

COMPARATIVE PHARMACOKINETICS OF A SINGLE AND DOUBLE DOSE OF
A CONVENTIONAL OXYTETRACYCLINE FORMULATION IN SHEEP, TO
ALLOW FOR THERAPEUTIC OPTIMISATION.

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Master of Veterinary Medicine
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STATEMENT BY CANDIDATE

I declare that the dissertation, which I hereby submit for the degree MMedVet (Pharm) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another university.

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ABSTRACT

In the veterinary industry, long acting oxytetracycline formulations are loosely referred to as those formulations that only require a single dose at 20 mg/kg to achieve clinical cure and to be repeated after three days only if required. Short acting oxytetracycline formulations are recommended for use once a day for four days, at a dose of 10 mg/kg IV and 10mg/kg IM on day one, 10 mg/kg IM on day two and 5 mg/kg IM on days three and four.

The primary objective of this study is to demonstrate that, based on pharmacokinetics, a double dose of a conventional short acting, 135mg/ml formulation of oxytetracycline has a longer action than a single dose of the same formulation. As a secondary objective the efficacy and safety of a single, double dose of a conventional oxytetracycline formulation are compared to multiple, single doses of a conventional formulation as well against a single dose of a long-acting formulation. Factors that influence the duration of action of a parenteral oxytetracycline formulation are reviewed, as are the pharmacokinetic / pharmacodynamic relationship of oxytetracycline.

A single dose, randomized, two treatment, two sequence cross-over experimental design as described by Grizzle (1965) was selected for this study. The washout period between the two sequences was determined using at least 5 half-lives (11.1 hours x 5) of conventional oxytetracycline formulation, based on a study by Davey *et al* (1985). Although a wash-out period of 55.5 hours for a dose rate 20 mg/kg of oxytetracycline would have sufficed to ensure the absence of any residual drug in the central compartment of the experimental animals, it was decided to extend the washout period between treatment periods to 7 days (168 hours) for mainly practical reasons. Sample size determination was based on the rejection of the null hypothesis as described by Anderson and Hauck (1983). 5 animals per treatment group were selected.

The sheep were equally and randomly assigned to either the group that would receive the 10 mg/kg dose first (group 1), or the group that would receive the 20 mg/kg dose first (group 2). For the cross over treatment (phase 2), the animals remained in the groups they

were allocated to for phase 1, but group 1 received the 20 mg/kg dose and group 2 received the 10 mg/kg dose. The volume of oxytetracycline was calculated based on a product oxytetracycline content of 135 mg/ml.

The blood sample collection procedure was the same for phases (treatments) 1 and 2. Time 0 was the time of treatment. Samples were collected into 10ml lithium heparinized vacutainer glass tubes with 19G disposable needles at the following intervals (hrs): 0, 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24, 36, 48, 72, 96.

The oxytetracycline concentrations in plasma were determined using validated High Performance Liquid Chromatographic methodology.

The difference between the 2 sets of results emanating from phase 1 and phase 2 of the study are used as basis for presenting the results. Three pharmacokinetic parameters were used to compare the 2 treatments: C_{max} (maximum plasma concentration) , AUC_{inf} (Total area under the concentration curve) and T>0.5 (Time that the drug concentration remains above 0.5 µg/ml).

The geometric means of the results show that:

The 20mg/kg treatment maintains levels above 0.5µg/ml significantly longer than the 10 mg/kg treatment. (37.4 hours versus 24 hours; p value 0.0013)

The 20 mg/kg dose reaches a significantly higher concentration than does the 10 mg/kg dose. (6.59µg/ml versus 3.55µg/ml; p value 0.000000)

The 20mg/kg treatment has an AUC_{inf} which is greater than the 10 mg/kg treatment by a highly significant margin. (120.63 µg/ml*hr versus 71.63 µg/ml*hr; p value 0.000001)

In demonstrating that a conventional oxytetracycline formulation administered intramuscularly at double dose provides drug plasma concentrations above MIC for an average duration of 13 hours longer than a single dose, the primary objective of the study was achieved. The study demonstrated that a single dose at 20 mg/kg of a conventional

oxytetracycline formulation offers an acceptable alternative to conventional treatment regimes in terms of efficacy, target animal safety, as well as convenience to the user.

OPSOMMING

In die veterinêre industrie word losweg na langwerkende oksitetrasiklien formulasies verwys as daardie formulasies waar slegs `n enkele dosis teen 20 mg/kg benodig word om kliniese sukses te bewerkstellig. Die dosis mag na drie dae herhaal word slegs indien nodig. Kortwerkende oksitetrasiklien formulasies word aanbeveel vir gebruik een maal per dag vir vier dae teen 10 mg/kg intravenous en 10 mg/kg binnespiers op dag een, 10 mg/kg binnespiers op dag 2 en 5 mg/kg binnespiers op dae drie- en vier.

Die primêre doel van hierdie studie is om te wys dat `n dubbeldosis van `n konvensionele, kortwerkende formulase wat 135 mg / ml oksitetrasiklien bevat, `n langer werking het as `n enkele dosis van dieselfde formulase. Die studie word gegrond op oksitetrasiklien farmakokinetika. As sekondêre doel, word die doeltreffendheid en veiligheid van `n enkele, dubbeldosis inspuiting van `n konvensionele oksitetrasiklien formulase vergelyk met meervoudige, enkeldosis inspuitings van konvensionele oksitetrasiklien formulasies. Daar word oorsig gehou aangaande faktore wat die werksduur van `n parenterale oksitetrasiklien formulase beïnvloed, en ook word die farmakokinetiese / farmakodinamiese verwantskap van oksitetrasiklien in oënskou geneem.

`n Enkele dosis, lukrake, dubbelbehandeling, tweefase oorkruis studie-ontwerp (soos beskryf deur Grizzle (1965)) was vir hierdie studie gebruik. Die uitwasperiode tussen die twee fases was bereken deur ten minste 5 halflewes (11.1 uur x 5) van `n konvensionele oksitetrasiklien formulase, soos beskryf deur Davey *et al* (1985). Alhoewel `n uitwasperiode van 55.5 uur vir `n dosis van 20 mg/kg oksitetrasiklien genoegsaam sou wees om die afwesigheid van residuele oksitetrasiklien in die sentrale kompartement van die eksperimentele diere te verseker, is die uitwasperiode verleng na 7 dae (168 ure) vir praktiese doeleindes. Berekening van die hoeveelheid diere benodig is baseer op die verwerping van die null hipotese soos beskryf deur Anderson en Hauck (1983). 5 skape per groep is gekies.

Die skape is gelykmatig en lukraak toegeken aan die groepe wat òf die 10 mg/kg dosis (groep 1) òf die 20 mg/kg dosis (groep 2) sou ontvang. Vir die oorkruis behandeling (fase 2), is die diere in dieselfde groepe gehou as wat gegeld het vir fase 1, maar groep 1 het die 20 mg/kg dosis, en groep 2 die 10 mg/kg dosis ontvang. Die volume oksitetrasiklien is bereken volgens 'n produk oksitetrasiklien inhoud van 135 mg/ml.

Die bloedversamelings-proses was dieselfde vir fases 1 en 2. Tyd 0 was die tyd van behandeling. Bloedmonsters is in 10ml litium gehepariniseerde vakuüm (glas) buisies versamel met weggooibare 19G naalde. Die bloedmonsters is versamel met die volgende tussenposes (ure): 0, 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24, 36, 48, 72, 96.

Die oksitetrasiklien konsentrasies in die plasma is bepaal deur gevalideerde hoë-verrigting vloeistof kromatograaf (HPLC) metodologie.

Die verskil tussen die 2 stelle resultate vanuit fase 1 en- 2 is gebruik as basis vir die voorlegging van die resultate. 3 farmakokinetiese maatstawwe is gebruik om die 2 behandelings te vergelyk: C_{max} (maksimum plasma konsentrasie), AUC_{inf} (Totale area onder die konsentrasie kurwe) en $T_{>0.5}$ (Tyd wat die middel bo die vlak van 0.5 $\mu\text{g/ml}$ handhaaf).

Die geometriese gemiddeldes van resultate wys die volgende:

Die 20mg/kg behandeling handhaaf vlakke bo 0.5 $\mu\text{g/ml}$ vir 'n betekenisvolle langer tydsduur as die 10 mg/kg behandeling (37.4 uur teenoor 24 uur; p waarde 0.0013).

Die 20 mg/kg dosis bereik 'n betekenisvolle hoër konsentrasie in vergelyking met die 10 mg/kg dosis (6.59 $\mu\text{g/ml}$ teenoor 3.55 $\mu\text{g/ml}$; p waarde 0.000000).

Die 20mg/kg behandeling toon 'n AUC_{inf} wat betekenisvol groter is as die 10 mg/kg behandeling (120.63 $\mu\text{g/ml}\cdot\text{hr}$ teenoor 71.63 $\mu\text{g/ml}\cdot\text{hr}$; p waarde 0.000001).

Die primêre doel van die studie is bereik deur te bewys dat 'n konvensionele oksitetrasiklien formulاسie wat eenmalig binnespiers toegedien word teen 20 mg/kg

oksitetrasiklien plasma konsentrasies tot gevolg het wat gemiddeld 13 uur langer bo MIK gehandhaaf word in vergeleke met `n enkeldosis van dieselfde formulاسie. Die studie het bewys dat die eenmalige, dubbeldosis behandeling `n aanvaarbare alternatief bied tot konvensionele behandelings-metodes met betrekking tot doeltreffendheid, teiken-dier veiligheid, asook gerief vir die gebruiker.

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PHARMACOKINETIC TERMINOLOGY DESCRIPTION:

Area under the curve (AUC)	Along with T _{max} and C _{max} (see description below), AUC is the main pharmacokinetic parameters used to evaluate the bioavailability (absorption) of a drug (Adams <i>et al.</i> , 1995). AUC _{last} refers to drug absorption from the time of administration up to the last sampling point. AUC _{inf} refers to the total amount of drug absorbed, including drug absorbed after the last sampling point. This is achieved by extrapolation to infinity using the terminal slope of the time concentration curve (Beta). AUC _{inf} should not exceed AUC _{last} by more than 20% if AUC _{last} is to be accepted as being accurate (Chow & Liu, 2000).
Biological elimination half-life (T _{1/2β})	The time required for a specific drug to decrease its plasma concentration by one half after reaching pseudo-equilibrium (Toutain & Bousquet-Melou, 2004c; Giguere <i>et al.</i> 2006).
Mean Residence Time (MRT)	Mean residence time may be defined as the average time a molecule is in the body, or as the time needed for 63.2% of a drug to be eliminated. (Boothe, 2001 b)
Non-compartmental analysis	This type of analysis assumes that clearance of the product takes place directly from the plasma in a linear fashion. It is however, model-independent (Svensson, 2005) which means that the assumptions that are made, may not be correct. The benefit of this system is that mathematical computation of kinetic data varying with time need not be done with differential equations, but with integral equations, which are less demanding from a mathematical point of view as the model is not linked to any anatomical compartment as with compartment modelling (Giguère <i>et al.</i> 2006). Non-compartmental analysis is especially suited for bio-equivalence and other non-linear time / plasma

	<p>concentration studies, as key parameters such as C_{max} and T_{max} can be read directly from the plasma concentration versus time graphs generated. This method is also known as the SHAM method (slope /height/ area / moment) (Giguère <i>et al.</i> 2006). For this study, all pharmacokinetic parameters discussed, were determined using the non-linear analytical function of the programme WinNonLin 5.2 (Pharsight).</p>
Pharmacokinetic breakpoint (MIC ₉₀)	<p>That drug serum concentration which is needed to kill 90% of the bacteria being treated. The MIC₉₀ of an organism should be below or equal to the pharmacokinetic breakpoint of a drug (Jacobs, 1999).</p>
Pharmacokinetic/ pharmacodynamic Relationship of Antimicrobials	<p>This is the relationship between the pharmacokinetic profile of an antimicrobial, and the eventual outcome (efficacy) of treatment (Giguère <i>et al.</i> 2006). This relationship for each group of antimicrobial drugs is pathogen specific (McKellar <i>et al.</i> 2004).</p>
Plasma clearance (Cl)	<p>Clearance represents the volume of blood (ml) cleared of drug per unit of time. It expresses the overall ability of the body to eliminate a drug by scaling the drug elimination rate by the corresponding plasma concentration (Toutain & Bousquet-Melou, 2004b). The parameter is commonly used to calculate dosage intervals or rate of infusion of a drug in order to maintain a steady state.</p>
Significance of difference	<p>The 95% confidence level that confirms that two sets of data do in fact differ significantly from one another was determined by using NSCC 2004 (Kaysville, Utah)</p>

	<p>statistical software two-tailed student t-test where the significance is represented by the p (probability) value. When $p < 0.05$, the two sets of data differ significantly from one another (Weldon, 1986).</p>
T > 0.5	<p>0.5 represents the plasma concentration in $\mu\text{g/ml}$ that was deemed to be the pharmacokinetic breakpoint for oxytetracycline (Davey <i>et al.</i>, 1985; Kumar & Malik, 1998; Toutain & Raynaud, 1983).</p>
Volume of distribution (V_d)	<p>Denotes the magnitude of drug distribution. It represents a hypothetical volume of fluid that would be required to contain the amount of drug at a concentration equal to the drug concentration in the plasma (Giguère <i>et al.</i> 2006). V_d can also be calculated by dividing the total amount of drug in the body (mg), by the total area under the plasma concentration curve, multiplied by the overall elimination rate constant of the drug, obtained from the linear terminal phase of the semi-logarithmic disposition curve (Giguère <i>et al.</i> 2006). The parameter is a proportionality constant between total amount of drug in the body and plasma concentrations (Toutain & Bousquet-Melou, 2004d).</p>

CHAPTER 1. INTRODUCTION AND OBJECTIVE

The Tetracycline group of antimicrobials, which consist of tetracycline, oxytetracycline, doxycycline, chlortetracycline and minocycline, represent some of the most widely used antimicrobials in veterinary practice (Shifferli *et al.*, 1982; Riviere & Spoo, 1995). Much of their popularity has probably arisen from a combination of the class's broad spectrum of activity and its lower cost (Schifferli *et al.*, 1982). Another feature that could have been contributory to this wide scale use may be its availability of long-acting depot intramuscular formulation.

In the veterinary industry, long acting oxytetracycline formulations are loosely classified as those formulations that only require a single dose at 20 mg/kg to achieve clinical cure or require repetitive doses every third day (Toutain & Raynaud, 1983). In contrast short acting oxytetracycline formulations are recommended for use once a day for at least four days, at a dose of 10 mg/kg IV and 10mg/kg IM on day one, 10 mg/kg IM on day two and 5 mg/kg on day three and four (Toutain & Raynaud, 1983). When comparing the pharmacokinetics of different formulations of oxytetracycline, it has been consistently demonstrated that the long-acting formulations showed lower peaks, but longer periods of higher drug serum concentration in comparison to the conventional formulations (Schifferli *et al.*, 1982; Nouws *et al.* 1983; Toutain & Raynaud, 1983; Davey *et al.*, 1985).

Although the pharmacokinetic features of the different OTC formulations have been well characterised, little information is available to explain these differences. One possible hypothesis is that the solvents used in long-acting formulations were responsible for these effects viz. purely pharmaceutical. The other hypothesis centred on the extended period of drug serum concentration and speculates that it is a function of the increased dosage (20 mg/kg instead of 10 mg/kg used with conventional formulations) i.e. the prolonged mean residence time is due to a greater area under the moment curve achieved.

The primary objective of this study was to demonstrate that an increase in the dose of a conventional formulation of oxytetracycline would contribute towards prolonged drug

serum concentrations similar to that seen for the slow-release solvents used in the long acting formulations. In demonstrating this, conventional formulations would be rendered more versatile, and the negative effects associated with the use of slow-release (long-acting) solvents in a formulation could be minimized. As a secondary objective the efficacy and safety of a single and double dose of a conventional oxytetracycline formulation will be reviewed and compared to multiple, single doses of a conventional as well as against a single dose of a long-acting formulation.

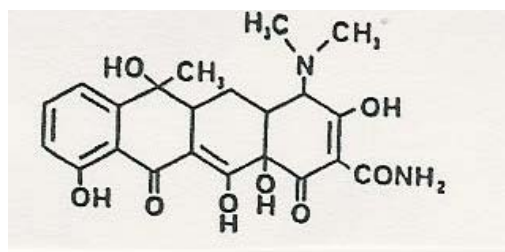
CHAPTER 2. LITERATURE REVIEW

2.1. BACKGROUND OF OXYTETRACYCLINE USE IN SOUTH AFRICA

Oxytetracycline is commonly used in veterinary medicine throughout the world (Riviere & Spoo, 1995) and this is especially true in countries with temperate climates such as South Africa where certain rickettsial blood parasites such as *Anaplasma marginale* and *Ehrlichia ruminantium* (which are both susceptible to oxytetracycline) are prevalent (Allsopp *et al.*, 2004; Potgieter & Stoltz, 2004). Oxytetracycline is available to the farmer in various parenteral formulations as an over-the-counter product in South Africa (Carrington, 2007).

At first only conventional injectable oxytetracycline formulations were used due to the limited number of solvents that were available during the third quarter of the 1900's. It quickly became apparent that, due to the extensive nature of South African farming enterprises, an oxytetracycline with a longer duration of action would be very helpful in eliminating the need to round up animals on a daily basis for repeat-treatments. Nowadays farmers have free access to both conventional and long acting formulations as over the counter stock remedies.

2.2. TETRACYCLINES

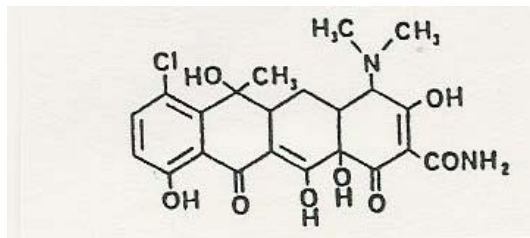


Picture obtained from HR Adams (1995) p 787

Fig 2.1: Chemical structure of tetracycline

Initially isolated from various species of *Streptomyces*, several semi-synthetic modifications have been made to the parent tetracycline molecule to yield variants with different pharmacokinetic and antimicrobial properties over the last 50 years. Tetracyclines (Fig. 2.1) are chemically characterised as 4-ringed amphoteric substances that are acidic

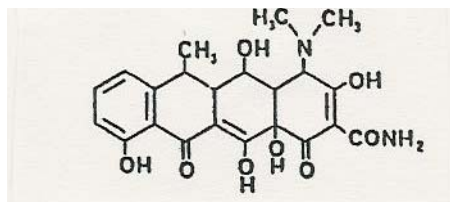
and hygroscopic. They easily form salts with acids and bases, and the most common forms marketed are the hydrochloride salt (Riviere & Spoo, 1995).



Picture obtained from HR Adams (1995) p 786

Fig 2.2: Chemical structure of chlortetracycline

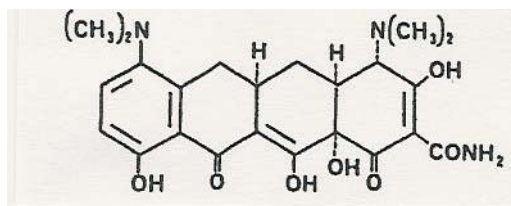
Chlortetracycline (Fig 2.2) was the first tetracycline to be discovered in 1948 with formulation becoming available for clinical use in 1952 (Riviere & Spoo, 1995). The compound is still in use today as a feed or water additive for food producing animals (Riviere & Spoo, 1995).



Picture obtained from HR Adams (1995) p 790

Fig 2.3: Chemical structure of doxycycline

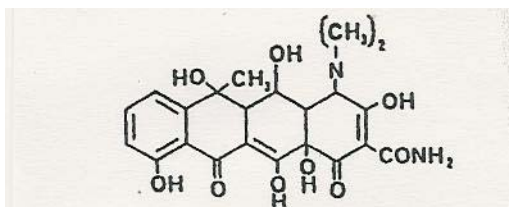
Doxycycline (Fig. 2.3) is a synthetic derivative of oxytetracycline and is characterised by a 5 – 10 fold increase in its lipophilic nature, which promotes this drug's tissue penetration, distribution and overall antimicrobial properties (Riviere & Spoo, 1995). Doxycycline is also unique in that it is excreted in the faeces as an inactive conjugate and has little impact on the microflora of the lower intestine (Riviere & Spoo, 1995).



Picture obtained from HR Adams (1995) p 791

Fig 2.4: Chemical structure of minocycline

Minocycline (Fig. 2.4) shares most of doxycycline's chemical properties, including greater lipophilicity which leads not only to better bio-availability and greater volumes of distribution, but it also accumulates inside bacterial cells (Riviere & Spoo, 1995).



Picture obtained from HR Adams (1995) p 787

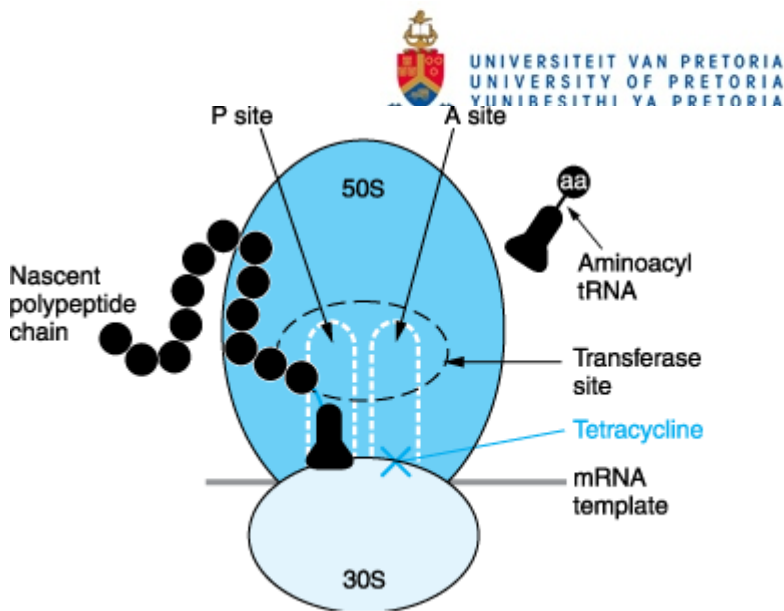
Fig 2.5: Chemical structure of oxytetracycline

Oxytetracycline (Fig 2.5) is the most widely used tetracycline in veterinary practise as parenteral, oral and topical preparations. It is used to treat canine and equine erlichiosis, pasteurellosis in calves and lambs and *Moraxella bovis*, amongst others (Riviere & Spoo, 1995). It is hygroscopic in aqueous solutions and only slightly soluble in water with a neutral pH. It forms salts with acids and bases, the salt most commonly used being hydrochloride (Giguère *et al.* 2006). Lipid solubility of oxytetracycline falls between the less lipid soluble chlortetracycline and the more lipid soluble doxycycline (Riviere & Spoo, 1995). Oxytetracycline readily binds to divalent and trivalent cations such as calcium and magnesium (Boothe, 2001).

2.3. GENERAL PHARMACOLOGICAL FEATURES OF OXYTETRACYCLINE

2.3.1. Mechanism of action

Oxytetracycline functions by binding to the 30S subunit of the ribosome, thereby preventing the translocation of amino acids from the transfer-RNA to the messenger-RNA (Boothe, 2001) and subsequently prevents the elongation of peptide chains (Fig 2.). By way of this mechanism of action, oxytetracycline is bacteriostatic (Boothe, 2001).



Picture obtained from Chambers *et al.* (2005)

Fig 2.6: Mechanism of action of the tetracyclines: The p- (peptidyl) site contains the nascent polypeptide chain. The tRNA charged with the next amino acid to be added to this chain normally moves into the A- (acceptor) site before complementary base pairing of the anticodon sequence of tRNA and codon sequence of the mRNA can take place. Tetracyclines will block the binding of the tRNA to the A-site, thereby inhibiting bacterial synthesis.

2.3.2. Spectrum, duration of activity and use of oxytetracycline in ruminants

Oxytetracycline is a broad spectrum antimicrobial, with good efficacy against both Gram-positive and Gram-negative organisms (with the exception of *Pseudomonas aeruginosa*) (Boothe, 2001; Giguère *et al.* 2006). In addition, oxytetracycline is also effective against Chlamydia, Haemobartinnella, Mycoplasma and Rickettsiae (Boothe, 2001). In South Africa, the latter is especially useful in the management of *Ehrlichia ruminantium* and *Anaplasma marginale* (Allsopp *et al.*, 2004; Potgieter & Stoltz, 2004). Oxytetracycline also has an *in vitro* post antibiotic effect as efficacy is still demonstrable even when bacteria are essentially exposed to subinhibitory concentrations (Giguère *et al.* 2006).

Factors that may contribute to the post antibiotic effect are (Giguère *et al.* 2006):

Reduced ability of the bacteria to adhere to the host cells.

Decreased toxin production by the microbes.

Increased phagocytosis of the offending microbes.

2.3.3. Formulations of parenteral oxytetracycline

To best understand the influence of formulation on the absorption and therefore the PK/PD relationship it is necessary to review the different parenteral oxytetracycline formulations that are currently available. Oxytetracycline is exceptional among the tetracycline group of antimicrobials as it is compatible with both conventional solvents with quick release characteristics, and other solvents demonstrating slower release characteristics (Riviere & Spoo, 1995). This has significant impact in veterinary medicine as conventional formulations (quick release) typically need to be administered once a day, whereas long acting formulations (slow release) need to be administered once only, to maintain effective plasma concentrations $> 0.5 \mu\text{g/ml}$ for 72 hours (Shifferli *et al.*, 1982, Toutain & Raynaud, 1983). Long acting formulations are usually repeated after 72 hours at the discretion of the clinician.

Most conventional, short-acting parenteral oxytetracycline formulations are used at a dose of 10 – 12 mg/kg once a day for three consecutive days. The perception is that these formulations generally cause less muscle irritation. As the patient is seen on a daily basis for 3 days, the monitoring of the convalescent phase is more effective. Disadvantages of these formulations include extra labour and patient stress associated with handling (Potgieter & Stoltz, 2004). Long-acting oxytetracycline formulations are used at a dose of 20 -21 mg/kg as a single treatment. Practically this is convenient for both the patient and farmer/veterinarian (Potgieter & Stoltz, 2004). Perceived increased muscle irritation and less effective monitoring of the patient represent its main disadvantages.

The type of formulation is dependent on the different vehicle systems and solvents used to keep oxytetracycline in solution (Riviere & Spoo, 1995). Several solvent systems are available for creating a longer duration of action of oxytetracycline and a few are reviewed next (Burger, A.P. Burchem Research, Per. Com). Manufacturing details regarding the inclusion of different solvent systems into oxytetracycline formulations are the intellectual property of Burchem Research and were not made available for further discussion.

Polyvinyl pyrrolidone (PVP) is a solvent without any “long-acting” properties and is usually included in conventional formulations, but is often also included in a long acting

formulation because of its excellent bio-compatibility and due to the fact that it contributes to the solvency power of the formulation. This solvent, with the addition of water only, allows a formulation with a concentration of 100 mg/ml.

Bio-incompatible organic solvents, such as vegetable oils, may be used in conventional or long-acting formulations as co-solvents but are usually very irritant and may cause severe tissue damage at the injection site. To some extent, these solvents rely on tissue damage for their longer action. The oxytetracycline is released slowly from the sequestered drug depot that had formed in the muscle tissue at the site of injection. Another contributing factor to the sustained release from these formulations is the additional active ingredient partitioning effect offered by the oil. Yet another factor playing a role in more sustained oxytetracycline plasma concentrations is a higher concentration being injected into the muscle (Riviere, Spoo, 1995).

Bio-compatible organic solvents include propylene glycol, 2-pyrrolidone and 2-methyl pyrrolidone. These solvents are credited with their longer actions due to the same reasons given for bio-incompatible organic solvents, although they appear to cause less tissue damage (Riviere & Spoo, 1995). The only long-acting oxytetracycline formulations allowed for use in veterinary medicine in the United States are those containing 2-pyrrolidone (Riviere & Spoo, 1995).

Propylene glycol has a medium solvency power and formulations up to 135 mg/ml are possible with this solvent. In the case of 2-pyrrolidone, that has a high solvency power, formulations in excess of 200 mg/ml are possible. The formulator has to keep in mind though, that oxytetracycline itself has intrinsic irritant properties and consequently the concentration of oxytetracycline per millilitre of product has to be limited. Currently, the highest concentration of oxytetracycline per ml of a registered formulation in South Africa is 230mg/ml (Carrington *et al.*, 2007).

Another aspect to oxytetracycline formulations is the salt form of the active moiety. Two salt forms are available viz. oxytetracycline hydrochloride (HCl) and oxytetracycline dihydrate. The salt form *per se* has no effect on antimicrobial efficacy other than affecting

the solubility of the oxytetracycline moiety. Oxytetracycline active material occurs as a dihydrate powder. It is insoluble in water and needs to be made soluble by adding HCl. As soon as the powder goes into solution, it loses the dihydrate fraction. The HCl fraction is only retained long enough for magnesium (which stabilizes the oxytetracycline molecule) to be added. To complete the formulation process, the solution needs to be alkalized again. This means that the oxytetracycline is present in the bottle on the shelf as oxytetracycline, not as oxytetracycline HCl or as oxytetracycline dihydrate.

An unexplored treatment scenario to optimize oxytetracycline efficacy is using a conventional, short acting formulation at a higher (double) dose as a once-off treatment. This dose may be repeated after two days only if necessary. It is envisaged that this should minimize some of the disadvantages of both the conventional and the long-acting formulations, thereby providing an alternative to both these treatment regimes.

2.3.4. Pharmacokinetics of Oxytetracycline in Cattle

2.3.4.1. Pharmacokinetics of conventional oxytetracycline formulations

In the near-absence of literature pertaining to the pharmacokinetics of conventional formulations of oxytetracycline in sheep via the intramuscular route, cattle data was reviewed on the assumption that *in vivo* dissolution would not differ greatly between the two domestic ruminant species. Table 2.1 below includes data regarding key pharmacokinetic parameters in calves where conventional oxytetracycline formulations were administered intramuscularly or / and intravenously.

Davey *et al.* (1985) administered a conventional formulation (Terramycin 100 – Pfizer) at double dose (20 mg/kg) into the semimembranosus muscles of 7 month- old calves. C_{max} achieved was 5.62 µg/ml at 8 hours after treatment. Serum oxytetracycline concentrations remained above 0.5 µg/ml for 51.5 hours.

Nouws *et al.* (1983) administered a conventional formulation (Engemycin 10 – Mycofarm BV, the Netherlands) at 10 mg/kg into the semitendineus muscles of 14 week- old calves.

C_{max} achieved was 4.82 µg/ml at 6 hours. The last sampling point where serum oxytetracycline concentrations were above 0.5 µg/ml was at 32 hours.

Although different manufacturers' formulations were used in these two studies, the double dose of a conventional formulation not only peaked higher, but maintained effective serum concentrations for longer than a single dose of a conventional formulation.

2.3.4.2. Pharmacokinetics of long acting oxytetracycline formulations

With previous studies demonstrating that clinically-effective plasma concentrations against most pathogens being approximately 0.5 µg/ml and the long-acting formulations in food animals having the advantage of a sustained clinically-effective serum and tissue concentrations without the expense and practical difficulty of frequent dosing, the long acting oxytetracycline formulations have become widely used in the veterinary industry (Toutain & Raynaud, 1983; Davey *et al.*, 1985; Riviere & Spoo, 1995; Kumar & Malik, 1998; Potgieter & Stoltz, 2004). Consequently, the pharmacokinetics of the long acting oxytetracyclines has been well documented (Table 2.1.)

Intramuscular administration of a long-acting formulation (20 mg/kg, Terramycin LA, Pfizer) in the bovine led to a maximal serum concentration of 2.3 µg/ml within 60 minutes and a half-life of elimination 21.8 hours. Serum levels > 0.5µg/ml were exceeded for 72 hours. Mean bioavailability was 51.5%. (Toutain & Raynaud, 1983).

Kumar & Malik, (1998) used a dose of 20 mg/kg intramuscularly (OTC-LA, Cipla) in 6-8 month-old calves and demonstrated an absorption half-life of 24 hours, a volume of distribution of 0.86 L/kg and a total body clearance of 76.1 ml/h/kg. The maximum serum concentration of 4.7 – 7.4 µg/ml was attained after 8 hours and the absolute bioavailability was established at 89% after 84 hours (Kumar *et al.*, 1998).

Table 2.1: Comparison of oxytetracycline pharmacokinetic parameters in the bovine as demonstrated by different investigators using different oxytetracycline formulations and different routes of administration : Treatments were administered once-off except for Toutain (83) conventional formulation*.

Formulation	Animal Age	Dose (mg/kg)	Route	Cmax (µg/ml)	Tmax	Last time point **	Reference
Long Acting	7 mths	20	IM	3.15	4 h	86.8	Davey (85)
	7 mths	20	IV	58	15 min	36	Davey (85)
	7 mths	20	IM	7.4	8 h	84	Kumar (98)
	6 wks	20	IM	3.01	4 h	24	Schifferli (82)
	7 mths	20	IM	2.3	1. h	72	Toutain (83)
	8 mths	20	IM	5.2	2.8 h	–	Craigmill (00)
Conventional	7 mths	20	IM	5.6	8 h	51.5	Davey (85)
	14wks	18.3	IM	4.8	6 h	32	Nouws (83)
	3 wks	7.5	IV	11.1	15 min	23	Nouws (83)
	7 mths	10 + 10	IM + IV	–	–	56*	Toutain (83)

* 10mg/kg IV followed by 10mg/kg IM 24 hours later. **Showing concentrations > 0.5 µg/ml (hours)

In Table 2.1 Oxytetracycline bioassays were done using *Bacillus cereus* as assay organism.

Studies with different formulations of injectable oxytetracycline at different doses, conducted in the bovine by different authors, are summarized in Table 2.1 by comparing certain key pharmacokinetic parameters. Comparing studies such as these may offer insight into certain pharmacokinetic trends for oxytetracycline. For example, it is shown that long-acting formulations reach peak serum concentrations slower, but maintain higher serum concentrations for longer periods than conventional formulations. The danger associated

with comparing studies such as these, is that variables that may influence results are not standardized

As mentioned above, it has also been questioned whether the long effect seen in cattle, with the long acting formulation, was due to the higher dose (20 mg/kg instead of 10mg/kg for the conventional dose) as opposed to a prolonged period of absorption. To illustrate the importance of formulation effect Davey *et al.* (1985) subsequently compared a conventional and long acting formulation at the 20 mg/kg dose (Table 2.1). In this study he was able to demonstrate that the time to the last concentration was dependant on the formulation and not just the dose administered.

2.3.5. Pharmacokinetics of Oxytetracycline in sheep.

2.3.5.1. Pharmacokinetics of conventional formulations:

At present no information is available on the pharmacokinetics of conventional oxytetracycline formulations in sheep via the intramuscular route.

2.3.5.2. Pharmacokinetics of long-acting formulations:

Craigmill *et al.* (2000) compared the pharmacokinetics of a long-acting formulation (Liquamycin LA – Pfizer) between cattle and sheep after intramuscular administration of the drug at 20 mg/kg. Key pharmacokinetic parameters emanating from the data generated by Craigmill *et al.* (2000) as well as those generated by Escudero *et al.* (1996) and Kaya *et al.* (2001) in sheep are summarized in Table 2.2 below:

Table 2.2: Oxytetracycline pharmacokinetic parameters in sheep as demonstrated by different investigators using long-acting oxytetracycline formulations administered via the intramuscular route: Treatments were administered as a single dose.

Product	Animal Age	Dose (mg/kg)	Route (IM)	Cmax (µg/ml)	Tmax (h)	AUC (µg*h/mL)	Last time point **	Reference
Liquamycin LA (Pfizer)	Adult	20	Cervical muscle	6.09	3.5	209	–	Craigmill (00)
Terramycin LA (Pfizer)	Adult	20	Semi-membranosus muscle	3.47	1.79	112	72	Escudero (96)
Primamycin LA (Pfizer)	Adult	20	Gluteal muscle	5.13	2	78.9	48	Kaya (01)

From this data, it would seem that a long-acting oxytetracycline formulation tends to peak sooner with a slightly higher Cmax in sheep than in cattle. In addition, Craigmill *et al.* (2000) reported that the extent of absorption, as reflected by AUC, was significantly higher for sheep than for cattle (209 µg*h/mL versus 168 µg*h/mL respectively).

2.3.6. Effect of physiological factors on oxytetracycline pharmacokinetics in ruminants.

Age

The work by Nouws *et al.* (1983) regarding the influence of age differences on oxytetracycline pharmacokinetics in ruminants must also be considered before evaluating oxytetracycline plasma concentrations in animals of different ages. A conventional formulation containing 100 mg/ml oxytetracycline (Engemycin 10- Mycofarm BV, the Netherlands) was used in this study. Both intravenous administration at 7.54 mg/kg, and intramuscular administration at 17 mg/kg in 3 week-old and 14 week-old calves respectively showed volumes of distribution greater than adult cows. The 3 week-old calf group showed a Vd of 2.48 l/kg and the 14 week-old group 1.83 l/kg. Corresponding values in cows were 0.8 l/kg (lactating and dry cows). From this study, Nouws *et al.* (1983)

concluded that the recommended dose of oxytetracycline for pre-weaning calves should be twice the recommended dose of cows in order to obtain similar oxytetracycline concentration time profiles and therefore biophasic concentrations.

Based on the results of Nouws *et al.* (1983), Burrows *et al.* (1987) commented that the larger volume of distribution and lower plasma concentrations seen in neonates may indeed indicate a need for an increased dosage at the earlier ages, but that justifying a dose based on plasma concentrations may not be appropriate, as oxytetracycline tissue concentrations may be more reflective of anti-bacterial action.

Nouws *et al.* (1983) and Burrows *et al.* (1987) also speculated that the larger volume of distribution of oxytetracycline in neonates may be due to the larger percentage of body water, including blood volume relative to body size shown in neonates.

Besides the clinical implications of these findings on potential dosage adjustment in neonates, it is important to consider the influence of age when planning pharmacokinetic studies with oxytetracycline in ruminants. Very little pharmacokinetic data that compare the exact same formulation of oxytetracycline administered at lower (10 mg/kg) and higher (20 mg/kg) doses are available. Although Nouws *et al.* (1983) did some work in this regard, the group of calves in this study that received the lower dose were younger (3 weeks) than the group of calves that received the higher dose (14 weeks).

Injection sites:

Nouws and Vree (1983), compared oxytetracycline disposition in a group of calves following the administration of a conventional formulation at doses ranging from 17 to 18 mg/kg bodyweight, via the following different routes: Subcutaneous in the lateral neck, intramuscular in the lateral neck, the shoulder (*Musculi triceps brachii*) and the buttock (*Musculi semitendineus*). The maximum plasma concentrations with the subcutaneous and intramuscular routes were obtained within 4 to 8 hours after administration. The highest concentrations were achieved after injection into the shoulder ($6.9 \mu\text{g/ml} \pm 0.82$), while the other routes (subcutaneous and intramuscular) yielded similar maximum plasma concentrations ($5 - 5.5 \mu\text{g/ml}$). Bioavailability was most rapid for the intramuscular

shoulder route (98% of administered dose at 52 hours post injection) whereas the intramuscular neck route achieved similar figures after 76 hours. The poorest bioavailability (83% of administered dose) was yielded via the intramuscular buttock route (Nouws & Vree, 1983).

pH of body fluids:

With oxytetracycline being an amphoteric compound (Riviere & Spoo, 1995), it is unlikely to become ion-trapped in either acidic or basic (ruminant saliva) body fluids according to the principles of ion trapping and the Hendelson Hasselbach equation (Adams, 1995) after parenteral administration. This statement is supported by the relatively good volume of distribution as described under 2.3.4.

2.4. PK / PD RELATIONSHIPS OF OXYTETRACYCLINE

2.4.1. General

The pharmacokinetic / pharmacodynamic (PK/PD) relationship is the science of attempting to correlate drug plasma time-concentration profiles, at the biophase and the clinical outcome (Giguère *et al.* 2006). When the pharmacokinetic (PK) behaviour is linked to pharmacodynamic (PD) activity and MIC of the pathogenic bacteria, a better understanding of dose effect relationships can be obtained. More recently this concept has become important in determining both the dose and dosing intervals of specific antimicrobials (Jacobs, 1999).

To better understand the concept, one must first consider the MIC of bacteria, which represents the minimal drug concentration that inhibits bacterial growth *in vitro*. Since the aim of therapy is the clearance of the causative agent at the biophase, a drug must attain this effective MIC at the biophase to produce its characteristic effect, (pharmacodynamic effect). Factors that may influence the ability of the drug to attain effective concentrations at the site of infection include: a) drug dissolution and absorption, b) drug bio-transformation, c) transport of the active drug from the blood to the biophase and d) free drug movement into the bacterial cell (Giguère *et al.* 2006). In veterinary medicine, this issue is further complicated by the variable inter-species disposition even when animals

receive the same drug dose, as this leads to variations in biophasic availability and consequently variations in response (Adams, 1995).

Schifferli *et al.* (1982) presented a table demonstrating the correlation between MIC values for various bacterial species and the minimal plasma drug concentrations (C_{\min}) required to show antimicrobial efficacy, taking into account not only a 50% protein binding in the case of oxytetracycline, but in addition building an uncertainty factor (referred to as a safety factor by Schifferli) of 2 into the correlation (Refer to Table 2.3). The uncertainty factor takes some physiological and pathological factors into consideration, such as tissue perfusion at the site of infection, diminished vascularization in chronic inflammation, pH changes at the infection site and density of the infective organism. If an uncertainty factor of 2 is employed, the result is that the required drug plasma concentration (in addition to the calculated elevation due to 50% protein binding) is increased four times.

Table 2.3 and 2.4 show the relationship between some pathogens' MIC values and the minimum plasma concentration that oxytetracycline needs to attain in order to be effective against the relevant pathogen(s):

Table 2.3: MIC values of susceptible strains of pathogenic bacteria for calves and the correlation with minimum plasma concentrations (C_{min}) of oxytetracycline, taking into account 50% protein binding and an uncertainty (safety) factor of 2 (Schifferli, 1982):

Pathogenic bacteria	MIC ($\mu\text{g/ml}$)	C_{min} ($\mu\text{g/ml}$) or MIC x 4
<i>Bacillus anthracis</i>	0.12	0.5
<i>Listeria monocytogenes</i> ; <i>Streptococcus pneumoniae</i>	0.25	1
<i>Staphylococcus aureus</i> ; Beta-haemolytic streptococci <i>Arcanobacter pyogenes</i> ; <i>Pasteurella sp.</i> <i>Haemophilus sp.</i> <i>Clostridium perfringens</i> <i>Bacteroides sp.</i> <i>Fusobacterium sp.</i>	0.5	2
<i>Streptococcus</i> 'group viridans' <i>Mycoplasma sp.</i>	1	4
<i>Klebsiella pneumoniae</i> <i>Salmonella sp.</i>	2	8
<i>E. coli</i>	4	16

Table 2.4: South African MIC₉₀ values (Van Vuuren *et al.*, 2007) of strains of pathogenic bacteria and the correlation with minimum plasma concentrations (C_{min}) of oxytetracycline, using the Schifferli (1982) principle of considering a 50% protein binding and an uncertainty (safety) factor of 2:

Pathogenic bacteria	MIC ₉₀ (µg/ml)	C _{min} (µg/ml)
<i>Escherichia coli</i>	128	512
<i>Salmonella enterica</i>	64	256
<i>Salmonella typhimurium</i>	128 (MIC ₈₀ = 8)	512 (MIC ₈₀ = 32)
<i>Mannheimia haemolytica</i>	64 (MIC ₈₀ = 1)	256 (MIC ₈₀ = 4)
<i>Staphylococcus aureus</i> (Bovine milk)	2	8
<i>Beta-haemolytic streptococci including S. agalactiae and S. dysgalactiae</i>	2	8

Compared to oxytetracycline pharmacokinetics, very little information is available regarding its pharmacodynamic relationship. Consequently, this will be dealt with in a theoretical fashion, after reviewing the PK / PD relationships of other antimicrobials, with possible extrapolation to oxytetracycline.

2.4.2. Mechanism of action of different compounds and how it is expressed in the PK/PD relationship of that particular compound.

There are a large number of antimicrobial agents with varying mechanisms of action, ranging from (amongst others) microbial cell wall inhibition (β-lactams), microbial cell membrane inhibitors (polymixin antibiotics) microbial DNA inhibitors (fluorinated quinolones, nitroimidazoles and sulphonamides) and those that interfere with ribosomal activity (tetracyclines, macrolides, aminoglycosides).

Of these antimicrobials, the β-lactams, polypeptides, fluorinated quinolones, nitroimidazoles and macrolides (high doses) are bactericidal, whereas tetracyclines, macrolides (lower doses) and the sulfonamides are bacteriostatic. Exceptions to the rule

amongst those antimicrobials with ribosomal activity are the aminoglycoside and aminocyclitols, which are bactericidal at all doses and may be linked to more efficient inhibition of the process of protein synthesis. (Boothe, D.M. 2001)

Although the available antimicrobial drugs produce their beneficial activity from a wide range of mechanisms they still only fall into two major patterns: Time dependent activity (TDA), and concentration dependent activity (CDA) (Jacobs, 1999). Time dependent killing refers to the duration of exposure to an antibiotic that will lead to the death of a pathogen. Post antibiotic effect with this type of antimicrobial is minimal (Jacobs, 1999). With CDA, prolonged post-antibiotic activity is often present, persisting at drug concentration levels below MIC (Jacobs, 1999). The fluorinated quinolones, nitromidazoles and aminoglycosides fall into the latter group, with the macrolides, clindamycin and β -lactam antibiotics being in the former (Jacobs, 1999). No mention is made of tetracyclines although it is generally accepted that they are TDA antimicrobials. It would therefore appear that most bactericidal compounds (with the exception of β -lactams and polymixins) are concentration dependent.

2.4.3. Parameters used to describe pk / pd relationships.

With the PK/PD relationships of the major antimicrobials being studied very closely, numerous pharmacokinetic parameters have been developed to best optimise drug dosages. One of the major PK/PD parameters that correlate clinical / bacteriologic efficacy of the CDA group of antimicrobials is the AUC/MIC ratio where AUC represents a 24 hour area under unbound serum drug concentration curve. In immunocompetent animals, this ratio should be ≥ 25 and in immunocompromised animals ≥ 100 (Jacobs, 2001). This parameter represents total eradication of a specific bacterial population (Giguère *et al.*, 2006). Peak drug concentration to MIC ratio (C_{max}/MIC) is also used and should be ≥ 8 (Jacobs, 1999; McKellar *et al.*, 2004). It is indicative of bacterial re-growth ability, or the status of resistance of that population against a specific drug (McKellar, 2004).

The major PK/PD parameter that correlates with the clinical and bacteriologic efficacy of TDA antimicrobials, is the time that drug serum concentration exceeds the MIC of a

specific pathogen ($T > MIC$). As would be expected $T > MIC$ varies depending on the drug, pathogen MIC and infection site. Generally, however, the drug serum $T > MIC$ should be maintained for 40-50% of the dosing interval for the drug to have the desired clinical effect (Jacobs, 1999; Jacobs, 2001).

For the management of infection in the general populations, PK/PD breakpoints can be calculated by using the formula $AUC/25$ for both CDA and TDA. (Jacobs, 2001). In practical terms, the concentration that inhibits 90% of the bacterial strains (MIC_{90}) for a specific pathogen should be below its PK / PD breakpoint in order for the drug to be useful empirically (Jacobs, 2001).

2.4.4. Pk / pd relationship of oxytetracycline.

As mentioned above (2.4.2.) the macrolide group of antimicrobials belong to the time dependent group of antimicrobials in terms of its pharmacodynamic activity. Literature pertaining to the pharmacodynamic activity of oxytetracycline is very scarce, and it is assumed that this compound would possess similar pharmacodynamic properties to the macrolides, due to the fact that the two groups of antimicrobials both interfere with protein synthesis albeit at different binding sites (Riviere & Spoo, 1995; Spoo & Riviere, 1995).

Tetracyclines are bacteriostatic by binding with the 30S subunit of the bacterial ribosome and interfering with the binding of aminoacyl-tRNA to the messenger RNA complex. Even mammalian ribosomes are susceptible to the protein inhibiting effect of tetracyclines (catabolic effect) but the affinity that tetracyclines have for mammalian ribosomes, are less than its affinity for bacterial ribosome. The bacteriostatic effect of the macrolides is by virtue of the compound(s) binding with the 50S subunit of the bacterial ribosome and interfering with the binding of aminoacyl-tRNA (Boothe, 2001). Plasma concentrations of oxytetracycline have been measured for many years, and yet the clinical significance of these concentrations remains unclear. Despite the fact that it is generally accepted that an oxytetracycline-protein complex is reversible, only the free drug fraction in the plasma is considered to be available for action against pathogens (Kunin, 1966). In the case of oxytetracycline, 50% is bound to plasma proteins in cattle, which leaves only 50% of the

drug free in the plasma. Ideally, the free drug concentration should not fall below the effective concentration at any time during treatment (Pillaud, 1973).

2.4.5. Pk / pd relationship of oxytetracycline in sheep.

As mentioned, literature pertaining to oxytetracycline pharmacokinetics in sheep with the use of conventional formulations via the intramuscular route are lacking. Kaya *et al.* (2001) used a conventional formulation in sheep, but via the intravenous route. Craigmill *et al.* (2000), Escudero *et al.* (1996) and Kaya *et al.* (2001) studied oxytetracycline kinetics in sheep after intramuscular administration of long-acting formulations (Refer to 2.3.5.2).

Referring to 2.4.3, pharmacodynamic parameters such as AUC/MIC and C_{max}/MIC is relevant only to CDA. The main pharmacodynamic parameter for TDA antimicrobials such as oxytetracycline is time (T) > MIC, the MIC in this instance being 0.5 µg/ml. Based on the pharmacokinetic data generated by Escudero *et al.* (1996) and Kaya *et al.* (2001), long-acting formulations of oxytetracycline maintain effective concentrations for 72 hours (Escudero *et al.*, 1996) and 48 hours (Kaya *et al.* 2001). However, if the Schifferli *et al.* (1982) principle of incorporating 50% protein binding and an additional uncertainty factor of 2 is considered, the MIC value for most bacteria shown in Table 2.3 is elevated to 2 µg/ml. In this situation, effective drug concentrations would be maintained for < 25 hours, based on the data generated by Craigmill *et al.* (2000) and Kaya *et al.* (2001). Table 2.4 shows the South African MIC data of certain pathogens (Van Vuuren *et al.*, 2007). If the Schifferli *et al.* (1982) principle is applied to these figures, the drug would not reach effective concentrations at any stage against any of the bacteria shown in this table.

2.5. SAFETY OF OXYTETRACYCLINE IN RUMINANTS

2.5.1. General

As with all drugs the tetracyclines are associated with many adverse reactions in domestic animals. Many adverse reactions have been described in domestic animals and humans: Pseudomembranous colitis in horses; gastro-intestinal intolerance in dogs, gastrointestinal disturbances in ruminants and equine following use of oral oxytetracycline preparations, (also with parenteral doxycycline /minocycline), teeth discolouration and phototoxicity in humans (Riviere & Spoo, 1995) and hepatotoxicity secondary to impaired renal clearance of oxytetracycline. The main ruminant side effects are described below.

2.5.2. Cardiac effects

Gross *et al.* (1981) found that increased peripheral resistance and aortic pressure in combination with a decreased stroke volume (attributed to endogenous histamine release) was evident following intravenous administration of oxytetracycline formulations, as a result of the specific solvents used in the formulations. Even solvents without long-acting properties, such as PVP, have been described to cause these side effects. With no dose response relationships being demonstrated it is unclear if the conventional treatment regime, which involves the administration of the least amount of solvents at one time point, would cause less severe cardiac effects than a higher dose of the same formulation as described under 2.3.3.

2.5.3. Calcium chelation

Oxytetracycline is known to have the potential to chelate calcium ions which may result in hypocalcaemic collapse, especially after intravenous administration of an oxytetracycline bolus (Boothe, 2001). Therefore the lower the dose of oxytetracycline measured in mg/kg bodyweight, the less the potential of precipitating such a reaction. In this regard, the conventional treatment regime of administering 10 mg/kg oxytetracycline intramuscularly once a day for 3 to 4 days is probably the safest available treatment regime of the 3 options described under 2.3.3. Long acting formulations are usually not recommended for

intravenous administration and consequently the calcium chelation effect is not an issue with these formulations.

2.5.4. Primary /Secondary nephrotoxicity

Primary nephrotoxicity occurs following oxytetracycline degradation due to excessive oxidation (Dukes & Aronson, 2000). Secondary nephrotoxicity may occur due to increased serum urea concentration subsequent to the catabolic effect of oxytetracycline. However, there is only potential for such a reaction at massive doses of oxytetracycline (Riviere & Spoo, 1995).

2.5.5. Tissue damage at the injection site

Tissue damage at the injection site is common with the use of parenteral oxytetracycline formulations. Tissue (muscle) damage due to parenteral medications has animal welfare, as well as economic implications (downgrading of the carcass at the abattoir). There is, however, a correlation between the type of solvent used, and the extent of muscle damage (Refer to 2.3.3). Solvents with slow-release properties (eg. propylene glycol) cause more muscle damage than solvents without slow-release properties (for example PVP). When using a higher dose of the conventional formulation, tissue damage should be less compared with the damage caused by the slow-release solvents in the long-acting formulations, providing the recommendation of not injecting a volume exceeding 20 ml at one site is adhered to (Because conventional formulations have lower concentrations of oxytetracycline than long-acting formulations, a larger volume will have to be administered to achieve the target dose rate of 20 mg/kg).

2.6. CONCLUSION

From a safety and efficacy point of view and using pharmacokinetic theory, a once-off double dose of oxytetracycline seems a viable option. Therefore it is the aim of this study to ascertain if this new therapeutic recommendation will be an effective, safe and convenient treatment alternative.

CHAPTER 3. MATERIALS AND METHOD

3.1. INTRODUCTION

The formulation of Ecomycin Dual Purpose (135 mg/ml oxytetracycline) is a conventional formulation and remains the intellectual property of the manufacturer, Burchem Research (Pty) Ltd.

The formulation does not contain any long-acting vehicles or solvents such as 2-pyrrolidone.

3.2. EXPERIMENTAL PROCEDURES

3.2.1. Experimental design

Comparative pharmacokinetic studies generally either employ parallel study designs, or cross-over designs (Grizzle, 1965). In a parallel design, experimental subjects in the one group receive only one of the two treatments involved in the study, while the subjects in the other group receive the alternative treatment only. In a cross-over study design, the two groups of experimental subjects which will be used to generate comparative pharmacokinetic data each receive both of the treatments in a cross-over fashion involving two treatment periods. Cross-over studies are usually preferred as the statistic power of a parallel designed study is lower due to inter-subject variation. With a parallel design, a larger sample size is thus required to overcome this disadvantage. Conversely the advantage of the cross-over design is that less subjects per group are required since the effect of inter-subject variation is minimized.

An inherent problem with cross-over design studies is that undetected drug may be carried over from one treatment to the next. In pharmacokinetic studies this problem is largely eliminated by the fact that any carry-over drug will be detected when the zero-time blood samples are analyzed. In addition, the half-life of the active ingredient is well researched and was taken into account when the “wash-out” period between the two treatments was

determined. The duration of at least 5 half-lives, based on a study by Davey *et al.* (1985) was used to determine the period between treatments.

For this study a single dose, randomized, two treatment, two sequence cross-over experimental design as described by Grizzle (1965) was selected. The reference treatment comprised of a single, intramuscular administration of a conventional formulation of oxytetracycline at 10 mg/kg, and the test treatment comprised of a single, intramuscular administration of the exact same conventional formulation of oxytetracycline at 20 mg/kg. The washout period between the two sequences was also determined using 5 half-lives (11.1 hours x 5) of conventional oxytetracycline formulation, based on a study by Davey *et al.* (1985). Although a wash-out period of 55.5 hours for a dose rate 20 mg/kg of oxytetracycline would have sufficed the washout period was further strengthened by expanding it to 7 days.

3.2.2. Sample size determination

The sample size selected is important as it has a direct impact on the rejection of the null hypothesis as described by Anderson and Hauck (1983). The noncentrality parameter (δ) was calculated as described by Anderson and Hauck (1983).

Anderson and Hauck (1983) recognized that two treatments will never yield results which are exactly alike due to biological variation and measurement error involved. They determined that limits for each treatment regime should be clinically determined and specified. Should two treatments differ by less than the specified limits, the regimes are deemed to be equivalent. They define the null hypothesis, which states that there is no difference between the two treatment regimes, as:

$$H_0 : \mu_e - \mu_s \leq A \quad \text{or} \quad \mu_e - \mu_s \geq B$$

Rejection of the null hypothesis (the objective of this study) is defined as:

$$H_A : A < \mu_e - \mu_s < B$$

Where μ_e and μ_s are the mean values for subjects receiving experimental and standard treatment regimes. A and B are the specified limits, with $A = -B$. Assuming a half-life of 11.1 hours (Davey *et al.*, 1985) and not requiring a deviation $> 20\%$, A will have a value of 2.22.

The equation used for estimating the noncentrality parameter (δ) was (Anderson, Hauck, 1983):

$$\delta = \frac{\frac{1}{2}(B - A)}{S \times \sqrt{\frac{2}{n}}}$$

Based on a non-centrality parameter of -0.305 , a sample size of 4.992 or 5 animals was determined, using the following formula (Anderson, Hauck, 1983):

$$n = \frac{8 \times \delta^2 \times \sigma^2}{(B - A)^2}, \text{ where } \sigma^2 \text{ is the variance and 8 is a constant.}$$

In order to detect any differences outside specified limits, a sample size of 5 was therefore considered adequate, when used in a cross-over study which increased n to 10 animal units.

3.2.3. Experimental animals

10 healthy, 3 year-old Dohne-Merino wethers with body weights varying between 45.5 and 62 kg, were selected. Blood was collected for the determination of haematocrit, white cell count (Cellydyne 3700 apparatus, Abbott method) and differentiation (manual counting) as well as urea and creatinine determination. Plasma samples were collected for liver enzyme determination [Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) (Nexet Chemistry Analyser, Bayer SA.; Alfa Wasserman method). A clinical examination was also performed on each sheep. Heart rates, respiratory rates and body temperatures were recorded, as well as any observations regarding habitus (behaviour).

3.2.4. Housing and feeding

Animals were accommodated in two separate roofed pens of approximately 8 m² each. They were adapted to grower pellets from day -7, and fed grower pellets for the duration of the trial. The feed was free of oxytetracycline. Fresh water was provided to the animals by means of concrete water troughs with automatic ball valves.

3.2.5. Allocation

The sheep varied in weight from 45.5 kg to 62 kg at the start the study. The sheep were equally and randomly assigned to either the group that would receive the 10 mg/kg dose first (group 1), or the group that would receive the 20 mg/kg dose first (group 2). Group 1 consisted of animals numbered 1 – 5, whereas group 2 consisted of animals 6, 7, 9 10 and 12. Weights were again determined before the second phase of the study was initiated. Weights of the 10 animals then varied between 48 and 60.5 kg. The animals remained in the groups they were allocated to before phase 1, but group 1 received the 20 mg/kg dose and group 2 received the 10 mg/kg dose.

3.2.6. Treatment

Table 3.1: Animal numbers, group allocation, weights and doses used in phases 1 and 2:

Animal number	Group	Weight (kg) Phase 1	Calculated dose (ml) Phase 1	mg/kg	Weight (kg) Phase 2	Calculated dose (ml) Phase 2	mg/kg
1	1	52	3.85	10	52	7.7	20
2	1	53.4	3.96	10	52.5	7.78	20
3	1	55.5	4.11	10	52.5	7.78	20
4	1	49.3	3.65	10	49	7.26	20
5	1	62	4.59	10	60.5	8.96	20
6	2	54.5	8.08	20	51	3.78	10
7	2	55	8.14	20	57	4.22	10
9	2	45.5	6.74	20	50	3.7	10
10	2	47	6.96	20	48	3.56	10
12	2	57	8.44	20	60	4.44	10

The sheep were treated with either the 10 mg/kg dose or the 20 mg/kg dose as described in table 3.1. The quantity of oxytetracycline was calculated based on a product oxytetracycline content of 135 mg/ml. Sheep were monitored for local pain reaction upon injection.

3.2.7. Duration of blood sample collection

According to Pabst & Jaeger (1990), sampling should continue for between 3 to 5 biological half-lives in order to obtain correct pharmacokinetic parameters from blood concentration curves. Davey *et al.* (1985) measured the half-life of a conventional formulation administered at 20 mg/kg at 11.1 hours. Five times the biological half-life of 11.1 equals 55.5 hours. In spite of this, it was decided to continue sampling until 96 hours, when plasma oxytetracycline should be undetectable.

Taking into account the half-lives and C_{max} that Davey *et al.* (1985) achieved, 14 bleeding points were decided on, including the pre-treatment baseline bleeding point. At least 3 points were during the absorption phase, 3 during the distribution phase and 3 during the elimination phase. 10 ml lithium heparinized tubes were used for collection, in order to ensure that enough plasma was available for analysis.

The blood sample collection procedure was the same for phases (treatments) 1 and 2. Time 0 was the time of treatment. Samples were collected into 10 ml heparinized vacutainer glass tubes with 19G disposable needles at the following intervals (hrs): 0, 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24, 36, 48, 72, 96. At each bleeding point, an extra sample was collected from a randomly chosen animal for quality control purposes. A minimum of 5 ml of blood was collected.

3.2.8. Analysis of oxytetracycline concentrations in plasma.

The blood was centrifuged for 15 minutes at a speed of 3000 rpm. The plasma was then separated from the cellular component by means of 5 ml syringes and 21G needles. A new

syringe and needle was used for every sample. The plasma samples were then placed in polyurethane plastic tubes in which they were kept at -15°C until analysed by high performance liquid chromatographic method (Appendix 1). A brief description of the method is given:

Sample preparation

For the analysis, samples (1000 μl) were pipetted into micro test tubes with 100 μl of the doxycycline internal standard. To the mixture 250 μl of a 15% tetrachloroacetate was added and the sample subjected to vortex for 15secs. The tube was subsequently incubated in an ice bath. The supernatant was used in all subsequent analyses.

High performance liquid chromatography

The method used for this study proved to be very specific for oxytetracycline, the accuracy ranged between 92 – 102%, and the average precision ranged from 83 – 112%. Reproducibility for blank plasma samples spiked with three levels of oxytetracycline showed a Coefficient of Variation (CV) of $< 20\%$. Extraction efficiency was 90%. A Beckman System Gold HPLC consisting of an autosampler module 508, programmable solvent module 126, diode array detector module (DAD) 168, and System GoldTM software package, was used (Beckman Instruments, Fullerton, California, USA). Separation was achieved with a Lunar column. The mobile phase consisted of 600 ml 0.05M potassium dihydrogen phosphate, 200ml methanol and 200ml acetonitrile. 100 μl of the samples were injected onto the HPLC column in an isocratic run at 1 ml/min. Detection of oxytetracycline and doxycycline (internal standard) was carried out at 365 nm. The total runtime per sample was 10 minutes. Control values showed regression coefficients greater than 0.99 for each analytical run. The LLQ was established at 0.300 $\mu\text{g}/\text{ml}$ and LOD at 0.100 $\mu\text{g}/\text{ml}$. The linear relationship between concentration and peak area was demonstrated for the total concentration range between 0.5 and 20 $\mu\text{g}/\text{ml}$.

3.2.9. Pharmacokinetic analysis

Non-compartmental analysis of the plasma concentration versus time data of oxytetracycline for extravascular input (Model 200) was also performed using

WinNonLon 4.2 (Pharsight coporation, California) using non-compartmental analysis. The area under the plasma concentration versus time curve (AUC, zero-moment) and the first non-normalized moment (AUMC) were calculated according to the linear trapezoidal method from time zero to the last sample time point (Gibaldi & Perrier, 1982). Extrapolation of AUC to infinity (AUCinf) was performed using the slope of the terminal phase (β). The mean residence time (MRT, first moment) was derived from AUC/AUMC. Maximum plasma concentration (Cmax) and time to Cmax (Tmax) were read directly from the individual plasma concentrations. The time plasma oxytetracycline concentration exceeded the MIC of 0.5 $\mu\text{g/ml}$ was obtained by reading off the individual plasma concentration versus time curves.

3.2.10. Statistical Methodology

Significance of difference between treatment groups for key pharmacokinetic parameters was performed for a crossover design with sequence, period, subject and treatment effects. Significance was set at $p < 0.05$ (Weldon, 1986). For antimicrobials with time dependent pharmacodynamic activity, therapeutic success depends on the time above the MIC (Jacobs, 1999).

Superiority between the two treatment groups was determined with NCSS statistical software using a paired t-test after establishing the absence of a significant period difference. The following primary pharmacokinetic parameters were evaluated:

AUC, representing the total amount of drug absorbed; Cmax, representing the maximum oxytetracycline concentrations reached in the experimental animals in both treatment groups; and the time that the plasma levels of this time dependent antimicrobial persisted above the MIC, which is 0.5 $\mu\text{g/ml}$ in the case of oxytetracycline (Toutain, 1983).

CHAPTER 4. RESULTS

4.1. CLINICAL EXAMINATION AND CLINICAL PATHOLOGY

Observations recorded showed that all the experimental animals were physiologically sound and suitable to be included in the study. Haematological evaluation included haematocrit, and differential white cell counts. Kidney function was evaluated by determining plasma concentrations of urea and creatinine. Liver function was evaluated by determining the plasma levels of ALP and AST. The results obtained by analysing the whole blood and serum collected from the experimental animals before the study was initiated, ranged within the acceptable limits as used by the clinical pathology laboratory involved. The laboratory involved was “Pathcare”, run by Dr. RP Mulligan and Partners.

4.2. OXYTETRACYCLINE PLASMA CONCENTRATIONS

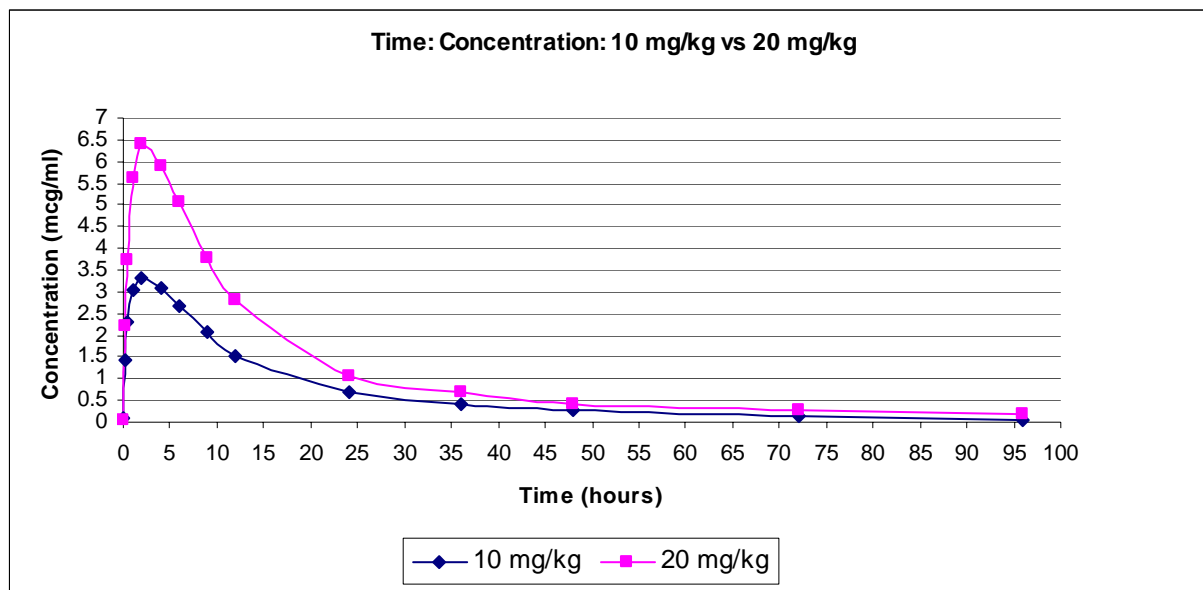
The oxytetracycline concentrations in plasma for both treatment groups for each treatment period are presented in Tables 4.1 and 4.2. The mean plasma concentration versus time profile is presented in Figure 4.1, while the individual and mean pharmacokinetic parameters are presented in Table 4.3.

Table 4.1: Oxytetracycline plasma concentration results: Group 1 Phase 1 (10 mg / kg) and phase 2 (20 mg/kg)

Time post treatment (h)	Animal number					Mean	SD	Animal number					Mean	SD
	(Phase 1)							(Phase 2)						
	1	2	3	4	5			1	2	3	4	5		
0.00	ND	ND	ND	ND	ND	NA	NA	ND	ND	0.17	0.16	ND	0.17	0.00
0.25	0.97	1.20	1.89	2.47	0.45	1.40	0.79	0.28	2.88	2.08	2.51	2.33	2.02	1.01
0.50	1.34	2.20	2.75	3.92	0.83	2.20	1.21	0.96	4.05	3.41	3.63	3.56	3.12	1.23
1.00	2.17	3.47	3.82	4.71	1.32	3.10	1.35	2.50	5.35	5.20	5.71	5.34	4.82	1.31
2.00	2.82	3.89	3.40	4.08	2.32	3.30	0.73	4.67	6.72	5.96	6.19	6.29	5.97	0.77
4.00	2.94	3.43	2.63	3.04	3.36	3.30	0.32	6.63	6.75	5.79	5.86	6.23	6.25	0.44
6.00	2.81	2.75	2.01	2.27	3.36	3.08	0.52	5.98	5.79	4.94	4.72	5.38	5.36	0.54
9.00	2.04	2.36	1.41	1.58	2.88	2.64	0.59	4.47	4.67	3.41	3.58	4.32	4.09	0.56
12.00	1.55	1.19	1.10	1.12	2.18	2.05	0.45	3.32	3.58	2.23	2.42	3.34	2.98	0.61
24.00	0.54	0.81	0.52	0.48	0.87	0.64	0.18	1.01	1.47	0.80	0.88	1.19	1.07	0.27
36.00	0.28	0.55	0.36	0.35	0.43	0.39	0.10	0.54	0.95	0.48	0.53	0.75	0.65	0.20
48.00	0.24	0.30	0.23	0.21	0.36	0.27	0.06	0.29	0.52	0.24	0.37	0.49	0.38	0.12
72.00	0.20	ND	0.18	0.16	0.28	0.21	0.05	0.20	0.21	0.13	0.29	0.34	0.23	0.08
96.00	ND	ND	0.19	0.19	ND	0.19	0.001	0.25	ND	0.31	0.16	0.21	0.23	0.07

Table 4.2: Oxytetracycline plasma concentration results: Group 2 Phase 1 (20 mg / kg) and phase 2 (10 mg/kg)

Time post treatment (h)	Animal number					Mean	SD	Animal number					Mean	SD
	(Phase 1)							(Phase 2)						
	6	7	9	10	12			6	7	9	10	12		
0.00	ND	ND	ND	ND	ND	NA	NA	0.18	0.44	ND	ND	ND	0.30	0.17
0.25	2.25	2.56	1.68	2.77	2.76	2.41	0.46	1.21	1.47	1.04	1.17	2.39	1.46	0.54
0.50	4.51	3.96	3.11	5.07	5.14	4.36	0.84	2.03	2.10	2.07	1.63	4.18	2.40	1.01
1.00	7.25	6.48	5.46	7.55	5.44	6.44	0.98	2.94	2.53	3.20	2.22	4.05	2.99	0.70
2.00	7.62	6.52	6.25	7.65	6.21	6.85	0.73	3.21	2.75	3.89	2.80	3.94	3.32	0.57
4.00	6.01	5.47	5.38	4.69	5.97	5.50	0.53	3.03	3.18	3.34	2.80	3.32	3.13	0.22
6.00	5.11	4.30	4.36	4.87	5.00	4.73	0.37	2.68	3.14	2.63	2.36	2.58	2.68	0.29
9.00	3.36	3.60	3.57	3.43	3.31	3.45	0.13	1.67	2.99	1.89	1.87	1.91	2.06	0.53
12.00	2.74	2.63	2.93	2.65	2.46	2.68	0.17	1.58	2.24	1.34	1.33	1.43	1.58	0.38
24.00	1.28	0.91	0.91	0.97	1.02	1.09	0.18	0.65	1.07	0.53	0.63	0.62	0.70	0.21
36.00	0.90	0.64	0.64	0.58	9.67	0.71	0.13	0.48	0.58	0.43	0.43	0.39	0.46	0.07
48.00	0.54	0.38	0.38	0.40	0.47	0.48	0.09	0.34	0.39	0.24	0.28	0.24	0.30	0.07
72.00	0.41	0.28	0.28	0.29	0.32	0.35	0.07	0.27	0.24	ND	0.17	ND	0.23	0.05
96.00	0.32	0.16	0.16	0.27	0.14	0.22	0.08	0.20	ND	ND	ND	ND	0.20	NA



*Graphs depicting the oxytetracycline concentration : time curve for each individual animal are presented in Appendix 2.

Fig 4.1: Graphic presentation of the mean oxytetracycline plasma concentrations generated by the intramuscular injection of the experimental oxytetracycline formulation at 10 mg/kg, and 20 mg/kg in sheep. The reference MIC was 0.5 µg/ml oxytetracycline.

4.3. PHARMACOKINETIC RESULTS

Table 4.3: Individual and mean pharmacokinetic results of the reference (10 mg/kg) and test (20 mg/kg) treatments administered intramuscularly in sheep.

Animal	AUCinf (µg/ml*h)		AUClast (µg/ml*h)		Cmax (µg/ml)		Tmax (h)		T½ λ (h)		T > 0.5 (h)	
	10 mg/kg	20 mg/kg	10 mg/kg	20 mg/kg	10 mg/kg	20 mg/kg	10 mg/kg	20 mg/kg	10 mg/kg	20 mg/kg	10 mg/kg	20 mg/kg
1	78.67	133.20	53.90	107.20	2.94	6.63	2.00	2.00	38.60	36.17	23.75	47.75
2	65.97	130.90	57.66	125.90	3.89	6.75	4.00	2.00	18.69	15.04	35.75	47.75
3	78.88	99.90	53.62	92.02	3.82	5.96	2.00	2.00	45.60	45.68	23.75	35.75
4	62.49	108.50	56.91	100.40	4.70	6.19	4.00	2.00	54.23	31.52	11.75	35.75
5	95.77	133.80	70.70	122.00	3.36	6.29	0.50	2.00	33.09	30.14	23.50	35.75
6	84.37	153.70	66.08	124.20	3.2	7.62	4.00	4.00	51.37	43.93	23.75	35.50
7	87.23	138.30	76.91	109.70	3.18	6.52	2.00	4.00	23.22	32.89	35.75	47.75
9	55.09	110.50	51.12	103.50	3.88	6.25	1.00	2.00	15.48	30.03	23.75	23.75
10	59.69	114.50	53.68	107.30	2.79	7.65	1.00	2.00	24.96	40.56	23.75	35.75
12	59.96	112.40	54.08	107.00	4.17	6.21	6.00	2.00	14.15	26.73	23.75	35.75
Mean	72.81	123.57	59.47	109.92	3.78	6.61	2.65	2.40	31.94	33.27	24.93	38.13
SD	13.92	16.80	8.70	10.96	0.63	0.59	1.76	0.84	14.89	8.99	6.82	7.60

The significance of difference between the two treatment groups is based upon the difference in mean values of key pharmacokinetic values between the two groups: The reference group (10 mg/kg) is compared with the test group (20 mg/kg). The mean values are represented in Table 4.4 below:

Table 4.4: Selected pharmacokinetic parameters presented as geometric means, for the two doses tested, with their p-value and power obtained using a student T-test

Pharmacokinetic parameter	Reference (10 mg/kg)	Test (20 mg/kg)	P value*	Power
T > 0.5 µg/ml (hr)	24.00	37.40	0.001308	0.81
C _{max} (µg/ml)	3.55	6.59	0.000000	1.00
AUC _{inf} (µg/ml*hr)	71.63	120.63	0.000001	1.00
AUC / MIC	119	219	0.000000	1.00

*All data was normally distributed with equal variance

For the selected pharmacokinetic parameters:

T > 0.5µg/ml: The 20 mg/kg treatment maintains levels above 0.5µg/ml significantly longer than the 10 mg/kg treatment.

C_{max}: The 20 mg/kg dose reaches a significantly higher C_{max} than does the 10 mg/kg dose.

AUC_{inf}: The 20 mg/kg dose has an AUC_{inf} which is greater than the 10 mg/kg dose by a highly significant margin.

AUC / MIC: Assuming a MIC of 0.5 µg/ml, the 20 mg/kg dose is a significantly higher ratio than the 10 mg/kg dose.

CHAPTER 5. DISCUSSION

5.1. EXTENT OF EXPOSURE / PEAK EXPOSURE

AUC represents the extent of exposure. AUC_{last} for both treatment groups is more than 85 % of AUC_{inf} indicating that AUC_{last} was a true reflection of extent of absorption. (Chow, 2000). The extent of exposure of oxytetracycline as measured by AUC_{last} and AUC_{inf} was nearly 2 fold greater in animals treated at 20 mg/kg compared to 10 mg/kg indicating dose proportionality for these two doses. This study showed that the AUC_{inf} and AUC_{last} are respectively 76.2% and 84.7% greater for the 20mg/kg dose compared with the 10 mg/kg dose. In addition, the AUC for time spent above 0.5 $\mu\text{g/ml}$ is more than double for the 20 mg/kg dose, compared to the 10 mg/kg dose.

As described in Chapter 2 the optimal AUC/MIC ratio represents the ability of the antimicrobial to totally eradicate a specific population of microbes (Giguère *et al.* 2006). The mean AUC/MIC ratios calculated were 119 and 219 for the 10 mg/kg and 20 mg/kg groups respectively. The target ratio value for immunocompetent animals is ≥ 25 and for immunocompromised animals ≥ 100 (Jacobs, 2001). Should 0.5 $\mu\text{g/ml}$ be accepted as the true MIC for the majority of pathogens (evidence as presented in table 2.4 would suggest that this is not the case), both the 10 mg/kg and 20 mg/kg dose would be sufficient to eradicate oxytetracycline–susceptible microbes even in pediatrics / geriatrics, where immune responses may be compromised.

In order to compare a 20 mg/kg dose of a conventional formulation with a 20 mg/kg dose of a long-acting formulation, results obtained by Davey *et al.* (1985) were used. The AUC/MIC ratios for both formulations used in Davey's study were similar (332. and 335.4, respectively). The results show that both formulations would have similar effects in eradicating a susceptible bacterial population, should the conventional formulation be used at double dose. It is unclear why the conventional formulation used by Davey *et al.* (1985) achieved such a significantly higher AUC than the test formulation used in this study (332 versus 219). It could possibly be due to the solvents used in that formulation,

differences in sample collection time points or differences in species used. However, if data generated by Davey *et al.* (1985) using a conventional formulation in cattle is extrapolated from the 20 mg/kg used in the study to 10 mg/kg, the values obtained show the same tendency as those obtained in this study (AUC for the 20 mg/kg dose is twice that of the 10 mg/kg dose).

Peak exposure is represented by C_{max}. The peak exposure of oxytetracycline in 20 mg/kg group was on average nearly twice that measured in sheep treated at 10 mg/kg, again indicating dose proportionality between the two doses. C_{max}/MIC ratio is indicative of the ability of the antimicrobial to prevent re-growth of the bacterial population and pertains to the status of resistance of that specific population of pathogens (Giguère *et al.* 2006). However, no report on this relationship for oxytetracycline could be found. Nevertheless, the C_{max}/MIC ratios calculated for the 10 mg/kg and 20 mg/kg groups were 7.2 and 13.2, respectively.

The target value for this ratio is ≥ 8 (Jacobs, 2001). The 10 mg/kg dose did not achieve this target indicating that resistant pathogens may present a problem in terms of re-growth when this dose is used. The 20 mg/kg dose surpassed the target value and it would be advisable to use this dose when doubt exists regarding the status of resistance of a bacterial population.

Comparison of a conventional and a long-acting formulation, administered intramuscularly at 20 mg/kg (with reference to the study by Davey *et al.*, 1985) indicated ratios of 13 and 6.6, respectively. These results would indicate that the conventional formulation would theoretically prevent re-growth of a bacterial population and that, due to the higher C_{max} achieved, would be the treatment of choice over a long-acting formulation when used against a resistant population of bacteria.

Since the time to peak exposure (T_{max}) was 2.4 hrs for the 20 mg/kg group and 2.65 hrs for the 10 mg/kg group (viz a 10% difference), the difference in the rate of onset of antimicrobial effect is expected to be negligible in clinical terms. Time to peak exposure

would be important in acute life-threatening diseases and under these conditions intravenous treatment is preferred.

5.2. TIME OF EXPOSURE

The dosing interval of the test product can be calculated based on the principle presented by Jacobs (1999), which states that antimicrobial efficacy of a time-dependent antibiotic is correlated with the time that plasma concentrations are maintained above the MIC of a particular organism with 0.5 µg/ml used as reference MIC (Shifferli *et al.*, 1982; Toutain & Raynaud, 1983). The time above 0.5 µg/ml was significantly longer in sheep treated at 20 mg/kg compared to 10 mg/kg. An average of 25.2 hours above 0.5 µg/ml was achieved for the 10mg/kg treatment and a mean time of 38.4 hours above 0.5 µg/ml for the 20 mg/kg treatment.

A difference in effective plasma concentration for an additional 13 hours is deemed clinically significant. If data generated by Davey *et al.* (1982) using a conventional formulation in cattle is extrapolated from the 20 mg/kg used in the study to 10 mg/kg, the values obtained show the same tendency as those obtained in this study (time > 0.5 µg/ml is approximately 12 hours longer for the higher dose).

Although Toutain & Raynaud (1983) achieved $T_{>0.5 \mu\text{g/ml}}$ of 56 hours with a conventional formulation, he administered 10 mg/kg IV and 10 mg/kg IM after 24 hours.

If the plasma concentration of oxytetracycline is to be maintained above the MIC of 0.5 µg/ml for 50% of the dosing interval to be effective, it follows that at 10 mg/kg, this formulation should be repeated after $25.2 \text{ hrs} \times 2 = 50.4$ hours, or in practical terms, 2 days. Toutain & Raynaud (1983) followed a treatment regime whereby it was shown that oxytetracycline levels >0.5 µg/ml can be maintained with a conventional formulation for 72 hours by IM administration of 10 mg/kg, followed by 5 mg/kg after 24 hours, and 5 mg/kg after 48 hours. With regard to the 20 mg/kg treatment, the dosing interval should be $38.4 \text{ hours} \times 2 = 76.8$ hours, or in practical terms 3 days (if necessary).

Furthermore, the recommended dose for the test product does not take into account additional factors that could be considered as suggested by Schifferli *et al.* (1982), which include 50% protein binding for oxytetracycline, as well as all the factors encompassed by the safety factor that could be incorporated into the calculation in order to determine accurate dose rates. This seems to affect most, if not all the recommended doses of currently registered oxytetracycline formulations, especially those registered as long-acting formulations.

If South African MIC₉₀ values for some pathogenic bacteria are considered (refer to table 2.4), the efficacy of oxytetracycline is in a worse state than suggested by Schifferli's work done in 1982. At 0.5 µg/ml ((once again excluding factors that may negatively influence drug performance as suggested by Schifferli *et al.* (1982)), oxytetracycline would be effective against only 8.3% of *Escherichia coli* isolates, 16.6% effective against *Enterococcus faecium*, 4.1% effective against *Salmonella enterica* and 70% effective against *Mannheimia haemolytica*. 68% of *Staphylococcus aureus* isolated from cows' milk would be killed, as would 64.9% of beta-haemolytic streptococci. That being said, the MIC₉₀ of a drug includes resistant strains of which even one strain can skew the results. MIC₅₀ values for the drug may be less ominous than MIC₉₀ values. More research with regard to the correlation of drug plasma concentrations and drug concentration at the biophase is necessary.

5.3. T_{1/2λ}

The time taken for each treatment to decrease its initial concentration by half is almost identical for both treatments ((33.9 hours for the 10mg/kg treatment (but with almost twice the variance of the 20 mg/kg treatment) and 33.3 hours for the 20 mg/kg treatment)). The difference is not statistically significant. The data supports the assumption that, when the formulation is used at proportional doses, the T_{1/2λ} would not differ significantly.

5.4. SAFETY

The C_{max} attained with a single intramuscular injection at 20 mg/kg dose of a conventional formulation of oxytetracycline averaged 6.59 μ g/ml in this study. This is well below the C_{max} 's achieved with conventional formulations administered intravenously (Refer to table 2.1). Although the intramuscular and intravenous routes cannot be compared, the difference in blood concentrations between the two routes show that overdosing with this regime (double dose of a conventional formulation) is highly improbable and due to this fact, the possible nephrotoxic effects of oxytetracycline due to the catabolic effect of the drug (refer to 2.5) are negated because massive doses of oxytetracycline are necessary to precipitate nephrotoxicity. Calcium chelation is a possibility with the intravenous use of oxytetracycline, and is not relevant with intramuscular treatment regimes. Tissue irritation with the intramuscular use of a conventional oxytetracycline formulation is perceivably less compared with formulations containing long-acting solvents (refer to 2.5). However, due to the higher volume of drug administered (due to the lower concentration of active ingredient in conventional formulations), more than one injection site should be used during administration. This is good practice with any parenterally administered drug and should not be perceived as a disadvantage.

CHAPTER 6. CONCLUSIONS

Based strictly on drug plasma concentrations, the results of this study show that the 20 mg/kg dose reaches effective concentrations for longer than the 10 mg/kg dose. The difference in time above 0.5 µg/ml and the difference in AUC between the two treatments were statistically significant. The author is convinced that in a clinical situation, the extra 13.2 hours above 0.5 µg/ml attained with the 20 mg/kg dose will be even more biologically significant both in terms of convenience to the clinician, as well as limitation of the stress of handling inflicted upon an already compromised patient.

It should be borne in mind that the 0.5 µg/ml MIC for oxytetracycline against most pathogens dates back to 1982. The latest South African MIC values for oxytetracycline against selected pathogens show that dosage rates based on the 0.5 µg/ml value may not be clinically successful anymore, especially when considering the Schifferli *et al.* (1982) principle which suggests much lower drug concentrations at the bio-phase compared with drug concentrations in the plasma. Compared with a long-acting formulation, a once-off, double dose treatment with a conventional formulation would be superior in terms of efficacy when used against a resistant bacterial population due to a significantly higher C_{max}. Due to reasons discussed under point 5.4 above, this treatment regime is deemed safe in target species.

The objective of this study was fulfilled by showing that the administration of a single, 20 mg/kg dose of a conventional oxytetracycline formulation is an effective and safe alternative treatment regime to either a single injection of a long-acting formulation at 20 mg/kg, or multiple injections of a conventional formulation at 10 mg/kg each.

Studies to aid in further optimization of oxytetracycline treatment regimes in sheep may include a comparative study where plasma concentrations attained by a long-acting oxytetracycline formulation are compared with those attained by a conventional oxytetracycline formulation. Both formulations should be administered intramuscularly at

20 mg/kg as a single dose, using sheep of similar ages and weights, of the same gender and kept under identical conditions.

CHAPTER 7. REFERENCES

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APPENDIX 1:

**Validation of the Analytical Procedure for the Determination of Oxytetracycline in
Ovine Plasma.**