to 21 Term 21	-	I/N	-
9. Burrows *et al* (2002)	Valnemulin Acetylisovaleryl tylosin	In feed	- -1 to +6 Term 19	4-11 4-11 Term 19	I/N	-
10.Yamamoto *et al* (1986)	Josamycin	In feed	-1 to +3-+7 Term 36	-	I/N x2	-
11.Kubo *et al* (1990)	Lincomycin	In feed	0 to 7 Term 27	-	I/N x2	-
12.Ross *et al* (1990)	Danofloxacin	In water	-2 to +10 Term 10 & 20	-	I/T	-
13.Kuwano *et al* (1992)	Ofloxacin	In feed	0 to 7 Term 35	-	I/N	-

Key: I/N = intra-nasal; I/T = intra-tracheal; Mh = *M. hyorhinis*; Ag = *Acholeplasma granularum*;
Pm = *P. multocida*; Ph = *P. haemolytica*; App = *A. pleuropneumoniae*; Bb = *Bordetella bronchiseptica;*
b.i.d. = twice daily

Table 5 - Comparison of antimicrobial efficacy judged by lung lesion reduction (%) for the prevention and treatment of enzootic pneumonia under controlled conditions

Antimicrobial	Ref.	Dose (mg/kg bwt/day)	Inclusion rate in feed (ppm)	Prevention Lesion reduction (%)	Treatment Lesion reduction (%)
Chlortetracycline (CTC)	1	22	550ppm	95	36
Tiamulin	2	5	100ppm	35	-
		10	200ppm	58	-
	5	10	200ppm	-	92 (day 15) 57 (day 29)
	4	20	G b.i.d.	-	98
	3	50	1000ppm	-	69
Tiamulin + CTC	6	5 + 15	100 + 300	97	-
	7a	2 + 22	38.5 +440	-	76
	7b	5 + 20	100 + 400	-	76
Valnemulin	8a	10	200	45	-
		15	300	43	-
		20	400	71	-
	8b	10	200	79	-
	9	10	200	-	38
Valnemulin + CTC	7a	1.25 + 22	25 + 440	-	90
		2.5 + 22	50 + 440	-	93
	7b	1.25 + 20	25 + 400	-	86
		3.75 +20	75 + 400	-	90
Tylosin	4	100	G b.i.d.	-	95
Tylosin + sulphadimidine	6	5 + 15	100 + 300	52	-

Table 5 contd. on next page

Table 5 (contd.) - Comparison of antimicrobial efficacy judged by lung lesion reduction (%) for the prevention and treatment of enzootic pneumonia under controlled conditions

Acetylisovaleryl tylosin	9	2.5 2.5 5	50 50 100	45 - -	- 51 32
Josamycin	10	2.5 5	50 100	12 70	- -
Tilmicosin	7b 7a	15 20	300 400	- -	60 60
Lincomycin	11	4.4 8.8	88 176	26 41	- -
Lincomycin + CTC	7b	5 + 20	100 + 400	-	24
Enrofloxacin	5	7.5 10	150 200	- -	92 (day 15) 92 (day 15) 79 (day 29)
Danofloxacin	12	3.4	W25	94 (day 10) 86 (day 20)	- -
Ofloxacin	13	0.6 2.5 10	12.5 50 200	51 82 100	- - -

Key: W = in water; G = by gavage; b.i.d = twice daily

Chlortetracycline was highly effective in the prevention of EP, which was also the author's own findings (Burch and Morgan – unpublished data) but less so for the treatment of EP. Combinations with the pleuromutilins were highly efficacious for both the prevention and treatment of EP complicated with bacteria.

Tiamulin and valnemulin both showed dose/response effects for the prevention of EP and also a good response was achieved with a second strain more sensitive to valnemulin. Tiamulin at very high levels was also very effective in treating EP. Tylosin at very high levels of 100mg/kg bodyweight was effective, but a related compound acetylisovaleryl tylosin showed an effect at $1/40^{th}$ of the level. The pleuromutilins, macrolides and lincosamides showed only an intermediate preventative effect in comparison with chlortetracycline and the fluoroquinolones. Enrofloxacin was effective in the treatment of EP and danofloxacin and ofloxacin were very effective for prevention. Interestingly, the lesion reduction deteriorated with time after treatment in many cases, suggesting further lesion development once medication had been removed, presumably because the mycoplasma started to grow again and complete immunity had not been fully established during the treatment and observation period to control it.

Comparative pharmacokinetic information

The available antimicrobial pharmacokinetic data has been collated from a number of sources but is not always complete. Lung concentration data and correlating plasma levels are presented, where available, for the various antimicrobials. In-feed administration of antimicrobials can have a detrimental effect on levels achieved in plasma if the substances are metabolised rapidly in the liver. Many compounds can still be detected in the lung because they concentrate there due to positive diffusion gradients, e.g. the macrolides and pleuromutilins. The substances excreted substantially by the kidneys such as the tetracyclines, lincomycin and the fluoroquinolones usually have detectable blood and lung levels.

Table 6 - Comparison of blood and lung levels achieved by various dosages of antimicrobial

Antimicrobial	Reference	Dose (mg/kg/day) estimated	Inclusion level (ppm)	Blood/plasma level (µg/ml)	Lung level (µg/g)
Chlortetracycline	Asanuma *et al* (1986)	20	400	0.25	0.55
Tiamulin	Product data Ibayashi *et al* (1994)	5.5 11 5.5	110 220 110	<0.3 <0.3 -	1.46 1.99 0.63
Valnemulin	Product data	3.75 10	75 200	<0.05 <0.05	0.04 0.23
Tylosin	Ibayashi *et al* (1994)	5.5	110	-	<0.05
Acetylisovaleryl tylosin	Ibayashi *et al* (1994)	5.5	110	-	0.14
Tilmicosin	Thomson *et al* (1994)	20	400	0.18	1.97
Lincomycin	Ibayashi *et al* (1994) DeGeeter *et al* (1980)	5.5 5.5 11	110 110 220	- 0.16 0.14	0.85 0.66 1.13
Enrofloxacin	Product data	5	150	0.24	0.65

Key: < = below limit of quantification

Pharmacokinetic/pharmacodynamic relationships

Most classic PK/PD antimicrobial relationships have been established by plasma concentration data, either Cmax (maximum concentration reached in plasma after administration) and/or area under the curve during a 24hour period (AUC24hr) and these figures are divided by the MIC. These relationships have been well described by Toutain (2003). For bactericidal antibiotics such as the aminoglycosides, a figure of 8-10hr has been quantified for the Cmax divided by the MIC to give an effective treatment of an infection. For fluoroquinolones, a figure of ≥100hr for the AUC/MIC (AUIC) has been established for effective bactericidal treatment and <30 for bacteriostasis. For bacteriostatic antimicrobials. these figures have not been determined. With in-feed or water

administration, the intake and absorption of the antimicrobial is relatively constant over a 24hour period and the blood level and lung level is relatively constant, in comparison with a bolus dose given either orally or by injection. The AUCs are therefore calculated by either antimicrobial blood or lung level times 24 hours and are summarised in Tables 7 and 8.

Table 7 - Comparison of the antimicrobial blood levels and AUC24hr with the MIC50 and MIC90 against *M. hyopneumoniae*

Antimicrobial	Level (ppm)	MIC50 (µg/ml)	MIC90 (µg/ml)	Blood level (µg/ml)	AUC (µg/ml/hr)	AUIC50	AUIC90
Chlortetracycline	550	0.39	1.56	0.25	6	15	4
Tilmicosin	400	0.1	0.39	0.18	4.32	43	11
Lincomycin	220	0.025	0.1	0.14	3.36	134	33.6
Enrofloxacin	150	0.025	0.05	0.24	5.76	230	115

Table 8 - Comparison of the antimicrobial lung levels and AUC24hr with the MIC50 and MIC90 against *M. hyopneumoniae*

Antimicrobial	Level (ppm)	MIC50 (µg/ml)	MIC90 (µg/ml)	Lung level (µg/ml)	AUC (µg/ml/hr)	AUIC50	AUIC90
Chlortetracycline	550	0.39	1.56	0.55	13.2	34	8
Tiamulin	220	0.04	0.08	1.99	47.76	1194	597
Valnemulin	200	0.0025	0.005	0.23	5.52	2208	1104
Tylosin	110	0.1	0.25	<0.05	1.2	12	5
Tilmicosin	400	0.1	0.39	1.97	47.28	473	121
AIV tylosin	100	0.0125	0.0125	0.14	3.36	269	269
Lincomycin	220	0.025	0.1	1.13	27.12	1085	271
Enrofloxacin	150	0.025	0.05	0.65	15.6	624	312

From the blood level data, enrofloxacin (fluoroquinolone) achieved an AUIC of over 100hr for 90% of the isolates and its therapeutic effect was 79-92% for the treatment of a complicated EP. The other products had very low AUIC figures, although all of them showed some effect in the prevention or treatment of EP, especially chlortetracycline with a preventative effect of 95%. Unfortunately, the MIC for the isolate used was not given.

With regard to lung concentrations, enrofloxacin showed an AUIC 90 of 312hr, or approximately three times the AUIC90 figure for blood, and this is the approximate ratio lung to blood that is found with this substance. All the other products had high AUIC90 figures, except tylosin and chlortetracycline. Valnemulin's AUIC figures were very high because of the very low MIC figures.

In the report by Morgan *et al* (1996), the MICs of the two *M. hyopneumoniae* used in the studies were given. The effect and lung PK/PD relationships can be examined further.

Table 9 - Comparison of AUIC lung for valnemulin and reduction in lung lesions (prevention)

Trial 1	**Valnemulin Level (ppm)**	**Extrapolated lung level (µg/ml)**	**AUC lung (µg/ml/hr)**	**AUIC lung**	**Lung lesion reduction (%)**
MIC 0.016(µg/ml)	100	0.12	2.88	180	0
	200	0.23	5.52	345	45
	300	0.35	8.28	518	43
	400	0.46	11.04	690	71
Trial 2					
MIC 0.0078(µg/ml)	200	0.23	5.52	708	79

Valnemulin's AUIC lung of 345 – 518hr gave 43-45% reduction in lung lesions and 690-708hr gave 71-79% reduction in lung lesions in a prevention trial, suggesting that an AUIC lung of 1000hr would be required to achieve complete prevention.

In a study using tiamulin for the treatment of EP, Goodwin (1979) demonstrated a 69% reduction in lesions using 50mg/kg bodyweight or approximately 1000ppm in feed. He also described the MIC of the organism used and the minimum mycoplasmacidal concentration (MMC). The MIC was 0.11-0.15µg/ml and the MMC was 0.45-0.9µg/ml. For the sake of the calculations, an average figure is used - 0.13 and 0.68µg/ml (MMC/MIC ratio 5).

Table 10 - Comparison of AUIC lung for tiamulin and the MIC and MMC of *M. hyopneumoniae* and lung lesion reduction

	Tiamulin Level (ppm)	**Extrapolated lung level (µg/ml)**	**AUC lung (µg/ml/hr)**	**AUIC lung**	**Lung lesion reduction (%)**
MIC 0.13	1000	9.05	217.2	1670	69
MMC 0.68	1000	9.05	217.2	319	69

When the AUC lung is divided by the MMC, a similar AUIC figure to enrofloxacin is achieved which is a bactericidal antimicrobial. However, the strain used was the reference 'J' strain and the MIC is almost 5-10 times higher than other authors, presumably because of the very high inoculum titre used, of 10^9 organisms/ml rather than the more usual 10^4-10^5.

Discussion and Conclusions

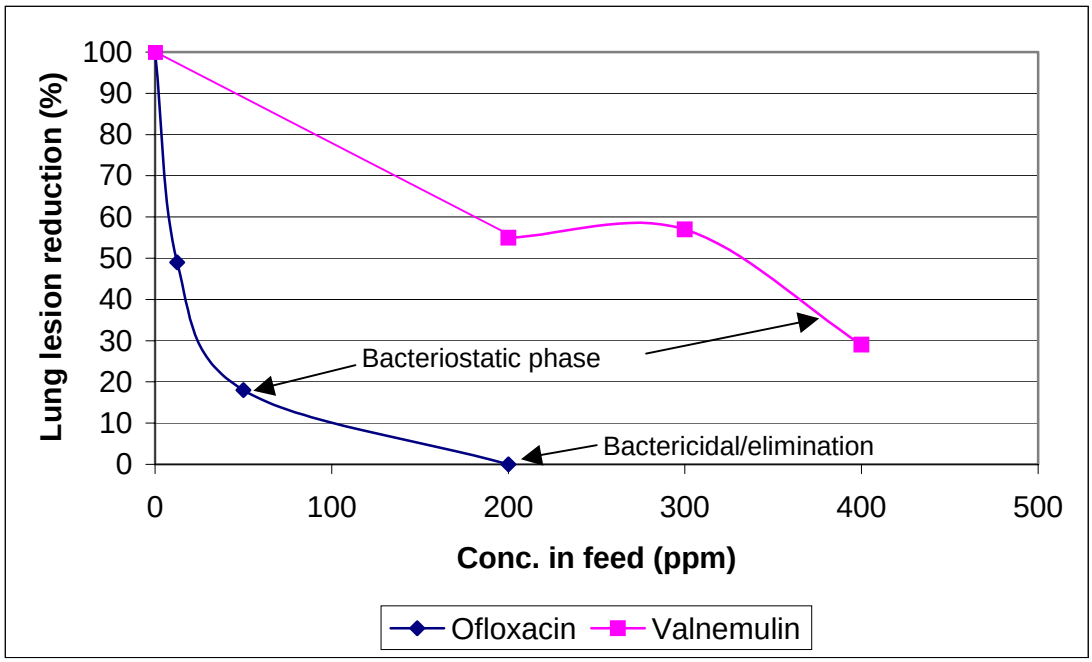
Enzootic pneumonia is a complex disease to treat. It is also difficult to draw conclusions from the MIC results, trial results and PK/PD relationships. *M. hyopneumoniae* is both difficult and slow to grow *in vitro* and the MIC results may vary from investigator to investigator. Broth or agar plates can have an impact, as well as inoculum strength, duration of incubation, end point used and antimicrobial used. Some antimicrobials appear to be pH sensitive and others are unstable in broth on incubation over several days. In some cases, e.g. after prolonged incubation with tylosin, the MIC continues to drift upwards until it reaches the MMC (Goodwin, 1979).

Infection challenge models also vary substantially, not so much by route of administration of the infection, but by timing of treatment. Prevention studies are relatively straight forward in that treatment starts around the time of infection. However, observation periods after treatment are very variable. As lesions can take over four weeks to form, the timing of treatment studies is more complex. Should they start before four weeks after infection during the lesion development or when the lesions are becoming well-established, but may be naturally regressing after another 3-4 weeks of treatment and observation? They are not standardised. There is an obvious overlap in timing between many of the studies but, in effect, this is what is happening in a field infection, where there are all stages of lesion development at any one time depending on whether or when the pig succumbs to the disease.

The effects of the medicinal products can be divided into three simple phases, bacteriostatic, bactericidal and eliminatory and can be further subdivided into prevention and treatment. The fluoroquinolones are considered

primarily bactericidal antimicrobials. Their bacteriostatic phase may be quite narrow in dosage terms before they become bactericidal and elimination is possible, as demonstrated in the case of ofloxacin. In the case of valnemulin, a pleuromutilin, it is primarily considered as a bacteriostatic antibiotic, so its bacteriostatic phase should be much wider (see Graph 2). In a prevention study it is unlikely that immunity to *M. hyopneumoniae* will have much of an effect in the first four weeks, hence the lesions continue to develop after removal of the antimicrobial unless the organism is killed.

Graph 2 - Comparative effect of ofloxacin (bactericidal) and valnemulin (bacteriostatic) in the prevention of EP



It can be seen that bacteriostatic antimicrobials such as tetracyclines, pleuromutilins, macrolides and lincosamides would find it harder to reach bactericidal levels and especially a mycoplasma elimination level, as very high inclusion levels would be required. It would appear that the majority of these groups give an intermediate (bacteriostatic) effect, although CTC appears to be highly effective in preventing lesions developing (95%), but not nearly as effective for treatment (36%), as it is primarily a bacteriostatic effect and a more bactericidal effect is required.

Many of the treatment studies are almost late prevention, starting under four weeks post-infection. Good responses were achieved with the fluoroquinolone enrofloxacin (92%) in a mixed infection, but this was reduced after an observation period of 14 days, to 79%, suggesting that it was having a bactericidal effect, but had not eliminated the infection and it then continued to develop. Once treatment has been established, consolidated lesions may alter some of the pharmacokinetic parameters, e.g. concentration in the lesion. It is interesting that the responses to a combination of pleuromutilins and

chlortetracycline (tiamulin + CTC 76%; valnemulin + CTC 86-93%) gave improvements over chlortetracycline alone (36%). Burch *et al* (1986) reported a clinical superiority in the field with tiamulin and chlortetracycline over chlortetracycline alone and Kitadai *et al* (1998) demonstrated an increased bactericidal effect when valnemulin and chlortetracycline were combined in a killing curve study with *P. multocida*, which might explain this phenomenon.

Relating pharmacokinetic parameters to effect has also been difficult. There has been debate over the significance of antimicrobial lung levels in comparison with blood levels. *M. hyopneumoniae* live primarily on the cell surface and are especially concentrated on the bronchiolar epithelium. Thus the relevance of lung concentrations, which are presumably intracellular in the alveolar cells, is unclear and levels in the blood or extracellular fluids are probably more significant. This may not be the case in the more necrotising pneumonic lesions associated with *A. pleuropneumoniae* where there may be significant disruption of cellular integrity. Determining concentrations of some antimicrobials at the site of infection is also quite difficult because they are below the limit of quantification or detection in some cases. However, they may still be above the MICs for *M. hyopneumoniae* e.g. valnemulin's MIC90 is 0.0049µg/ml and acetylisovaleryltylosin's is <0.0125µg/ml. The limit of detection in plasma or tissues is in the order of 0.02-0.05µg/ml. The lung concentration however can act as a surrogate marker for what is going on at different sites and has the advantage of magnifying the antimicrobial concentration, usually by positive diffusion gradients, to a detectable limit. Often this relationship data is available. For example, enrofloxacin's lung/serum ratio is about 2.7 (Baytril premix product literature) and tiamulin's is about 19 (Forster *et al*, 1982) in the pig. In theory, then the AUIC lung should be divided by the lung/serum ratio and the MMC/MIC ratio to give a comparable AUIC serum figure from which one can predict what effect the antimicrobial is having, whether bacteriostatic, bactericidal or even potentially eliminatory.

Calculations and example

Formula: AUC lung / MIC / (Lung/serum ratio) / (MMC/MIC ratio) = AUIC ratio

Using tiamulin 50mg/kg (Goodwin, 1979) 1000ppm in feed for **treatment** of EP

217.2 / 0.025 / 19 / 5 = AUIC ratio 91 = 69% reduction in lesions for treatment, a bactericidal effect.

Using tiamulin 10mg/kg (Schuller *et al*, 1977) 200ppm for **prevention** of EP

43.4 / 0.025 / 19/ 5 = AUIC ratio 18 = 58% reduction of lesions for prevention, a bacteriostatic effect.

Much more work is required to prove this hypothesis and it is hoped that it will lead to further investigation by researchers in this field and clarification of the mode of action of bacteriostatic antimicrobials in the prevention and treatment of swine enzootic pneumonia.

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