Pharmacokinetic and pharmacodynamic relationships of Denagard and other antibiotics for respiratory and enteric infections in pigs

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Introduction

Understanding how antibiotics work is core to how we use them in order to effectively treat infections in pigs and maintain their health and welfare. Additionally, it can give us some ideas on how to preserve their efficacy and avoid the downward spiral into antimicrobial resistance. It is highly likely that there will not be any new antibiotics of any great significance in the near future and that we will have to make do with what we have.

Pharmacokinetics (PK) is the study of the absorption, distribution, metabolism and excretion of an antimicrobial drug in the body of the pig. Concentrations in blood or plasma are the most common method of determining these parameters. However, it is only part of the story, as many infections in pigs occur in the gastrointestinal tract, so we need to know what concentrations we can find there. Lung infections are also important and therefore are the plasma concentrations of a drug the most significant measurement? The same question can be asked for any tissue or compartment such as the joint to treat arthritis.

Pharmacodynamics (PD) is the study of the effect of the antibiotic on the organism, mainly bacteria but also mycoplasma. The minimum inhibitory concentration **(MIC)** of the antibiotic is the common parameter to use to determine the susceptibility of that organism against the drug. However, some antibiotics are bactericidal so that concentrations around the MIC will kill the bacterium, therefore that figure is also the minimum bactericidal concentration **(MBC)**. Other antibiotics are bacteriostatic and are primarily inhibitory so at the MIC it stops the organism multiplying but does not necessarily kill it and a much higher concentration is required to do so. A normal operating immune system is required to help remove the bacterium. If the patient/pig is immuno-suppressed due to a virus infection such as porcine reproductive and respiratory syndrome (PRRS) virus or porcine circovirus type 2 (PCV2), many antibiotics show reduced efficacy. Some bacteriostatic antibiotics though can become bactericidal from 2 or more times the MIC but often it is several times higher. It is down to the antibiotic and the particular affected or involved bug.

Looking at the PK/PD relationships of the drugs and bugs help us to understand, which antibiotic to use, at which dose and by what route against a specific infectious agent.

Antibiotic use in pigs

Antibiotics have been and even still are widely used in pig production, for mainly prevention and treatment of disease but they are still used in certain countries for growth promotion. In the UK, it was estimated that over 50% of antimicrobials are used in pigs but in the US there is approximately a 30% split between pigs, poultry and cattle, which are also intensively reared in feedlots.

In the EU growth promoters have been banned since 2006 under the *'precautionary principle'* and there is increasing pressure to further restrict antimicrobial use in veterinary medicine following further human health resistance concerns. This was initiated by the isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs (MRSA CC398). There is also concern over the growing levels of bacteria in veterinary medicine that are producing extended-spectrum beta-lactamases (ESBLs), which are resistant to 3rd and 4th generation cephalosporins and their possible resistance transfer via plasmids in *Escherichia coli* to man. Currently, the risk of resistance transmission would appear to be

small but work is likely to be ongoing to quantify this. In the meantime, it has caused much political uncertainty in the EU and this is spilling over to other countries like the US, who have restricted the use of cephalosporins and are considering banning the use of antibiotics for growth promotion. Trading-partner countries that export to the EU are also taking note of these developments.

The use of antibiotics in pigs and poultry are particularly under the microscope, so the more we understand them and how they work, the better we can defend their use and use them responsibly.

Pharmacokinetics

A number of basic PK parameters are used when trying to relate to the PD of a certain antibiotic (see Figure 1).

Figure 1. Basic pharmacokinetic parameters
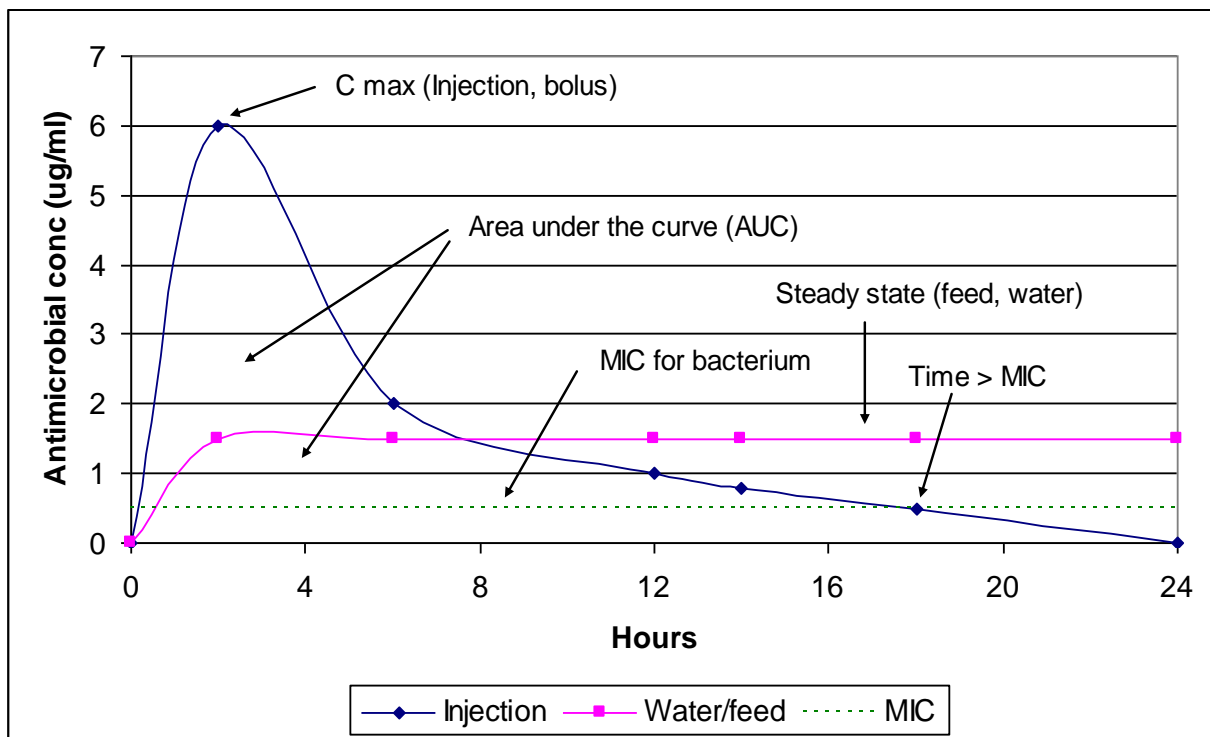
When a drug is injected or given as an oral bolus there is usually a maximum plasma concentration **(Cmax),** which occurs at a certain time **(Tmax)**. The height of the curve and the speed it reaches Cmax is dependent on the absorption rate. There is a distribution phase to other tissues. Normally, lipid soluble drugs have a high volume of distribution, as they can penetrate cells and tissues more readily. This is seen with tiamulin, as it penetrates and concentrates in lung tissue to a high level. Then there is a decline in the curve, depending on the excretion of the drug. The main routes of excretion are via the kidneys; the sulphonamides, aminoglycosides, penicillins and cephalosporins are primarily excreted by that route and the liver usually after metabolism and excretion via the bile system into the gut. Some drugs are deactivated but some produce highly microbiologically active metabolites such as enrofloxacin converts to ciprofloxacin, so it has a strong gut antibacterial effect. Tiamulin is also excreted this way. Aminoglycosides (neomycin, apramycin, gentamicin etc) and fluoroquinolones (enrofloxacin, marbofloxacin etc) are bactericidal antibiotics and kill bacteria comparatively quickly in a matter of hours. As a result the Cmax may be used in relation to the MIC as a guide to its efficacy. **A ratio of 10: 1 (Cmax/MIC)** frequently has a lethal effect on the bacterium.

When drugs are given orally, if they are absorbed, peak plasma concentrations are frequently lower than by injection and the curves are commonly flatter due to drug intake in multiple doses via feed or water during the day, rather than in one single dose. The area under the curve **(AUC)** is a very important parameter for most antibiotics to determine their efficacy in comparison with PD. This applies to most antibiotics, whether bactericidal, like the fluoroquinolones, aminoglycosides, penicillins, trimethoprim/sulphonamide combinations and bacteriostatic drugs, like the pleuromutilins (tiamulin and valnemulin), macrolides (tylosin, tilmicosin, tulathromycin etc), lincosamides (lincomycin) and even tetracyclines. The AUC is divided by the MIC **(AUC 24h/MIC)** and usually a **figure of 100-120h** provides a bactericidal effect for bactericidal drugs or the **AUC/MBC** for bacteriostatic drugs can also be used. This is approximately **4-5 times the average plasma concentration** over the MIC/MBC depending on the type of antibiotic.

Time above the MIC **(T>MIC)** is often used for the penicillins and cephalosporins. A time of **>10h** is required to kill Gram-positive bacteria such as *Streptococcus suis* but for Gram-negative bacteria such as *E. coli* the time of **24h** is required. For bacteria such as *Actinobacillus pleuropneumoniae*, although Gram negative, the time may be more similar to Gram positives at >10h.

Many drug plasma concentrations do not cover the whole 24h period but many show a post-antibiotic effect **(PAE)** where the concentration falls in the bacterium but it does not start growing again for a number of hours. Especially, those antibiotics that work on the ribosome, such as the macrolides, tetracyclines and pleuromutilins, can exert this effect.

The PK of tiamulin by injection gives an interesting example of the complexities of tissue distribution and antibiotic use (see Figure 2).

Figure 2. The pharmacokinetics of tiamulin administered at 15mg/kg bwt - concentrations in plasma lung and colon contents (McKellar et al, 2004)
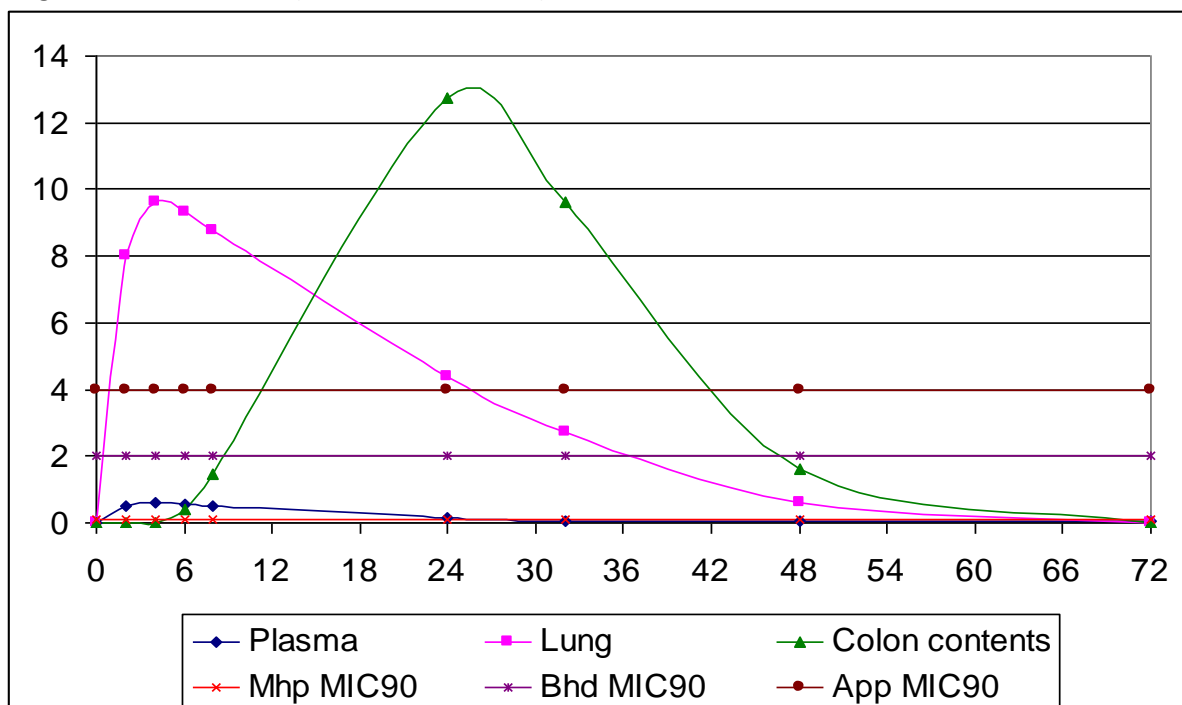

Plasma concentrations are generally quite low reaching a Cmax of 0.61µg/ml. The concentrations in lung mirror the plasma concentrations but the Cmax is much higher at 9.6µg/ml (times 15.7). The concentration in the colon contents is delayed, as the drug needs to be metabolised and excreted via

the liver/bile but also reaches very high levels with a Cmax of 12.75µg/ml (times 20.9). Recent work by Klein et al (2012) have shown in baby pigs <7 days of age similar tissue concentration effects but has also shown that tiamulin concentrates in bronchial fluid with a Cmax at 5.9 times the plasma Cmax. It also penetrates into joint fluid but at a lower concentration than plasma (64%).

The biologically effective concentrations in plasma are those after subtractions for plasma-protein binding have been made. Some compounds bind strongly, such as doxycycline (>90%) and ceftiofur and valnemulin >70%. The majority of antibiotics bind between 30-50%, such as tetracycline, enrofloxacin, amoxicillin, trimethoprim and tiamulin. Florfenicol and lincomycin are considered low binders at <20% in pigs.

When drugs are given orally in feed or water they exert their own PK characteristics in the gut (see Figure 3.

Figure 3. Drug flow of a non-absorbed liquid compound from the stomach via the small intestine to the colon (Burch, 2012)
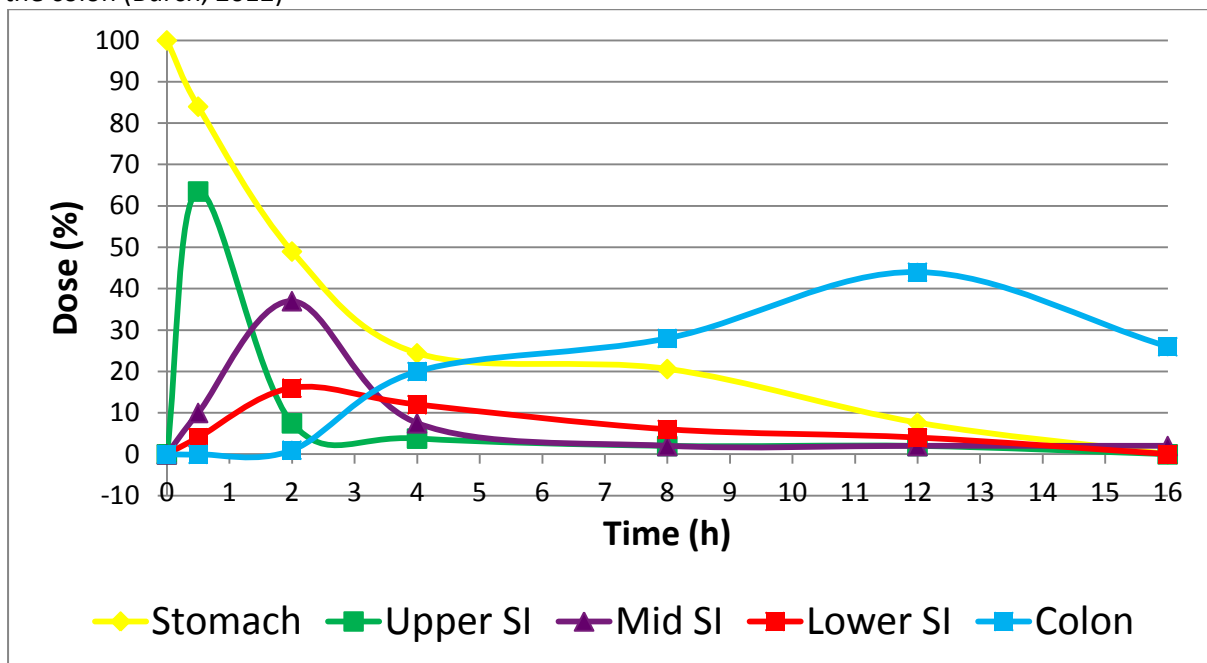
There is a high Cmax and quick flow from the stomach to the duodenum with liquids e.g. with liquid antibiotic formulations. This flows as a wave along the small intestine down to the ileum and after 2 hours starts to accumulate in the colon. The AUCs for the small intestine components and the colon are approximately in a ratio of **1: 3.4**. That is, if the drug is not absorbed or broken down, like the aminoglycosides or colistin, then it will concentrate in the colon at 3.4 times higher than the small intestine. If it is included in feed at 100ppm and not absorbed, approximately 167ppm will accumulate in the colon (feed to faeces ratio of **1: 1.67**) and 50ppm will be in the small intestine (29% of colon contents concentration). This is helpful when modelling gut concentrations.

Feed does interfere with some drugs absorption (see Figure 4).

Figure 4. Effects on bioavailability of drugs from fed and fasted pigs (Nielsen, 1997)
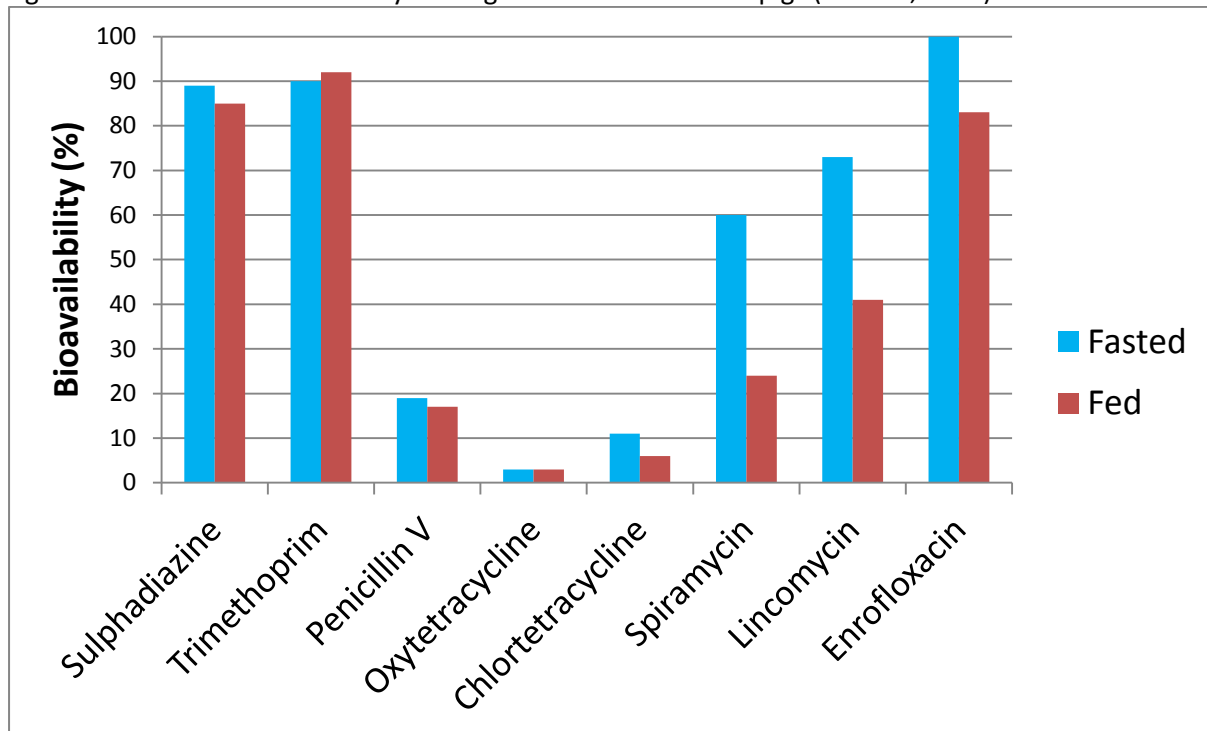

The bioavailability of tiamulin in feed is affected also (Schreiber & Wanner, 1990) by as much as 65%, so lower lung concentrations are achieved when given in feed in comparison with water medication but higher colon concentrations are achieved when given in feed, dose for dose.

Pharmacodynamics

The commonest laboratory test for antimicrobial sensitivity is the sensitivity disc method (Kirby-Bauer test). This gives a simple visual result with a zone of inhibition around the disc. The diameter of the zone can be measured and an approximate MIC can be estimated. Most of the methodology has been standardised by the Clinical and Laboratory Standards Institute (CLSI). It is a little crude and the most common method is determined by growing the organism in broth media or plates impregnated with doubling dilutions of antibiotic. The MBC can be determined by sub-culturing the broth on to antibiotic-free plates to see if there are any live organisms present. Further work can be carried out to examine the killing rate of the antibiotic and whether it is concentration dependent or time dependent or both. Aminoglycosides and fluoroquinolones are mainly concentration dependent, penicillins and cephalosporins are time dependent and most others are co-dependent on time and concentration like the pleuromutilins and macrolides.

When 10 or more isolates of a particular organism are grown the **MIC 50, MIC 90 and MIC range** can be determined. This helps the clinician determine the likelihood whether a particular organism will be sensitive to an antibiotic or not. It can also show the susceptibility pattern or resistance pattern of the bacterial organism. The more isolates (100 etc) the more accurate the pattern. The epidemiological breakpoint or '*wild-type*' pattern can be observed. Sometimes there is a one step mutation to resistance e.g. tylosin and *Brachyspira hyodysenteriae* or sometimes there are more than one step e.g. in the case of fluoroquinolones and *E. coli* and tiamulin and *B. hyodysenteriae* there is a two-step mutation pattern (see Figure 5). When an antimicrobial has been used for a while a resistance pattern is established and it can also indicate a drug usage pattern.

Figure 5. Susceptibility pattern of *B. hyodysenteriae* isolates in relation to tiamulin concentrations in the colon (Karlsson et al, 2002)
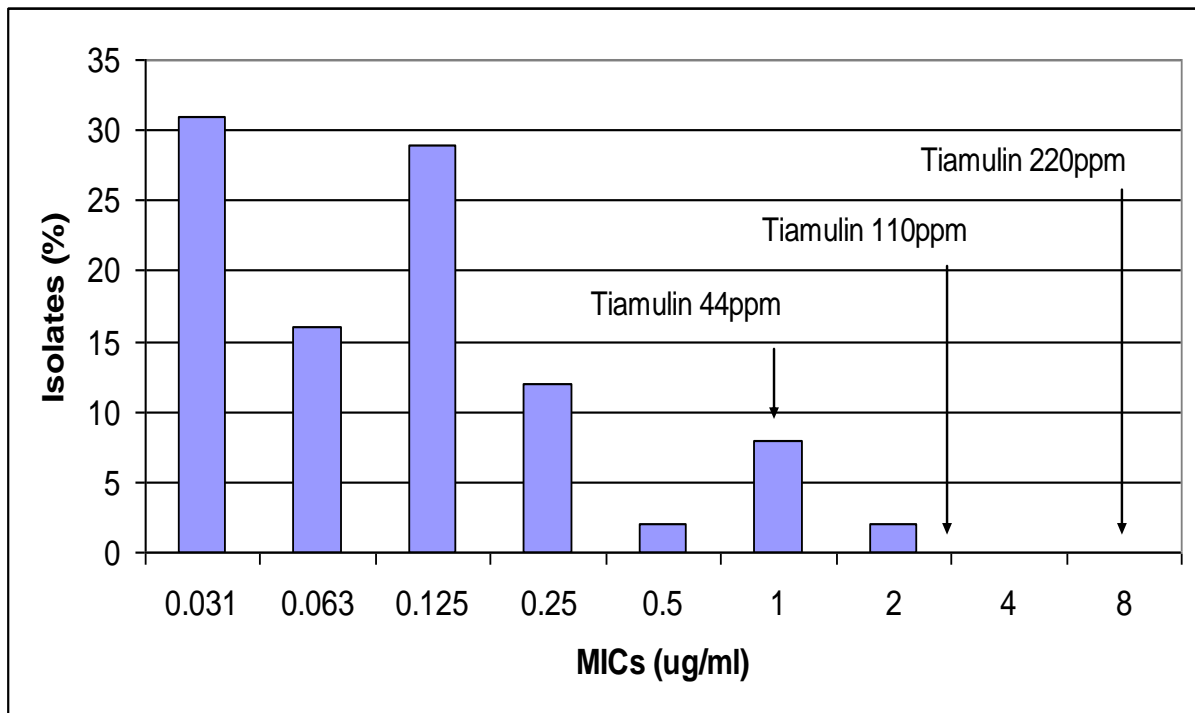

This approach also highlighted the value of high dose rates to prevent mutants developing as well as controlling the susceptible isolates. This is termed the mutant prevention concentration **(MPC)** and the window between MIC and mutant MIC is the mutant selection window **(MSW).** As tiamulin is bactericidal against *B. hyodysenteriae* at approximately 2 times the MIC the 220ppm colon contents concentration is likely to be above the MPC. This may be a reason why tiamulin resistance has developed slowly against *B. hyodysenteriae* over the last 30 years. This approach is being commonly used in man to prevent mutations occurring and reducing resistance development, particularly with the fluoroquinolones.

PK/PD relationships

The PK/PD relationships for tiamulin and *B. hyodysenteria*e have been demonstrated in Figure 5 and it would also apply to *B. pilosicoli* infections, as they are also found in the colon.

Regarding respiratory tract infections it is not so straightforward with tiamulin (see Figure 6).

Figure 6. Plasma and lung concentrations of tiamulin in comparison with tiamulin MICs of *M. hyopneumoniae* and other respiratory bacteria pathogens
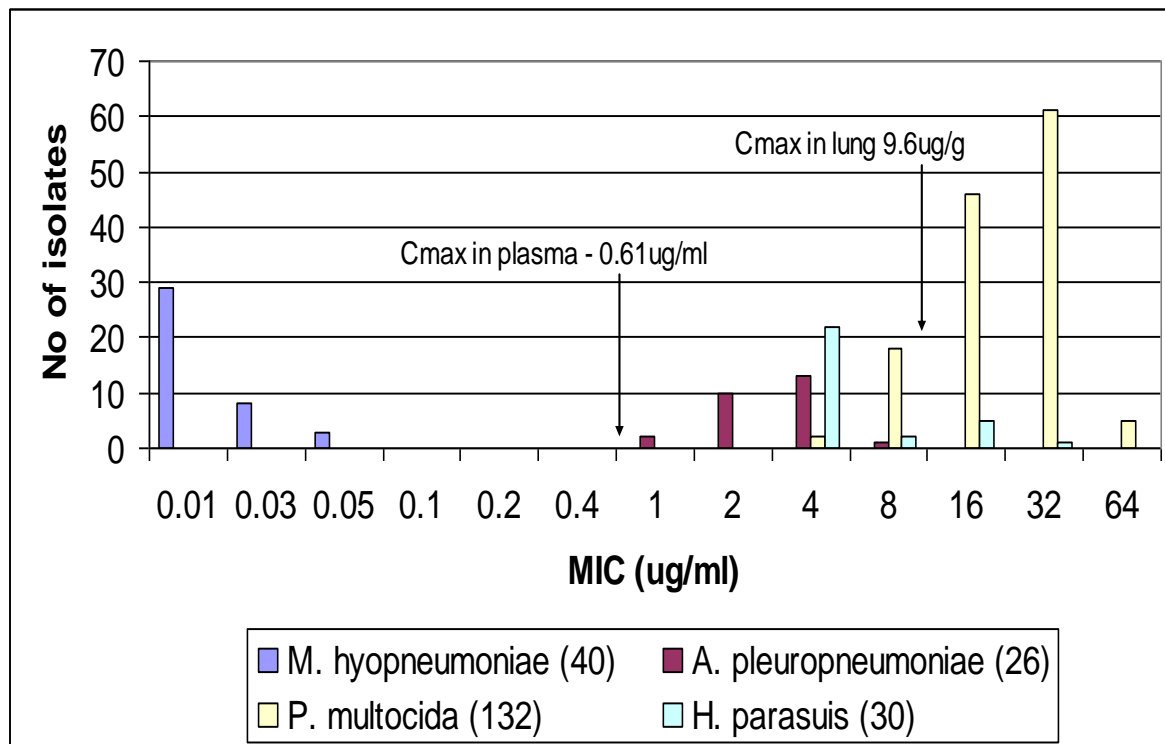
Using the tiamulin injection data (McKellar et al, 2004) suitable plasma concentrations can be achieved to inhibit *Mycoplasma hyopneumoniae* but not the respiratory bacteria such as *A. pleuropneumoniae*, *Haemophilus parasuis* or *Pasteurella multocida*, which all have far higher MICs. Another PK action is likely to be having an impact. It is considered unlikely that it is directly lung concentration, as the bacteria are located outside the lung cell. It might be bronchial fluid, which is considered important for most antibiotics. It is considered also likely that it is the concentration of tiamulin in polymorphs or macrophages, which engulf the bacteria that is playing a key role. Nielsen & Szancer (1998) showed very high concentrations of tiamulin accumulate in polymorphs. This has been postulated for other antibiotics such as tilmicosin and tulathromycin.

Another key swine disease that tiamulin is indicated for is the treatment of ileitis caused by *Lawsonia intracellularis*. This organism lives inside the ileal epithelial cell so a drug has to be able to penetrate cells and therefore needs to be lipid soluble. Tiamulin is particularly active against *L. intracellularis* in vitro (Wattanaphansak et al, 2009) and is also effective in vivo (see Figure 7) as is valnemulin another pleuromutilin antibiotic.

Figure 7. Tiamulin and valnemulin ileal concentrations in comparison with the intracellular MICs of *L. intracellularis* (Wattanaphansak et al, 2009)
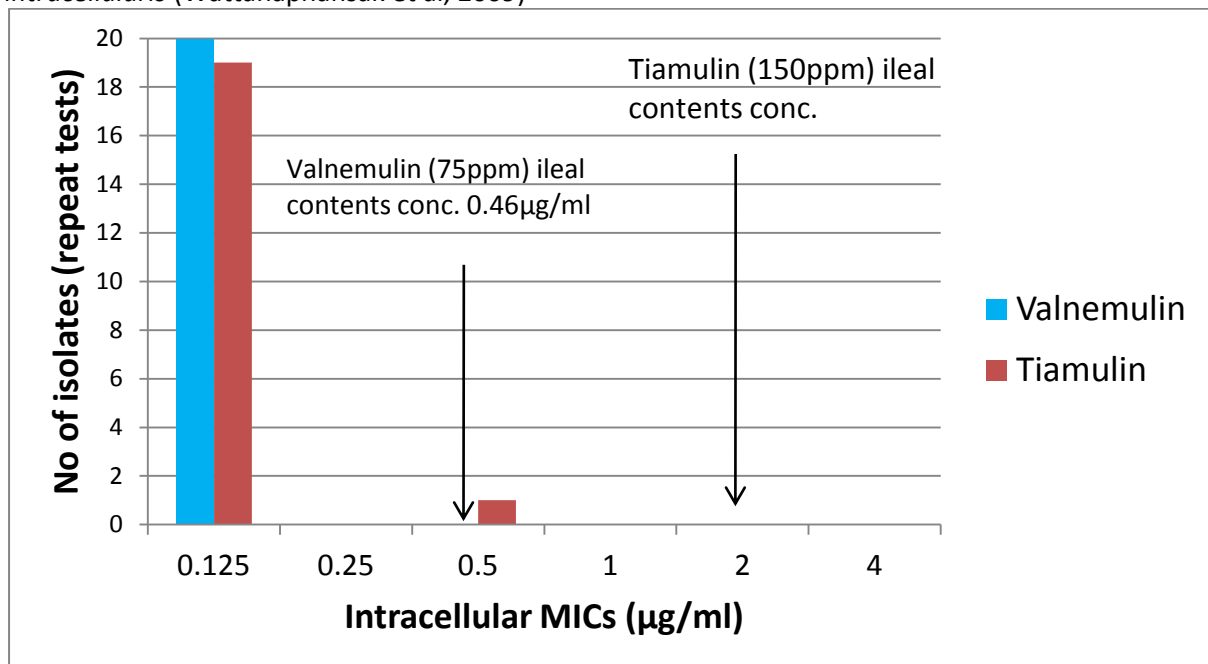
Conclusions

An understanding of the pharmacokinetics of a drug is very helpful in the understanding of how it works and coupled with the pharmacodynamic data should enable clinicians to make better choices regarding the type of drug and the dose needed to treat pigs.

We cannot always use human PK/PD relationships, as we mainly use different drugs that are primarily bacteriostatic but they may be bactericidal at higher concentrations. Hopefully, we are not faced with the medical task of treating immuno-compromised patients to the same extent, now that PCV2 is controlled.

However, the PK/PD relationships give us a guide how to use antibiotics more effectively and possibly avoid bacterial mutants and resistance development.

The relationship of PK to antimicrobial susceptibility patterns is also helpful to understand what is going on from previous exposure and what determined the resistance patterns. Hopefully, this will encourage more responsible use and help direct future antimicrobial use policy in a more practical and sensible way.

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