Review

Pharmacokinetic/Pharmacodynamic Relationships of Antimicrobial Drugs used in Veterinary Medicine

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The rise in incidence of antimicrobial resistance, consumer demands and improved understanding of antimicrobial action has encouraged international agencies to review the use of antimicrobial drugs. More detailed understanding of relationships between the pharmacokinetics (PK) of antimicrobial drugs in target animal species and their action on target pathogens [pharmacodynamics (PD)] has led to greater sophistication in design of dosage schedules which improve the activity and reduce the selection pressure for resistance in antimicrobial therapy. This, in turn, may be informative in the pharmaceutical development of antimicrobial drugs and in their selection and clinical utility. Pharmacokinetic/PD relationships between Area Under the Concentration time curve from zero to 24h (AUC_{0-24}) and Minimum Inhibitory Concentration (MIC), maximum plasma concentration (C_{max}) and MIC and time during which plasma concentrations exceed the MIC have been particularly useful in optimising efficacy and minimising resistance.

Antimicrobial drugs have been classified as concentration-dependent where increasing concentrations at the locus of infection improve bacterial kill, or time-dependent where exceeding the MIC for a prolonged percentage of the inter-dosing interval correlates with improved efficacy. For the latter group increasing the absolute concentration obtained above a threshold does not improve efficacy. The PK/PD relationship for each group of antimicrobial drugs is 'bug and drug' specific, although ratios of 125 for AUC_{0-24}:MIC and 10 for C_{max} :MIC have been recommended to achieve high efficacy for concentration-dependent antimicrobial drugs, and exceeding MIC by 1-5 multiples for between 40% and 100% of the inter-dosing interval is appropriate for most time-dependent agents. Fluoroquinolones, aminoglycosides and metronidazole are concentration-dependent and beta lactams, macrolides, lincosamides and glycopeptides time-dependent. For drugs of other classes there is limited and conflicting information on their classification.

Resistance selection may be reduced for concentration-dependent antimicrobials by achieving an $AUC_{0\text{-}24}$:MIC ratio of greater than 100 or a C_{max}:MIC ratio of greater than eight.

The relationships between time greater than MIC and resistance selection for time-dependent antimicrobials have not been well characterised.

Introduction

Antimicrobial drugs have revolutionised human and veterinary medicine through the provision of effective and inexpensive means of treating, and in some circumstances preventing, bacterial infectious disease. In veterinary medicine intensified production methods have led to an increase in the spread of disease where animals were kept in confined air spaces, or in the case of fish, in confined water spaces. Antimicrobial drug prophylaxis and therapy have permitted maintenance of animals in these husbandry systems without the adverse impact on animal health and welfare which bacterial disease would have otherwise inflicted. Furthermore, it has long been recognised that antimicrobial drugs confer growth-promoting effects, even when administered to healthy animals at dosage rates lower than those effective in treating clinical disease (Stokestad & Jukes 1949,1950). However, three major factors have now encouraged a review of antimicrobial drug use in animals. Firstly, the inexorable selection of bacteria resistant to available drugs in animals and 'more particularly' in man (House of Lords 1998, World Health Organisation 2001). Added to this there is a growing body of evidence that bacteria selected for resistance in animals can be transmitted to man where they may cause disease or transmit the genetic material responsible for resistance to human pathogenic bacteria (MAFF 1998, Smith *et al.*, 1999, Saenz *et al.,* 2000). Moreover, the acquisition of bacterial resistance has outstripped the ability of pharmaceutical companies to produce new products with mechanisms of action which overcome resistance, as is evidenced by the growing number of virtually untreatable bacterial infections in man (World Health Organisation, 2001). Nevertheless, it is clear that the majority of human resistant infections which are difficult to treat are derived from bacteria selected for resistance in man and not in animals (World Health, Organisation 2001).

Secondly, the sophistication of the consumer in developed markets has meant that production systems which are demonstrably favourable to animal health and welfare, and where audit can confirm minimal chemotherapeutic intervention, are gaining popularity. While this conflicts somewhat with major market forces whereby consumers effectively 'buy cheap', it is undoubtedly a

trend which will continue and will be spurred by increased affluence, traceability and information availability. This socio-economic pressure has already caused a rapid move away from the use of antimicrobial drugs as growth promoters in Europe, where they have been labelled as a crutch for poor production methods but where the hard evidence implicating them in human health problems is scant (Bates *et al.*, 1994, Klare *et al.*, 1995, Report from the Commission on Antimicrobial Feed Additives, Stockholm, 1997). Thirdly, there is the realisation over the last decade that the relationship between the concentration of an antimicrobial in an animal or man and its effect on the target pathogen is not simple. Thus, dosage strategies have been developed which may increase the efficacy or reduce the selection pressure for resistance associated with the antimicrobial for which they are tailored (Hyatt *et al*., 1995).

Pharmacokinetic/pharmacodynamic (PK/PD) interactions for antimicrobial drugs result in gross observable readouts in administered animals including clinical improvement, growth promotion and adverse reactions (toxicity). Clinical improvement is associated with the antimicrobial causing bacterial cell death or inhibiting bacterial cell growth and thus facilitating its removal by the host immune system (Prescott & Walker, 2000). Growth promotion is also effected by the interaction between drug and bacteria and thus a change in bacterial populations and in their metabolic profile (Lev & Forbes, 1959; Eyssen & De Somer, 1967). Adverse reactions may be the result of the antimicrobial affecting commensal bacterial populations and thus overgrowth by pathogenic organisms and for instance the development of enterocolitis (English & Roberts, 1983). Alternatively, the antimicrobial drug may directly affect the host animal causing specific organ or system pathology (Yeary, 1975, Appel & Neu, 1977). The PK/PD interaction may also result in the selection of bacteria resistant to the administered antimicrobial or to other antimicrobials demonstrating common resistance mechanisms (Blaser *et al.*, 1987, Baquero *et al.*, 1997).

The utility of PK/PD information is demonstrable in the development of new antimicrobials, the more specific selection of appropriate antimicrobials from formularies, the design of optimal dosage strategies and the reduction in selection of antimicrobial resistance (Gunderson *et al.*, 2001). The

development of new drugs for use in veterinary medicine has generally been based on dosage titration against model infections of bacterial disease supported by subsequent field investigations using the selected dosage (The rules governing medicinal products in the European Union 1999). Models utilising the dose titration approach provide very useful but limited information since the only variable generally tested is absolute dosage and the pattern of drug disposition is essentially fixed. Furthermore, a selected bacterial population is tested, with a fixed susceptibility to the antimicrobial. Dose titration has been shown to discriminate clinical outcome (mortality) poorly, whereas bacterial shedding may correlate well with dose (Yancey *et al.,* 1990). Field studies based solely on monitoring clinical outcome are flawed by the 'Pollyanna effect', whereby the efficacy of very good drugs is underestimated and the efficacy of poor drugs is overestimated. Thus, Marchant *et al.,* 1992) demonstrated a clinical success of 89% in children with otitis media treated with an antimicrobial which conferred 100% bacterial eradication. A clinical success of 74% was achieved with an antimicrobial conferring only 27% bacteriological cure, thus suggesting apparent success on a treatment which was essentially no better that placebo. Integration of the subtle differences in plasma disposition and bacterial sensitivities of closely related antimicrobials will allow more specific selection of antimicrobials from formularies and thus more effective individualisation of treatment strategies (Gunderson *et al.,* 2001). In veterinary medicine such subtleties in dosage selection are in their infancy but they are clearly appropriate and achievable targets. Perhaps the greatest utility for PK/PD integration is in the design of optimal dosage strategies. Thus not only can the absolute amount of drug required to treat an infection be determined but the way in which the drug is delivered to produce a plasma (or tissue) profile optimum to effect removal of the causal bacteria can also be characterised (Sarasola *et al*., 2002). Integration of kinetic and dynamic characteristics of antimicrobial drugs has been used widely virtually since their first introduction. However more sophisticated PK/PD modelling (see later for details) is now being utilised to predict optimum dosages and may provide a versatile tool to determine optimum administration strategies. Optimisation of dosage strategy may impact on the selection of antimicrobial resistance in two

ways. It is implicit that the optimal strategy to remove the offending pathogen delivers the minimal appropriate dosage (effective to eradicate the target pathogen), thus commensals are exposed to the minimal selection pressure for resistance. Furthermore PK/PD parameters can be determined which minimise the selection window (see later) for resistance associated with the target pathogen (Blondeau *et al.*, 2001).

Pharmacokinetic / Pharmacodynamic Relationships

In defining PK/PD relationships the most useful pharmacokinetic parameters are the Area Under the Plasma Concentration time curve (AUC) from 0 time to 24h, the maximum plasma Concentration (C_{max}) achieved and Time (T) during which concentrations exceed a defined pharmacodynamic threshold. The most useful pharmacodynamic parameter is the Minimum Inhibitory Concentration (MIC) (Hyatt *et al.*, 1995). The MIC is the lowest concentration of antimicrobial which inhibits the growth of the target bacteria and is generally determined by growing the bacteria in doubling dilutions of the antimicrobial for 24h and assessing inhibition of growth by the resultant turbidity of the growth media (Walker, 2000a). The sensitivity of this method can be improved by using overlapping doubling dilutions although this is impractical for routine screening of large numbers of organisms when determining MIC_{50} and MIC_{90} (see later).

However, as stated earlier the relationship between antimicrobial concentration achieved in the target animal and the effect on the offending bacterial pathogen is not simple. Efficacy may depend upon achieving concentrations in plasma several fold higher than the MIC of the pathogen (Blaser *et al.*, 1987). Alternatively it may be dependent on maintaining concentrations in plasma just above the MIC for a prolonged time (Craig, 1998a) and, for some drugs and pathogens, a combination of concentration and time of exposure may be important (co-dependency). Furthermore, resistance may develop more rapidly when insufficient concentration or time of exposure to the drug is achieved (Baquero & Negri, 1997; Blondeau *et al.*, 2001). In quantitative terms the PK/PD parameters which have been most extensively investigated, and for which the most robust information is currently available, are $AUC_{0\text{-}24}$:MIC, C_{max}:MIC and T greater than MIC (T>MIC); (Hyatt *et al.*, 1995).

The relationship between AUC_{0-24}:MIC and efficacy has been well demonstrated in a mouse model of a gram negative bacterial infection in which treatment with fluoroquinolones reduced mortality from approximately 100% at a low AUC_{0-24}:MIC ratio to almost zero at a ratio of greater than 100 (Craig, 1998b). The AUC_{0-24}:MIC ratio has also been shown to relate to bacteriological cure in seriously ill people treated with ciprofloxacin in which a ratio of greater than 125 resulted in almost 80% reduction in the number of patients from which pathogenic bacteria could be isolated (Schentag, 2000). In the same study, when a ratio of less than 125 was achieved bacteriological cure was achieved in only about 50% of patients. Extrapolation of these results to veterinary clinical situations should be done with caution since models utilise neutropenic mice and seriously ill human patients are also likely to be neutropenic. In infected but immuno-competent domestic animals similar relationships almost certainly exist but the ratios are likely to differ since antimicrobial drugs and immune mechanisms act in concert. A clear relationship between C_{max}:MIC, and favourable clinical response, has been demonstrated in human patients with gram-negative infections treated with aminoglycoside antibiotics. A C_{max}:MIC ratio of 2 resulted in favourable clinical response in about 50% of patients, whereas a ratio of 12 produced a positive response in approximately 90% of patients (Moore *et al.*, 1987). A relationship has also been demonstrated between time above MIC and bacteriological cure in people with *otitis media* treated with beta-lactam antibacterial drugs. Bacteriological cure increased from about 40% to 80% as the time above MIC increased from 10% to 100% of the interdosing interval (Craig & Andes 1996). Each of the above studies was carried out in models of human bacterial disease, or in human patients, and each is complicated by the fact that there is co-variance between C_{max}, AUC_{0-24} and T >MIC. In other words, as the maximum concentration achieved in plasma increases so does the AUC and T >MIC.

In order to study the relationship between C_{max} and T >MIC in a representative animal model of respiratory disease, and to reduce the influence of co-variance of PK/PD markers, a study utilising bolus and infusion delivery of danofloxacin was carried out. Danofloxacin was administered to calves experimentally infected with *Mannheimia (Pasturella) haemolytica* by strategies conferring

either a high C_{max}:MIC ratio (bolus injection) or a long T greater than MIC (i.v. infusion) but at the same absolute dose and producing similar AUC:MIC ratio in both treatments. The high C_{max}:MIC ratio conferred statistically better clinical and bacteriological cure than the long T >MIC (Sarasola *et al*., 2002). The above studies and others (see later under individual classes) permit the classification of antimicrobial drugs according to their optimum activity. They may be concentration – dependent (time – independent) whereby the antimicrobial drugs kill bacteria to a greater extent at increasing exposure concentration. Alternatively, they may be time – dependent (concentration – independent) whereby bacteria are killed to the same extent once a threshold concentration has been reached, but the antimicrobial drugs are more effective when concentrations above the MIC are maintained for a longer proportion of the interdosing interval. These classifications are both antibacterial and pathogen specific, and there may be drugs which are co–dependent on concentration and time (Gunderson *et al.,* 2001). A general classification of antimicrobial agents for which information is available is given in Table 1. It should be appreciated that many of the data utilised in classifying these agents are derived from human studies and that, for some drugs, this information is conflicting. In this regard the fact that some show apparent time and concentration dependence may be due to co-variance of the pharmacokinetic parameters.

Pharmacokinetic Issues

Perhaps the most important pharmacokinetic consideration is where to measure drug concentrations from which pharmacokinetic data are derived. It is axiomatic that the antimicrobial should reach the locus of the offending bacteria to be effective and, therefore, it could be assumed that tissue or cellular concentrations would be the most appropriate for determining the required kinetic parameters. Nevertheless, plasma concentrations have been shown to be the best predictors of clinical success even for most tissue infections (Schentag, 1989, Cars, 1997, Toutain *et al.*, 2003). This is because extravascular fluid drug penetration is generally complete and plasma concentration of non-protein bound drug, therefore, provides an accurate measure of tissue concentrations. Where a specific anatomical or pathological barrier exists, for example in the central nervous system or in

an abscess, or where bacteria are intracellular (e.g Mycoplasma, Chlamydophila) it may be more appropriate to use concentrations derived from that site (Toutain *et al.*, 2003). However, this may also be true for drugs which preferentially accumulate inside cells, such as the macrolides, for which plasma concentrations do not appear to correlate with observed efficacy (Gladue *et al.*, 1989, Nightingale, 1999). Of the available veterinary antimicrobials fluoroquinolones, macrolides, lincosamides and trimethoprim display the greatest capacity for intracellular penetration. For these agents intracellular disposition may also be important since penetration of lysosomes and phagolysosomes, as well as cytosolic distribution, may be required to affect bacteria within specific subcellular loci (Tulkens, 1990). Since only free drug is pharmacologically active, plasma pharmacokinetic parameters should be corrected to reflect the extent of protein binding. However, for practical purposes, this appears to be of importance only for drugs which are highly protein bound with a free fraction of less than 20% in plasma (Toutain *et al.*, 2003) and may, therefore, be of relevance for clindamycin, cloxacillin, doxycycline and some sulphonamides (Hardman *et al.*, 1996). Protein binding of drugs varies between animal species and it is important to consider binding in the target animal.

Pharmacodynamic Issues

Many pharmacodynamic factors affect antimicrobial activity and these may be important both *in vitro*, where the drug activity is being assessed, and *in vivo*, where activity is the intent. In determining MIC, or other *in vitro* pharmacodynamic parameters, pH and presence of oxygen in the environment of the organism are critical to its growth; as are the bacterial load applied to the test system and the phase of growth of the bacteria being tested (Walker, 2000a). Such factors should be standardised according to internationally accepted methodologies (National Committee on Clinical Veterinary Standards, NCCLS, 1999). Nevertheless, it is important to realise that MIC data may still be somewhat inaccurate since MIC's are normally determined using doubling dilutions raising the possibility of an almost 100% error between two sequential readouts. Running parallel tests, with overlapping concentrations, can reduce the error (Forrest *et al.*, 1997; AliAbadi & Lees, 2002, 2003;

AliAbadi *et al.,* 2003)). Another important consideration is that MICs are routinely determined in a culture broth, and not in the environment in which the bacteria grow *in vivo* such as blood, extracellular fluid, intracellular environment, urine and milk, or in the presence of pus, exudate or detritus. The pH, aerobic or anaerobic environment and growth phase are also important *in vivo* and, in particular, in specific anatomical or physiological situations such as the mammary gland and urinary tract and in pathological situations, such as severe inflammation or abscessation (Ziv & Rasmussen, 1975; Verklin & Mandell, 1977; Strausbaugh & Sande, 1978; Luscombe & Nicholls, 1988).

In vitro pharmacodynamic assessment of activity may underestimate the activity of an antimicrobial drug achieved *in vivo* because of the Post-antibiotic Effect (PAE) and Post-antibiotic Leukocyte enhancement (PALE) (Prescott & Walker, 2000). The PAE describes the persistent suppression of bacterial growth following removal of an antimicrobial from the locus of the bacteria (Fuursted 1987). The occurrence and magnitude of PAE are dependent on the micro-organism, the type and concentration of drug to which the micro-organism has been exposed, and the duration of exposure. The PAE is generally longer *in vivo* than *in vitro* (Renneberg & Walder, 1989). The mechanisms of action of the PAE are varied and include, for beta-lactams, the length of time that the organism takes to synthesise new penicillin binding proteins. For aminoglycosides the length of time taken for the drug to dissociate from the ribosome and to diffuse from its site of activity, and then for protein synthesis to recommence confers a PAE. Beta lactams express PAE for gram positive bacteria only (except for carbapenems which may also have a gram negative PAE). Antimicrobial drugs which inhibit DNA or protein synthesis, tend to impart long PAE against gram negative bacteria (Prescott & Walker, 2000). Macrolides, fluoroquinolones and aminoglycosides consistently show PAE for gram negative bacteria (Lutsar *et al.,* 1998; Dudley, 1991; Craig, 1998b). The efficacy of spaced administrations of concentration – dependent antimicrobials may be associated with their PAE. The PALE describes the increased susceptibility to phagocytosis and intracellular killing demonstrated

by bacteria following exposure to an antimicrobial agent. Drugs, which produce the greatest PAE also, tend to produce the greatest PALE.

Resistance Selection

Conventional wisdom suggests that underdosing with an antimicrobial drug rapidly selects for resistance and this has been confirmed using PK/PD markers where an $AUC_{0\text{-}24}$:MIC ratio of 100 or greater for all antimicrobial drugs was shown to reduce selection for resistance (Thomas *et al.*, 1998). More detailed examination for the fluoroquinolones indicates that an independent risk of resistance selection is conferred when an $AUC_{0\text{-}24}$:MIC greater than 100 or a C_{max}:MIC greater than 8 are not achieved (Blaser *et al.*, 1987, Forrest *et al.*, 1993). Furthermore, underexposure to one fluoroquinolone can confer resistance to the whole class and it must be anticipated that similar risks will apply to other drug groups sharing resistance mechanisms. Some caution is required in extrapolating this ratio from severely ill and presumably immuno-compromised humans to immuno-competent (but bacterially infected) animals. Repeated exposure to suboptimal drug concentrations is now recognised as the single most important factor for the emergence of resistance (Burgess, 1999). Optimal dosing strategies confer appropriate drug concentration and time of exposure for the target pathogen which is likely to have a specific or narrow range of susceptibility (MIC) on which the dosage is based. However, commensal bacteria may express quite different sensitivities, which could result in their underexposure to the delivered drug, and thus the selection of resistant populations, which could then transfer resistance genes to pathogens (Baquero *et al.*, 1997). Thus, optimal dosing strategies for specific pathogens may not be those which minimise selection in the whole animal. Using the example of the fluoroquinolones, it is possible to conceptualise a concentration window, exposure to which is likely to select for resistance. Resistance in fluoroquinolones may be conferred by two successive mutations, on bacterial gyrase enzymes (Hooper & Wolfson 1993). The first mutation reduces susceptibility to fluoroquinolones without conferring full resistance. In a treated animal the plasma concentration of fluoroquinolone declines from the C_{max} to concentrations below which even the wild type bacteria are unaffected. However, as

drug concentrations fall there is a period during which the first step (gyrase) mutants have a selective advantage over the wild type, and during which the population of these mutants increases. As this population grows so does the probability of the second mutation occurring and thus the selection of double mutants which are fully resistant. The concentration between that where the first step mutants are killed [the Mutant Prevention Concentration MPC (Blondeau *et al.*, 2001)] and that at which wild type bacteria survive is termed the selection window (Fig.1) (Catry *et al.*, 2003). The disposition pharmacokinetics of an antimicrobial drug can affect the size of the selection window as can the administration strategy for its delivery. An optimum PK/PD ratio can be determined to reduce the selection window and thus resistance development.

Optimal Dosage

For drugs whose optimal efficacy can be related to the AUC_{0-24}:MIC ratio, optimum dosage can be determined if appropriate PK and PD data are known as shown in equation 1 (Toutain *et al.*, 2003). This equation provides an absolute dose per day but does not indicate how that dose should be divided for optimal efficacy.

Equation 1

$$\text{Dose per day} = \frac{\text{AUIC} \times \text{MIC} \times \text{Cl}}{\text{fu} \times \text{F} \times 24\text{h}}$$

where AUIC = AUC/MIC ratio for optimal efficacy
MIC = Minimum Inhibitory Concentration
CI = Clearance per day
fu = Free fraction of drug in plasma *(ignore if minimal binding)*
F = Bioavailability

Where prior study information is available, a sigmoidal E_{max} relationship for bacterial count versus *ex vivo* AUC_{0-24}:MIC may be utilised. In this model the dose can be adjusted to provide a specific desired effect which may be bacteriostasis, bactericidal activity or bacterial eradication as shown in equations 2 and 3 and in Fig 2 (AliAbadi & Lees, 2001). In fact, equation 2 yields the same result as equation 1.

Equation 2

$$DO = DE \times \frac{AUC_{24h}/MIC\ ex\ vivo}{AUC_{24h}/MIC\ in\ vivo} \times \frac{MIC_{90}}{MIC_E}$$

where:

DO	=	Optimal dose
DE	=	Dose used experimentally
MIC_{90}	=	MIC for 90% of organisms
MIC_E	=	MIC for organism used experimentally
$AUC_{24h}/MIC_{in\ vivo}$	=	Ratio provided by dose DE *in vivo*
$AUC_{24h}/MIC_{ex\ vivo}$	=	Ratio provided by required effect *ex vivo*
Required effect (see equation 3 and fig 2)	=	Bacteriostasis Bactericidal activity Elimination of bacteria

Equation 3

$$E = E_{max}\ C_c^N/EC_{50}^N + C_c^N$$

E	=	Antibacterial effect measured as change in $\log_{10}$ CFU
E_{max}	=	Maximum antibacterial effect
EC_{50}	=	$AUIC_{24h}$ of drug that results in 50% of maximum antibacterial effect
C_c	=	$AUIC_{24h}$ of drug in effect compartment (eg serum)
N	=	Hill coefficient steepness of $AUIC_{24h}$ vs effect (eg bacteriostasis)

Ex vivo antibacterial effect = $AUIC_{24h}$ in serum, exudate, transudate required for bacteriostasis, bactericidal activity, bacterial eradication

$AUIC_{24}$ bacteriostasis E = O (no change in bacterial count)
$AUIC_{24}$ bactericidal E = -3 (3log or 99.9% reduction in bacterial count)

$AUIC_{24}$ Bacterial elimination, reduction to limit of detection 10 CFU/ml

A weighted AUC(WAUC) has also been applied in dosage optimisation (Corvaisier *et al.,* 1998). This incorporates the total time for which the plasma drug concentrations exceed the MIC (equation 4) and can be used for both concentration and time-dependent drugs.

Equation 4

$$WAUC_{(h)} = \frac{AUC_{h} \times T>MIC_{(h)}}{MIC \quad (T>MIC)_{max\,(h)}}$$

$WAUC_{(h)}$ = Area under the concentration time curve weighted for total time which plasma drug concentration exceeds the MIC

$(T>MIC)_{max} = 24_{h}$

Population pharmacokinetic methods were developed to study the kinetics of drugs in target human populations, generally during phase III clinical trials. Since early drug development kinetic trials utilise, often normal, healthy, young, male volunteers they may not identify inter and intra individual variation in the target population (Aarons, 1992, Sheiner & Ludden, 1992). Techniques were therefore developed which utilise sparse data sets typically with many patients but few observations per patient (Samara & Grannerman, 1997). In veterinary medicine population pharmacokinetics have been used to study inter-individual variation and the influence of diverse pathophysiological factors in circumstances where spares data per individual was available (Martin-Jimenez *et al*, 1998, Whittem *et al*, 2000). It has also clear utility for monitoring and predicting tissue residues (Martin-Jiminez & Riviere, 1998, Whittem, 1999). The potential to integrate population pharmacokinetics with dynamics for antimicrobial drugs has recently been demonstrated in dogs administered marbofloxacin before cataract surgery. A limited number of aqueous humor samples and blood samples were collected from 63 dogs during surgery. Marbofloxacin concentrations were measured and data analysed using population pharmacokinetic parameters. Pharmacodynamic surrogate markers were then used to predict *in vivo* antimicrobial activity. (Reiner *et al*, 2003). The diversity of pathological physiological and developmental states apparent in veterinary patients make population kinetics very attractive for PK PD studies since sub-populations requiring dosage alterations should be identified.

SPECIFIC AGENTS (for experimental data see Table 2.).

Beta Lactams

The available evidence indicates that beta lactams are essentially time-dependent, whereby the time that the drug concentration remains above the MIC is the greatest determinant of likely efficacy (Eagle *et al.,* 1950, Roosendaal *et al.,* 1986). However, there is still considerable debate on how much above the MIC the plasma concentration of the antimicrobial should be maintained, and for what proportion of the interdosing interval the concentration should be maintained at the desired level. There is unlikely to be a single answer to each of these questions since they are probably drug, pathogen and locus specific. As a rule of thumb, exceeding the MIC by 1-5 multiples for between 40% and 100% of the dosage interval is considered appropriate if not highly specific (Gunderson *et al*., 2001). In deep seated infections penetration of the bacterial locus may depend on high blood concentrations since distribution is normally by simple diffusion and thus affected by the drug concentration gradient. As a consequence, distribution and activity of time-dependent drugs may therefore be associated with the AUC_{0-24} and C_{max} (Lavoie & Bergeron, 1998). Achieving concentrations several times higher than the MIC may also be important to limit resistance selection (see above).

In order to achieve the plasma concentration profile optimal for efficacy, several strategies may be adopted. It may be possible to select beta lactams with longer terminal serum half lives and this is a potential target for pharmaceutical companies producing newer beta lactams. Alternatively, they could be formulated in repository dosage types such as the procaine or benzathine forms Fig 3. Although the benzathine formulations were conceived before the time-dependent nature of beta lactam activity was understood, by serendipity, they conferred the appropriate concentration time profiles of the active beta lactams (Prescott, 2000). Concern has been expressed that concentrations of beta lactams formulated with benzathine penicillin failed to exceed the MIC of relevant target pathogens and, although this is evidently true, they were generally part of more complex formulations containing procaine also. It may be therefore that the release profile associated with

combined procaine and benzathine formulations did confer an appropriate plasma concentration time profile for target pathogens. Products containing benzathine penicillin are no longer authorised in the EU but are available elsewhere. It is also possible to extend the residence time of beta lactam antibacterials by co-administering them with agents such as probenecid, which inhibit their active secretion in the kidney tubules (Fig 4) (Sarasola & McKellar, 1992). The most obvious step, which the practitioner can take to improve the concentration time profile of the beta lactams, is to administer dosages more frequently. Administration by continuous infusion would provide an even more accurate method for achieving optimum plasma concentrations but is unlikely to prove practical except perhaps in intensive care or during anaesthesia (Sarasola & McKellar, 1993).

Aminoglycosides

The aminoglycosides are concentration-dependent drugs for the gram-negative bacteria against which they are generally used (Moore *et al.*, 1987), although they may demonstrate some concentration-independent activity when used as adjunctive therapy against gram-positive bacteria. The C_{max}:MIC ratio has been shown to be the most useful PK/PD parameter for predicting efficacy of aminoglycosides, and increasing the C_{max}:MIC ratio correlates with clinical response. A C_{max}:MIC ratio of greater than 10 has been recommended for single daily dosing, although some care should be taken to avoid toxicity. Since toxicity to aminoglycosides is related to the trough concentration of drug it is likely that single daily dosing will allow concentrations to fall during the trough period below the threshold which would cause toxicity (Marra *et al.*, 1996). This may not be the case for animals with impaired renal function, although aminoglycosides should generally be contraindicated in these animals, or should be administered with extended interdosing interval (Riviere, 2000). A relationship between the C_{max}:MIC ratio and the emergence of resistance exists for aminoglycosides since it has been shown for netilmicin, used to treat either *Escherichia coli* or *Staphylococcus aureus* infections, that regrowth is prevented if the C_{max}:MIC ratio exceeds eight (Blaser *et al.*, 1987).

Fluoroquinolones

A great deal of information is now available on the PK/PD relationships for fluoroquinolones used in human medicine. They conform to concentration dependency against gram negative bacteria and the C_{max}:MIC ratio has been shown to have particular utility in determining their optimal activity (Drusano *et al.,* 1993). This has subsequently been confirmed in a *Mannheimia (Pasteurella) haemolytica* model of respiratory disease in cattle (Sarasola *et al.,* 2002). A C_{max}:MIC ratio of greater than eight and an AUC_{0-24}:MIC ratio of greater than 100 have been shown to prevent bacterial regrowth during treatment and are thus recommended to prevent resistance selection (Dudley, 1991, Thomas *et al.,* 1998). The impact which MIC has on the attainment of desirable PK/PD ratios (AUC_{0-24}:MIC of 125) is clearly demonstrated for orbifloxacin (Fig 5), and difloxacin in dogs where it is apparent that optimal AUC_{0-24}:MIC is achievable only for organisms with low MIC (0.12µg/ml or less) with recommended dose rates (Walker, 2000b). When pharmacokinetic and pharmacodynamic data are available the PK/PD ratios can be determined (Tables 3&4). These data demonstrate the substantial differences which experimental methodology can make in determination of pharmacokinetics and the impact that this and the sensitivity of the pathogen makes to the derived PK/PD ratios. Few of the determined results indicate optimal ratios, and this raises the question of whether optimal ratios derived from *in vitro* studies, or by extrapolation from man, can be directly applied to domestic animals. Some of the newer fluoroquinolones which have been developed in human medicine, with good activity against gram positive bacteria and some against anaerobes, have been shown to possess concentration-independent activity against these pathogens (Ibrahim *et al.,* 1999). Furthermore, fluoroquinolones have been shown to retain activity against gram positive bacteria at lower AUC_{0-24}:MIC ratios than for gram negative bacteria (Lacy *et al.,* 1999, Lister & Sanders, 1999, Peterson *et al.,* 1999), which is paradoxical given their generally lower MIC's for gram negative compared with gram positive bacteria.

Macrolides, Glycopeptides, Lincosamides and Metronidazole

Relatively little information is available on the macrolides, glycopeptides, lincosamides and metronidazole and most must be extrapolated from either mouse models or *in vitro* simulations. Macrolides are thought to be time-dependent drugs for which the time greater than MIC most closely relates to efficacy. Erythromycin has been demonstrated to have greatest activity against *Streptococcus pneumoniae* when the time greater than MIC exceeds 60% of the interdosing period (Vogelman *et al.,* 1988). The macrolide, azithromycin, used in humans only, has been reported to possess concentration-dependent activity when the AUC_{0-24}:MIC ratio is optimised (Craig *et al.,* 1992). There is debate regarding the activity of macrolides for which the plasma concentrations appear insufficient to confer good efficacy against bacteria where even wild type rarely have MIC's which would make them susceptible. One suggestion is that activity is related to high concentrations achieved in cells and at tissue sites of infection. Clarithromycin has been shown to achieve concentrations 1-30 fold higher in lung epithelial lining and 200-1000 fold higher in alveolar macrophages than in plasma (Patel *et al.,* 1996, Rodvoed *et al.,* 1997). Release from these intracellular sites may subject bacteria to prolonged exposure appropriate for a time-dependent drug (Gladue *et al.,* 1989). Whether the reservoir capacity of the cellular deposits of macrolides is sufficient to endorse this hypothesis has been contested (Toutain *et al.,* 2003). The macrolides have been shown to express extended PAE, which may support their activity (Craig, 1998b).

The glycopeptide vancomycin has greatest *in vitro* activity when the time greater than MIC is achieved for the whole interdosing interval (Larsson *et al.,* 1996). Nevertheless, in a murine peritonitis model of *S.pneumoniae* and *Staphylococcus aureus* time greater than MIC, C_{max}:MIC and AUC_{0-24}:MIC were all shown to correlate with efficacy suggesting that for glycopeptides there may be co-dependency on time and concentration (Knudsen *et al.,* 2000). Metronidazole has been shown to have some concentration-dependent activity in an anaerobic *in vitro* model of *Trichomonas vaginalis* infection (Nix *et al.,* 1995). The lincosamide clindamycin is time-dependent in an *in vitro*

S. pneumoniae model (Lewis *et al.*, 1999) although there is little information on the *in vivo* activity of these antimicrobials.

Conclusions

The outstanding utility of PK/PD integration is readily apparent from the human medical literature. Whilst there may be quantitative differences between the optimal ratios determined for man and domestic animals it is certain that there are qualitative similarities. The laudable progress towards optimising (and minimising) the use of chemotherapeutics in animals means that PK/PD integration for antimicrobial drugs for veterinary research is a priority and provides an opportunity which should be embraced as such with enthusiasm, urgency and vigour.

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Table 1

Concentration – Dependent	Time- Dependent	Co-dependent
Aminoglycosides	Beta- lactams	Beta-lactams[1]
Fluoroquinolones	Macrolides (except azithromycin)	Fluoroquinolones[2]
Metronidazole (vs anaerobes)	Clindamycin	Glycopeptides
	Vancomycin	

General classification of antimicrobial drugs for which information is available on concentration or time-dependent killing activity.

[1] In relation to reduction in resistance selection pressure.

[2] Some with anaerobic activity

Table 2 Pharmacodynamic data from selected in vitro animal and human studies

Antibiotic	Organism	Parameter	Source	Reference
Beta Lactams				
Penicillin G	*Diplococcus pneumoniae*	T>MIC	Mouse + rabbit	Eagle et al 1950
Ampicillin	*Escherichia coli*	AUC_{0-24} : MIC (To prevent resistance + regrowth)	IVPDM	White et al 1989
Cephalosporins	*Sreptococcus pneumoniae* *Enterobacteriaceae* *Streptococcus spp* *Staphylococcus*	T>MIC T>MIC 60-70% T>MIC 60-70% T>MIC 40-50%	In vitro, murine Animal models Animal models Animal models	Frimodt-Møller et al 1986 Craig 1995
Cephazolin	*E.coli*	T>MIC 100%	Neutropenic murine	Vogelman et al 1988
Aminoglycosides				
Gentamicin	*Pseudomonas aeruginosa, E.coli*	AUC_{0-24} : MIC and T>MIC	Neutropenic murine	Vogelman et al 1988
Gentamicin Netilmicin	*Klebsiella pneumoniae*	AUC_{0-24} MIC and T>MIC	Neutropenic murine thigh and lung infection	Leggett et al 1989
Amikacin	*K.pneumoniae, E.coli, P.aeruginosa*	T>MIC – normal renal function AUC_{0-24} – impaired renal function	Neutropenic murine	Craig et al 1991
Gentamicin Amikacin	*E.coli, Klebsiella sp*	C_{max} : MIC>10	Human retrospective	Moore et al 1987
Aminoglycosides	Gram negative bacteria	C_{max} : MIC>8 T>4 x MIC	Human retrospective	Deziel-Evans et al 1986
Fluoroquinolones				
Ciprofloxacin	*P.aeruginosa* *S.pneumoniae* *S.aureus* *S.pneumoniae, S.aureus* *P.aeruginosa*	C_{max}:MIC>8 AUC_{0-24} :MIC>100 AUC_{0-24} :MIC>35 AUC_{0-24} :MIC>57 C_{max}:MIC 15-40 C_{max}:MIC20-50	IVPDM IVPDM IVPDM IVPDM Serum ultrafiltrate human	Dudley et al 1991 Madaras-Kelly et al 1996 Wright et al 1998 Hoang et al 1998 Hyatt et al 1994
Levofloxacin	*S.pneumoniae* *Bacillus fragilis* *S.pneumoniae, S.aureus*	AUC_{0-24} :MIC>35 AUC_{0-24} :MIC>50 C_{max}:MIC>10	IVPDM IVPDM Human skin and UTI infections	Wright et al 1998 Peterson et al 1998 Preston et al 1998
Macrolides, Glycopeptides, Lincosamides and Metronidazole				
Erythromycin	*S.pneumoniae*	T>MIC 60%	Neutropenic murine	Vogelman et al 1988
Clarithromycin	*S.pneumoniae*	T>MIC	Neutropenic murine	Ebert et al 1991
Azithromycin	*S.pneumoniae*	AUC_{0-24} :MIC	Neutropenic murine	Craig et al 1992
Vancomycin	*S.aureus*	T>MIC~100%	IVPDM	Larsson et al 1996
Vancomycin	*S.pneumoniae, S.aureus*	T>MIC, C_{max}:MIC AUC_{0-24} :MIC	Murine peritonitis	Knudsen et al 2000
Metronidazole	*Trichomonus vaginalis*	C:MLC>10-25	IVPDM anaerobic	Nix et al 1995
Clindamycin	*S.pneumoniae*	T>MIC	IVPDM	Lewis et al 1999

Table adapted from Gunderson *et al*., 2001).
IVPDM - *in vitro* pharmacodynamic model
MLC - minimum lethal concentrations

Table 3

AUC_{0-00}:MIC ratios for fluoroquinolones in dogs (Walker, 2000b).

	Dose[a]	AUC[b] (µg.h/ml)	MIC $_{90}$[c] (µg/ml)	AUC/MIC (h)	MIC $_{90}$ (µg.h/ml)	AUC/MIC (h)	MIC $_{90}$ (µg.h/ml)	AUC/MIC (h)
			Staphylococcus intermedius		*Escherichia coli*		*Klebsiella pneumoniae*	
Enrofloxacin	5.0mg/kg	15.7	0.12-0.5	131-31	0.03-0.125	523-126	0.06-0.12	262-130
Orbifloxacin	2.5mg/kg	14.3	0.5	29	0.5	29	0.25	57
Difloxacin	5.0mg/kg	14.5	1.0	15	0.25	58	0.5	29
Marbofloxacin	2.0mg/kg	22.0	0.25	88				

a) All doses given per os.
b) AUC area under the curve from 0-∞ (not 0-24h).
c) MIC_{90} more than 20 isolates, range is given where data generated from more than one study.

Table 4

AUC_{0-24}:MIC ratios for fluoroquinolones in dogs (Pirro *et al*.,1999).

	Dose	AUC_{0-24} (µg.h/ml)	AUC_{0-24}/MIC_{90} (h)	
			S.intermedius	*E.coli*
Enrofloxacin	5.0mg/kg	8.7	70	146
Orbifloxacin	2.5mg/kg	12.7	51	102
Difloxacin	5.0mg/kg	9.3	37	73
Marbofloxacin	2.0mg/kg	13.1	52	105

MIC_{90} n=25
Pirro *et al.*, (1999) - Pharmacodynamic (MIC) data
Heinen (2002) - Pharmacokinetic (AUC)data

Figure 1

The selection window for an antimicrobial lies between the concentration at which wild type bacteria survive exposure and the mutant prevention concentration which confers bacterial eradication. The size of the selection window is affected by a drug's pharmacokinetics as demonstrated for the drug (or formulation) represented by the bold and dashed lines. (Baquero & Negri, 1997, Drlica, 2001, Catry *et al.,* 2003).

Figure 2

Sigmoidal E_{max} relationship for bacterial count versus *ex vivo* $AUIC_{0-24}$ in a goat (AliAbadi & Lees, 2001).

Figure 3

Concentration of penicillin in blood after administration of equivalent doses of sodium, procaine and benzathine penicillins (Bogan, 1983).

Figure 4

Concentrations of ampicillin in horses after the administration of an intravenous bolus dose (10mg/kg) of ampicillin sodium with or without probenecid (75mg/kg). The MIC of representative equine pathogens and T>MIC are given. (Adamson *et al*., 1985, Hirsh & Jang 1987, Sarasola & McKellar, 1992).

Figure 5

$C_{max:}$MIC and AUC_{0-24}:MIC ratios for orbifloxacin where C_{max} = 2.33 μg/ml and $AUC_{0-\infty}$ = 14.3 μg.h/ml. (Walker, 2000b).

Figure 1

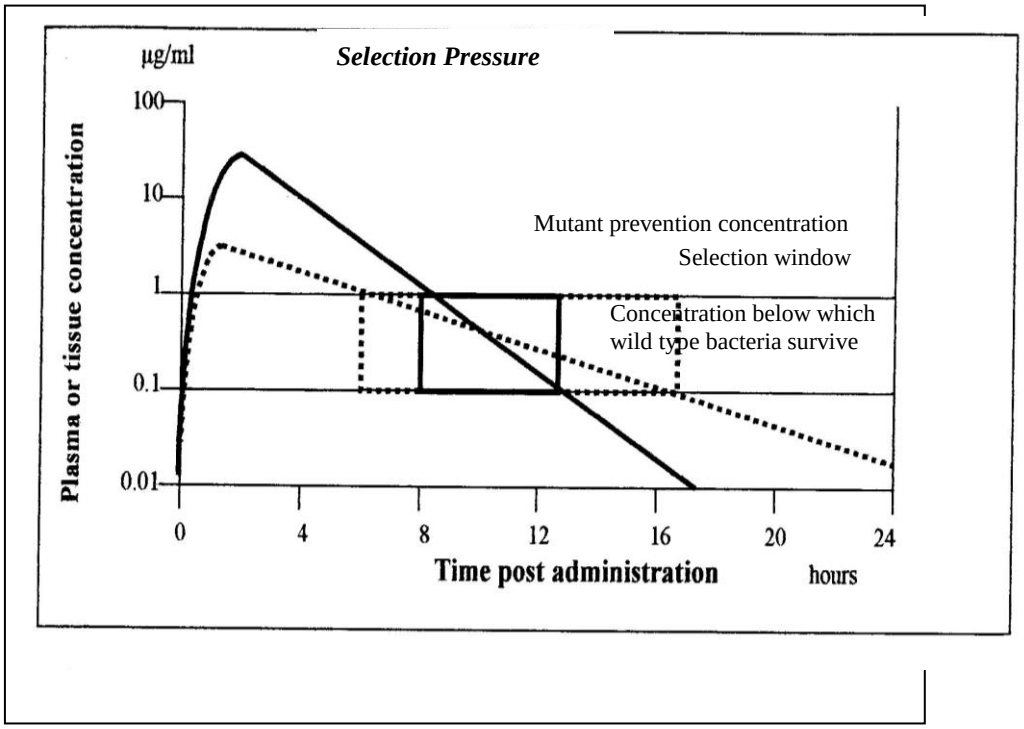

µg/ml
Selection Pressure
100
10
1
0.1
0.01
Plasma or tissue concentration
Mutant prevention concentration
Selection window
Concentration below which wild type bacteria survive
0
4
8
12
16
20
24
Time post administration
hours

Figure 2

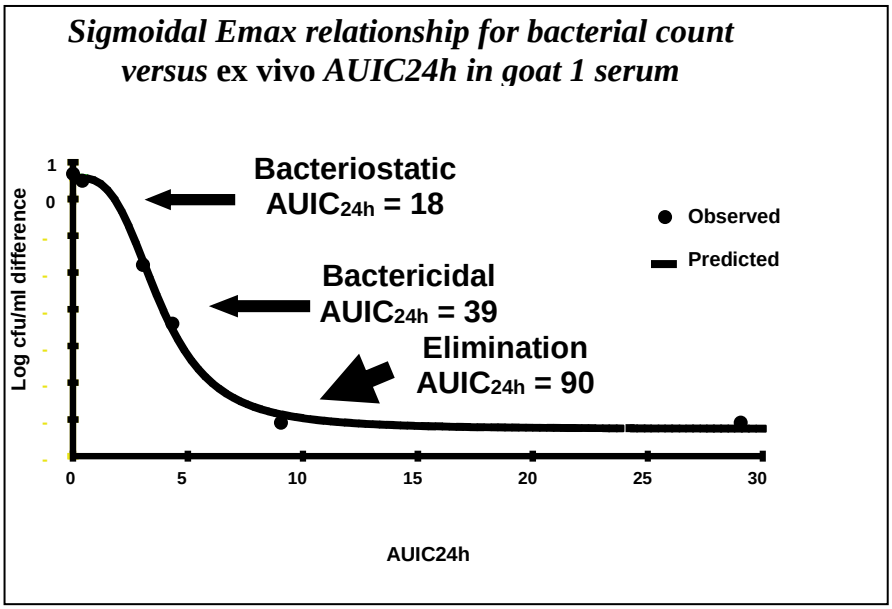

Sigmoidal Emax relationship for bacterial count versus ex vivo AUIC24h in goat 1 serum
Bacteriostatic
AUIC24h = 18
Bactericidal
AUIC24h = 39
Elimination
AUIC24h = 90
Observed
Predicted
Log cfu/ml difference
1
0
0
5
10
15
20
25
30
AUIC24h

Figure 3

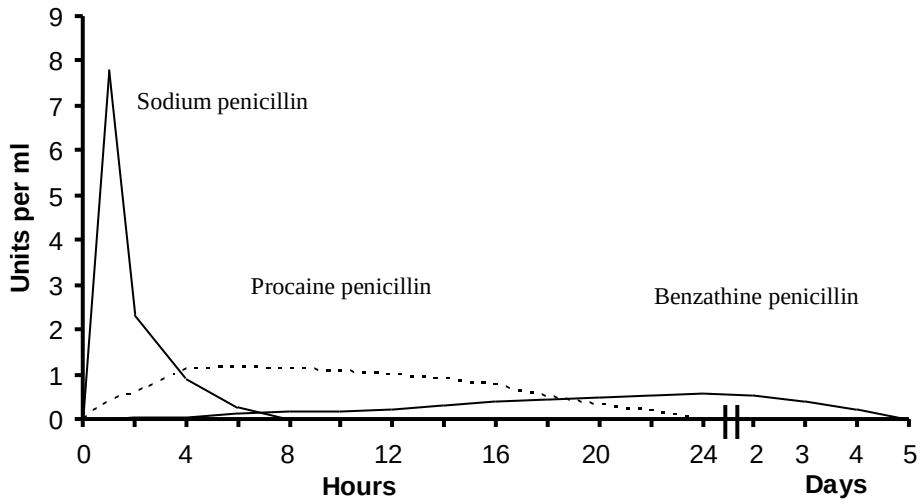
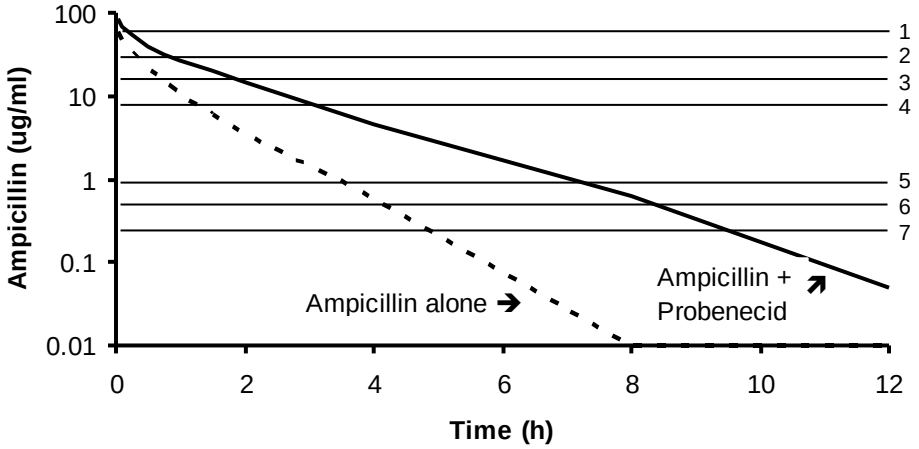

Units per ml
9
8
7
6
5
4
3
2
1
0
Sodium penicillin
Procaine penicillin
Benzathine penicillin
0
4
8
12
16
20
24
2
3
4
5
Hours
Days

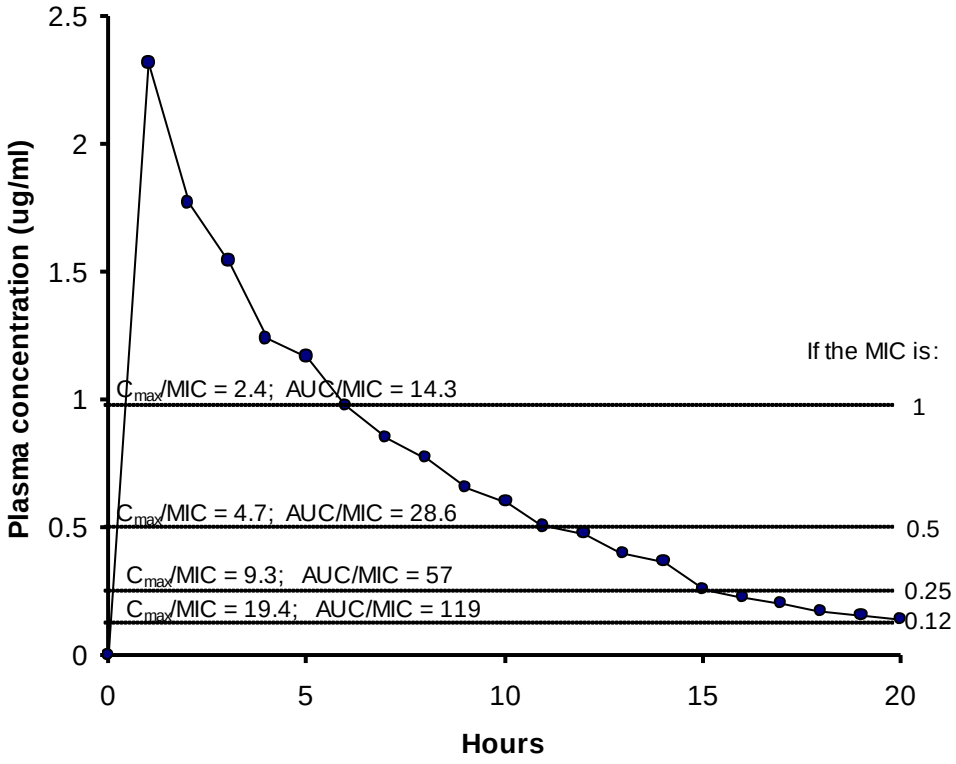
Figure 4



Bacterium	Representative MIC	Time >MIC (approximate) (h)	
		Ampicillin alone	Ampicillin + Probenecid
Escherichia coli	64*	0	0.5
Actinobacillus sp.	32**	0.5	1.5
Rhodococcus equi	16**	1.0	2.5
Coagulase +ive *Stapylococcus*	8**	1.5	3.5
Fusobacterium necrophorus	1*	3.5	7.5
Corynebacterium pseudotuberculosis	0.5**	4.5	9.0
Streptococcus zooepidemicus	0.25**	5.5	10.0

* Hirsh & Jang, 1987
** Adamson *et al*, 1985

Figure 5


Plasma concentration (ug/ml)
2.5
2
1.5
1
0.5
0
0
5
10
15
20
Hours
If the MIC is:
Cmax/MIC = 2.4; AUC/MIC = 14.3
1
Cmax/MIC = 4.7; AUC/MIC = 28.6
0.5
Cmax/MIC = 9.3; AUC/MIC = 57
0.25
Cmax/MIC = 19.4; AUC/MIC = 119
0.12