Is the current dose of a conventional oxytetracycline formulation adequate for the management of infections in sheep?

M G Snyman[a], V Naidoo[a*], C de Bruin[b] and G E Swan[a]

ABSTRACT

In the veterinary industry, short-acting or conventional oxytetracycline formulations are recommended for use once a day for 4 days, at a dose of 10 mg/kg. With the large degree of antimicrobial resistance reported, the efficacy of this dose was assessed using pharmacodynamic modelling. The specific parameters evaluated were based on the time-dependent activity of the tetracycline class of antimicrobials according to the total time above minimal inhibitory concentration (T > MIC) and the ratio of the total exposure in 24 hours, represented by area under the curve (AUC_{24}), to the minimal inhibitory concentration (AUC_{24}:MIC). The current pharmacokinetic study examined whether the prevailing antimicrobial resistance could be overcome by doubling the recommended conventional dose. Using reported MIC data for South Africa and elsewhere, modelling indicated the presence of a large degree of resistance. In general, doubling the dose only overcame resistance of 2 bacterial species in South Africa.

Key words: conventional formulation, oxytetracycline, pharmacodynamic, pharmacokinetic, sheep.

Snyman M G, Naidoo V, de Bruin C, Swan G E **Is the current dose of a conventional oxytetracycline formulation adequate for the management of infections in sheep?** *Journal of the South African Veterinary Association* (2008) 79(4): 171–174 (En.). Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa

INTRODUCTION

Antimicrobials of the tetracycline group including oxytetracycline (4S,4aR,5S,5aR, 6S,12aS-4-dimethylamino-1,4,4a,5,5a,6, 11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methylene-1,11-dioxonaphthacene-2-carboxamide;5β-hydroxytetracycline) are amongst the most widely used antimicrobials in production animal medicine, as a result of their broad spectrum and favourable pricing in the market[1,9,10]. As a class the tetracyclines impede bacterial protein synthesis at the 30S subunit of the ribosome, are generally considered bacteriostatic agents and function in a time-dependent manner[3]. Tetracyclines are only effective in stopping bacterial multiplication via protein synthesis inhibition if the total period of exposure is sufficient[3].

At present 2 types of over-the-counter (OTC) formulations (conventional and long-acting) are available for use in South Africa. For the conventional formulations the recommended dose is 10 mg/kg for a minimum of 4 days while the long-acting formulations are used at 20 mg/kg as a once-off treatment. One of the problems with this dosing regimen is the historic nature of the dose. In the past, a serum concentration of 0.5 μg/m was generally accepted as the minimum inhibitory concentration (MIC) of oxytetracycline[4,6,11]. This historic breakpoint MIC fails to consider the degree of resistance in South Africa, which is unfortunately largely unknown. If the recent survey by van Vuuren *et al.*[12] is any indication, a large degree of resistance is already present. It was reported that only 8, 10.5 and 30 % of *Escherichia coli, Salmonella enterica* and *Enterococcus faecium,* respectively, were responsive to a MIC of 0.5 μg/m oxytetracycline[12]. This implies that a large proportion of animals may be exposed to ineffective therapy.

It is therefore necessary to ascertain whether the current dosage regimen of oxytetracycline is still effective. Instead of performing clinical studies, it has become more common to examine the pharmacokinetic-pharmacodynamic (PK/PD) relationship as a means of establishing the optimum treatment regime. In this method the *in vivo* pharmacokinetics (PK) achieved following dosing is related to the MIC obtained *in vitro*[9]. For the tetracyclines the major pharmacodynamic (PD) parameter that correlates clinical/bacteriologic efficacy is the AUC_{24}:MIC ratio (AUC_{24} represents a 24 hour area under the serum drug concentration curve) and the time above the MIC (T > MIC). The primary objective of this study was to compare the serum concentration profiles of a single dose of a conventional oxytetracycline formulation (reference dose) with a double dose (test dose) of the same formulation and to determine whether a better PK/PD relationship can be achieved at the higher dose to overcome resistance.

MATERIALS AND METHODS

Pharmacokinetic study

Animal husbandry and treatment. Ten healthy, 3-year-old Dohne-Merino wethers (45.5 to 62 kg) were used.

Experimental animals were subjected to clinical and clinical pathological examination in order to ensure that only healthy animals were used in the study. Animals were allowed an adaptation period of 2 weeks in 2 separate roofed pens of approximately 8 m^2 each, prior to the start of the study. Animals were maintained on commercial grower pellets (Monti Foods complete ruminant feed; Registration No. V11966) confirmed free of oxytetracycline, with water being available *ad lib.*

The study was performed using a randomised, 2-treatment, 2-sequence cross-over experimental design, with a washout period of 7 days between the phases. Animals were treated with oxytetracycline (Ecomycin Dual Purpose 13.5 % m/v, Afrivet) in 2 groups (n = 5) at a dose of 10 and 20 mg/kg. Blood samples were collected from the jugular vein into 10 m lithium heparinised glass vacutainer tubes with 19 G disposable needles prior to dosing and at 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24, 36, 48, 72 and 96 hours (h) after dosing. After collection, samples were centrifuged for 15 minutes at 3000 rpm. The plasma was subsequently separated from the cellular component and stored at –15 °C until analysed.

Analysis of oxytetracycline concentrations in plasma. For the analysis, 1000 μl samples were pipetted into micro test tubes with

[a]Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.
[b]Berea Veterinary Clinic, 12 Scherwitz Road, Berea, East London, 5214 South Africa.
*Author for correspondence.
E-mail: vinny.naidoo@up.ac.za
Received: July 2008. Accepted: October 2008.

100 μl of doxycycline as internal standard. To this mixture, 250 μl of 15 % tetrachloroacetate was added and the sample vortexed for 15 seconds. The tubes were then incubated in an ice bath. The supernatant was used in all subsequent analyses.

A Beckman System Gold HPLC consisting of an autosampler module 508, programmable solvent module 126, diode array detector module (DAD) 168, and System Gold™ software package, was used (Beckman Instruments, Fullerton, California, USA). Separation was achieved with a Lunar column. The mobile phase consisted of 600 m 0.05 M potassium di-hydrogen phosphate, 200 m methanol and 200 m acetonitile. One hundred microlitres of the samples were injected onto the HPLC column at 1 m /min in an isocratic run. 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 lower limit of quantification (LLQ) was established at 300 ng/m and a linear relationship between concentration and peak area was demonstrated for the total concentration range between 0.1 and 20 μg/m .

Pharmacokinetic model. Non-compartmental analysis of the plasma concentration *versus* time data of oxytetracycline for extravascular input (Model 200) was performed using WinNonLin Version 5.1 (Pharsight corporation). The area under the plasma concentration *versus* time curve (AUC, zero-moment) and the 1st non-normalised moment (AUMC) were calculated according to the trapezoidal method from time zero to the last sample time[7]. Extrapolation of AUC to infinity (AUC_{inf}) was performed using the slope of the terminal phase (β). The mean residence time (MRT, 1st moment) was derived from AUC/AUMC. Maximum plasma concentration (C_{max}) and time to C_{max} (T_{max}) were read directly from the individual plasma concentrations.

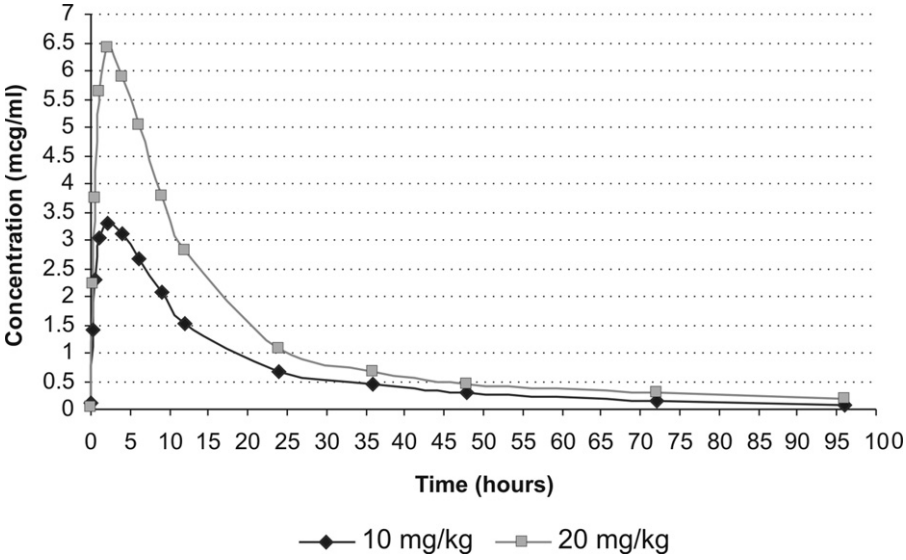

Fig. 1: **Mean oxytetracycline plasma levels generated by the intramuscular injection of a conventional oxytetracycline formulation at 10mg/kg** (reference dose) **and 20mg/kg** (test dose) **in sheep.**

Pharmacodynamic analyses

To ascertain the efficacy of the product, the ratio of AUC truncated to the first 24 hours (AUC_{24}) to MIC was also established, as recent reports suggest that a ratio of AUC_{24}:MIC above 25 is indicative of efficacy of tetracycline[2]. For the purpose of comparison the South African and international MICs were used[8,12]. The time above MIC (T > MIC) was also determined. Although not specifically used as a parameter for the tetracyclines evidence tends to suggest that the tetracyclines have a similar PK/PD relationship to the penicillins[5]. For purposes of efficacy it has been established that the T > MIC needs to be maintained for 50 % of the dosing interval[2].

RESULTS AND DISCUSSION

The mean oxytetracycline plasma concentration *versus* time profile and mean pharmacokinetic parameters for the 10 mg/kg and 20 mg/kg doses are presented in Fig. 1 and Table 1, respectively.

The AUC_{inf}, AUC_{last} and C_{max} for the 20 mg/kg dose were approximately twice that of the 10 mg/kg dose, indicating linearity in the extent of absorption and an expected greater exposure to oxytetracycline at the higher dose. Furthermore, the similarity in the terminal half-life between the doses suggests that the rate of absorption was not affected by the larger dose volume associated with the higher dose.

When the T > MIC was determined against the historic plasma concentration of 0.5 μg/m , the 10 mg/kg dose would have a duration of action of at least 24 hours, while at 20 mg/kg dose the duration will be 38 hours. For time-dependent

Table 1: **Individual and mean pharmacokinetic results of the reference** (10 mg/kg) **and test** (20 mg/kg) **treatments administered intramuscularly in sheep.**

Animal	AUC_{inf} (µg/m *h)		AUC_{last} (µg/m *h)		C_{max} (µg/m)		T_{max} (h)		T½λ (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
1	78.67	133.20	53.90	107.20	2.94	6.63	2.00	2.00	38.60	36.17
2	65.97	130.90	57.66	125.90	3.89	6.75	4.00	2.00	18.69	15.04
3	78.88	99.90	53.62	92.02	3.82	5.96	2.00	2.00	45.60	45.68
4	62.49	108.50	56.91	100.40	4.70	6.19	4.00	2.00	54.23	31.52
5	95.77	133.80	70.70	122.00	3.36	6.29	0.50	2.00	33.09	30.14
6	84.37	153.70	66.08	124.20	3..2	7.62	4.00	4.00	51.37	43.93
7	87.23	138.30	76.91	109.70	3.18	6.52	2.00	4.00	23.22	32.89
9	55.09	110.50	51.12	103.50	3.88	6.25	1.00	2.00	15.48	30.03
10	59.69	114.50	53.68	107.30	2.79	7.65	1.00	2.00	24.96	40.56
12	59.96	112.40	54.08	107.00	4.17	6.21	6.00	2.00	14.15	26.73
Mean	72.81	123.57	59.47	109.92	3.78	6.61	2.65	2.40	31.94	33.27
SD	13.92	16.80	8.70	10.96	0.63	0.59	1.76	0.84	14.89	8.99

Table 2: **South African MIC_{90} values of susceptible strains of pathogenic bacteria compared with the T > MIC[12].**

Pathogenic bacteria	MIC_{90} (g/m)	T > MIC		AUC:MIC	
		10 mg	20 mg	10 mg	20 mg
Escherichia coli	128	0	0	0.34	0.62
Salmonella enterica	64	0	0	0.67	1.23
Salmonella typhimurium	128	0	0	0.34	0.62
Mannheimia haemolytica	64 (MIC_{80} = 1)	0	0	0.67	1.2
Staphylococcus aureus	2	10	18[a]	21.5	39.5[a]
Beta-haemolytic streptococci including *S. agalactiae* and *S. dysgalactiae*	2	10	18[a]	21.5	39.5[a]

[a] Represents species that are considered responsive to oxytetracycline according to current pharmacodynamic rules.

Table 3: **Pharmacodynamic parameters compared with the MIC as published in *Antimicrobial Therapy in Veterinary Medicine*[8].**

Bacteria		T > MIC		AUC_{24}:MIC	
	MIC*	10 mg/kg	20 mg/kg	10 mg/kg	20 mg/kg
Actinomyces spp.	1	18[a]	24[a]	43[a]	79[a]
Arcanobacterium pyogenes	16	0	0	2.6875	4.9375
Bacillus anthracis	4	0	7	10.75	19.75
Clostridium perfringens	32	0	0	1.34375	2.46875
Corynebacterium renale	4	0	7	10.75	19.75
Campylobacter fetus	2	8	17[a]	21.5	39.5[a]
Campylobacter jejuni	>64	0	0	<0.66	<1.22
Clostridium spp.	8	0	0	5.375	9.875
Corynebacterium pseudotuberculosis	<25	>24	>24	>172	>316
Erysipelothrix rhusiopathiae	0.25	48[a]	72[a]	172[a]	316[a]
Escherichia coli	>64	0	0	<0.67	<1.23
Fusobacterium necrophorum	4	0	5	10.75	19.75
Histophilus somni	2	8	17[a]	21.5	39.5[a]
Leptospira spp.	4	0	5	10.75	19.75
Listeria monocytogenes	1	18[a]	24[a]	43[a]	79[a]
Mycoplasma ovipneumoniae	0.5	28[a]	48[a]	86[a]	158[a]
Mannheinemia haemolytica	>16	0	0	<2.68	<4.93
Mycoplasma agalactiae	0.5	28[a]	48[a]	86[a]	158[a]
Salmonella spp.	>16	0	0	<2.69	<4.94
Staphylococcus aureus	>64	0	0	<0.67	<1.23

*The MIC listed are not specific to sheep, but represent reported MIC to oxytetracycline.
[a]Represents species that are considered responsive to oxytetracycline according to current pharmacodynamic rules.

antimicrobials this would relate to a higher efficacy. These results indicate that at a microbial sensitivity of 0.5 μg/m , the recommended dose of 10 mg/kg once a day for 4 days would be adequate and that at the higher dose of 20 mg/kg, only 2 treatments with an interval of 48 h would most likely suffice.

However, the MIC of 0.5 μg/m plasma for oxytetracycline is historic and fails to consider current levels of resistance. Table 2 shows South African MIC_{90} values of susceptible strains of pathogenic bacteria compared with the T > MIC[12].

Using actual MIC values, both *E. coli* and *Salmonella* spp. showed complete resistance, while the MIC required for mastitic strains of staphylococci and streptococci shows efficacy against these bacteria of 10 and 18 hours for the 10 and 20 mg/kg doses, respectively (Fig. 1). With the tetracyclines being similar to the penicillins in PD responsiveness, the plasma concentrations achieved needed to be above MIC levels for 50 % of the dosing interval. If this were so the double dose would be able to overcome the resistance shown by the latter organisms if administered daily, while the 10 mg/kg dose would be ineffective. A similar trend was seen when evaluating the AUC_{24}:MIC.

Table 3 shows the MICs published for other organisms, and the pharmacokinetic parameters of both the 10 mg/kg and 20 mg/kg doses are compared with these MICs.

When evaluating efficacy values for other organisms, a large degree of resistance is once again evident for the 10 mg/kg dose, with only *Erysipelothrix rhusiopathiae, Listeria monocytogenes, Actinomyces* spp. and *Mycoplasma* spp. being responsive. It is evident regarding the South Africa strains that increasing the dose increases efficacy marginally *viz.* the increase in dose would allow for additional efficacy against *Campylobacter fetus* and *Histophilus somni.*

CONCLUSION

Based on drug plasma concentrations, the results of this study show that the 20 mg/kg dose would be more effective than the 10 mg/kg dose. For the specific organisms discussed above, resistance may be overcome by increasing the dose of oxytetracycline. A change in dose of a drug when used in production animals represents extra-label use, and consequently a new withdrawal period will have to be set by the clinician.

REFERENCES

1. Adams H R 1995. *Veterinary pharmacology and therapeutics* (7th edn). Iowa State University Press, Ames
2. Agwuh K N, MacGowan A 2006 Pharmacokinetics and pharmacodynamics of tetracyclines including glycylcyclines. *Journal of Antimicrobial Chemotherapy* 58: 256–265
3. Boothe D M, 2001 Antimicrobial Drugs. In Kersey R, Kilmer (eds) *Small animal clinical pharmacology and therapeutics* (1st edn). W B Saunders, Philadelphia: 150–173
4. Davey L A, Ferber M T, Kaye B, 1985 Com-

parison of the serum pharmacokinetics of a long acting and conventional oxytetracycline injection. *Veterinary Record* 117: 426–429
5. Jacobs M R 1999 Pharmacodynamic approach to antimicrobial treatment for respiratory infections Online at: www.ansorp.org/isaar/1999/symposium/11_Jacobs.pdf (accessed June 2006)
6. Kumar R, Malik J K 1998 Some pharmacokinetic parameters and dosage regimes for a long-acting formulation of oxytetracycline in 6–8-month-old male calves. *Veterinary Research Communications* 22: 533–544
7. Gibaldi M, Perrier D 1982 *Drugs and pharmaceutical sciences in pharmacokinetics* (2nd edn). M Marcel Dekker, New York: 145–198
8. Giguere S, Prescott J F, Baggot J D, Walker RD, Dowling P M 2006 *Antimicrobial therapy in veterinary medicine* (4th edn). Blackwell Publishing, Oxford, UK
9. Reviere J E, Spoo J 1995 Tetracycline/aminoglycocide antibiotics. In Adams H R, (ed.) *Veterinary pharmacology and therapeutics* (7th edn). Iowa State University Press, Ames: 797–813
10. Schifferli D, Galleazzi R L, Nicolet J, Wanner M 1982 Pharmacokinetics of oxytetracycline and therapeutic implications in veal calves. *Journal of Veterinary Pharmacology and Therapeutics* 5: 247–257
11. Toutain P L, Raynuad J P 1983 Pharmacokinetics of oxytetracycline in young cattle: comparison of conventional vs long-acting formulations. *American Journal of Veterinary Research* 44: 1203–1209
12. Van Vuuren M, Picard J, Greyling J 2007 *SANVAD (South African National Veterinary Surveillance and Monitoring Programme for Resistance to Antimicrobial Drugs).* ISBN: 978-1-86854-673-2