AN ABSTRACT OF THE DISSERTATION OF

Triporn Wattananat for the degree of Doctor of Philosophy in Pharmacy presented on December 1, 2003.

Title: Pharmacokinetic Analysis of Antimicrobials and an Anthelmintic Agent in Alpacas and Llamas with Theoretical Applications.

Abstract approved:

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J. Mark Christensen

The pharmacokinetics of two antimicrobials were investigated in alpacas. Six healthy alpacas were each administered a single dose of 10 mg/kg of oxytetracycline by IV injection and IM injection. In addition, a single dose of 20 mg/kg of florfenicol by IV administration was given to alpacas in a separate study. The pharmacokinetic parameters of oxytetracycline and florfenicol in alpacas were compared to the results previously obtained in llamas. There were significant differences between llamas and alpacas in several of oxytetracycline pharmacokinetic parameters but there were no significant differences in all of florfenicol pharmacokinetic parameters in these two animals. It can be concluded that llamas and alpacas have different oxytetracycline disposition kinetics while they have similar disposition kinetics of florfenicol.

The pharmacokinetics of clorsulon, a narrow-spectrum anthelmintic agent, was investigated in llamas following oral administration at a single dose of 14 mg/kg. The plasma levels of clorsulon produced by this dose was lower than the values reported in the clorsulon pharmacokinetic studies carried out in sheep and goats following oral administration at a single dose of 7 mg/kg. This suggests the entire dose of clorsulon is not

absorbed in llamas.

Since the differential equations describing one-compartment system with first-order input and two-compartment system after IV administration with nonlinear elimination kinetics cannot be solved, there is no mathematical expression for the AUC for drugs following these models. The AUC values calculated from the proposed preliminary AUC equations for drugs following these models were compared to the AUC calculated using the trapezoidal rule method based on computer-generated data using the fourth-order Runge-Kutta method. Except for a few exceptions, the predicted AUC from the proposed equations matched the values calculated from the theoretically generated data.

Pharmacokinetic Analysis of Antimicrobials and an Anthelmintic Agent in Alpacas and Llamas with Theoretical Applications

by

Triporn Wattananat

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Triporn Wattananat, Author

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CONTRIBUTION OF AUTHORS

Dr. J. Mark Christensen was involved in all aspects of this work. Nancy Hollingshed assisted with providing the alpacas and llamas, administering the drug and supervising the blood sampling process.

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PHARMACOKINETIC ANALYSIS OF ANTIMICROBIALS AND AN ANTHELMINTIC AGENT IN ALPACAS AND LLAMAS WITH THEORETICAL APPLICATIONS

CHAPTER 1

INTRODUCTION

The primary goal of pharmacokinetics is to generate the mathematical parameters that quantitate physiological processes as an aid to better understand a drug's disposition in the body. Based on the parameters obtained, the proper dosage regimen for each animal species can be determined.

Information of pharmacokinetic of antimicrobials in alpacas is very limited so the appropriate dosage regimen of antimicrobials in alpacas has not been defined yet. Drug dosing for the treatment of bacterial infections in alpacas are frequently based on dosing used in ruminants.

Chapter 2 presents the pharmacokinetics of oxytetracycline which is a widely used broad spectrum antibiotic in veterinary medicine. The objective of this study is to investigate the pharmacokinetics of oxytetracycline in alpacas following intravenous and intramuscular administrations. The study was conducted in six healthy alpacas following a single administration of an intravenous injection of a solution of oxytetracycline and an intramuscular injection of a sustained action product of oxytetracycline at a dose of 10 mg/kg body weight. Both compartmental and noncompartmental analyses were performed to determine the pharmacokinetic parameters. In order to evaluate the absorption process that occurs after intramuscular injection of the sustained action oxytetracycline product, deconvolution and Loo-Riegelman approaches were utilized. The proper dosage regimens were proposed based on the pharmacokinetic parameters obtained in conjunction with the minimum inhibitory concentration of oxytetracycline against commonly known pathogens.

Chapter 3 contrasts the pharmacokinetics of oxytetracycline in alpacas to llamas following intravenous and intramuscular administration at a single dose of 10 mg/kg. The pharmacokinetic parameters obtained from chapter 2 were compared to the results previously obtained in llamas and determined if the two animals handle oxytetracycline in the same manner.

Chapter 4 presents the pharmacokinetics of florfenicol in alpacas following intravenous administration of a single dose of 20 mg/kg. The study was carried out on six healthy alpacas after intravenous administration. Both compartmental and noncompartmental analyses were performed to determine the pharmacokinetic parameters. The proper dosage regimen for florfenicol in alpacas was proposed based on the pharmacokinetic parameters obtained to maintain the minimum inhibitory of florfenicol against pathogenic bacteria.

Chapter 5 is the comparison of pharmacokinetic parameters of florfenicol in alpacas with the results previously obtained in llamas and determines if the disposition kinetics of florfenicol in these two animals is similar.

Chapter 6 presents the pharmacokinetics of clorsulon which is a narrow-spectrum anthelmintic agent. Clorsulon was approved by the US Food and Drug Administration for the treatment of liver flukes in cattle at the recommended dose of 7 mg/kg. The preliminary efficacy study of clorsulon in llamas showed that clorsulon at the recommended dose was not effective against liver flukes. This clorsulon pharmacokinetic study was conducted in five healthy llamas following oral administration at a single dose of 14 mg/kg. Both compartmental and noncompartmental analyses were performed to determine the pharmacokinetic parameters.

Chapter 7 presents the evaluation of preliminary AUC equations of drugs following one-compartment system with first-order input and two-compartment system after IV administration with nonlinear elimination kinetics. The plasma concentration-time curves of drugs following these models were generated using the fourth-order Runge-Kutta method. The AUC value calculated from the preliminary equations were compared to the value calculated using trapezoidal rule method.

CHAPTER 2

PHARMACOKINETICS OF OXYTETRACYCLINE IN ALPACAS AFTER INTRAVENOUS AND LONG-ACTING INTRAMUSCULAR ADMINISTRATION

Triporn Wattananat, J. Mark Christensen, and Bradford B. Smith

ABSTRACT

Oxytetracycline is a widely used broad spectrum antibiotic for the treatment of various infections in veterinary medicine. The purpose of this study was to investigate the pharmacokinetics of oxytetracycline in alpacas following intravenous and intramuscular administration at a single dose of oxytetracycline at a dosage of 10 mg/kg body weight. After the IV dose, the plasma concentration-time curves were best fitted to a three-compartment open model. The apparent volume of distribution of the central compartment (Vc) was 0.16 ± 0.05 L/kg, while the volume of distribution at steady state (Vss) was 1.24 ± 0.16 L/kg. The harmonic mean elimination half-life was 8.88 ± 1.43 hours. The total body clearance was 0.13 ± 0.02 L/kg/h. After IM administration, a two-compartment open model with two parallel first-order input functions best described the plasma concentration-time profiles. The rapid absorption rate constant was 0.36 ± 0.17 min^{-1} (21.5 ± 10.0 h^{-1}) while the slow absorption rate constant was 0.0003 ± 0.0001 min^{-1} (0.016 ± 0.003 h^{-1}). According to the pharmacokinetic parameters obtained, the proper dosage regimen for alpacas is 8 mg/kg every 12 hours with IV injection and an initial loading dose of 20 mg/kg and a maintenance dose of 10 mg/kg every 48 hours with LA-IM formulation will achieve a trough concentration of 2.26 and 3.04 μg/mL, respectively, at steady state. These dosing schedules will allow plasma concentrations above the MIC of 2 μg/mL for most microorganisms.

INTRODUCTION

Various bacterial infections in alpacas have been reported in the literature. Those diseases include Alpaca fever, anthrax, brucellosis, blackleg, botryomycosis, John's disease, meningoencephalitis, etc., as shown in Table 2.1 (Thedford and Johnson, 1989; Fowler, 1998).

Tetracycline was discovered during the systematic screening of microorganisms obtained from soil specimens for antimicrobial properties. The tetracycline group of antibiotic includes chlortetracycline and oxytetracycline which are naturally produced by *Streptomyces aureofaciens* and *Streptomyces rimosus*, respectively, whereas doxycycline, minocycline, methacycline, tetracycline, rolitetracycline, and demeclocycline are semisynthetic derivatives (Hardman and Limbird, 1999). The hydronaphthacene nucleus containing four fused 6-membered rings forms the basic structure from which the various tetracyclines are made. Their structural formulas are shown in Figure 2.1 and Table 2.2.

Tetracycline antibiotics have a broad spectrum of activity against aerobic and anaerobic gram positive and gram negative bacteria, including *Rickettsia*, *Coxiella burnetii*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Chlamydia*, *Staphylococcus aureus*, *Bacillus anthraces*, and *Neisseria spp.* The lipophilic congeners, minocycline and doxycycline are more active than the hydrophilic ones such as oxytetracycline, tetracycline, and demeclocycline (Lambert and O'Grady, 1992).

The bacteriostatic action of tetracyclines is due to their ability to inhibit bacterial protein synthesis. By binding to the 30S ribosomal subunit, tetracyclines prevent access of t-RNA to the acceptor (A) site on the m-RNA ribosome complex, so that the elongation of the amino acid chain during protein synthesis in bacteria is terminated (Hardman and Limbird, 1999). This bacteriostatic effect of tetracycline becomes cidal at extremely high concentrations (Bush *et al.*, 2000).

Tetracyclines are generally regarded as relatively safe, but they produce a fairly large number of adverse effects which may be related to their severely irritant nature (gastrointestinal disturbance, nausea, vomiting after oral administration, tissue damage at injection site) and toxic effects on liver and kidney cells (Prescott, 2000). Stevenson (1980) reported acute tubular necrosis in two dogs after being given an intravenous

overdose of oxytetracycline (130 mg/kg body weight). Severe tubular damage (a Fanconi-like syndrome) may occur as a result of the toxic degradation products, anhydrotetracycline and epianhydrotetracycline (Prescott, 2000; Stevenson, 1980; Moalli *et al.*, 1996; Alexander *et al.*, 1984). Nephrotoxicosis was also reported in feedlot calves (Alexander *et al.*, 1984) and neonatal foal (Vivrette *et al.*, 1993). Severe damage of structure and function of the small intestine and excessive accumulation of fat in the liver have been reported in rats treated for 3 days or longer with high doses of oxytetracycline (400 mg/kg body weight) (De Jonge, 1973).

In addition, increased sensitivity of the skin to sunlight and superinfection may occur with tetracyclines, particularly with tetracyclines that are poorly absorbed when given orally. The use of tetracycline is not recommended during pregnancy or breast feeding since it may cause permanent discoloration of the teeth and may slow the growth of the infant's teeth and bones as tetracyclines pass into breast milk.

Oxytetracycline was isolated from actinomycete, *Streptomyces rimosus* and is present in drug products as either the amphoteric base compound, the hydrochloride salt, or as a quaternary ammonium salt complex. The activity of oxytetracycline against common pathogenic bacteria is presented in Table 2.3 (Lambert and O'Grady, 1992).

In human plasma, 20-35% of oxytetracycline is protein bound (Lambert *et al.*, 1992) and it distributes widely throughout the body into tissues and body fluids including sputum, urine, peritoneal and pleural fluids. The primary route of elimination for oxytetracycline is the kidney via glomerular filtration as parent drug (Hardman and Limbird, 1999; Lambert and O'Grady, 1992).

Table 2.1 Bacterial diseases in alpacas.

Disease	Bacteria	Clinical signs
Alpaca fever	*Streptococcus zooepidimicus*	Anorexia, recumbency, and fever as high as 41.2°C (102.6°F)
Anthrax	*Bacillus anthraces*	Anorexia, stomachstasis, colic, hematuria, and hemorrhagic diarrhea
Brucellosis	*Brucella melitensis*	Abortion
Blackleg	*Clostridium chauvei*	High fever, serohemorrhagic swellings, gas formation in the heavy muscles of the body and limbs
Botryomycosis	*Staphylococcus aureus*	Dermal abscesses
Leptospirosis	*Leptospira spp.*	Hemolytic anemia
Colibacillosis	*Escherichia coli*	Diarrhea, weight loss, abdominal distention, no fever, pica, and debility
John's disease	*Mycobacterium paratuberculosis*	Weight loss, poor growth, hypoproteinemia, and terminally diarrhea
Meningoencephalitis	*Escherichia coli*	Weakness, lethargy, neurological signs consisting of inability to stand, opisthotonus, and depression
Enterotoxemia Type A	*Clostridium perfringens*	Recumbency with the head stretched forward, eyes closed, ears directed backward

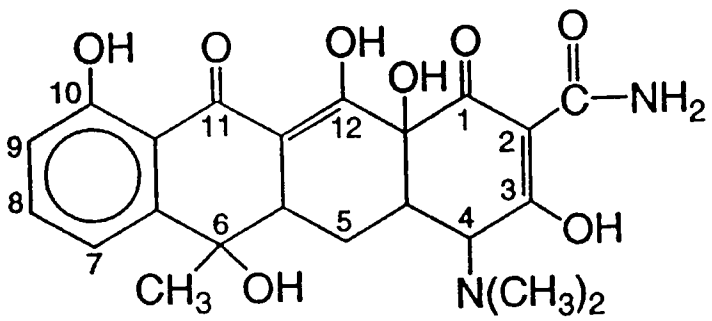

Figure 2.1 Chemical structure of tetracycline.

Table 2.2 Structural formulas of the tetracyclines.

Congener	Substituent(s)	Position(s)
Chlortetracycline	— Cl	(7)
Oxytetracycline	— OH, — H	(5)
Demeclocycline	— OH, — H; — Cl	(6; 7)
Methacycline	— OH, — H; $=CH_2$	(5; 6)
Doxycycline	— OH, — H; $-CH_3$, — H	(5; 6)
Minocycline	— H, — H; $-N(CH_3)_2$	(6; 7)

Table 2.3 Susceptibility of common pathogenic bacteria to oxytetracycline (MIC: μg/mL).

Bacteria	MIC (μg/mL)
Staphylococcus aureus	2-8
Streptococcus pyogenes	0.25-1
Streptococcus pneumoniae	0.1-0.25
Neisseria gonorrheae	1-2
Haemophilus influenzae	4-16
Escherichia coli	2-16
Klebsiella pneumoniae	16-128
Enterobacter	8-Resistant
Pseudomonas aeruginosa	64-128
Bacillus fragilis	0.5-64

Pharmacokinetic studies of oxytetracycline have been conducted in many animal species such as goats, sheep, camels, llamas, dogs, rats, hens, cattle, etc. The pharmacokinetic parameters reported in those animals are listed in Table 2.4.

Interspecies allometric analysis of 44 drugs across various animal species by Riviere *et al.* (1997) showed the relationship between elimination half-life of oxytetracycline and body weight by the allometric equation $HL = 2.572W^{0.227}$ with the correlation coefficient (R^2) of 0.74 where HL is the half-life in hours and W is the body weight in kilograms (kg). In addition, Baggot (2001) reported the allometric relationship between clearance of oxytetracycline and body weight of various mammalian species as $CL = 7.96W^{0.73}$ with R^2 equals to 0.978 where CL is the clearance in mL/min. The values of half-life and clearance of oxytetracycline predicted from these equations are 6.66 hours and 2.56 mL/kg/min, respectively, as determined by the mean body weight of alpacas of 66 kg.

Table 2.4 Pharmacokinetic parameters of oxytetracycline in some animal species.

Animals	Goats[1]	Goats[1]	Sheep[2]	Sheep[2]	Sheep[3]
Dose (mg/kg)	10	20	20	20	20
Route	IV	IM (LA)	IV	IM (LA)	IM (LA)
Parameters					
Ka (h^{-1})		0.51 ± 0.05			2.41 ± 2.35
λz (h^{-1})	0.11 ± 0.00	0.02 ± 0.00	0.21 ± 0.01	0.05 ± 0.004	0.03 ± 0.02
$t_{1/2, Ka}$ (h)		1.42 ± 0.17			0.67 ± 0.48
$t_{1/2, \lambda z}$ (h)	6.46 ± 0.24	28.0 ± 2.81	3.29*	14.1*	28.0 ± 9.10
MRT (h)	7.90 ± 0.30	37.5 ± 3.49	4.99 ± 0.27	23.0 ± 1.86	35.9 ± 9.71
CL_T (L/kg/h)	0.16 ± 0.00	0.19 ± 0.01	1.30 ± 0.01		0.18 ± 0.03**
tmax (h)		3.20 ± 0.23		3.03 ± 0.48	1.79 ± 0.61
Vss (L/kg)	1.23 ± 0.03		0.78 ± 0.02		
Vz (L/kg)	1.44 ± 0.04				7.01 ± 1.72
Vc (L/kg)	0.45 ± 0.01		0.20 ± 0.01		
F (%)		79.4		93 ± 4	

* = harmonic mean; ** = CL/F (L/kg/h)

1=(Escudero *et al.*, 1994); 2=(Moreno *et al.*, 1998); 3=(Escudero *et al.*, 1996)

Table 2.4 (Continued)

Animals	Llamas[4]	Llamas[4]	Camels[5]	One-humped camels[6]	One-humped camels[6]
Dose (mg/kg)	10	10	3	5	10
Route	IV	IM (LA)	IM	IV	IM (LA)
Parameters					
Ka (h^{-1})		7.14 ± 6.3	4.21 ± 0.50		
λz (h^{-1})	0.04 ± 0.02	0.02 ± 0.01	0.10 ± 0.01	0.09 ± 0.02	0.02 ± 0.01
$t_{1/2, Ka}$ (h)		0.22 ± 0.120	0.17 ± 0.02		
$t_{1/2, \lambda z}$ (h)	16.2 ± 6.24*	30.2 ± 9.15*	7.00 ± 0.35	7.7 ± 1.9*	31.3 ± 9.2*
MRT (h)	20.6 ± 6.30	47.8 ± 11.22		7.7 ± 2.8	26.6 ± 2.3
CL_T (L/kg/h)	0.04 ± 0.01	0.04 ± 0.02		0.08 ± 0.02	
tmax (h)		7.50 ± 3.67	1.10 ± 0.05		
Vss (L/kg)	0.82 ± 0.09			0.71 ± 0.17	
Vz (L/kg)		0.18 ± 0.73		0.82 ± 0.19	
Vc (L/kg)	0.11 ± 0.04			0.06 ± 0.04	
F (%)		97.8			93.7 ± 32.0

* = harmonic mean

4=(Al-Ghazawi, 1998); 5= (El-Gendi *et al.*, 1983); 6=(Oukessou *et al.*, 1992)

Table 2.4 (Continued)

Animals	Dairy cows[7]	Dairy cows[7]	Veal calves[8]	Veal calves[8]	Calves[9]
Dose (mg/kg)	5	5	40	20	40
Route	IV	IM	IV (LA)	IM (LA)	IM (LA)
Parameters					
Ka (h^{-1})		0.028		0.41 ± 0.14	0.75 ± 0.46
λz (h^{-1})	0.08 ± 0.02	0.077			0.029 ± 0.004
$t_{1/2, Ka}$ (h)		25		1.86 ± 0.52	2.2 ± 1.8
$t_{1/2, \lambda z}$ (h)	9.46 ± 1.40	9	93.3 ± 20.9	98.5 ± 24.3	23.9 ± 3.2
MRT (h)					
CL_T (L/kg/h)	0.09 ± 0.01		0.14 ± 0.15	0.13 ± 0.01	
tmax (h)				6.26 ± 0.96	
Vss (L/kg)					
Vz (L/kg)	0.92 ± 0.13		18.14 ± 4.52	18.5 ± 4.52	
Vc (L/kg)	0.12 ± 0.02				
F (%)				103.2 ± 9.4	

7=(Nouws *et al.*, 1985); 8=(Meijer *et al.*, 1993); 9=(TerHune and Upson, 1989)

Table 2.4 (Continued)

Animals	Calves[10]	Calves[10]	African elephant calves[11]	African elephant calves[11]
Dose (mg/kg)	20	20	18.2	18.2
Route	IV (LA)	IM (LA)	IV (LA)	IM (LA)
Parameters				
Ka (h^{-1})				
λz (h^{-1})			0.039 ± 0.003	0.023 ± 0.002
$t_{1/2, Ka}$ (h)		16.7 ± 1.04		
$t_{1/2, \lambda z}$ (h)	7.8 ± 0.4	24.4 ± 1.1	18.31 ± 1.3	30.9 ± 2.7
MRT (h)	11.2 ± 0.6	35.3 ± 1.5		
CL_T (L/kg/h)	0.076 ± 0.003	0.076 ± 0.003	6.17 ± 1.01	6.11 ± 0.74
tmax (h)		8.0 ± 0.4		
Vss (L/kg)	0.86 ± 0.07	2.7 ± 0.2		
Vz (L/kg)			2.69 ± 0.48	4.57 ± 0.88
Vc (L/kg)			2.41 ± 0.54	3.56 ± 2.22
F (%)		89.1 ± 4.2		

10=(Kumar and Malik,1998); 11=(Bush *et al.*, 2000)

Table 2.4 (Continued)

Animals	Cattle[12]	Cattle[12]	Buffalo[13]	Buffalo[13]	Horses[14]
Dose (mg/kg)	10	20	22	22	6.6
Route	IV	IM (LA)	IV	IM	IV
Parameters					
Ka (h^{-1})		0.864			
λz (h^{-1})	0.13 ± 0.03	0.032 ± 0.003		0.09-0.14***	0.11 ± 0.02
$t_{1/2, Ka}$ (h)		0.8	0.003-0.004***	0.042-0.066***	
$t_{1/2, \lambda z}$ (h)	5.88*	21.83*	2.82-3.61***	10.5-16.5***	6.08*
MRT (h)					7.27 ± 1.97
CL_T (L/kg/h)			0.061-0.087***		0.13 ± 0.02
tmax (h)					
Vss (L/kg)					0.95 ± 0.25
Vz (L/kg)	0.94 ± 0.18	0.94 ± 0.18	0.28-0.45***	1.18-2.15***	
Vc (L/kg)					
F (%)				51.0-76.5***	

* = harmonic mean; *** = reported in range

12=(Toutain and Raynaud, 1983); 13=(Varma and Paul, 1983); 14=(Dowling and Russell, 2000)

Table 2.4 (Continued)

Animals	Horses[14]	Rabbits[15]	Rabbits[15]	Pigs[16]	Pigs[17]
Dose (mg/kg)	6.6	10	10	10	20
Route	IM	IV	IM	IV	IM (LA)
Parameters					
Ka (h^{-1})	0.03 ± 0.012		0.32 ± 0.15		
λz (h^{-1})		0.53 ± 0.16			
$t_{1/2, Ka}$ (h)	23.15 ± 6.36		2.09 ± 0.92	0.118 ± 0.004	
$t_{1/2, \lambda z}$ (h)	22.08*	1.32 ± 0.40		5.86 ± 0.21	
MRT (h)	35.87 ± 9.816	1.62 ± 0.66	5.15 ± 2.10		31.3 ± 4.3
CL_T (L/kg/h)		0.43 ± 0.14	0.58 ± 0.16**	0.22 ± 0.02	
tmax (h)	1.87 ± 1.58		1.5		4.0 ± 2.7
Vss (L/kg)		0.67 ± 0.27			
Vz (L/kg)		0.86 ± 0.29		1.84 ± 0.18	
Vc (L/kg)		0.19 ± 0.12			
F (%)	83.1 ± 15.0				

* = harmonic mean; ** = CL/F (L/kg/h)

14=(Dowling and Russell, 2000); 15=(McElroy *et al.*, 1987); 16=(Pijpers *et al.*, 1990); 17=(Archimbault *et al.*, 1994)

Table 2.4 (Continued)

Animals	Piglets[18]	Dogs[19]	Hens[20]	Rats[21]	Rats[21]
Dose (mg/kg)	20	5	10	20	20
Route	IV	IV	IV	IV (LA)	IM (LA)
Parameters					
Ka (h^{-1})					
λz (h^{-1})				0.03 ± 0.003	0.04 ± 0.02
$t_{1/2, Ka}$ (h)		0.12 ± 0.03			
$t_{1/2, \lambda z}$ (h)	14.1 ± 2.85	6.02 ± 1.51		27.26 ± 3.54	22.0 ± 10.1
MRT (h)			6.84 ± 0.63		
CL_T (L/kg/h)	0.25 ± 0.03	0.25 ± 0.08	0.10 ± 0.08	0.131 ± 0.041	0.58 ± 0.04
tmax (h)					
Vss (L/kg)			0.71 ± 0.06		
Vz (L/kg)	5.18 ± 1.67	2.10 ± 0.42	0.68 ± 0.05	0.79 ± 0.22	5.09 ± 1.66
Vc (L/kg)	0.29 ± 0.05	0.24 ± 0.05	0.15 ± 0.01		
F (%)					

** = CL/F (L/kg/h)

18=(Mevius *et al.*, 1986); 19=(Baggot *et al.*, 1977); 20=(Moreno *et al.*, 1996); 21=(Curl *et al.*, 1988)

Table 2.4 (Continued)

Animals	Foals[22]	Rainbow trout[23]	Rainbow trout[23]	Human[24]
Dose (mg/kg)	60	60	60	500 mg
Route	IV (LA)	IV	IM	Oral
Parameters				
Ka (h^{-1})				0.718 ± 0.080
λz (h^{-1})		0.008 ± 0.001		0.135 ± 0.017
$t_{1/2,\ Ka}$ (h)			1	
$t_{1/2,\ \lambda z}$ (h)	7.30*	89.5 ± 8.7	94.7 ± 16.0	5.22 ± 0.65
MRT (h)	10.6 ± 3.58			
CL_T (L/kg/h)	0.19 ± 0.06	0.016 ± 0.001		
tmax (h)			4	2.9 ± 0.28
Vss (L/kg)	2.17 ± 0.24			
Vz (L/kg)	2.19 ± 0.47	2.10 ± 0.30		
Vc (L/kg)	0.94 ± 0.19	0.09 ± 0.04		0.23 ± 0.02
F (%)			85	

* = harmonic mean

22=(Papich *et al.*, 1995); 23= (Grondel *et al.*, 1989); 24=(Wojcicki *et al.*, 1985)

Abbreviations used in Table 2.4:

Ka = absorption rate constant, $t_{1/2, Ka}$= absorption half-life, λz = elimination rate constant, $t_{1/2, \lambda z}$ = elimination half-life, MRT = mean residence time, CL_T = total body clearance, t_{max} = time when maximum concentration was obtained, Vss = apparent volume of drug distribution at steady state, Vz = terminal volume of distribution, Vc = apparent volume of the central compartment, and F = bioavailability.

Information on the pharmacokinetics of oxytetracycline in alpacas is speculative as drug disposition in alpacas is considered to be equivalent to llamas so the appropriate dosage regimen of oxytetracycline in alpacas has not been defined yet. The purpose of this study was to investigate the disposition and pharmacokinetics of oxytetracycline in alpacas following intravenous and intramuscular administration. Based on the pharmacokinetic parameters obtained in the study, a proper dosing regimen will be developed in order to improve dosing of oxytetracycline and drug treatment of alpacas.

MATERIALS AND METHODS

Drugs and chemicals:

Oxytetracycline hydrochloride (Biomycin C®, Boehringer Ingelheim Animal Health, Inc., St. Joseph, Missouri, USA, 100 mg/mL) was used for intravenous (IV) administration, while long-acting intramuscular (LA-IM) administration of oxytetracycline was by Liquamycin®LA-200 (Pfizer Animal Health, Exton, Pennsylvania, USA, 200 mg/mL). Oxytetracycline hydrochloride, trichloroacetic acid, disodium EDTA, citric acid monohydrate, and N, N-dimethyl formamide (HPLC grade) were obtained from SIGMA Chemical Co. (St. Louis, Missouri, USA). Sodium citrate anhydrous was obtained from SPECTRUM (New Brunswick, New Jersy, USA). Potassium nitrate was obtained from Mallinckrodt (Paris, Kentucky, USA). Methanol (HPLC grade) was obtained from Fisher Scientific (Fair Town, New Jersy, USA).

Animals:

Six healthy adult alpacas, three females and three gelded males in the Veterinary Medicine Animal Isolation Laboratory at Oregon State University, weighing between 59.6-71.9 kg, age 4-8 years, were used in the study. All animals received a routine checkup including vaccination and deworming prior to the study. Routine health treatments were completed at least two weeks and no longer than a month before the start of the study. Grass hay and water were available *ad libitum*.

Administration of drugs and sampling protocol:

Initially a single dose of 10 mg/kg body weight of Biomycin C® was administered intravenously through the jugular vein. A week later, the same animals were given the same dose of Liquamycin®LA-200 into the semimembranosus hind leg muscle.

Blood samples were collected from the jugular vein before drug administration and at 10, 20, and 30 minutes and 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after IV administration. The same blood sampling time scheme was performed after IM administration except additional samples were taken at 48 and 72 hours. Before taking the next sample, 8 mL of blood was drawn through the catheter and discarded to remove the heparin saline flush of the previous blood sample, ensuring a proper blood sample was drawn. After each blood sample was collected, the catheter was flushed with heparinized normal saline. All blood samples were transferred to evacuated tubes coated with 15.0 mg EDTA powder and mixed gently. Plasma was separated by centrifugation at 1,500 x g for 15 minutes and stored at -20°C until assayed.

Analytical method:

The plasma concentrations of oxytetracycline were measured by High Performance Liquid Chromatography (HPLC) with a system P2000 pump, a SP 8880 autosampler, a Spectra 100 variable wavelength detector and a ChromJet integrator (Spectra-Physics, San Jose, California, USA) using the method of Escudero *et al.* (1994). A sample of plasma (400 µL) was deproteinated with 100 µL of 15% trichloroacetic acid in methanol. The mixture was vortexed for 1 minute, placed on ice for 15 minutes, and centrifuged at 10,000 x g for 10 minutes. Samples (100 µL) of the supernatant were injected onto C_{18} column

(Phenomenex, Torrance, California, USA) and scanned by the ultraviolet detector whose wavelength was set at 357 nm. The mobile phase was composed of 30% N, N-dimethyl formamide and 70% water, containing 0.013 M Sodium citrate, 0.01 M potassium nitrate, 0.001 M disodium EDTA, and 0.05 M citric acid monohydrate with the pH adjusted to 3.5.

Calibration curves for the quantification of oxytetracycline in alpaca plasma samples were obtained by plotting the peak areas against the concentrations of oxytetracycline prepared as standard solutions. The calibration curves were determined by linear regression analysis to be linear. Correlation coefficients of calibration curves were greater than 0.999. The concentrations of oxytetracycline in alpaca plasma samples were calculated from the calibration curve using inverse prediction.

The overall percent coefficient of variation (% CV) of the oxytetracycline assay was 3.26%. The % CV of oxytetracycline concentration in prepared standard solutions is shown in Table 2.5. The limit of quantification was 0.24 μg/mL.

Table 2.5 The percent coefficient of variation (% CV) of oxytetracycline concentration in prepared standard solutions.

Actual conc. (μg/mL)	Average of predicted conc. (μg/mL)	SD	% theoretical conc.	%CV
0.4	0.425	0.156	106.3	36.71
0.8	0.748	0.168	93.50	22.46
2.0	1.972	0.168	98.60	8.519
4.0	4.003	0.279	100.1	6.970
8.0	7.966	0.255	99.58	3.201
16.0	15.99	0.431	99.94	2.695
20.0	20.21	0.537	100.1	2.657
40.0	39.81	0.738	99.53	1.854
60.0	59.74	0.957	99.57	1.602

Grand mean of % theoretical conc. = 99.8
Grand SD of mean % theoretical conc. = 3.25
Overall %CV = 3.26

Pharmacokinetic analysis:

The plasma concentration-time curves of oxytetracycline following IV and IM administration were fitted for each individual alpaca by both compartmental and noncompartmental approaches with WinNonlin Professional Version 3.2 software (Pharsight Corporation, Mountain View, California, USA) using a weighting factor of 1/plasma concentration. The optimum number of first-order rate processes in the predictive equation was selected on the basis of the minimal Akaike's information criterion (Wagner, 1993; Yamaoka *et al.*, 1978). Oxytetracycline plasma concentration-time profiles after IV administration were best described by a linear three-compartment open model while that of IM administration were fitted using two-compartment open model with two parallel first-order input functions.

For compartmental analysis, the pharmacokinetic parameters were calculated from the equation best describing the plasma concentration-time profiles. The area under the curve ($AUC_{0-\infty}$) was calculated from the coefficients and exponential constants of the equation explaining the data.

$$Cp = \sum_{i=1}^{n} C_i e^{(-\lambda i * t)} \tag{1}$$

$$AUC_{0-\infty} = \sum_{i=1}^{n} \frac{C_i}{\lambda_i} \tag{2}$$

where Cp is the plasma concentration, λ_i is the exponential constant, C_i is the coefficient, and n is the number of exponential terms in the equation.

The total body clearance (CL_T) was calculated according to the following equation.

$$CL_T = \frac{Dose}{AUC_{0-\infty}} \tag{3}$$

MRT is the mean residence time and is equal to

$$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}} \tag{4}$$

$AUMC_{0-\infty}$is the area under first moment versus time curve, and was calculated from

$$AUMC_{0-\infty} = \sum_{i=1}^{n} Ci/\lambda_i^2 \tag{5}$$

The apparent volume of distribution at steady state (Vss) was calculated according to

$$Vss = CL_T \times MRT \tag{6}$$

Half-lives were calculated according to

$$t_{1/2,\lambda i} = \frac{0.693}{\lambda_i} \tag{7}$$

The bioavailability (F) of oxytetracycline after IM injection was estimated according to the equation

$$F = \frac{AUC_{IM} / Dose_{IM}}{AUC_{IV} / Dose_{IV}} \tag{8}$$

For noncompartmental analysis, t_{max} and C_{max} were determined from the observed data. The MRT and terminal half-lives were calculated as stated above. The area under the curve ($AUC_{0-\infty}$) and the area under the first moment curve ($AUMC_{0-\infty}$) were calculated by the trapezoidal rule up to the last sampling time point (AUC_{0-t}, $AUMC_{0-t}$) and extrapolated to infinity ($AUC_{t-\infty}$, $AUMC_{t-\infty}$) using the equations

$$AUC_{t-\infty} = \frac{Ct}{\lambda z} \tag{9}$$

$$AUMC_{t-\infty} = \frac{t * Ct}{\lambda z} + \frac{Ct}{\lambda z^2} \tag{10}$$

Therefore,
$$AUC_{0-\infty} = AUC_{0-t} + AUC_{t-\infty} \tag{11}$$

and
$$AUMC_{0-\infty} = AUMC_{0-t} + \frac{t * Ct}{\lambda z} + \frac{Ct}{\lambda z^2} \tag{12}$$

The terminal volume of distribution (Vz) was calculated by the following equation.

$$Vz = \frac{CL_T}{\lambda z} \tag{13}$$

RESULTS

The plasma concentration-time curves for each alpaca were individually fitted following both IV and LA-IM administration. Tables 2.6 and 2.7 show the plasma

concentrations of oxytetracycline in each alpaca after IV and LA-IM administration given at a single dosage of 10 mg/kg body weight. Also listed in Tables 2.6 and 2.7 are the average oxytetracycline concentrations with their standard deviations at each time point. The semilogarithmic plot of individual plasma concentrations of oxytetracycline after IV and LA-IM administration are presented in Figures 2.2 and 2.3, respectively, while the mean plasma concentration of oxytetracycline for six alpacas following IV and LA-IM administrations along with the standard deviations are depicted in Figures 2.4 and 2.5, respectively.

The plasma concentration-time profile after IV administration showed declining oxytetracycline plasma concentrations that followed a tri-exponential manner, as demonstrated in Figure 2.6, according to the equation

$$Cp = C_1 e^{-\lambda 1 * t} + C_2 e^{-\lambda 2 * t} + C_3 e^{-\lambda 3 * t} \quad (14)$$

where Cp is the plasma concentration of oxytetracycline at time t, C_1, C_2, and C_3 are the coefficients, and λ_1, λ_2, and λ_3 are the exponential constants for the triexponential equation describing the oxytetracycline plasma concentration-time curve.

For LA-IM administration, none of the one- and two-compartment open models with first-order or zero-order input produced a good fit to the absorption phase of the plasma concentration-time profiles, indicating that the absorption process is atypical. Loo-Riegelman and deconvolution techniques were applied to the data to better understand the absorption process. The results are presented in Figures 2.7, 2.8, 2.9, and 2.10, respectively. Based on these two approaches, the absorption of oxytetracycline from the intramuscular injection site occurred in two parallel phases. Initially, a rapid absorption phase having a mean absorption rate constant (Ka_1) of 0.36 ± 0.17 min^{-1} (21.5 ± 10.0 h^{-1}) predominates followed by the appearance of a slower absorption phase having a mean absorption rate constant (Ka_2) of 0.0003 ± 0.0001 min^{-1} (0.016 ± 0.003 h^{-1}). Oxytetracycline plasma concentration–time curves were fitted to a two-compartment open model with two parallel first-order inputs, as demonstrated in Figure 2.11, according to the equation

$$Cp = Ae^{-\lambda 2 * t} - Be^{-\lambda 1 * t} + Ce^{-Ka2 * t} - De^{-Ka1 * t} \quad (15)$$

where Cp is the plasma concentration at time t. A, B, C, and D are the coefficients for a tetra-exponential equation. λ_1 and λ_2 are rapid and slow disposition rate constants, respectively. Ka_1 is the first-order absorption rate constant for the rapid absorption phase and Ka_2 is the first-order absorption rate constant for the slow absorption phase.

Pharmacokinetic parameters of oxytetracycline after IV and LA-IM administrations obtained from compartmental and noncompartmental analysis are summarized in Tables 2.8, 2.9, 2.10, and 2.11, respectively.

Table 2.6 Plasma concentrations of oxytetracycline (μg/mL) in six alpacas at each sampling time point after 10 mg/kg IV administration.

Time (min)	Plasma oxytetracycline concentrations (μg/mL)							
	Alpaca 1	Alpaca 2	Alpaca 3	Alpaca 4	Alpaca 5	Alpaca 6	Average	SD
10	32.5704	27.2641	28.9542	38.2415	40.5733	30.5350	33.0231	5.2982
20	18.3692	17.3973	18.1057	21.9797	19.0170	17.9367	18.8009	1.6455
30	13.3840	13.3082	12.5237	15.8013	13.1418	12.1520	13.3852	1.2779
60	8.2271	8.3398	8.1486	9.7249	9.6022	7.9958	8.6731	0.7763
90	6.9461	6.4965	7.1930	7.6647	7.3286	6.7014	7.0550	0.4277
120	5.2924	5.6748	5.5396	6.8233	6.3227	5.5692	5.8703	0.5805
180	4.2508	4.1711	4.1851	4.5919	4.7794	3.8494	4.3046	0.3317
240	3.3763	3.3290	3.7678	4.2149	4.4134	3.5208	3.7704	0.4525
360	2.8006	2.6208	2.7540	3.2209	3.4627	2.9915	2.9751	0.3173
480	2.2692	2.0030	2.5581	2.6625	2.6055	2.1445	2.3738	0.2728
720	1.5275	1.6185	1.3260	1.9718	1.9662	1.5509	1.6602	0.2583
1440	0.8940	0.5588	0.4860	0.8285	0.8673	0.6869	0.7203	0.1706

Table 2.7 Plasma concentrations of oxytetracycline (μg/mL) in six alpacas at each sampling time point after 10 mg/kg LA-IM administration.

Time (min)	Plasma oxytetracycline concentrations (μg/mL)							
	Alpaca 1	Alpaca 2	Alpaca 3	Alpaca 4	Alpaca 5	Alpaca 6	Average	SD
10	1.5678	1.5231	0.5864	1.7079	0.7254	0.7471	1.1429	0.5069
20	1.6594	1.6260	0.6005	1.7185	1.0877	1.0562	1.2914	0.4481
30	1.8971	1.6277	0.6621	1.8370	1.2262	1.1109	1.3935	0.4792
60	1.9465	1.8346	0.7711	1.9966	1.6699	1.3274	1.5910	0.4687
90	1.9467	1.8457	1.1430	2.0699	1.6805	1.5169	1.7004	0.3356
120	1.9635	1.8980	1.3474	2.3096	1.7034	1.7130	1.8225	0.3209
180	1.9663	1.9031	1.3911	2.3026	1.7422	1.5271	1.8054	0.3273
240	1.9868	1.9311	1.4431	2.2289	1.8806	1.4980	1.8281	0.3022
360	1.5579	1.9425	1.5057	2.1218	1.9502	1.3576	1.7393	0.3050
480	1.4060	1.7141	1.4000	2.0601	2.0722	1.2293	1.6470	0.3604
720	1.2877	1.6026	1.2218	1.9486	1.3118	1.1540	1.4211	0.3006
1440	1.2186	1.1900	0.8423	1.5867	1.1996	0.8503	1.1479	0.2771
2880	0.9325	0.6514	0.8084	0.8762	0.7151	0.6962	0.7800	0.1106
4320	0.6200	0.4298	0.5123	0.4874	0.5046	0.4845	0.5064	0.0627

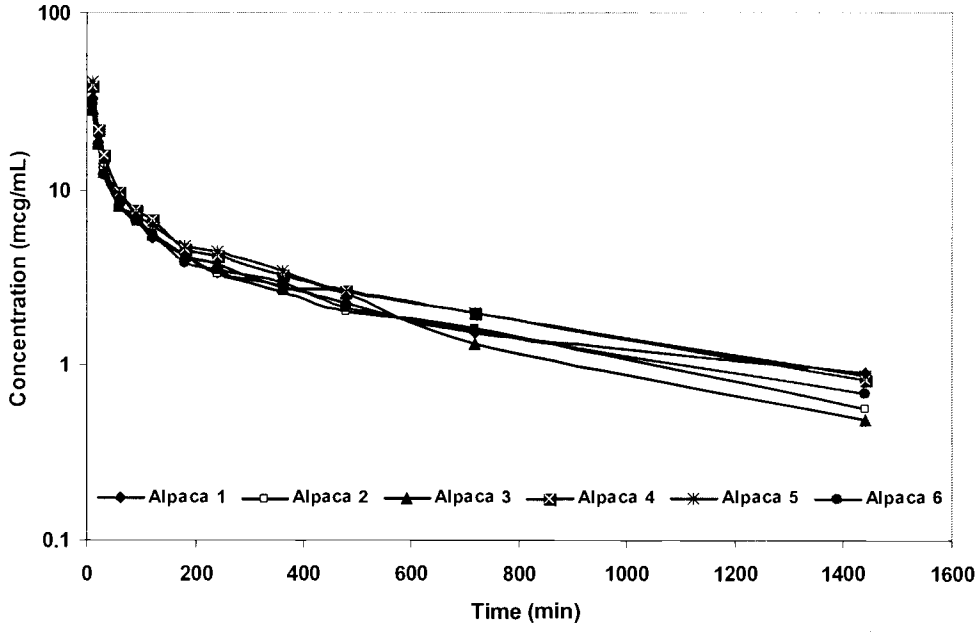

Figure 2.2 Semilogarithmic plot of individual oxytetracycline plasma concentration-time curve after a 10 mg/kg single dose IV administration.

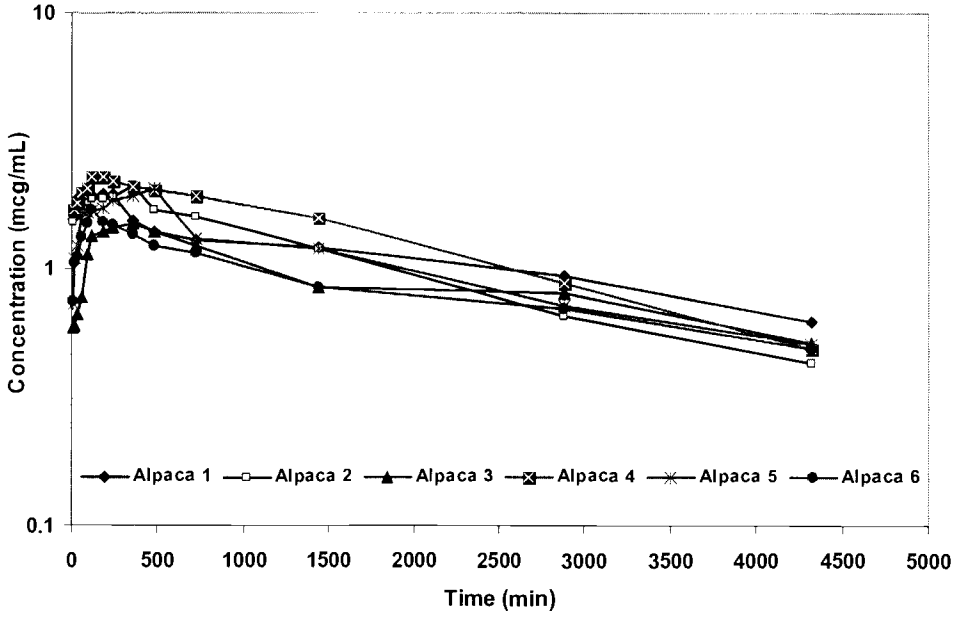

Figure 2.3 Semilogarithmic plot of individual oxytetracycline plasma concentration-time curve after a 10 mg/kg single dose LA-IM administration

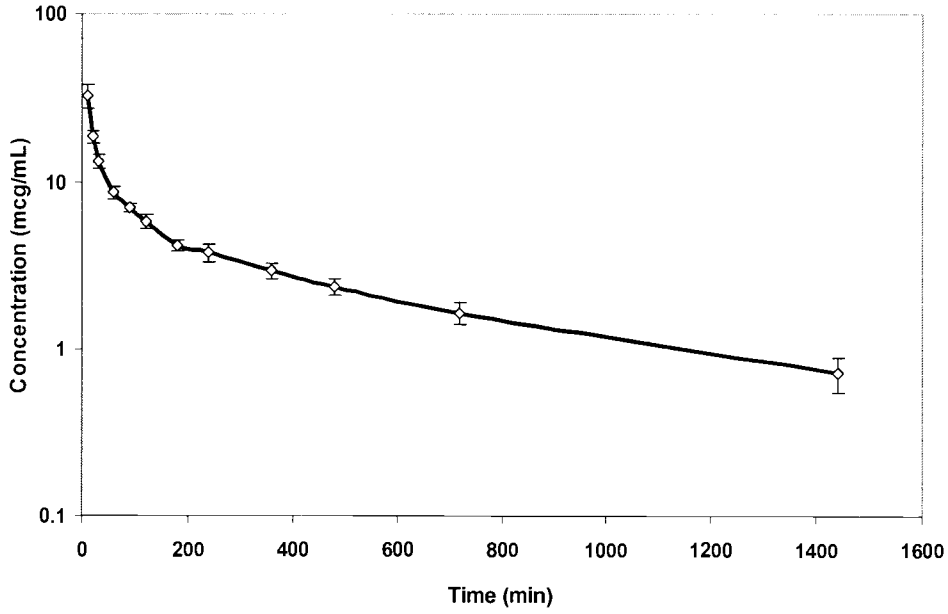

Figure 2.4 Semilogarithmic plot of mean oxytetracycline plasma concentration-time curve along with standard deviations after a single dose of 10 mg/kg was administered intravenously.

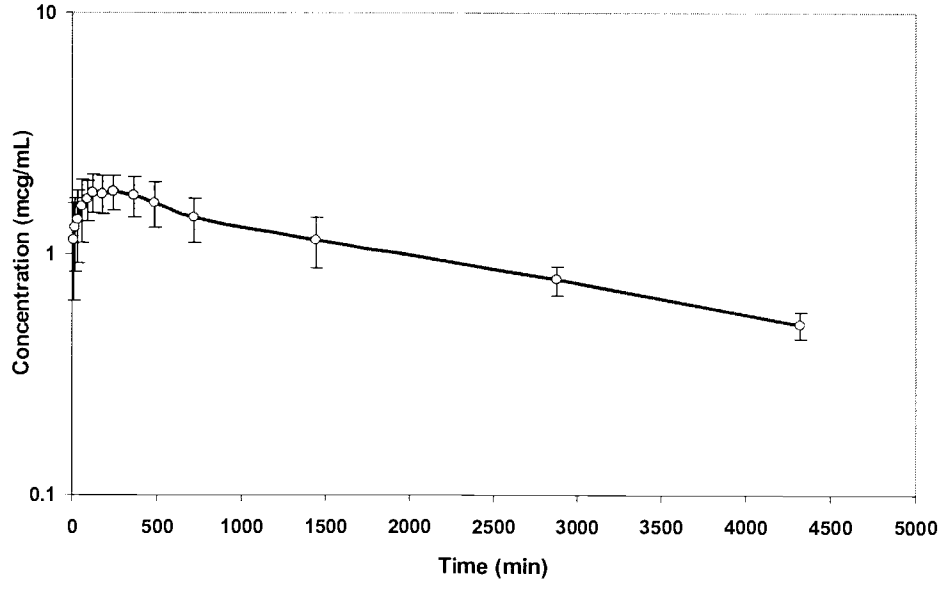

Figure 2.5 Semilogarithmic plot of mean oxytetracycline plasma concentration-time curve along with standard deviations after a single dose of 10 mg/kg was administered intramuscularly.

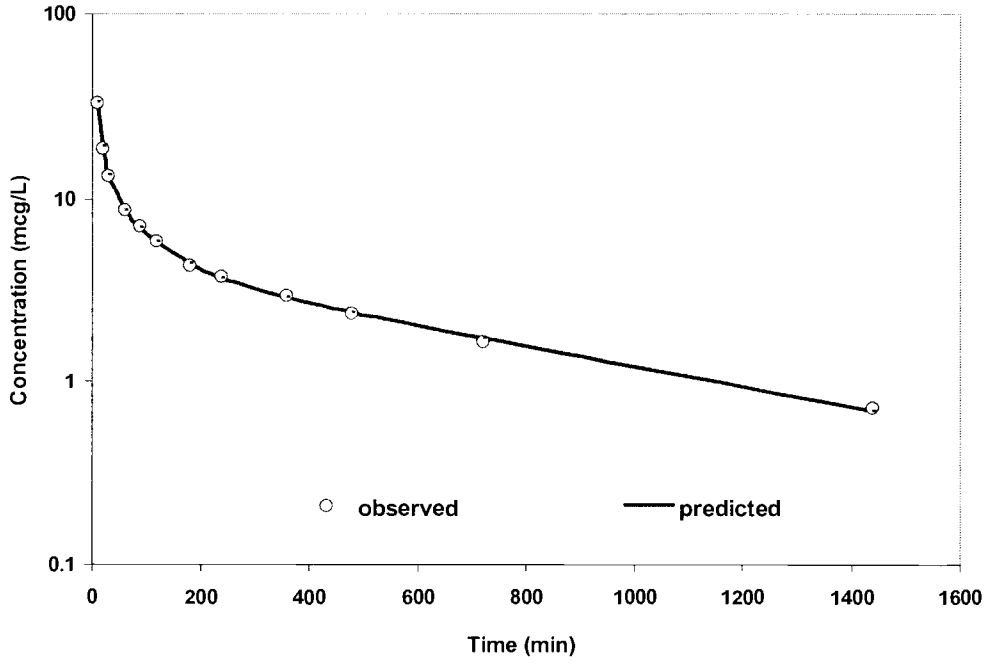

Figure 2.6 Semilogarithmic plot of average plasma concentration-time curve of oxytetracycline in alpacas after IV administration at a single dose of 10 mg/kg.

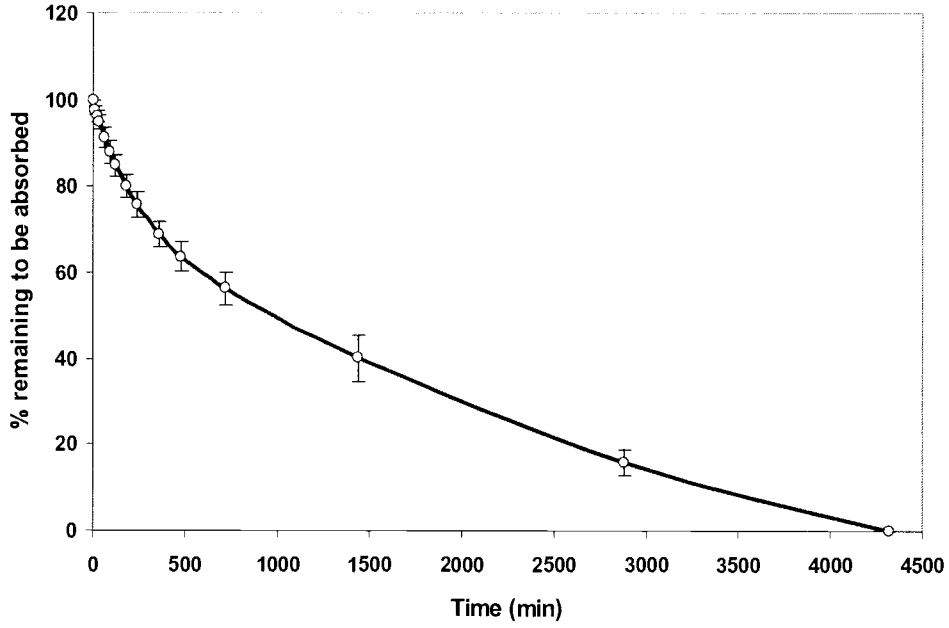

Figure 2.7 Plot of percent remaining to be absorbed versus time (min) after 10 mg/kg LA-IM administration using Loo-Riegelman approach.

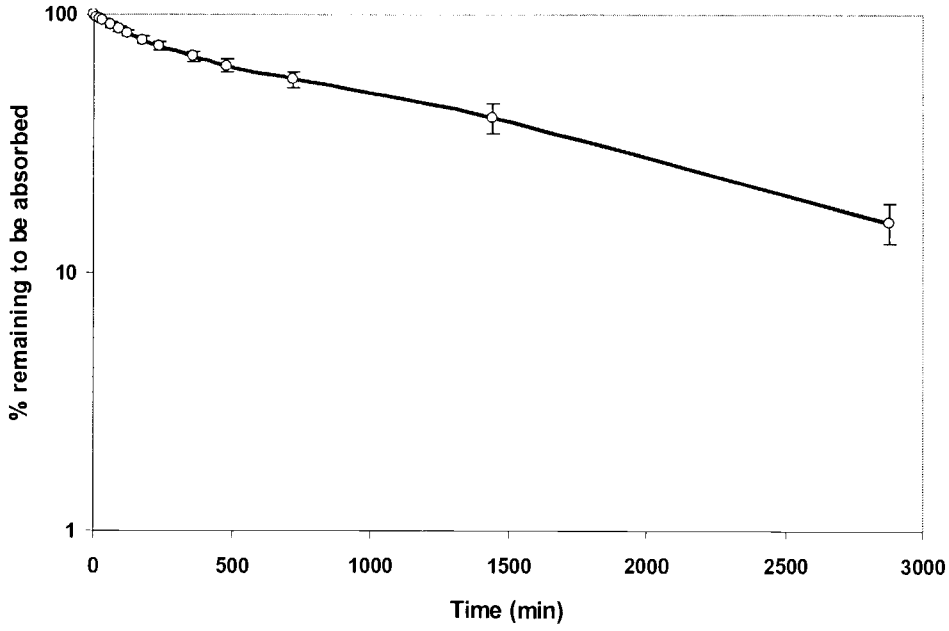

Figure 2.8 Semilogarithmic plot of percent remaining to be absorbed versus time (min) after 10 mg/kg LA-IM administration using Loo-Riegelman approach.

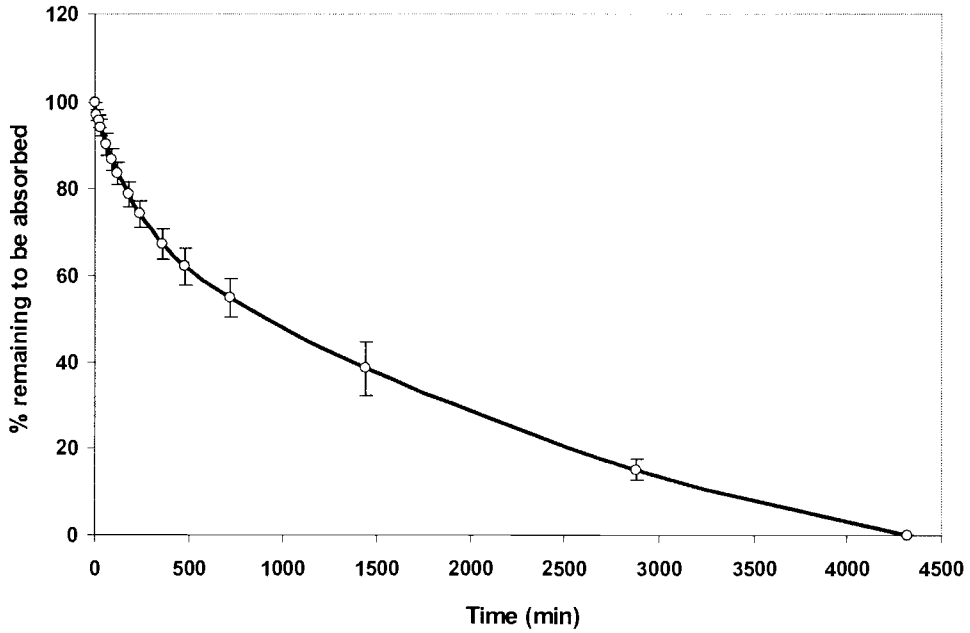

Figure 2.9 Plot of percent remaining to be absorbed versus time (min) after 10 mg/kg LA-IM administration using deconvolution approach.

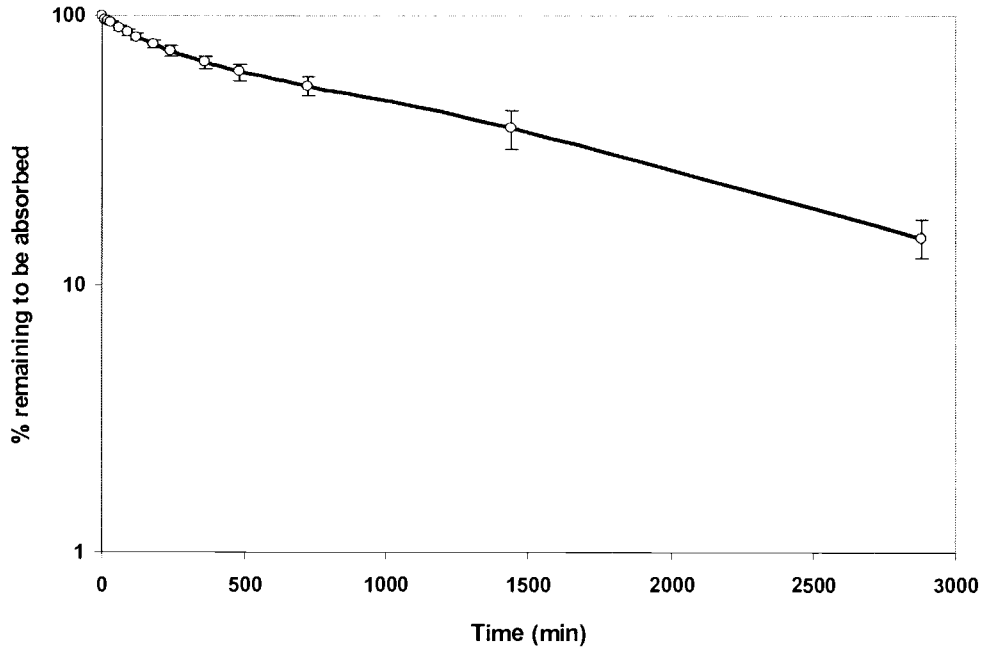

Figure 2.10 Semilogarithmic plot of percent remaining to be absorbed versus time (min) after 10 mg/kg LA-IM administration using deconvolution approach.

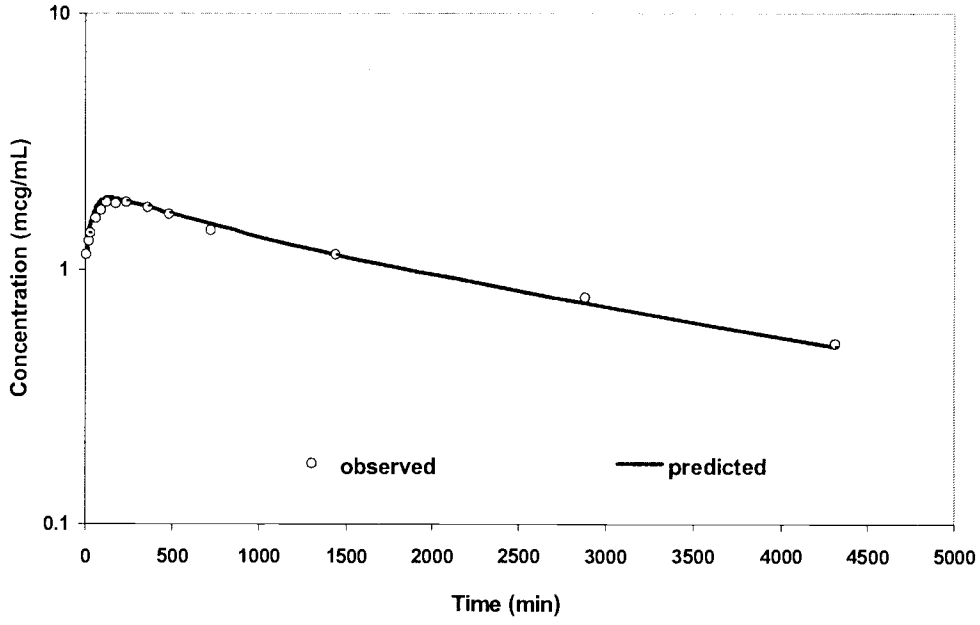

Figure 2.11 Semilogarithmic plot of average plasma concentration-time curve of oxytetracycline in alpacas after LA-IM administration at a single dose of 10 mg/kg.

Table 2.8 Pharmacokinetic parameters of oxytetracycline after 10 mg/kg IV administration using compartmental analysis.

Parameters	Alpaca 1	Alpaca 2	Alpaca 3	Alpaca 4	Alpaca 5	Alpaca 6	Average	SD
C_1 (μg/mL)	55.5	34.8	43.3	38.7	117	51.9	57.0	30.8
C_2 (μg/mL)	9.97	9.35	8.24	9.94	10.8	8.58	9.48	0.96
C_3 (μg/mL)	3.62	3.97	5.08	4.70	5.20	4.13	4.45	0.64
λ_1 (min^{-1})	0.102	0.084	0.094	0.072	0.151	0.101	0.101	0.027
λ_2 (min^{-1})	0.0120	0.0123	0.0140	0.0119	0.0146	0.0124	0.0128	0.0012
λ_3 (min^{-1})	0.00102	0.00134	0.00167	0.00121	0.00129	0.00128	0.00130	0.00021
$t_{1/2, \lambda 1}$ (min)	6.81	8.24	7.38	9.68	4.60	6.89	6.90*	1.69
$t_{1/2, \lambda 2}$ (min)	57.9	56.6	49.5	58.5	47.5	55.7	53.9*	4.62
$t_{1/2, \lambda 3}$ (min)	679	518	414	575	537	542	533*	85.8
AUC (μg•min/mL)	4923	4148	4082	5419	5557	4434	4761	638
CL_T (mL/kg/min)	2.03	2.41	2.45	1.85	1.80	2.26	2.13	0.28
MRT (min)	721	552	455	628	573	583	585	87.5
Vc (mL/kg)	145	208	1767	188	74.7	155	158	46.6
Vss (mL/kg)	1464	1331	1116	1189	1031	1314	1241	159

* = harmonic mean

Table 2.9 Pharmacokinetic parameters of oxytetracycline after 10 mg/kg IV administration using noncompartmental analysis.

Parameters	Alpaca 1	Alpaca 2	Alpaca 3	Alpaca 4	Alpaca 5	Alpaca 6	Average	SD
λz (min^{-1})	0.0011	0.0014	0.0016	0.0013	0.0013	0.0013	0.00133	0.00016
$t_{1/2,\lambda z}$ (min)	608	486	421	535	515	540	515*	63.0
MRT (min)	727	514	448	590	617	605	583	95.2
AUC (μg•min/mL)	4964	4221	4144	5529	5667	4552	4846	652
CLz (mL/kg/min)	2.01	2.37	2.41	1.81	1.76	2.20	2.09	0.28
Vz (mL/kg)	1831	1692	1508	1391	1357	1690	1578	189

* = harmonic mean

Table 2.10 Pharmacokinetic parameters of oxytetracycline after 10 mg/kg LA-IM administration using compartmental analysis.

Parameters	Alpaca 1	Alpaca 2	Alpaca 3	Alpaca 4	Alpaca 5	Alpaca 6	Average	SD
A (μg/mL)	0.57	0.53	0.46	0.88	0.85	0.10	0.56	0.29
B (μg/mL)	0.79	0.79	1.50	1.14	1.35	1.05	1.10	0.29
C (μg/mL)	1.43	1.76	1.34	1.80	1.50	1.50	1.55	0.18
D (μg/mL)	1.21	1.50	0.30	1.54	1.00	0.55	1.02	0.51
Ka_1 (min^{-1})	0.49	0.37	0.48	0.50	0.10	0.21	0.36	0.17
Ka_2 (min^{-1})	0.0002	0.0003	0.0002	0.0003	0.0003	0.0003	0.0003	0.0001
$t_{1/2,\,Ka1}$ (min)	1.41	1.87	1.44	1.39	6.92	3.25	1.93*	2.18
$t_{1/2,\,Ka2}$ (min)	3465	2068	2888	2576	2665	2415	2613*	472
λ_1 (min^{-1})	0.059	0.010	0.012	0.016	0.010	0.031	0.025	0.019
λ_2 (min^{-1})	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.0002
$t_{1/2,\,\lambda1}$ (min)	11.7	69.3	57.7	58.2	69.2	22.5	31.1*	24.8
$t_{1/2,\,\lambda2}$ (min)	630	532	410	578	545	538	530*	72.7
AUC (μg•min/mL)	7645	5574	5725	7560	6304	5264	6345	1031
CL/F (mL/kg/min)	1.308	1.794	1.747	1.323	1.586	1.899	1.614	0.278

* = harmonic mean

Table 2.11 Pharmacokinetic parameters of oxytetracycline after 10 mg/kg LA-IM administration using noncompartmental analysis.

Parameters	Alpaca 1	Alpaca 2	Alpaca 3	Alpaca 4	Alpaca 5	Alpaca 6	Average	SD
t_{max} (min)	240	360	360	120	480	120	280	145
C_{max} (μg/mL)	1.99	1.94	1.51	2.31	2.07	1.71	1.92	0.28
λz (min^{-1})	0.0002	0.0004	0.0003	0.0004	0.0003	0.0002	0.0003	0.0001
$t_{1/2, \lambda z}$ (min)	2890	1936	2188	1752	2376	3460	2308*	638
CLz (mL/kg/min)	1.89	2.13	2.42	1.70	2.08	2.49	2.12	0.30
MRT (min)	3685	2815	4287	2535	3327	4802	3575	865
AUC (μg•min/mL)	5299	4698	4138	5898	4807	4010	4808	711
Vz (mL/kg)	1715	1520	1510	1304	1600	1918	1595	208
F (%)	107	111	99.9	107	84.8	88.1	99.6	10.9

* = harmonic mean

DISCUSSIONS AND CONCLUSION

The pharmacokinetic behavior of oxytetracycline in alpacas after IV administration of a single dose of 10 mg/kg was best described by a three-compartment open model on the basis of minimal AIC. This conclusion is in agreement with the results of previous studies carried out on various animal species such as one-humped camels (Oukessou *et al.*, 1992), rats (Curl *et al.*, 1988), dairy cows (Nouws *et al.*, 1985), dogs (Baggot *et al.*, 1977), piglets (Mevius *et al.*, 1986), and rainbow trout (Black *et al.*, 1991). A two-compartment open model gave the best fit in goats (Escudero *et al.*, 1994), dairy goats (Rule *et al.*, 2001), hens (Moreno *et al.*, 1996), and pigs (Pijpers *et al.*, 1990). The rapid distribution phase of oxytetracycline after IV administration to alpacas had a mean half-life of 6.90 ± 1.69 min (0.12 ± 0.03 h). A slower distribution phase had a half-life of 53.9 ± 4.62 min (0.91 ± 0.08 h). The elimination half-life of 533 ± 85.8 min (8.88 ± 1.43 h) was similar to that reported for dairy cows (9.46 ± 1.40 h) by Nouws *et al.* (1985), but it is a longer half-life compared to 7.70 ± 1.90 h published for one-humped camels (Oukessou *et al.*, 1992). For alpacas, the apparent volume of distribution of the central compartment (Vc) was 0.16 ± 0.05 L/kg whereas 0.12 ± 0.01 L/kg and 0.24 ± 0.05 L/kg were reported in dairy cows (Nouws *et al.*, 1985) and dogs (Baggot *et al.*, 1977), respectively. The apparent volume of distribution at steady state (Vss) for the alpaca was 1.24 ± 0.16 L/kg, while 0.71 ± 0.17 L/kg was found in one-humped camels (Oukessou *et al.*, 1992).

The total body clearance of 0.13 ± 0.02 L/kg/h (2.13 ± 0.28 mL/kg/min) in alpacas was about the same as compared with 0.13 ± 0.04 L/kg/h in rats (Curl *et al.*, 1988), 0.10 ± 0.08 L/kg/h in hens (Moreno *et al.*, 1996), and 0.13 ± 0.02 L/kg/h in horses (Dowling and Russell, 2000), whereas 0.08 ± 0.02 L/kg/h was reported in one-humped camels (Oukessou *et al.*, 1992).

With long-acting IM administration, a two-compartment open model with two parallel first-order inputs best described the plasma concentration-time profiles of oxytetracycline in alpacas. The value of the smallest disposition rate constant (λz) usually designated as the elimination rate constant was significantly different from the elimination rate constant obtained from IV alpaca oxytetracycline data (p-value < 0.05). The value of

the absorption rate constant of the slower absorption phase (Ka_2) was similar to the value of the elimination rate constant obtained from the terminal slope of the oxytetracycline plasma concentration-time curve after IM administration in alpacas, indicating that the long-acting formulation followed a flip-flop model. Thus the apparent elimination phase of the long-acting IM formulation appears to be dependent on the very long absorption process of oxytetracycline from the injection site. This phenomenon was also reported in goats (Escudero *et al.*, 1994), veal calves (Schifferli *et al.*, 1982), cattle (Toutain and Raynaud, 1983), sheep (Moreno *et al.*, 1998), and horses (Dowling and Russell, 2000).

Bioavailability of oxytetracycline in alpacas after LA-IM administration was 99.6 ± 10.9 %, compared to 93.0 ± 4.0 %, 93.7 ± 32 %, 103 ± 9.4 %, and 83 ± 15 % reported in sheep (Moreno *et al.*, 1998), one-humped camels (Oukessou *et al.*, 1992), veal calves (Meijer *et al.*, 1993), and horses (Dowling *et al.*, 2000), respectively.

In alpacas, most of the susceptible bacteria have MIC ≥ 2 μg/mL. In order to maintain the oxytetracycline plasma concentrations greater than 2 μg/mL, 8 mg/kg of IV injection should be given every 12 hours and a loading dose of 20 mg/kg and an maintenance dose of 10 mg/kg of LA-IM formulation should be administered every 48 hours. A steady state trough concentration of 2.26 and 3.04 μg/mL will be achieved after IV and LA-IM administrations, respectively, as determined by superposition method.

Table 2.12 Recommended dosage regimens of oxytetracycline in alpacas.

Formulation	Dose (mg/kg)	Dosing interval (h)	Trough steady state concentration (μg/mL)	Cost for 5 days ($)
IV	8	12	2.26	3.30
LA-IM	20 (LD) 10 (MD)	48	3.04	2.00

LD = Loading dose
MD = Maintenance dose

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CHAPTER 3

COMPARATIVE PHARMACOKINETICS OF OXYTETRACYCLINE IN LLAMAS AND ALPACAS FOLLOWING INTRAVENOUS AND LONG-ACTING INTRAMUSCULAR ADMINISTRATION

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ABSTRACT

This chapter explores the pharmacokinetics of oxytetracycline in alpacas following a single dose of 10 mg/kg body weight given by intravenous injection and intramuscular administration of a long acting formulation and compares the pharmacokinetic parameters with the results previously obtained in llamas. After IV administration, a three-compartment open model best described the plasma concentration-time profiles of both llamas and alpacas. There were significant differences between these two animals in several of the pharmacokinetic parameters. With long-acting IM administration, a one-compartment open model best described the plasma concentration-time curves in llamas whereas a two-compartment open model gave a better fit to the plasma concentration-time profiles in alpacas. The biphasic absorption was shown in both animals. Since the disposition of oxytetracycline in llamas and alpacas is different, the dosage regimen of oxytetracycline should be defined differently in order to get the same therapeutic effect in llamas and alpacas.

INTRODUCTION

New World camelids, also called South American camelids (SACs), are classified as 4 species, *Lama glama* (Llama), *Lama pacos* (Alpaca), *Lama guanicoe* (Guanaco), and *Vicugna vicugna* (Vicuna). Both the llama and the alpaca exist only as domestic species whereas the guanaco and the vicuna are wild species. The llama is the largest in size of the four SACs (Fowler, 1998).

Llamas serve as pack, driving, and companion animals. They are used for breeding stock, Show, producing fiber (wool), and livestock guardians. Well-trained llamas can be utilized as pet therapy with elderly and disabled people because of their calming effect (Birutta, 1997 and Fowler, 1998). Alpacas are mainly used for producing commercial fiber production (Fowler, 1998).

Although llamas and alpacas are different in size and physical characteristics, they are able to interbreed and produce fertile offspring. It is questionable whether they are one or two separate species. If they are treated as a different species, it is certain that drug disposition in the two animals is different. Therefore, dosage regimens should be defined differently in order to get the same therapeutic effect.

The objective of this study is to compare the pharmacokinetics of oxytetracycline in the alpaca with results previously obtained in the llama (Al-Ghazawi, 1998) and determine if llamas and alpacas drug disposition is similar.

RESULTS

The plasma concentration-time profiles of oxytetracycline following a single dose of 10 mg/kg IV administration in both llamas and alpacas were best fitted to a three-compartment open model. The comparison of mean plasma concentrations versus time along with standard deviations after an IV dose in both animals is shown in Table 3.1.

With IM administration of a long acting formulation (LA-IM), a one-compartment open model best described the plasma concentration-time curves in llamas whereas a two-

compartment open model gave a better fit to the plasma concentration-time profiles in alpacas. To better understand the absorption process after the IM dose, Loo-Riegelman and deconvolution methods were used and biphasic absorption phases were found in both llamas and alpacas. Two parallel first-order absorption phases were demonstrated in both animals. The comparison of average oxytetracycline plasma concentrations along with standard deviations after LA-IM dose in llamas and alpacas is depicted in Table 3.2.

Mean plasma concentration-time curves of oxytetracycline in llamas and alpacas following IV and LA-IM administration at a single dose of 10 mg/kg body weight are shown in Figures 3.1 and 3.2, respectively. Noncompartmental analysis of the plasma concentration-time curves of oxytetracycline in llamas and alpacas after IV and LA-IM dosing was also performed. The mean pharmacokinetic parameters for llamas and alpacas following IV and LA-IM administration obtained from compartmental and noncompartmental analysis are listed in Tables 3.3, 3.4, 3.5, and 3.6, respectively. The Student's *t*-test and Mann-Whitney U-test were used to determine significant differences (p-value < 0.05) between pharmacokinetic parameters and the results are also shown in Tables 3.3, 3.4, 3.5, and 3.6.

Table 3.1 Comparison of oxytetracycline plasma concentrations (mean ± SD, μg/mL) in llamas and alpacas after a single dose (10 mg/kg) IV administration.

Time (min)	Llamas	Alpacas
10	44.11 ± 5.33	33.02 ± 5.30
20	32.37 ± 3.50	18.80 ± 1.65
30	24.33 ± 2.79	13.39 ± 1.28
60	15.84 ± 0.89	8.67 ± 0.78
90	12.72 ± 1.16	7.06 ± 0.43
120	11.10 ± 1.03	5.87 ± 0.58
180	10.11 ± 0.90	4.31 ± 0.33
240	8.55 ± 0.85	3.77 ± 0.45
360	7.08 ± 0.67	2.98 ± 0.32
480	6.54 ± 0.47	2.37 ± 0.27
720	6.35 ± 0.94	1.66 ± 0.26
1440	3.76 ± 1.53	0.72 ± 0.17

Table 3.2 Comparison of oxytetracycline plasma concentrations (mean ± SD, μg/mL) in llamas and alpacas after a single dose (10 mg/kg) LA-IM administration.

Time (min)	Llamas	Alpacas
10	1.69 ± 1.10	1.14 ± 0.51
20	3.08 ± 0.10	1.29 ± 0.45
30	3.63 ± 0.94	1.39 ± 0.48
60	3.76 ± 0.95	1.59 ± 0.47
90	3.96 ± 0.84	1.70 ± 0.34
120	4.19 ± 1.03	1.82 ± 0.32
180	4.33 ± 0.95	1.81 ± 0.33
240	4.60 ± 1.11	1.83 ± 0.30
360	4.75 ± 1.28	1.74 ± 0.31
480	4.36 ± 1.10	1.65 ± 0.36
720	4.89 ± 1.64	1.42 ± 0.30
1440	3.90 ± 1.24	1.15 ± 0.28
2880	2.49 ± 0.79	0.78 ± 0.11
4320	1.25 ± 0.41	0.51 ± 0.06

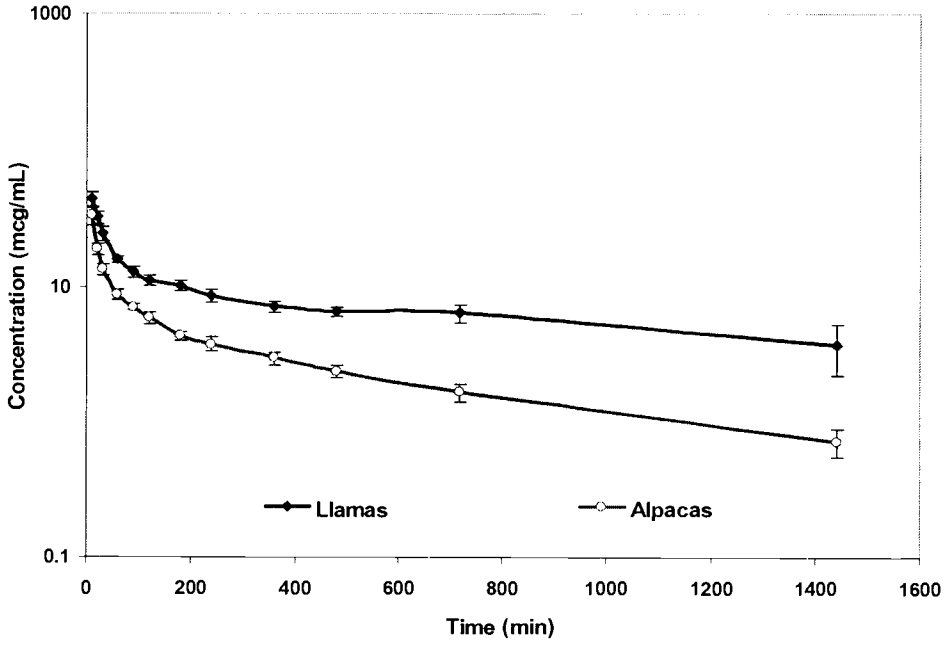

Figure 3.1 Semilogarithmic plot of average plasma concentration-time curves of oxytetracycline in llamas and alpacas after IV administration at a single dose of 10 mg/kg.

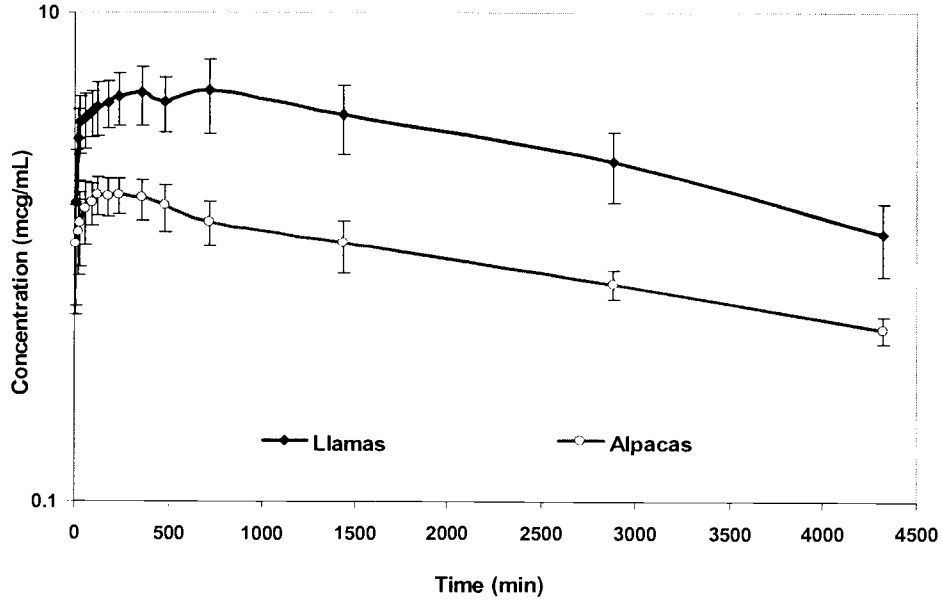

Figure 3.2 Semilogarithmic plot of average plasma concentration-time curves of oxytetracycline in llamas and alpacas after LA-IM administration at a single dose of 10 mg/kg.

Table 3.3 Pharmacokinetic parameters of oxytetracycline (mean ± SD) after a single dose (10 mg/kg) IV administration using compartmental analysis.

Parameters	Llamas	Alpacas	Level of significance	p-value	Number of subjects required to be statistical significant different
C_1 (μg/mL)	72.4 ± 30.9	57.0 ± 30.8	NS	0.204	31
C_2 (μg/mL)	18.1 ± 11.1	9.48 ± 0.96	P < 0.05	0.025	
C_3 (μg/mL)	9.35 ± 0.69	4.45 ± 0.64	P < 0.05	< 0.0005	
λ_1 (min^{-1})	0.08 ± 0.05	0.10 ± 0.03	NS	0.238	31
λ_2 (min^{-1})	0.022 ± 0.015	0.013 ± 0.001	NS	0.221	238
λ_3 (min^{-1})	0.0007 ± 0.0001	0.0013 ± 0.0002	P < 0.05	< 0.0005	
$t_{1/2, \lambda 1}$ (min)	8.27* ± 4.59	6.90* ± 1.69	NS	0.221	41
$t_{1/2, \lambda 2}$ (min)	45.5* ± 29.6	53.9* ± 4.62	NS	0.397	32
$t_{1/2, \lambda 3}$ (min)	1025* ± 362	533* ± 85.8	P < 0.05	0.022	
MRT (min)	1425 ± 517	585 ± 87.5	P < 0.05	0.010	
CL_T (mL/kg/min)	0.62 ± 0.16	2.13 ± 0.28	P < 0.05	< 0.0005	
Vc (mL/kg)	113 ± 38.8	158 ± 46.6	P < 0.05	0.049	
Vss (mL/kg)	818 ± 95.5	1241 ± 159	P < 0.05	< 0.0005	
AUC (μg•min/mL)	17195 ± 2517	4761 ± 638	P < 0.05	0.010	

* = harmonic mean

Table 3.4 Pharmacokinetic parameters of oxytetracycline (mean ± SD) after a single dose (10 mg/kg) IV administration using noncompartmental analysis.

Parameters	Llamas	Alpacas	Level of significance	p-value
λz (min^{-1})	0.0007 ± 0.0003	0.0013 ± 0.0002	$P < 0.05$	< 0.0005
$t_{1/2, \lambda z}$ (min)	975* ± 374	515* ± 63.0	$P < 0.05$	0.019
CLz (mL/kg/min)	0.62 ± 0.22	2.09 ± 0.28	$P < 0.05$	< 0.0005
Vz (mL/kg)	842 ± 120	1578 ± 189	$P < 0.05$	< 0.0005
MRT (min)	1236 ± 378	583 ± 95.2	$P < 0.05$	0.010
AUC (μg•min/mL)	17953 ± 7153	4846 ± 652	$P < 0.05$	0.010

* = harmonic mean

Table 3.5 Pharmacokinetic parameters of oxytetracycline (mean ± SD) after a single dose (10 mg/kg) LA-IM administration using compartmental analysis.

Parameters	Llamas	Alpacas	Level of significance	p-value
Ka_1 (min^{-1})	0.119 ± 0.105	0.359 ± 0.167	$P < 0.05$	0.007
Ka_2 (min^{-1})	0.0031 ± 0.0029	0.0003 ± 0.0001	$P < 0.05$	0.005
$t_{1/2, Ka1}$ (min)	5.81* ± 11.8	1.93* ± 2.18	$P < 0.05$	0.035
$t_{1/2, Ka2}$ (min)	222* ± 365	2613* ± 472	$P < 0.05$	< 0.0005
Ke (min^{-1})	0.0005 ± 0.0002	0.0013 ± 0.0002	$P < 0.05$	0.010
$t_{1/2, Ke}$ (min)	1531* ± 600	530* ± 72.7	$P < 0.05$	0.010
AUC (µg•min/mL)	16818 ± 4480	6345 ± 1031	$P < 0.05$	0.010

* = harmonic mean

Table 3.6 Pharmacokinetic parameters of oxytetracycline (mean ± SD) after a single dose (10 mg/kg) LA-IM administration using noncompartmental analysis.

Parameters	Llamas	Alpacas	Level of significance	p-value	Number of subjects required to be statistical significant different
C_{max} (μg/mL)	5.11 ± 1.61	1.92 ± 0.28	$P < 0.05$	0.010	
t_{max} (min)	450 ± 220	280 ± 145	NS	0.076	9
λz (min^{-1})	0.0004 ± 0.0001	0.0003 ± 0.0001	NS	0.093	8
$t_{1/2, \lambda z}$ (min)	1812* ± 549	2308* ± 638	NS	0.092	11
CLz (mL/kg/min)	0.63 ± 0.25	2.12 ± 0.30	$P < 0.05$	< 0.0005	
Vz (mL/kg)	1748 ± 730	1595 ± 208	NS	> 0.25	73
MRT (min)	2867 ± 673	3575 ± 865	NS	0.076	10
AUC (μg•min/mL)	17348 ± 4937	4808 ± 771	$P < 0.05$	0.010	

* = harmonic mean

DISSCUSSIONS AND CONCLUSION

There were statistically significant differences between llamas and alpacas in several of the oxytetracycline pharmacokinetic parameters. After IV administration, the elimination rate constant (0.0007 ± 0.0001 VS 0.0013 ± 0.0002 min^{-1}), elimination half-life (1025 ± 362 VS 533 ± 86 min), area under the curve (17195 ± 2517 VS 4761 ± 638 μg•min/mL), total body clearance (0.62 ± 0.16 VS 2.13 ± 0.28 mL/kg/min), terminal volume of distribution (842 ± 120 VS 1578 ± 189 mL/kg), and apparent volume of distribution at steady state (818 ± 96 VS 1241 ± 159 mL/kg) were statistically significantly different (p-value < 0.05) between llamas and alpacas, respectively. The elimination rate constant and clearance of oxytetracycline tend to be higher and the elimination half-life tends to be longer than the values found in llamas.

With LA-IM administration, absorption rate constants and peak plasma concentrations (5.11 ± 1.61 VS 1.92 ± 0.28 μg/mL) were statistically significantly different (p-value < 0.05) between llamas and alpacas, but the time to peak plasma oxytetracycline concentrations (450 ± 220 VS 280 ± 145 min) were similar.

Based on the data obtained, llamas and alpacas handle oxytetracycline distinctly differently since the disposition of oxytetracycline after IV and LA-IM administration differs significantly. In order to achieve MIC > 2 μg/mL in both animals, the proposed dosage regimens are different. For IV dose, administering 4 mg/kg of oxytetracycline every 24 hours to llamas will give the trough plasma concentration at steady state of 2.26 μg/mL while administering 8 mg/kg of oxytetracycline every 12 hours will achieve the trough plasma concentration at steady state of 2.26 μg/mL in alpacas.

With LA-IM administration, injecting 10 mg/kg of oxytetracycline every 60 hours to the llama will give trough plasma concentration at steady state of 2.24 μg/mL whereas administering LA-IM formulation with loading dose of 20 mg/kg and a maintenance dose of 10 mg/kg every 48 hours will achieve trough plasma concentration at steady state of 3.04 μg/mL in alpacas.

Table 3.7 Comparison of recommended dosage regimens of oxytetracycline in llamas and alpacas.

Animals	Formulation	Dose (mg/kg)	Dosing interval (h)	Cost for 5 days ($)
Llamas	IV	4	24	1.60
	LA-IM	10	60	2.25
Alpacas	IV	8	12	3.30
	LA-IM	20 (LD) 10 (MD)	48	2.00

LD = Loading dose
MD = Maintenance dose

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CHAPTER 4

PHARMACOKINETICS OF FLORFENICOL IN ALPACAS FOLLOWING INTRAVENOUS ADMINISTRATION

Triporn Wattananat, J. Mark Christensen, and Bradford B. Smith

ABSTRACT

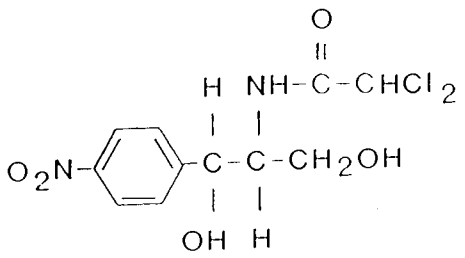
Florfenicol, a fluorinated derivative of thiamphenicol, is a widely used broad spectrum antibiotic for the treatment of various infections in veterinary medicine. Florfenicol has the advantages over chloramphenicol and thiamphenicol in that it shows a broader spectrum of antibacterial activity without carrying the risk of inducing aplastic anemia. The purpose of this study was to investigate the pharmacokinetics of florfenicol in alpacas following intravenous administration at a single dose of 20 mg/kg body weight. After the IV dose, the plasma concentration-time curves were best described using a two-compartment open model with first-order elimination. The apparent volume of distribution of the central compartment (Vc) was 0.170 ± 0.107 L/kg, while the volume of distribution at steady state (Vss) was 0.652 ± 0.217 L/kg. The harmonic mean elimination half-life was 2.63 ± 0.20 hours. The total body clearance was 0.218 ± 0.055 L/kg/h. According to the pharmacokinetic parameters obtained, the proper dosage regimen for alpacas is 8 mg/kg IV every 12 hours which will achieve a trough concentration of 0.36 μg/mL, at steady state dosing.

INTRODUCTION

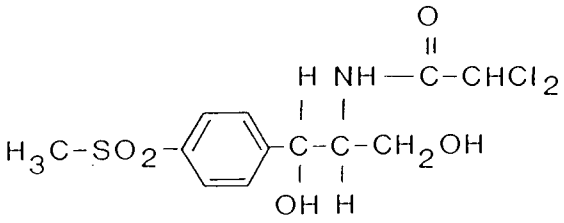
Florfenicol is a common antimicrobial of the phenicol class of antibiotics with chloramphenicol and thiamphenicol. Florfenicol is a structural analog of thiamphenicol in which a hydroxyl group has been substituted with fluorine, as shown in Figure 4.1 (Prescott, 2000). Like thiamphenicol, florfenicol does not carry the risk of inducing aplastic anemia that is associated with chloramphenicol because it lacks a nitro group on the aromatic ring. It also has a broader antibacterial spectrum with activity against some chloramphenicol-resistant strains of bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhimurium*, and *Staphylococcus aureus*, etc. (Neu and Fu, 1980) that can produce chloramphenicol acetyl transferase, an enzyme responsible for plasmid-mediated bacterial resistance to chloramphenicol and thiamphenicol (Prescott, 2000; Syriopulou *et al.*, 1981). Because of the substitution of a fluorine atom for the 3-hydroxyl group, florfenicol is able to maintain its antimicrobial activity in the presence of chloramphenicol acetyl transferase enzyme (Hoar *et al.*, 1998). The activity of florfenicol against common pathogenic bacteria is presented in Table 4.1 (Neu and Fu, 1980; Aguirre *et al.*, 1994; Marshall *et al.*, 1996; Ayling *et al.*, 2000a; Prescott, 2000), while the susceptibility of resistant bacteria to florfenicol is shown in Table 4.2 (Ho *et al.*, 2000; Neu and Fu, 1980; Aguirre *et al.*, 1994; Marshall *et al.*, 1996; Ayling *et al.*, 2000b; Prescott, 2000).

The bacteriostatic action of florfenicol is due to its ability to inhibit bacterial protein synthesis by irreversible binding to the 50S subunit of the bacterial ribosome and interfering with the formation of peptides by blocking the action of peptidyl transferase (Kapusnik *et al.*, 1996; Prescott, 2000). This bacteriostatic effect of florfenicol can become bactericidal at high concentrations (Prescott, 2000).

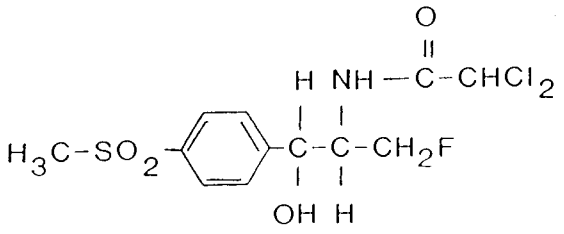
Florfenicol has a relatively high apparent volume of distribution, 5.11 L/kg, 5.15 L/kg, 3.41 L/kg, 0.95 L/kg, and 0.77 L/kg have been reported in broiler chickens (Afifi and El-Sooud, 1997), Egyptian goats (Atef *et al.*, 2000), Muscovy ducks (El-Banna, 1998), calves (De Craene *et al.*, 1997), and cattle (Lobell *et al.*, 1994), respectively.



Chloramphenicol



Thiamphenicol



Florfenicol

Figure 4.1 Chemical structures of chloramphenicol, thiamphenicol, and florfenicol.

The plasma protein binding of florfenicol is low. In veal calves, 22-26% of the drug binds to plasma proteins (Adams *et al.*, 1987) while 22.45%, 19.9%, and 18.5% binding to plasma proteins was reported in Egyptian goats (Atef *et al.*, 2000), Muscovy ducks (El-Banna *et al.*, 1998), and broiler chickens (Afifi and El-Sooud, 1997), respectively.

De Craene *et al.* (1997) showed that florfenicol penetrated well into cerebrospinal fluid well with a relative availability of 46% to plasma. Therefore, florfenicol has

extensive distribution in tissue as its high volume of distribution and low protein binding would suggest. In calves, 50% of the drug is eliminated unchanged in the urine (Varma *et al.*, 1986).

Florfenicol is solely for use in veterinary medicine. Long-term florfenicol administration might cause reversible bone marrow suppression through its effects on erythroid cells (Prescott, 2000). Transient diarrhea or inappetence has been reported in cattle which usually return to normal within a few days after the end of treatment (Prescott, 2000). Local irritation at the injection site was also reported for the drug (Schering-Plough, 1996).

Table 4.1 Susceptibility of common pathogenic bacteria to florfenicol.

Bacteria	MIC (μg/mL)
Actinobacillus pleuropneumoniae	1
Haemophilus somnus	0.5
Pasteurella haemolytica	1
Pasteurella multocida	0.5
Streptococcus dysgalactiae	2
Streptococcus uberis	2
Mycoplasma mycoides	2
Haemophilus influenzae	0.8
Neisseria gonorrhoeae	3.1
Seromanas veronii	1
Fusobacterium mecrophorum	0.25
Bacteroides melaninogenicus	0.25

Table 4.2 Susceptibility of resistant bacteria to florfenicol.

Bacteria	MIC (μg/mL)
Escherichia coli	4-8
Enterobacter cloacae	12.5
Klebsiella pneumoniae	3.1
Providencia	12.5
Serratia marcescens	> 200
Pseudomonas aeruginosa	64-100
Salmonella typhimurium	3.1
Proteus rettgeri	12.5
Proteus vulgaris	12.5
Acinetobacter	100
Shigella sonnei	3.1
Citrobacter freundii	12.5
Staphylococcus aureus	3.1
Bacteroides melaninogenicus	12.5
Salmonella typhi	3.1
Mycoplasma bovis	16
Vibrio spp.	25

Pharmacokinetic studies of florfenicol have been conducted in some animal species such as goats, dairy cows, cattle, calves, ducks, chicken, pigs, etc. The pharmacokinetic parameters reported in those animals are listed in Table 4.3.

Table 4.3 Pharmacokinetic parameters of florfenicol in some animal species.

Animals	Broiler chickens[1]	Broiler chickens[2]	Muscovy ducks[3]	Pigs[4]	Calves[5]
Dose (mg/kg)	30	30	30	20	20
Route	IV	IV	IV	IV	IV
Parameters					
λ_1 (h^{-1})	3.84 ± 0.12		2.10 ± 0.18	5.60 ± 2.21	4.17 ± 4.04
λ_z (h^{-1})	0.24 ± 0.12		0.10 ± 0.01	0.19 ± 0.01	0.23 ± 0.06
$t_{1/2, \lambda 1}$ (h)	0.18 ± 0.01		0.326 ± 0.004	0.64 ± 0.39	0.38 ± 0.32
$t_{1/2, \lambda z}$ (h)	2.89 ± 0.14	2.68 ± 0.45	7.18 ± 0.14	2.63 ± 0.51	3.18 ± 1.01
MRT (h)					3.92 ± 1.34
Vss (L/kg)	5.11 ± 0.69	1.15 ± 0.41	5.15 ± 0.10	0.95 ± 0.07	0.95 ± 0.20
Vc (L/kg)	1.46 ± 0.08	0.89 ± 0.16	1.59 ± 0.01	1.17 ± 0.32	
CL_T (L/kg/h)	1.61 ± 0.14	0.73 ± 0.12	0.61 ± 0.01	0.31 ± 0.02	0.22 ± 0.05

1=(Afifi *et al.*, 1997); 2=(Shen *et al.*, 2002); 3=(El-Banna *et al.*, 1998); 4=(Liu *et al.*, 2003); 5=(De Craene *et al.*, 1997)

Table 4.3 (Continued)

Animals	Veal calves[6]	Veal calves[7]	Cattle[8]	Lactating cows[9]	Egyptian goats[10]
Dose (mg/kg)	11	22	20	20	20
Route	IV	IV	IV	IV	IV
Parameters					
λ_1 (h^{-1})	5.60 ± 2.21	6.55 ± 1.76	15.4** (4.38-30.7)		
λ_z (h^{-1})	0.19 ± 0.01	0.24 ± 0.04	1.50** (0.94-2.32)	0.23 ± 0.03	
$t_{1/2, \lambda 1}$ (h)	0.13** (4.48-16.3)	0.10** (0.08-0.17)			0.17 ± 0.02
$t_{1/2, \lambda z}$ (h)	3.71** (3.46-4.11)	2.87** (2.30-3.40)	2.65* (2.42-3.03)	2.93* (3.0 ± 0.37)	0.94 ± 0.05
MRT (h)			3.3** (3.1-4.1)	2.15 ± 0.167	1.04 ± 0.06
Vss (L/kg)	0.87** (0.74-1.03)	0.75** (0.65-0.80)	0.77** (0.68-0.85)	0.35 ± 0.10	3.41 ± 0.30
Vc (L/kg)			0.27** (0.18-0.47)		
CL_T (L/kg/h)	0.17** (0.15-0.18)	0.17** (0.17-0.24)	0.23** (0.19-0.26)	0.16 ± 0.04	3.31 ± 0.33

* = harmonic mean

** = median value (range)

6=(Adams *et al.*, 1987); 7=(Varma *et al.*, 1986); 8=(Lobell *et al.*, 1994); 9=(Soback *et al.*, 1995) ; 10=(Atef *et al.*, 2000);

Table 4.3 (Continued)

Animals Dose (mg/kg) Route Parameters	Goats[11] 20 IV	Atlantic salmon[12] 10 IV	Loggerhead sea turtles[13] 20 IV	Human[14*] 500 mg IV
λ_1 (h^{-1})		0.17	13.9	
λ_z (h^{-1})		0.08	0.09-0.35	
$t_{1/2, \lambda 1}$ (h)	0.27 ± 0.01		0.05	
$t_{1/2, \lambda z}$ (h)	2.61 ± 0.15	14.7	2-7.8	2.1
MRT (h)	3.18 ± 0.23			
Vss (L/kg)	1.68 ± 0.11	1.32	10.5-60	
Vc (L/kg)				
CL_T (L/kg/h)	0.55 ± 0.07		3.6-6.3	

* = thiamphenicol

11=(Atef *et al.*, 2001); 12=(Horsberg, 1996); 13=(Stamper *et al.*, 2003); 14=(Ferrari, 1984)

Abbreviations used in Table 4.3:

λ_1 = distribution rate constant, $t_{1/2, \lambda 1}$ = distribution half-life, λz = elimination rate constant, $t_{1/2, \lambda z}$ = elimination half-life, MRT = mean residence time, CL_T = total body clearance, Vss = apparent volume of drug distribution at steady state, and Vc = apparent volume of the central compartment.

Information on the pharmacokinetics of florfenicol in alpacas is speculative so the appropriate dosage regimen of florfenicol in alpacas has not been defined yet. A dose of 20 mg/kg body weight was selected for the florfenicol pharmacokinetic study in alpacas based on the recommended dose for cattle. The purpose of this study was to investigate the pharmacokinetics of florfenicol in alpacas following intravenous administration. Based on the pharmacokinetic parameters obtained, the proper dosing regimen can be developed in order to improve the overall health and drug treatment of alpacas.

MATERIALS AND METHODS

Drugs and chemicals:

Nuflor®, 300 mg florfenicol/mL (Schering-Plough Animal Health Corp, Kenilworth, New Jersy, USA) was used for intravenous injection. Ethyl paraben was obtained from SIGMA Chemical Co. (St. Louis, Missouri, USA). Acetonitrile (HPLC grade) and ethyl acetate (HPLC grade) were obtained from Fisher Scientific (Fair Lawn, New Jersy, USA). Monobasic potassium phosphate and sodium hydroxide were obtained from SPECTRUM (New Brunswick, New Jersy, USA).

Animals:

Six healthy adult alpacas, three females and three gelded males in the Veterinary Medicine Animal Isolation Laboratory at Oregon State University, weighing between 59.6-71.9 kg, age 4-8 years, were used in the study. All animals received a routine checkup

including vaccination and deworming prior to the study. Routine health treatments were completed at least two weeks prior to the start of the study. Grass hay and water were available *ad libitum.*

Administration of drugs and sampling protocol:

Nuflor® was administered to the animals via jugular vein as a single injection at a dose of 20 mg/kg bodyweight. Blood samples were collected via the jugular vein immediately prior to drug administration and at 10, 20, 30 minutes and 1, 1.5, 2, 3, 4, 6, 8, 12, 24 hours after IV administration. Before taking a blood sample, 8 mL of blood was drawn and discarded. After each 10 mL blood sample was collected, the catheter was flushed with heparinized normal saline. All blood samples were transferred to evacuated tubes coated with 15.0 mg EDTA powder and mixed gently. Plasma was separated by centrifugation at 1,500 x g for 15 minutes and stored at -20°C until assayed.

Analytical method:

The plasma concentrations of florfenicol were measured by High Performance Liquid Chromatography (HPLC) using a modified technique of Lobell *et al.* (1994). The HPLC system consisted of an autosampler (WISP model 712, Waters, Division of Millipore, Milford, MA, USA), a pump (model 590, Waters, Division of Millipore, Milford, MA, USA), a variable wavelength ultraviolet absorption detector (Spectroflow model 783, Applied Biosystems, Ramsey, NJ, USA), and an integrator (model CR 501, Chromatopac, Shimadzu Scientific Instruments, Inc., Columbia, MD, USA).

A sample of plasma (500 µL) was added to each test tube containing the internal standard, 1.6 µg of ethyl paraben, and was mixed for 30 seconds using vortex mixer. Half a milliliter of 0.2 M phosphate buffer (pH 7.0) was then added to each test tube and vortexed for 10 minutes. Two milliliters of ethyl acetate were added and the contents of the tubes were mixed by end-over-end rotation for 10 minutes. The upper ethyl acetate phase of each sample was transferred to another glass test tube and evaporated under vacuum at 40°C until complete dryness. The samples were reconstituted with 0.5 mL of HPLC mobile phase composed of 40% acetonitrile and 60% deionized water. Samples

(100 μL) of the solutions were injected onto C_{18} column (Phenomenex, Torrance, CA, USA) and scanned by the ultraviolet detector whose wavelength was set at 229 nm.

Calibration curves for the quantification of florfenicol in alpaca plasma were obtained by plotting the ratio of peak areas of florfenicol and ethyl paraben against the concentrations of florfenicol prepared as standard solutions. The calibration curves were determined to be linear. Correlation coefficients of calibration curves were by linear regression analysis greater than 0.999. The concentrations of florfenicol in alpaca plasma samples were calculated from the calibration curve using inverse prediction.

The overall percent coefficient of variation (% CV) of the florfenicol assay was 7.98%. The % CV of florfenicol concentration in prepared standard solutions is shown in Table 4.4. The limit of quantification was 0.19 μg/mL.

Table 4.4 The percent coefficient of variation (%CV) of florfenicol concentration in prepared standard solutions.

Actual conc. (μg/mL)	Average of predicted conc. (μg/mL)	SD	% theoretical conc.	%CV
0.19	0.169	0.072	88.02	42.6
0.48	0.514	0.088	107.1	17.1
0.96	1.128	0.100	117.5	8.87
1.92	1.870	0.126	97.40	6.74
3.84	3.533	0.154	92.01	4.35
4.80	4.364	0.056	90.92	1.28
9.60	9.245	0.167	96.30	1.81
19.2	18.84	1.351	98.13	7.17
28.8	30.27	1.179	105.1	3.89
48.0	48.25	0.821	99.16	1.70
57.6	58.10	0.500	100.4	0.86
96.0	95.34	0.474	99.66	0.50

Grand mean of % theoretical conc. = 99.3
Grand SD of mean % theoretical conc. = 7.93
Overall %CV = 7.98

Pharmacokinetic analysis:

The plasma concentration-time curves of florfenicol following IV administration for each individual alpaca were fitted by both compartmental and noncompartmental approaches with WinNonlin Professional Version 3.2 software (Pharsight Corporation, Mountain View, California, USA) using a weighting factor of 1/plasma concentration. The optimum number of first-order rate processes in the predictive equation was selected on the basis of the minimal Akaike's information criterion (Wagner, 1993; Yamaoka *et al.*, 1978). A linear two-compartment open model best described florfenicol plasma concentration-time profiles after IV administration.

For compartmental analysis, the pharmacokinetic parameters were calculated from the equation best describing the plasma concentration-time profiles. The area under the curve ($AUC_{0-\infty}$) was calculated from the coefficients and exponential constants of the equation explaining the data.

$$Cp = \sum_{i=1}^{n} C_i e^{(-\lambda i * t)} \tag{1}$$

$$AUC_{0-\infty} = \sum_{i=1}^{n} \frac{C_i}{\lambda_i} \tag{2}$$

where Cp is the plasma concentration, λ_i is the exponential constant, C_i is the coefficient, and n is the number of exponential terms in the equation.

The total body clearance (CL_T) was calculated according to the following equation.

$$CL_T = \frac{Dose}{AUC_{0-\infty}} \tag{3}$$

MRT is the mean residence time and is equal to

$$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}} \tag{4}$$

$AUMC_{0-\infty}$ is the area under first moment versus time curve, and was calculated from

$$AUMC_{0-\infty} = \sum_{i=1}^{n} Ci/\lambda_i^2 \tag{5}$$

The apparent volume of distribution at steady state (Vss) was calculated according to

$$Vss = CL_T \times MRT \tag{6}$$

Half-lives were calculated according to

$$t_{1/2,\lambda i} = \frac{0.693}{\lambda_i} \tag{7}$$

For noncompartmental analysis, the MRT and terminal half-live were calculated as stated above. The area under the curve ($AUC_{0-\infty}$) and the area under the first moment curve ($AUMC_{0-\infty}$) were calculated by the trapezoidal rule up to the last sampling time point (AUC_{0-t}) and extrapolated to infinity ($AUC_{t-\infty}$) using the equation

$$AUC_{t-\infty} = \frac{Ct}{\lambda z} \tag{8}$$

$$AUMC_{t-\infty} = \frac{t * Ct}{\lambda z} + \frac{Ct}{\lambda z^2} \tag{9}$$

Therefore, $$AUC_{0-\infty} = AUC_{0-t} + AUC_{t-\infty} \tag{10}$$

and $$AUMC_{0-\infty} = AUMC_{0-t} + \frac{t * Ct}{\lambda z} + \frac{Ct}{\lambda z^2} \tag{11}$$

The terminal volume of distribution (Vz) was calculated by the following equation.

$$Vz = \frac{CL_T}{\lambda z} \tag{12}$$

RESULTS

The plasma concentration-time curves were individually fitted following IV administration. Table 4.5 showed the plasma concentrations of florfenicol in each alpaca after IV administration after being given a single dose of 20 mg/kg body weight. Also listed in Table 4.5 are the average florfenicol concentrations with standard deviation at each time point. The semilogarithmic plot of individual plasma concentrations of florfenicol after IV administration are presented in Figure 4.2, while the mean plasma concentration of florfenicol for six alpacas following IV administration along with the standard deviations are depicted in Figure 4.3.

The plasma concentration-time profile after IV administration showed declining florfenicol plasma concentrations that followed a bi-exponential manner, as demonstrated in Figure 4.4, according to the equation

$$Cp = C_1 e^{-\lambda 1 * t} + C_2 e^{-\lambda 2 * t} \quad (13)$$

where Cp is the plasma concentration of florfenicol at time t, C_1 and C_2 are the coefficients, and λ_1 and λ_2 are the exponential constants for the biexponential equation describing the florfenicol plasma concentration-time curve.

Pharmacokinetic parameters of florfenicol after IV administration obtained from compartmental and noncompartmental analysis are summarized in Tables 4.5 and 4.6, respectively.

Table 4.5 Plasma concentrations of florfenicol (μg/mL) in six alpacas at each sampling time point after 20 mg/kg IV administration.

Time (min)	Plasma florfenicol concentrations (μg/mL)							
	Alpaca 1	Alpaca 2	Alpaca 3	Alpaca 4	Alpaca 5	Alpaca 6	Average	SD
10	91.1404	40.0395	85.6813	72.0959	40.4080	66.1389	65.9173	21.8423
20	32.0340	21.8530	30.8233	41.6416	24.2196	33.0037	30.5959	7.0288
30	24.1463	19.6291	25.3206	36.1268	18.9413	30.3027	25.7445	6.5572
60	13.5351	14.2844	19.8864	30.3027	14.5334	22.1140	19.1093	6.4840
90	11.3168	11.8977	16.2657	18.4044	10.2197	20.2799	14.7307	4.1636
120	10.6930	10.0460	11.0714	12.9738	7.4568	14.9922	11.2055	2.5747
180	7.9854	6.4433	8.5209	8.1346	4.4362	9.9332	7.4756	1.9011
240	5.4687	5.1026	4.0825	7.0334	2.6649	3.9844	4.7227	1.4986
360	2.8625	2.4499	2.5346	3.5247	1.9787	2.8871	2.7063	0.5199
480	1.9523	1.3660	1.7290	2.2148	1.0252	1.4530	1.6234	0.4295
720	09891	0.8971	0.8608	0.7612	0.6349	1.0532	0.8660	0.1520
1440	0.2044	0.2565	0.3401	0.3101	0.2901	0.4046	0.3847	0.1656

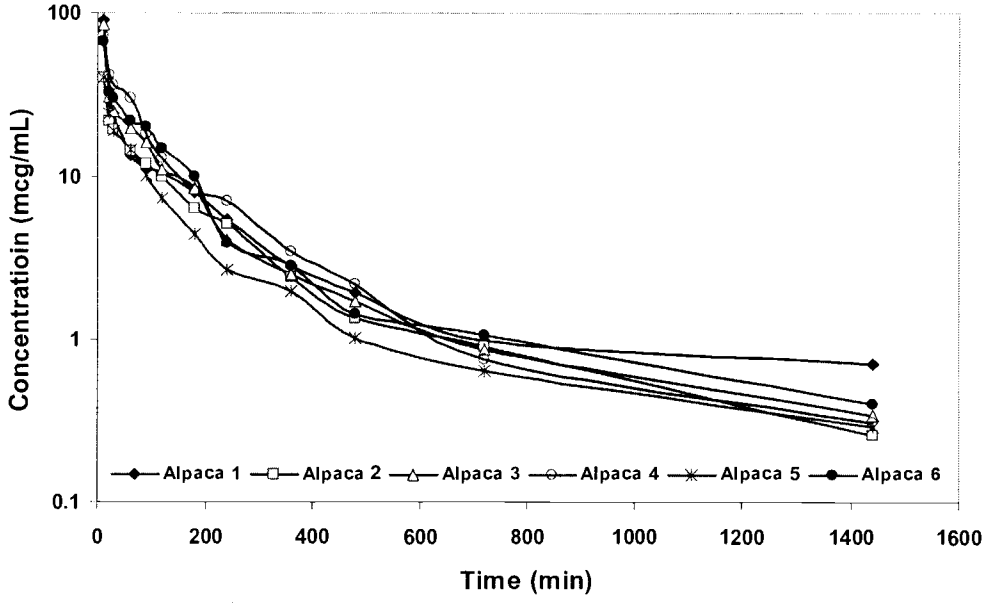


Figure 4.2 Semilogarithmic plot of individual florfenicol plasma concentration-time curves in alpacas after a 20 mg/kg single dose IV administration.

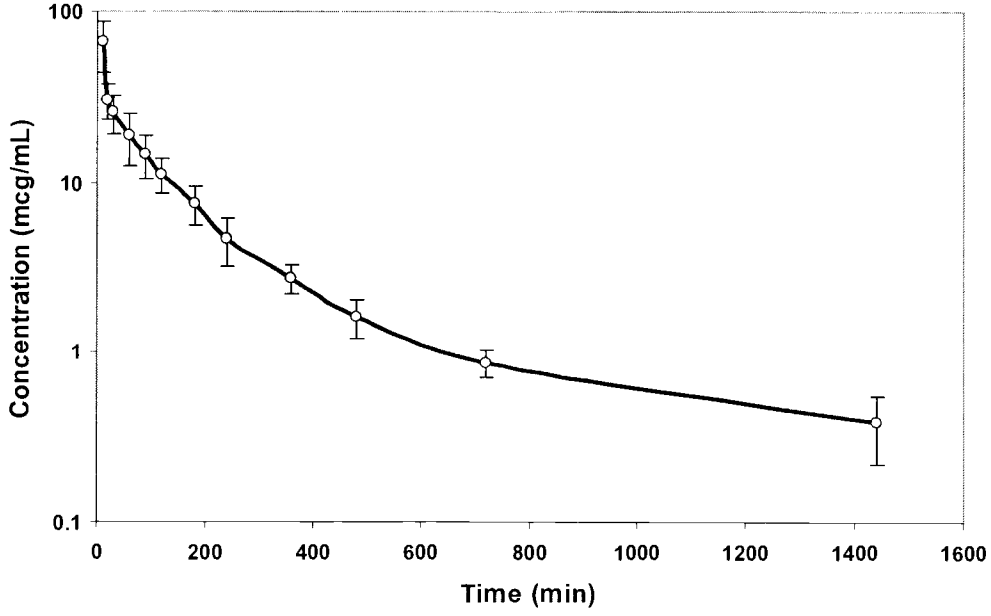


Figure 4.3 Semilogarithmic plot of mean florfenicol plasma concentration-time curve along with standard deviations in alpacas after a single dose of 20 mg/kg was administered intravenously.

Table 4.6 Pharmacokinetic parameters of florfenicol in alpacas after 20 mg/kg IV administration using compartmental analysis.

Parameters	Alpaca 1	Alpaca 2	Alpaca 3	Alpaca 4	Alpaca 5	Alpaca 6	Average	SD
C_1 (μg/mL)	291	67.2	313	79.6	46.9	130	155	118
C_2 (μg/mL)	17.4	16.1	21.3	25.3	12.6	23.5	19.4	4.82
λ_1 (min^{-1})	0.137	0.102	0.157	0.062	0.050	0.110	0.103	0.042
λ_2 (min^{-1})	0.0043	0.0040	0.0049	0.0047	0.0044	0.0042	0.0044	0.0003
$t_{1/2, \lambda 1}$ (min)	5.06	6.79	4.41	11.2	13.9	6.30	6.73*	3.75
$t_{1/2, \lambda 2}$ (min)	161	173	141	147	158	165	158*	11.7
MRT (min)	154	218	142	176	178	199	178	28.0
CL_T (mL/kg/min)	3.25	4.24	3.15	2.99	5.22	2.94	3.63	0.91
AUC (μg•min/mL)	6151	4715	6358	6677	3830	6799	5755	1204
Vc (mL/kg)	64.9	240	59.8	191	336	130	170	107
Vss (mL/kg)	501	925	447	527	930	585	652	217

* = harmonic mean

Table 4.7 Pharmacokinetic parameters of florfenicol in alpacas after 20 mg/kg IV administration using noncompartmental analysis.

Parameters	Alpaca 1	Alpaca 2	Alpaca 3	Alpaca 4	Alpaca 5	Alpaca 6	Average	SD
λz (min^{-1})	0.0037	0.0037	0.0052	0.0039	0.0040	0.0036	0.0040	0.0006
$t_{1/2, \lambda z}$ (min)	186	189	133	178	175	191	173*	21.7
MRT (min)	180	248	172	203	222	243	211	31.8
AUC (µg•min/mL)	6476	4971	6524	7215	3939	7117	6040	1306
CLz (mL/kg/min)	3.09	4.02	3.07	2.77	5.08	2.81	3.47	0.91
Vz (mL/kg)	835	1087	590	711	1269	781	879	253

* = harmonic mean

DISCUSSIONS AND CONCLUSION

The pharmacokinetic behavior of florfenicol in alpacas after IV administration of a single dose of 20 mg/kg was best described by a two-compartment model on the basis of minimal AIC. This conclusion is in agreement with the results of previous studies carried out on some animal species such as broiler chickens (Afifi *et al.*, 1997 and *Shen et al.*, 2002), Muscovy ducks (El-Banna *et al.*, 1998), and veal calves (Varma *et al.*, 1986). A three-compartment open model gave the best fit in cattle (Lobell *et al.*, 1994). A rapid distribution phase occurred in alpacas with a harmonic mean half-life of 6.73 ± 3.75 min (0.11 ± 0.06 h) while half-lives of 10.25 ± 0.94, 10.85 ± 1.96, 22.8 ± 1.96, and 38.4 ± 23.4 min were reported in Egyptian goats (Atef *et al.*, 2000), broiler chickens (Afifi *et al.*, 1997), holestein calves (De Craene *et al.*, 1997), and pigs (Liu *et al.*, 2003), respectively. In alpacas, the harmonic mean elimination half-life of florfenicol was 158 ± 11.7 min (2.63 ± 0.20 h) compared to 2.65 h in cattle (Lobell *et al.*, 1994), and 2.93 h in lactating cows (Soback *et al.*, 1995) and 2.87 h in veal calves (Varma *et al.*, 1986).

For alpacas, the apparent volume of distribution of the central compartment (Vc) was 0.17 ± 0.11 L/kg whereas 1.17 ± 0.32 L/kg, 1.46 ± 0.08 L/kg and 1.59 ± 0.01 L/kg were reported in pigs (Liu *et al.*, 2003), broiler chickens (Afifi *et al.*, 1997) and Muscovy ducks (El-Banna *et al.*, 1998), respectively. The apparent volume of distribution at steady state (Vss) in alpaca was 0.65 ± 0.22 L/kg, while 0.35 ± 0.10 L/kg, 5.11 ± 0.69 L/kg, and 5.15 ± 0.10 L/kg were found in lactating cows (Soback *et al.*, 1995), broiler chickens (Afifi *et al.*, 1997), and Muscovy ducks (El-Banna *et al.*, 1998), respectively.

The total body clearance of 3.63 ± 0.91 mL/kg/min (0.22 ± 0.05 L/kg/h) was similar to reported value of 0.22 ± 0.05 L/kg/h in calves (De Craene *et al.*, 1997) and 0.23 L/kg/h in cattle (Lobell *et al.*, 1994) compared to 3.31 ± 0.33 and 0.31 ± 0.02 L/kg/h reported values in Egyptian goats (Atef *et al.*, 2000), and pigs (Liu *et al.*, 2003), respectively.

Florfenicol has a broad spectrum of activity against a number of common pathogenic bacteria. The minimum inhibitory concentration (MIC) of florfenicol for *Pasteurella multocida* and *Pasteurella hemolytica*, which are primary pathogenic bacteria

causing bovine shipping fever, range from 0.25-5 μg/mL (Varma *et al.*, 1986), whereas the MIC for *Haemophilus somnus*, an important pathogen involving bacterial meningitis, was 0.25 μg/mL (De Craene *et al.*, 1997).

It is desirable to maintain florfenicol plasma concentrations above the MIC of 0.25 μg/mL during antibiotic therapy to eradicate infection. Using superposition method, it was determined that a 8 mg/kg dose delivered by IV injection should be given every 12 hours. The superposition method determined that a steady state trough concentration of 0.36 μg/mL will be achieved for IV administration.

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CHAPTER 5

COMPARATIVE PHARMACOKINETICS OF FLORFENICOL IN LLAMAS AND ALPACAS FOLLOWING INTRAVENOUS ADMINISTRATION

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ABSTRACT

This chapter compares the pharmacokinetics of florfenicol in alpacas following a single dose of 20 mg/kg body weight given by intravenous injection administration and with the results previously obtained in llamas. After IV administration, a two-compartment open model best described the plasma concentration-time profiles of both llamas and alpacas. There were no significant differences between these two animals in any of the pharmacokinetic parameters. Since the disposition of florfenicol in llamas and alpacas is not statistically significantly different, it can be concluded that they have similar disposition kinetics of florfenicol.

INTRODUCTION

Llamas and alpacas are members of South American camelids (SACs). They were introduced into North America in the nineteenth century as zoo animals (Fowler, 1998). The llama and the alpaca have become economically important animals of the four SACs (llama, alpaca, guanaco, and vicuna) (Smith, 1998).

Bacterial infections are common problems in llamas and alpacas (Fowler, 1998). The symptoms of illness rarely show until the infection has extensively progressed because they are very stoic animals (Burt, 1991). Since the information on the pharmacokinetics of antimicrobials in llamas and alpacas is very limited, the dosage regimen of any antimicrobials in these two animals has not been defined yet.

At present there are no drugs approved for use in the llama and the alpaca (Smith, 1998). Drug dosing for the treatment of bacterial infections are frequently based on dosing used in sheep and cattle (ruminants). In fact, camelids and ruminants evolutionally separated from each other 30-40 million years ago (Fowler, 1998). Drug dosing across species is not appropriate because different animal species might handle the same drug in a different manner.

The objective of this study is to compare the pharmacokinetics of florfenicol in the alpaca with results previously obtained in the llama (Al-Ghazawi, 1998) and determine if drug disposition between the two animals is similar.

RESULTS

The plasma concentration-time profiles of florfenicol following a single dose (20 mg/kg) IV administration in both llamas and alpacas were best fitted to a two-compartment open model. The comparison of mean plasma concentrations versus time along with standard deviations after IV dose in both animals is shown in Table 5.1 and Figure 5.1.

Noncompartmental analysis of the plasma concentration-time curves of florfenicol in llamas and alpacas after a 20 mg/kg IV dose was also performed. The mean

pharmacokinetic parameters (mean ± SD) for llamas and alpacas following IV administration obtained from compartmental and noncompartmental analysis are listed in Tables 5.2 and 5.3, respectively. The Student's *t*-test was used to determine significant differences (p-value < 0.05) between pharmacokinetic parameters and the results are also shown in Tables 5.2 and 5.3, respectively.

Table 5.1 Comparison of florfenicol plasma concentrations (mean ± SD, μg/mL) in llamas and alpacas after a single dose (20 mg/kg) IV administration.

Time (min)	Llamas	Alpacas
10	61.51 ± 10.1	65.92 ± 21.8
20	29.54 ± 5.84	30.60 ± 7.03
30	24.79 ± 5.32	25.75 ± 6.56
60	16.32 ± 4.39	19.11 ± 6.48
90	11.15 ± 2.58	14.73 ± 4.16
120	8.83 ± 2.28	11.21 ± 2.57
180	6.68 ± 1.95	7.58 ± 1.90
240	4.09 ± 1.55	4.72 ± 1.50
360	2.36 ± 1.38	2.71 ± 0.52
480	1.30 ± 0.70	1.62 ± 0.43
720	0.75 ± 0.69	0.87 ± 0.15
1440	0.32 ± 0.39	0.38 ± 0.17

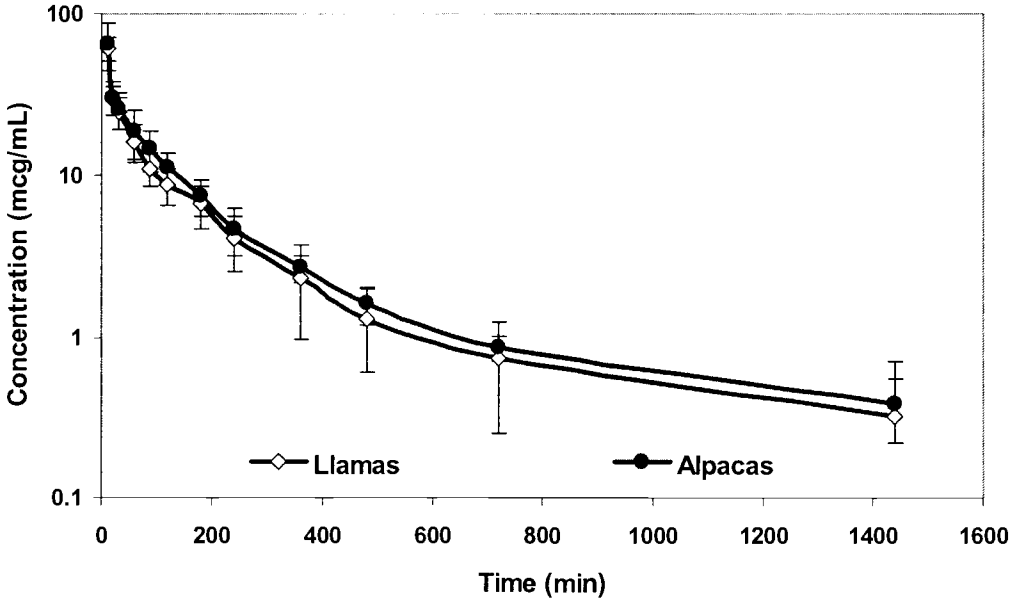

Figure 5.1 Semilogarithmic plot of average plasma concentration-time curves of florfenicol in llamas and alpacas after IV administration at a single dose of 20 mg/kg.

Table 5.2 Pharmacokinetic parameters of florfenicol (mean ± SD) after a single dose (20 mg/kg) IV administration using compartmental analysis.

Parameters	Llamas	Alpacas	Level of significance	p-value	Number of subjects required to be statistical significant different
C_1 (μg/mL)	107 ± 56.1	155 ± 118	NS	0.195	30
C_2 (μg/mL)	15.4 ± 4.57	19.4 ± 4.82	NS	0.088	11
λ_1 (min^{-1})	0.082 ± 0.030	0.103 ± 0.042	NS	0.173	24
λ_2 (min^{-1})	0.005 ± 0.002	0.004 ± 0.0003	NS	0.129	115
$t_{1/2, \lambda 1}$ (min)	8.47* ± 3.89	6.73* ± 3.75	NS	0.225	39
$t_{1/2, \lambda 2}$ (min)	147* ± 65.3	158* ± 11.7	NS	> 0.25	145
MRT (min)	174 ± 73.6	178 ± 28.0	NS	> 0.25	1550
CL_T (mL/kg/min)	4.04 ± 1.05	3.63 ± 0.91	NS	0.244	46
Vc (mL/kg)	185 ± 59.3	170 ± 107	NS	> 0.25	266
Vss (mL/kg)	706 ± 144	652 ± 217	NS	> 0.25	93
AUC (μg•min/mL)	4855 ± 1081	5755 ± 1204	NS	0.100	13

* = harmonic mean

Table 5.3 Pharmacokinetic parameters of florfenicol (mean ± SD) after a single dose (20 mg/kg) IV administration using noncompartmental analysis.

Parameters	Llamas	Alpacas	Level of significance	p-value	Number of subjects required to be statistical significant different
λz (min^{-1})	0.004 ± 0.002	0.004 ± 0.001	NS	> 0.25	232
$t_{1/2, \lambda z}$ (min)	166* ± 90.6	173* ± 21.7	NS	> 0.25	318
CLz (mL/kg/min)	4.14 ± 1.20	3.47 ± 0.91	NS	0.151	21
Vz (mL/kg)	965 ± 211	879 ± 253	NS	> 0.25	55
MRT (min)	186 ± 79	211 ± 32	NS	0.245	38
AUC (µg•min/mL)	5135 ± 1567	6040 ± 1306	NS	0.151	18

* = harmonic mean

DISCUSSIONS AND CONCLUSION

There were no significant differences between llamas and alpacas in all of the florfenicol pharmacokinetic parameters. According to noncompartmental analysis, elimination rate constant (0.0042 ± 0.0016 VS 0.0040 ± 0.0006 min^{-1}), harmonic mean of elimination half-life (166 ± 90.6 VS 173 ± 21.7 min), terminal clearance (4.14 ± 1.20 VS 3.47 ± 0.91 mL/kg/min), terminal volume of distribution (965 ± 211 VS 879 ± 253 mL/kg), mean residence time (186 ± 79 VS 211 ± 32 min), and area under the curve (5135 ± 1567 VS 6040 ± 1306 μg•min/mL) of llamas and alpacas were not statistically significant different. Based on the data obtained, llamas and alpacas handle florfenicol similarly since the disposition of florfenicol after IV administration is similar.

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CHAPTER 6

PHARMACOKINETICS OF CLORSULON IN LLAMAS AFTER ORAL ADMINISTRATION

Triporn Wattananat, Bradford B. Smith, and J. Mark Christensen

ABSTRACT

Clorsulon is a narrow-spectrum anthelmintic agent which possesses potent fasciolicidal activity. The purpose of this study was to investigate the pharmacokinetics of clorsulon in llamas following oral administration of clorsulon at a single dose of 14 mg/kg of body weight. After the oral dose, the plasma concentration-time curves were best described using a one-compartment open model with first-order input and first-order output. The first-order absorption rate constant (Ka) was 0.184 ± 0.135 $hour^{-1}$ whereas the first-order elimination rate constant was 0.015 ± 0.005 $hour^{-1}$. The harmonic mean elimination half-life was 39.2 hours. The maximum plasma concentration (C_{max}) was 0.706 ± 0.129 μg/mL and the time to reach maximum plasma concentration (tmax) was 24 hours.

INTRODUCTION

Various parasitic infections in llamas have been reported in the literature. Those diseases included trypanomiasis, toxoplasmosis, coccidiasis, fascioliasis, giardiasis, etc., as listed in Table 6.1 (Cheney and Allen, 1989; Rickard and Bishop, 1991; Rickard, 1994; Fowler, 1998).

Hepatic fascioliasis, or liver fluke infection, is an important problem that causes liver damage and death in llamas (Smith, 1998). Both acute and chronic fascioliasis have been reported (Rickard and Bishop, 1991; Rickard, 1994) but the chronic form is more often seen (Fowler, 1998). Fluke-infected animals will first show clinical signs of anorexia, weight loss, pale mucus membranes, and ill thrift, followed by chronic stasis of the bile due to bile duct obstructions by flukes. Thereafter, hepatic fibrosis occurs which causes intrahepatic hypertension. Also, adult flukes living in bile ducts suck the blood causing anemia (Fowler, 1998).

The liver fluke, *Fasciola hepatica*, matures in bile ducts of the llamas and their eggs pass down the bile ducts and are excreted with the feces. The eggs fall into water and the ciliated miracidium develop 10-12 days later. Then, the miracidium infect the snail which acts as the intermediate host of the liver fluke. Miracidium mature to become a cercaria in snails in 4.5-7 weeks. The cercaria leaves the snail and attaches to a plant and becomes a metacercaria which is the infective stage for llamas (Fowler, 1998). Llamas can get fluke infection if they graze in a pasture contaminated with metacercaria (Cheney and Allen, 1989). When metacercaria are ingested, immature flukes will be released into duodenum. They penetrate the intestinal wall and migrate to the liver and mature in the bile ducts (Fowler, 1998).

Presently, the only anthelmintic agents effective against liver flukes are clorsulon and albendazole (Rickard, 1994; Rew and Mckenzie, 2000).

Clorsulon (4-amino-6-trichloroethenylbenzene-1,3-disulfonamide) is characterized as a narrow-spectrum anthelmintic drug in the benzenedisulfonamide family (Rew and Mckenzie, 2000). The chemical structure of clorsulon is shown in Figure 6.1 (O'Neil *et al.*, 2001).

Table 6.1 Parasitism in llamas.

Disease	Parasites	Signs and Symptoms
Trypanomiasis	*Trypanosoma brucei* *Trypanosoma evansi*	Fever, depression , weakness, and edema
Toxoplasmosis	*Toxoplasma gondii*	Abortion
Coccidiosis	*Eimeria lamae* *Eimeria alpacae* *Eimeria punoensis* *Eimeria macusaniensis*	Enteritis and diarrhea
Sarcocystiasis	*Sarcocystis aucheniae* *Sarcocystis tilopodi*	Acute febrile disease resulting in abortion, mild myositis with myalgia
Giardiasis	*Giadiasia spp.*	Soft stool, diarrhea
Fascioliasis	*Fasciola hepatica*	Chronic stasis of the bile, hepatic fibrosis, elevation of intrahepatic blood pressure
Hydatid disease	*Echinococcus granulosus*	Malfunction of organ
Monieziasis	*Moniezia expansa* *Moniezia benedeni*	Diarrhea and unthriftiness
Cephenemyia	*Cephenemyia spp.*	Sneezing, coughing, nasal discharge, and difficult breathing

Table 6.1 (Continued)

Disease	Parasites	Signs and Symptoms
Encephalomalacia	*Parelaphostrongylus tenuis*	Local hemorrhage, head tilting, arching of the neck, incoordination, difficulty in getting up, and a gradual weight loss over several weeks
Sarcoptic mange	*Sarcoptes spp.*	Scaly, crusty lesion, loss of wool, intense pruritic reaction that leads to self trauma and excoriation
Chorioptic mange	*Chorioptes spp.*	Mild pruritus, lesions start on the feet and at the base of the tail, then spread to other parts of the body
Tick paralysis	Derrmacentor ticks	Weakening of the hind legs, unsteady gait, knuckling, and ataxia. The animal has difficulty chewing, swallowing, and breathing

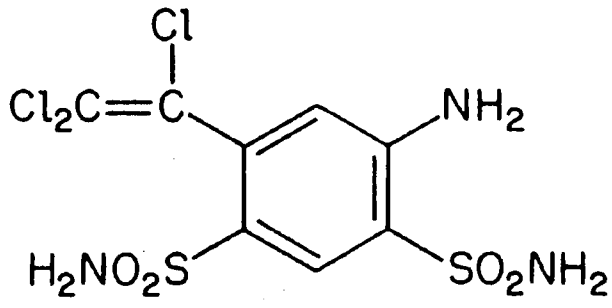

Figure 6.1 Chemical structure of clorsulon

Clorsulon is effective against adult liver flukes (>15 weeks) but have little activity against immature flukes (6- 8 weeks) (Prichard, 1986; Plumb, 1995; and Zimmerman *et al.*, 1986). The fasciolicidal activity of clorsulon has been studied in experimentally fluke-infected rats, sheep, goats, and calves and in naturally and experimentally fluke-infected cattle and sheep. In rats, a single oral dose of clorsulon from 12.5 to 100 mg/kg was 84-100% effective against mature flukes while the efficacy reduced to 78% when clorsulon was given to the rats at a single dose of 6.25 mg/kg (Schulman *et al.*, 1979). In sheep, the efficacy of clorsulon against *Fasciola hepatica* at the oral dose of 7 mg/kg body weight was reported to be 90-100% for mature flukes (Ostlind *et al.*, 1977; Fairweather and Boray, 1999), whereas Rehbein and Visser (1999) showed that 2 mg/kg of clorsulon administered subcutaneously killed 99% of adult flukes. Coles and Stafford (2001) reported an efficacy of 74 % in the treatment of fluke-infected lambs given 2 mg/kg clorsulon subcutaneously. In cattle, the efficacy of clorsulon against mature liver flukes at the oral dose of 7 mg/kg of body weight were reported to be 91-100% (Yazwinski *et al.*, 1985; Courtney *et al.*, 1985; Kilgore *et al.*, 1985; Zimmerman *et al.*, 1986). Malone *et al.* (1990) showed that the higher dose of clorsulon (35 mg/kg) was required to kill 99% of immature flukes in calves. In goats, clorsulon was 98-100 % effective against adult flukes when it was administered at a single oral dose of 7-15 mg/kg of body weight (Sundlof *et al.*, 1991).

The efficacy of clorsulon against other flukes has also been investigated. The drug is also effective against *Fascioloides magna* in white-tailed deer (Foreyt and Drawe, 1985)

but it is not as effective against *Fascioloides magna* as it is against *Fasciola hepatica* in cattle and sheep after being given the equivalent single oral doses of 7 mg/kg of body weight (Foreyt, 1988). Clorsulon also showed activity against *Echinostoma caproni* in ICR mice (Maurer *et al.*, 1996) but has poor efficacy against paraphistomes (Courtney *et al.*, 1985; Malone *et al.*, 1984, 1990) and has no effect on other parasites (Rew and Mckenzie, 2000).

The fasciolicidal activity of clorsulon is due to its ability to stop energy generation in the flukes via glucose metabolism by inhibition of two enzymes, phosphoglyceromutase and phosphoglycerate kinase (Schulman and Valentino, 1980; Martin, 1997; Fairweather and Boray, 1999; Rew and Mckenzie, 2000). The flukes die because clorsulon prevents its main source of metabolic energy to be generated.

Clorsulon is a very safe anthelmintic drug. The acute toxicity of the drug has been evaluated in mice, rats and sheep. In mice, the LD_{50} was 761 mg/kg intraperitoneally and more than 10,000 mg/kg orally, whereas an oral dose up to 10,000 mg/kg had no toxic effect in rats (Ostlind *et al.*, 1977). In sheep, doses up to 400 mg/kg have not produced toxicity (Plumb, 1995).

Lankas and Peter (1992) reported that clorsulon increased the pH and altered the electrolyte composition of urine in rats, which relates to the weak carbonic anhydrase inhibitory activity of the drug as reported by Chiu *et al.* (1985). This enzyme inhibitory effect which increases urinary pH and alter electrolyte excretion might cause urothelial cell hyperplasia in rats.

Clorsulon is available in suspension formulations for oral use or injectable form for subcutaneous administrations and also in combination with ivermectin as a product effective against nematodes, arthropods, and flukes (Rew and Mckenzie., 2000).

Clorsulon was approved by the US Food and Drug Administration for the treatment of liver flukes in cattle at the recommended dose of 7 mg/kg (Sundlof and Whitlock., 1992) but has not been approved yet in sheep or llamas.

Pharmacokinetic studies of clorsulon have been conducted in some animal species such as sheep, goats, and rats. The pharmacokinetic parameters reported in these animals are listed in Table 6.2.

Table 6.2 Pharmacokinetic parameters of clorsulon in some animal species.

Animals	Sheep[1]	Sheep[1]	Goats[1]	Goats[1]	Rats[2]
Dose (mg/kg)	7	7	7	7	5
Route	IV	oral	IV	oral	oral
Parameters					
λ_1 (h^{-1})	5.74 ± 1.95		4.35 ± 1.85		
λ_2 (h^{-1})	0.177 ± 0.037		0.289 ± 0.166		
λ_3 (h^{-1})	0.044 ± 0.015		0.073 ± 0.048		0.054-0.065
$t_{1/2, \lambda 1}$ (h)	0.12*		0.196*		
$t_{1/2, \lambda 2}$ (h)	3.93*		2.41*		
$t_{1/2, \lambda 3}$ (h)	15.80*	27.86*	10.00*	22.40*	
MRT (h)	11.5 ± 2.8	34.8 ± 5.0	6.8 ± 2.8	29.4 ± 8.5	
t_{max} (h)		15.2 ± 5.1		13.9 ± 7.3	2-4
MAT (h)		6.89 ± 4.28		22.8 ± 8.8	
Vss (L/kg)	0.567 ± 0.283		0.520 ± 0.259		
Vc (L/kg)	0.151 ± 0.034		0.135 ± 0.051		
CL_T (L/kg/h)	0.051 ± 0.018		0.080 ± 0.025		
F (%)		60.2 ± 0.26		55.3 ± 21.2	

* = harmonic mean

1= (Sundlof and Whitlock, 1992); 2= (Schulman *et al.*, 1979)

Abbreviations used in Table 6.2:

λ_1= rapid distribution rate constant, λ_2= slower distribution rate constant, λ_3= elimination rate constant, K_a = absorption rate constant, $t_{1/2, \lambda 1}$ = rapid distribution phase half-life, $t_{1/2, \lambda 2}$ = slower distribution phase half-life, $t_{1/2, \lambda 3}$ = elimination half-life sorption half-life, $t_{1/2, Ka}$ = absorption half-life, MRT = mean residence time, MAT = mean absorption time, CL_T = total body clearance, t_{max} = time when maximum concentration was obtained, Vss = apparent volume of drug distribution at steady state, Vc = apparent volume of the central compartment, and F = bioavailability.

Information on the pharmacokinetics of clorsulon in llamas is speculative based on information available from the cattle and sheep so the appropriate dosage regimen of clorsulon in llamas has not been defined yet. The purpose of this study was to investigate the pharmacokinetics of clorsulon in llamas following oral administration.

MATERIALS AND METHODS

Drugs and chemicals:

Curatrem®, 85 mg clorsulon/mL (Merck Sharp & Dohme, Rahway, New Jersy, USA) was used for oral administration. Ethyl paraben was obtained from SIGMA Chemical Co. (St. Louis, Missouri, USA). Acetonitrile (HPLC grade), n-hexane (HPLC grade), and ethyl acetate (HPLC grade) were obtained from Fisher Scientific (Fair Lawn, New Jersy, USA). Monobasic potassium phosphate and sodium hydroxide were obtained from SPECTRUM (New Brunswick, New Jersy, USA).

Animals:

Five healthy adult llamas in the Veterinary Medicine Animal Isolation Laboratory at Oregon State University, weighing between 88.6-161.8 kg were used in the study. All animals received a routine checkup including vaccination and deworming prior to the study. Routine health treatments were completed at least two weeks before the start of the

study. Grass hay and water were available *ad libitum*.

Administration of drugs and sampling protocol:

Curatrem® was administered orally to the animals via stomach tube at a single dose of 14 mg/kg bodyweight. Blood samples were collected via jugular vein catheters before drug administration and at 0.5, 1, 2, 3, 4, 6, 12, 24, 36, 48, and 72 hours after drug administration. Before taking the next sample, 8 mL of blood was drawn from the catheter extension and discarded to remove any of the heparinized saline flush of the previous blood sample, ensuring a proper blood sample was drawn. After each blood sample was collected, the catheter was flushed with heparinized normal saline. All blood samples were transferred to evacuated tubes coated with 15.0 mg EDTA powder and mixed gently. Plasma was separated by centrifugation at 1,500 x g for 15 minutes and stored at -20°C until assayed.

Analytical method:

The plasma concentrations of clorsulon were measured by High Performance Liquid Chromatography (HPLC) using a modified technique of Sundlof and Whitlock (1992). The HPLC system consisted of an autosampler (WISP model 712, Waters, Division of Millipore, Milford, MA, USA), a pump (model 590, Waters, Division of Millipore, Milford, MA, USA), a variable wavelength ultraviolet absorption detector (Spectroflow model 783, Applied Biosystems, Ramsey, NJ, USA), and an integrator (model CR 501, Chromatopac, Shimadzu Scientific Instruments, Inc., Columbia, MD, USA).

A sample of plasma (500 µL) was added to each test tube containing the internal standard, ethyl paraben, and was mixed with 1 mL of ethyl acetate for 1 minute using a vortex mixer and centrifuged at 10,000 x g for 10 minutes. The upper phase was transferred to a clean glass test tube and evaporated at 40°C under vacuum until complete dryness. A second extraction was performed by adding the same amount of ethyl acetate to the remaining phase and the extraction was performed in the manner similar to that previously described. The ethyl acetate layer obtained from the second extraction was added to the tube containing the dried first extract and the contents were evaporated to

dryness once again. To the resultant dry residue was added 1.5 mL of n-hexane and 2 mL of acetonitrile; this was shaken vigorously for 1 minute, and centrifuged at 10,000 x g for 10 minutes. Thereafter the upper (hexane) layer was discarded. A second extraction was performed using n-hexane and the hexane layer again discarded. The acetonitrile layer was dried under vacuum at 40°C. The dry residue was reconstituted with 0.5 mL of mobile phase containing 75% of 0.01 M potassium phosphate (pH 7.0) and 25% of acetonitrile. Samples (100 μL) of the solutions were injected onto a C_{18} column (Phenomenex, Torrance, CA, USA) and scanned by an ultraviolet detector whose wavelength was set at 265 nm.

Calibration curves for the quantification of clorsulon in alpaca plasma were obtained by plotting the ratio of peak areas of clorsulon and ethyl paraben against the concentrations of clorsulon prepared as standard solutions. The calibration curves were determined by linear regression analysis to be linear. Correlation coefficients of calibration curves were greater than 0.999. The concentrations of clorsulon in the llama plasma samples were calculated from the calibration curve using inverse prediction.

The overall percent coefficient of variation (%CV) of the clorsulon assay was 3.55 %. The % CV of clorsulon concentration in prepared standard solutions are shown in Table 6.3. The limit of quantification was 0.029 μg/mL.

Table 6.3 The percent coefficient of variation (%CV) of clorsulon concentration in prepared standard solutions.

Actual conc. (μg/mL)	Average of predicted conc. (μg/mL)	SD	% theoretical conc.	%CV
0.036	0.039	0.005	108.3	12.8
0.072	0.073	0.006	101.4	8.22
0.181	0.178	0.008	98.34	4.49
0.272	0.275	0.010	101.1	3.64
0.362	0.351	0.006	96.96	1.71
0.544	0.550	0.027	101.1	4.91
1.088	1.110	0.007	102.0	0.63

Grand mean of % theoretical conc. = 101.3
Grand SD of mean % theoretical conc. = 3.60
Overall %CV = 3.55

Pharmacokinetic analysis:

The plasma concentration-time curves of clorsulon for each individual llama were fitted by both compartmental and noncompartmental approaches with WinNonlin Professional Version 3.2 software (Pharsight Corporation, Mountain View, California, USA) using a weighting factor of 1/plasma concentration. The optimum number of first-order rate processes in the predictive equation was selected on the basis of the minimal Akaike's information criterion (Wagner, 1993 and Yamaoka *et al.*, 1978). A linear one-compartment open model best described clorsulon plasma concentration-time profiles after oral administration.

For compartmental analysis, the pharmacokinetic parameters were calculated from the equation best describing the plasma concentration-time profiles. The area under the curve ($AUC_{0-\infty}$) was calculated from the following equation.

$$AUC_{0-\infty} = \frac{A}{Ke} - \frac{A}{Ka} \quad (1)$$

where $A = \frac{F * D * Ka}{V(Ka - Ke)}$, Ka = first-order absorption rate constant, Ke = first-order elimination rate constant, V = apparent volume of distribution, and F = bioavailability. The total body clearance (CL_T) was calculated according to the following equation.

$$CL_T = \frac{Dose}{AUC_{0-\infty}} \tag{2}$$

MRT is the mean residence time and is equal to

$$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}} \tag{3}$$

where $AUMC_{0-\infty}$ is the area under first momentum curve, and was calculated from

$$AUMC_{0-\infty} = \frac{A}{Ke^2} - \frac{A}{Ka^2} \tag{4}$$

The apparent volume of distribution at steady state (Vss) was calculated according to

$$Vss = CL_T \times MRT \tag{5}$$

Absorption half-lives were calculated from

$$t_{1/2,Ka} = \frac{0.693}{Ka} \tag{6}$$

Elimination half-lives were calculated according to

$$t_{1/2,Ke} = \frac{0.693}{Ke} \tag{7}$$

tmax was calculated from

$$t\,\max = \frac{\ln(Ka / Ke)}{Ka - Ke} \tag{8}$$

For noncompartmental analysis, t_{max} and C_{max} were determined from the observed data. The MRT and terminal half-lives were calculated as stated above. The area under the curve ($AUC_{0-\infty}$) and the area under the first moment curve ($AUMC_{0-\infty}$) were calculated by the trapezoidal rule up to the last sampling time point (AUC_{0-t}) and extrapolated to infinity ($AUC_{t-\infty}$) using the equation

$$AUC_{t-\infty} = \frac{Ct}{\lambda z} \tag{9}$$

$$AUMC_{t-\infty} = \frac{t * Ct}{\lambda z} + \frac{Ct}{\lambda z^2} \quad (10)$$

Therefore, $$AUC_{0-\infty} = AUC_{0-t} + AUC_{t-\infty} \quad (11)$$

and $$AUMC_{0-\infty} = AUMC_{0-t} + \frac{t * Ct}{\lambda z} + \frac{Ct}{\lambda z^2} \quad (12)$$

The terminal volume of distribution (Vz) was calculated by the following equation.

$$Vz = \frac{CL_T}{\lambda z} \quad (13)$$

Since the pharmacokinetics of clorsulon following intravenous administration was not investigated in this study, some of the parameters will be reported along with F (fraction of dose absorbed). If F is known, the actual value of those parameters can be determined.

RESULTS

The plasma concentration-time curves of clorsulon following oral administration for each llama were individually fitted using WinNonlin Professional Version 3.2 software (Pharsight Corporation, Mountain View, California, USA). Table 6.4 shows the plasma concentrations of clorsulon in each llama after oral administration at a single dose of 14 mg/kg body weight. Also listed in Table 6.4 are the average clorsulon concentrations with their standard deviations at each time point. The semilogarithmic plot for each individual llama's plasma concentrations of clorsulon after oral administration are presented in Figure 6.2, while the mean plasma concentration of clorsulon for five llamas following oral administration along with standard deviation is depicted in Figure 6.3.

The plasma concentration-time profile after oral administration showed an absorption phase followed by declining clorsulon plasma concentrations that followed a mono-exponential manner, as demonstrated in Figure 6.4, according to the equation

$$Cp = \frac{F * D * Ka}{V(Ka - Ke)}\left[e^{-Ke*t} - e^{-Ka*t}\right] \quad (14)$$

where Cp is the plasma concentration of clorsulon at time t, D = given dose, V = volume of central compartment, Ka = first-order absorption rate constant, Ke = first-order elimination rate constant, and F = bioavailability.

Pharmacokinetic parameters of clorsulon after oral administration obtained from compartmental and noncompartmental analysis are summarized in Tables 6.5 and 6.6, respectively.

Table 6.4 Plasma concentrations of clorsulon (μg/mL) in five llamas at each sampling time point after 14 mg/kg single dose oral administration.

Time (h)	Clorsulon plasma concentrations (μg/mL)						
	Llama 1	Llama 2	Llama 3	Llama 4	Llama 5	Average	SD
0.5	0.0594	0.1027	0.1220	0.0877	0.0526	0.0849	0.0291
1	0.0971	0.2342	0.2086	0.2527	0.1221	0.1829	0.0693
2	0.2334	0.2854	0.2252	0.2727	0.2256	0.2485	0.0285
3	0.2720	0.3011	0.3797	0.3294	0.2869	0.3138	0.0425
4	0.3314	0.3236	0.4115	0.3940	0.3381	0.3597	0.0401
6	0.3851	0.3782	0.6072	0.5634	0.3762	0.4620	0.1137
8	0.4645	0.4104	0.6611	0.6431	0.4770	0.5312	0.1133
12	0.7092	0.4366	0.8106	0.6923	0.5984	0.6494	0.1408
24	0.7891	0.5177	0.8537	0.7128	0.6553	0.7057	0.1292
36	0.6547	0.4792	0.7748	0.6431	0.6091	0.6322	0.1060
48	0.5519	0.3811	0.6431	0.5334	0.4818	0.5182	0.0963
72	0.3607	0.2504	0.3621	0.3592	0.3381	0.3341	0.0478

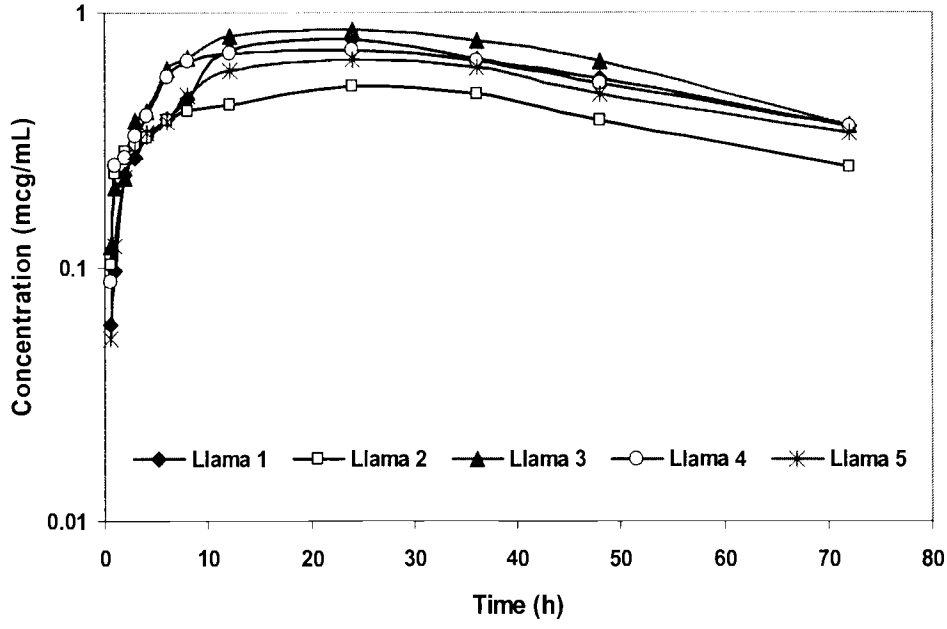

Figure 6.2 Semilogarithmic plot of individual clorsulon plasma concentration-time curve in llamas after a 14 mg/kg single dose oral administration.

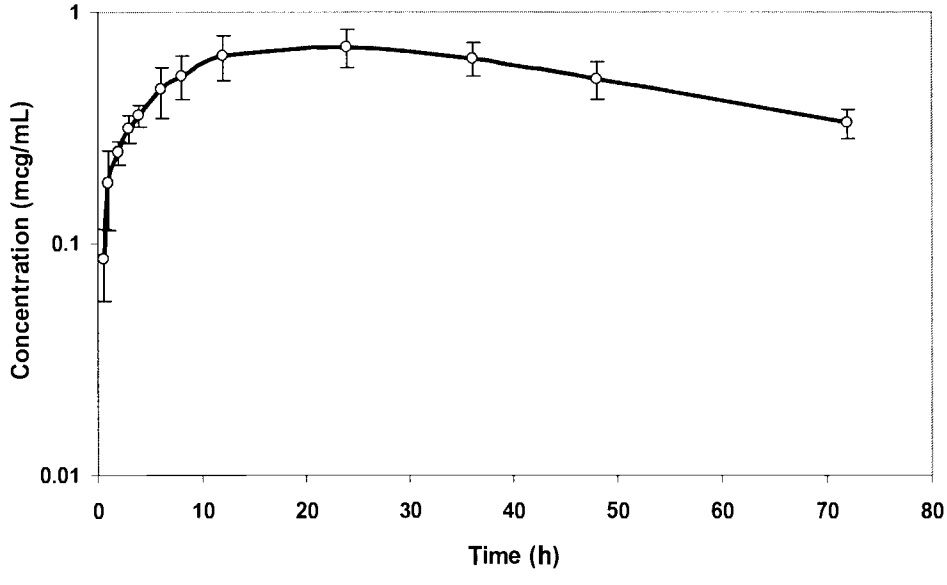

Figure 6.3 Semilogarithmic plot of average plasma concentration-time curve of clorsulon in llamas after oral administration at a single dose of 14 mg/kg.

Table 6.5 Pharmacokinetic parameters of clorsulon after oral administration (14 mg/kg) using compartmental analysis.

Parameters	Llama 1	Llama 2	Llama 3	Llama 4	Llama 5	Average	SD
K_a (h^{-1})	0.087	0.419	0.113	0.174	0.125	0.184	0.135
K_{el} (h^{-1})	0.020	0.006	0.019	0.013	0.014	0.015	0.005
T_{max} (h)	22.1	10.2	18.9	16.0	19.5	17.3	4.55
C_{max} (µg/mL)	0.748	0.445	0.890	0.735	0.652	0.694	0.163
$t_{1/2, Ka}$ (h)	7.98	1.66	6.11	3.98	5.53	3.77*	2.38
$t_{1/2, Kel}$ (h)	35.2	110.6	36.1	52.2	48.3	47.6*	31.2
MRT (h)	50.5	159.2	51.9	75.6	69.8	81.4	44.8
AUC (µg•h/mL)	58.7	75.6	66.6	68.5	60.1	65.9	6.83
CL_T/F (mL/kg/h)	0.239	0.185	0.210	0.204	0.233	0.214	0.022
Vc/F (mL/kg)	12.1	29.5	11.0	15.4	16.2	16.8	7.43

* = harmonic mean

Table 6.6 Pharmacokinetic parameters of clorsulon after oral administration (14 mg/kg) using noncompartmental analysis.

Parameters	Llama 1	Llama 2	Llama 3	Llama 4	Llama 5	Average	SD
C_{max} (μg/mL)	0.789	0.518	0.854	0.713	0.655	0.706	0.129
T_{max} (h)	24	24	24	24	24	24	0
λ_z (h^{-1})	0.017	0.018	0.022	0.016	0.016	0.018	0.002
$t_{1/2, \lambda z}$ (h)	41.4	38.6	32.2	42.7	43.0	39.1*	4.49
MRT (h)	68.5	64.8	56.4	69.4	71.4	66.1	5.92
$AUC_{0-\infty}$ (μg•h/mL)	61.9	42.5	63.1	62.2	56.9	57.3	8.65
CLz/F (mL/kg/h)	0.226	0.330	0.222	0.225	0.246	0.250	0.046
Vss/F (mL/kg)	15.5	21.4	12.5	15.6	17.6	16.5	3.26
Vz/F (mL/kg)	13.5	18.4	10.3	13.9	15.3	14.3	2.92

* = harmonic mean

DISCUSSIONS AND CONCLUSION

The pharmacokinetic behavior of clorsulon in llamas after oral administration of a single dose of 14 mg/kg of body weight was best described by a one-compartment open model on the basis of minimal AIC when fitting the data. This conclusion is in agreement with the results of previous studies carried out on sheep and goats (Sundlof and Whitlock, 1992). The harmonic mean elimination half-life in llama was 39.15 h whereas 27.86 h and 22.40 h were reported in sheep and goats, respectively (Sundlof and Whitlock, 1992). Schulman *et al.* (1982) reported an elimination half-life of 12.79 h and 10.68 h in old and young rats, respectively. Time to reach maximum concentration in the blood (t_{max}) for llamas was 24 h compared to 15.18 ± 5.12 h, 13.92 ± 7.32 h, 2-4 h in sheep (Sundlof and Whitlock, 1992), goats (Sundlof and Whitlock, 1992), and rats (Schulman *et al.*, 1982), respectively. Plumb (1995) reported t_{max} of 4 h in cattle after oral administration of clorsulon at a single dose of 7 mg/kg of body weight. The harmonic mean absorption half-life of clorsulon in llamas was 3.77 h and the maximum clorsulon concentration was 0.71 ± 0.13 μg/mL after being given a single oral dose of 14 mg/kg.

Although the pharmacokinetics of clorsulon following intravenous administration was not investigated in this study, Sundlof and Whitlock (1992) reported that a three-compartment open model best described the plasma concentration-time profiles of clorsulon in both sheep and goats after a single intravenous dose of 7 mg/kg of body weight. In goats, a single oral dose of clorsulon of 7 mg/kg was 98% effective against mature flukes (Sundlof *et al.*, 1991) while the efficacy of 90-100% was reported when the same dose of clorsulon was orally given to sheep infected with mature flukes (Ostlind *et al.*, 1977; Fairweather and Boray, 1999). The maximum clorsulon plasma concentration (Cmax) produced by a single oral dose of 7 mg/kg in goats and sheep were 1.19 ± 0.50 and 1.60 ± 0.47 μg/mL, respectively (Sundlof and Whitlock, 1992). These plasma values of clorsulon in goats and sheep after 7 mg/kg oral dose are higher than 0.71 ± 0.12 μg/mL plasma levels observed in llamas in this study after a 14 mg/kg oral dose. This suggests not the entire oral dose is absorbed in llamas.

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CHAPTER 7

EVALUATION OF THE AREA UNDER THE CURVE EQUATIONS FOR PHARMACOKINETIC SYSTEMS WITH NONLINEAR ELIMINATION

Triporn Wattananat and J. Mark Christensen

ABSTRACT

Since the differential equations describing one-compartment system with first-order input and two-compartment system after IV administration with nonlinear elimination kinetics cannot be solved, there is no mathematical expression for plasma concentration-time curves and consequently no simple expression for the area under the curve (AUC) for drugs following these models. Preliminary AUC equations were designed for drugs following these models and these equations predictions for AUC were calculated and were compared to AUC that were calculated using the trapezoidal rule method based on computer-generated data. The computer generated data for the concentration-time curves for the two nonlinear models were generated from the differential equations and numerically integrated using the fourth-order Runge-Kutta method. Except for a few exceptions the predicted AUC's from the proposed AUC equations matched the AUC's that were calculated from the theoretically generated numerically integrated data.

INTRODUCTION

The area under the plasma level versus time curve (AUC) is a measurement of the amount of drug absorbed to the systemic circulation. In linear pharmacokinetics, the AUC is linearly proportional to the dose administered for drugs eliminated by first-order kinetics. However, for drugs that are eliminated by capacity-limited processes or Michaelis-Menten kinetics, the AUC is not linearly proportional to the administered dose. The AUC increases overproportionally with increasing dose due to saturation of the clearance mechanism.

Michaelis-Menten kinetics can be described by the equation:

$$-\frac{dC}{dt} = \frac{Vm * C}{Km + C} \tag{1}$$

where – dC/dt is the rate of decline in drug concentration at time t, C is the drug concentration, Vm is the maximum rate of metabolism, and Km is Michaelis-Menten constant.

When the drug concentration is considerably greater than Km, C >> Km, equation (1) reduces to

$$-\frac{dC}{dt} = Vm \tag{2}$$

Under this condition, the elimination rate of the drug is independent of drug concentration, thus the drug elimination becomes a zero-order process.

When the drug concentration is much lower than Km, C << Km, equation (1) reduces to

$$-\frac{dC}{dt} = \frac{Vm}{Km} * C \tag{3}$$

Under this condition, the drug elimination becomes a first-order process.

At intermediate concentrations, the plasma concentration decline at a variable rate as a function of the varying plasma concentrations as described in equation (1). Currently, only the mathematical expression for describing the AUC for drugs following nonlinear pharmacokinetics or drugs obeying a one-compartment open model with single Michaelis-Menten elimination kinetics after intravenous (IV) administration is available. There are

no simple expressions for AUC for drugs following one-compartment model with first-order absorption or two-compartment model after IV administration eliminated from the central compartment in a nonlinear fashion.

Equations to predict AUC for drugs following one-compartment open model with first-order absorption and Michaelis-Menten elimination kinetics and a two-compartment open model with IV administration and Michaelis-Menten elimination kinetics have been proposed. How well these equations work and the boundary of their accuracy is unknown.

The objective of this study is to compare the AUC predicted by these equations for drugs following pharmacokinetic systems with nonlinear elimination to AUCs obtained using the trapezoidal rule method from data numerically generated plasma concentration-time curves using the differential equations by computer simulation and to test the limits of accuracy these proposed equations for AUC have.

THEORETICAL

One –Compartment Model

IV-bolus administration

The model for a drug which follows one-compartment system having only Michaelis-Menten elimination kinetics after single intravenous dose can be illustrated as shown in Figure 7.1.

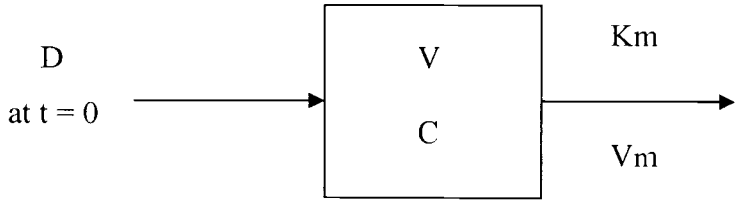


Figure 7.1 Schematic diagram of a drug following one-compartment open model with Michaelis-Menten elimination kinetics after intravenous bolus administration.

The rate of decline of drug concentration (C) with time (t) can be described by the following equation.

$$V * \frac{dC}{dt} = -\frac{Vm * C}{Km + C} \tag{4}$$

where V = the apparent volume of distribution (units = volume)

Vm = the maximum elimination rate (units = amount/time)

Km = the plasma concentration at which the rate of metabolism is one-half of its maximum (units = concentration)

Rearrangement of equation (4) yields equation (5).

$$\frac{dC}{dt} = \frac{-Vm' * C}{Km + C} \tag{5}$$

where Vm' = Vm/V

Inversion and rearrangement of equation (5) gives equation (6).

$$Cdt = -\frac{Km}{Vm'} dC - \frac{C}{Vm'} dC \tag{6}$$

Since at t = 0, C(t) = C_0 and at t = ∞, C(t) = 0, integration of equation (6) from time zero to infinity yields equation (7).

$$\int_0^{\infty} Cdt = AUC_{0-\infty} = \frac{KmC_0}{Vm'} + \frac{C_0^2}{2Vm'}$$

$$AUC_{0-\infty} = \frac{C_0}{Vm'}\left[Km + \frac{C_0}{2}\right]$$

$$AUC_{0-\infty} = \frac{D}{V * Vm'}\left[Km + \frac{D}{2V}\right]$$

$$AUC_{0-\infty} = \frac{D}{Vm}\left[\frac{D}{2V} + Km\right] \tag{7}$$

First-Order Absorption

The model for a drug that follows one-compartment system with only Michaelis-Menten elimination kinetics after single first-order input can be depicted as seen in Figure 7.2.

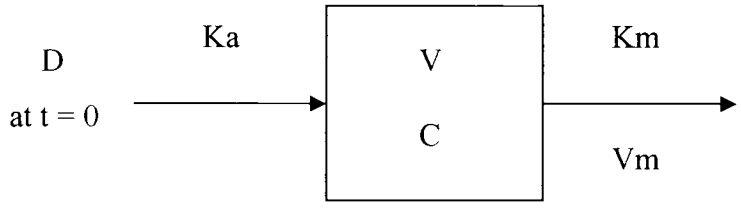


Figure 7.2 Schematic diagram of a drug following one-compartment open model with Michaelis-Menten elimination kinetics and first-order absorption.

The differential equation for the model in Figure 7.2 is given as equation (8).

$$V * \frac{dC}{dt} = KaFDe^{-Ka*t} - \frac{Vm * C}{Km + C} \tag{8}$$

Rearrangement of equation (8) yields equation (9).

$$\frac{dC}{dt} = \frac{KaFD}{V} e^{-Ka*t} - \frac{Vm'*C}{Km + C} \tag{9}$$

where Vm' = Vm/V

Ka = the apparent first-order absorption rate constant (units = time^{-1})

F = fraction of dose absorbed

Since both equation (8) and its arrangement, equation (9), cannot be integrated, there is no mathematical expression or AUC equation of drugs following this model. Al-Ghazawi (1998) proposed a preliminary equation for the calculation of AUC for drugs obeying one-compartment open model eliminated in nonlinear fashion as shown in equation (10).

$$AUC_{0-\infty} = \frac{KaFD}{V\left(Ka - \frac{Vm'}{Km + C_0}\right)}\left[\frac{Km + C_0/2}{Vm'} - \frac{1}{Ka}\right] \tag{10}$$

Two-Compartment Model

IV bolus administration

The model for a drug which follows one-compartment system having only Michaelis-Menten elimination kinetics from the central compartment after single intravenous dose can be illustrated as shown in Figure 7.3.

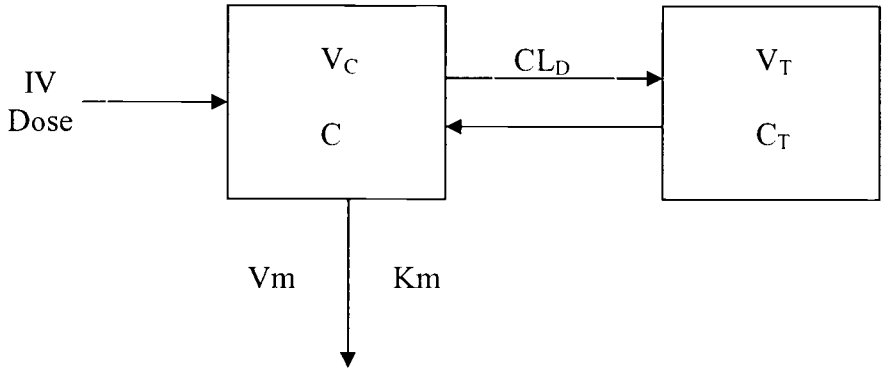


Figure 7.3 Schematic diagram of a drug following two-compartment system with Michaelis-Menten elimination kinetics from the central compartment after IV bolus administration.

The rate of decline of drug concentration (C) with time (t) can be described by the following equations.

$$V_C * \frac{dC}{dt} = \frac{-Vm * C}{Km + C} - CL_D * C + \frac{CL_D}{R} * C_T \qquad (11)$$

$$\frac{V_T}{R} * \frac{dC_T}{dt} = CL_D * C - \frac{CL_D}{R} * C_T \qquad (12)$$

where V_C = the apparent volume of distribution of the central compartment

V_T = the apparent volume of distribution of the tissue compartment

CL_D = the intercompartmental or distribution clearance (units = volume/time)

C = drug concentration in the central compartment

C_T = drug concentration in the tissue compartment

R = the tissue: plasma distribution ratio

It is assumed that an intravenous bolus dose of drug into the central compartment produces initial conditions of $C_0 = D/V_C$ and $C_T(0) = 0$.

Based on the computer simulations, Cheng and Jusko (1989) found that equation (13) from which V_C in equation (7) is substituted by Vss has been shown to be a meaningful AUC equation of drugs following two-compartment system with IV administration and Michaelis-Menten elimination kinetics from the central compartment.

$$AUC = \frac{D}{Vm}\left[\frac{D}{2Vss} + Km\right] \tag{13}$$

METHODS

Plasma concentration-time profiles after intravenous and oral administrations were generated by numerical integration of the differential equations (5), (9), (11), and (12) using WinNonlin Professional Version 3.2 software (Pharsight Corporation, Mountain View, California, USA).

For one-compartment system, simulations were performed by using the following values: Dose = 50, 100, 500, 1000, 2500, and 5000 mg, Vm = 5, 10, 20, and 40 mg/hr, Km = 1, 4, 10, and 20 mg/L, Ka = 0.3, 0.75, 1, 2, and 4 hr^{-1}, V = 44.8 L, and F = 1.

For two-compartment system, simulated data were carried out with dose = 50, 100, 500, 1000, 2500, and 5000 mg, Vm = 5, 10, 20, 40, and 50 mg/hr, Km = 1, 4, 10, and 20 mg/L, V_C = 25.6 L, V_T = 19.2 L, CL_D = 28.7 L/hr, and R = 1.

The areas under the plasma concentration-time curves were calculated by trapezoidal rule method and compared with those directly obtained from equations (7), (10), and (13).

Six doses were used in the simulations of the one- and two-compartment open model cases to assure that the pseudo-first-order, Michaelis-Menten, and initial pseudo-zero-order elimination behavior would be observed in the limiting low-dose, middle, and

high-dose cases. In addition, five different values of Ka were used to illustrate the effect of the absorption rate constant on the AUC.

To show how well equations (7), (10), and (13) can predict AUC in the different situations, the simulations of all combinations of dose, Vm, Km, and Ka were performed for both one- and two-compartment systems. The actual differences and the percentage of the differences in AUC estimation between equations (7), (10), (13) and the trapezoidal rule method were calculated using the AUC obtained from the trapezoidal rule method as the reference.

RESULTS

One-Compartment Model

IV bolus administration

The simulated plasma concentration-time data for all combinations of dose, Vm, and Km were generated using equation (5). Figure 7.4 shows the simulated plasma concentration-time profiles of six doses when Vm = 20 mg/hr and Km = 4 mg/L. The comparison of area under the simulated plasma concentration-time curves calculated directly from equation (7) and trapezoidal rule method along with the percentage of the differences in AUC estimation between these two methods are shown in Table 7.1.

The curves exhibit first-order behavior at the low dose and pseudo zero-order behavior at the early time of the medium and high dose when the drug plasma concentration is greater than Km. The percent difference in predicted AUCs from equation (7) and the numerically integrated AUCs using the trapezoidal rule range from 0.000049 % to 0.000736 %, showing the accuracy of equation (7) and the accuracy of the method to study the equations.

First-order absorption

The simulated plasma concentration-time data for all combinations of dose, Vm, Km, and Ka were generated using equation (9). Figures 7.5 and 7.6 show the simulated plasma concentration-time profiles when Vm = 20 mg/hr and Km = 4 mg/L, and Ka = 1 hr^{-1} at different doses while Figure 7.7 shows a plot of the simulated plasma concentration-time curves at different values of the absorption rate constant when dose = 500 mg, Vm = 20 mg/hr, and Km = 4 mg/L.

The comparison of area under the simulated plasma concentration-time curves calculated directly from equation (10) and trapezoidal rule method along with the percentage of the differences in AUC estimation between these two methods at the doses of 50, 100, 500, 1000, 2500, and 5000 mg are shown in Table 7.2, 7.3, 7.4, 7.5, 7.6, and7.7, respectively.

Based on the results shown in Tables 7.2 to 7.7, equation (10) yielded good approximation of the AUC except under the conditions of two cases; clearance ratio (Vm/Km) greater than 4 with Ka in the range of 0.3-0.75, and clearance ratio (Vm/Km) greater than 10 with Ka in the range of 1-2. Equation (10) gave good approximation when Ka is very fast with most of the values of dose, Vm, and Km used in this study. With the same value of Ka, the % difference in AUC estimation using equation (10) tends to be higher with decreasing dose, as seen in Figures 7.8, 7.9, and 7.10. Figure 7.11 demonstrates the effect of Ka on AUC with the same conditions of dose, Vm, and Km.

Two-Compartment Model

IV-bolus administration

The simulated plasma concentration-time data for all combinations of dose, Vm, and Km were generated using equations (11) and (12). Figure 7.12 shows the simulated plasma concentration-time profiles of six doses when Vm = 20 mg/hr, Km = 4 mg/L, CL_D = 28.7 L/hr, V_C = 25.6 L, V_T = 19.2 L, and R = 1. The comparison of area under the simulated plasma concentration-time curves calculated directly from equation (13) and the trapezoidal rule method along with the percentage of the differences in AUC estimation

between these two methods are shown in Table 7.8.

Based on the results shown in Table 7.8, equation (13) overall yielded better approximation for AUC than equation (10). However under the conditions of low dose (50 and 100 mg) with clearance ratio (Vm/Km) greater than 10, the % difference in AUC estimation larger than acceptable errors were observed.

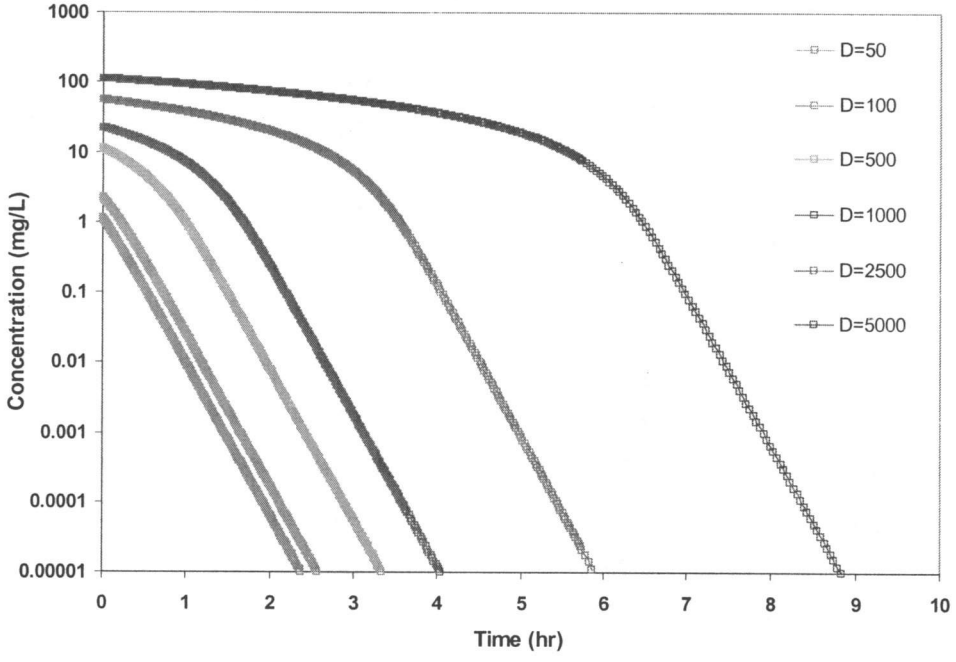


Figure 7.4 Simulated plasma concentration-time profiles for the one-compartment system following IV bolus administration using equation (2) at various doses when Vm= 20 mg/hr, Km = 4 mg/L, and V = 44.8 L.

Table 7.1 Comparison of AUC at different doses and clearance ratios (Vm/Km) of drug for one-compartment Michaelis-Menten system after IV bolus administration.

Dose (mg)	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 7) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
50	5	1	5	15.580357	15.580424	0.000427
		4	1.25	45.580357	45.580471	0.000250
		10	0.5	105.58036	105.58043	0.000068
		20	0.25	205.58036	205.58170	0.000653
50	10	1	10	7.7901786	7.7902294	0.000653
		4	2.5	22.790179	22.790272	0.000408
		10	1	52.790179	52.790284	0.000201
		20	0.5	102.79018	102.79031	0.000129
50	20	1	20	3.8950893	3.8951266	0.000478
		4	5	11.395089	11.395144	0.000361
		10	2	26.395089	26.395185	0.000257
		20	1	51.395089	51.395221	0.000372
50	40	1	40	1.9475446	1.9475519	0.000372
		4	10	5.6975446	5.6975855	0.000719
		10	4	13.197545	13.197607	0.000472
		20	2	25.697545	25.697644	0.000388

Table 7.1 (Continued)

Dose (mg)	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 7) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
50	50	1	50	1.558036	1.55804	0.000387
		4	12.5	4.558035	4.55807	0.000314
		10	5	10.55804	10.5581	0.000587
		20	2.5	20.55804	20.5812	0.000417
100	5	1	5	42.32143	42.3216	0.000447
		4	1.25	102.3214	102.322	0.000238
		10	0.5	222.3214	222.321	0.000135
		20	0.25	422.3214	422.324	0.000693
100	10	1	10	21.16071	21.1608	0.000482
		4	2.5	51.16071	51.1609	0.000412
		10	1	111.1607	111.161	0.000205
		20	0.5	211.1607	211.161	0.000131
100	20	1	20	10.58036	10.5804	0.000476
		4	5	25.58036	25.5805	0.000487
		10	2	55.58036	55.5806	0.000380
		20	1	105.5804	105.581	0.000209

Table 7.1 (Continued)

Dose (mg)	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 7) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
100	40	1	40	5.29017	5.29020	0.000558
		4	10	12.7901	12.7902	0.000735
		10	4	27.7901	27.7903	0.000494
		20	2	52.7901	52.7903	0.000381
100	50	1	50	4.23214	4.23215	0.000296
		4	12.5	10.2321	10.2322	0.000860
		10	5	22.2321	22.2322	0.000529
		20	2.5	42.2321	42.2323	0.000430
1000	5	1	5	2432.14	2432.14	0.000061
		4	1.25	3032.14	3032.14	0.000049
		10	0.5	4232.14	4232.15	0.000117
		20	0.25	6232.14	6232.17	0.000415
1000	10	1	10	1216.07	1216.07	0.000181
		4	2.5	1516.07	1516.07	0.000123
		10	1	2116.07	2116.07	0.000079
		20	0.5	3116.07	3116.07	0.000090

Table 7.1 (Continued)

Dose (mg)	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 7) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
1000	20	1	20	608.0357	608.0393	0.000603
		4	5	25.58035	25.58048	0.000402
		10	2	1058.035	1058.038	0.000231
		20	1	1558.035	1558.037	0.000128
1000	40	1	40	304.0178	304.0194	0.000509
		4	10	379.0178	379.0194	0.000425
		10	4	529.0178	529.0202	0.000459
		20	2	779.0178	779.0203	0.000324
1000	50	1	50	243.2142	243.2150	0.000297
		4	12.5	10.23214	10.23223	0.000447
		10	5	423.2142	423.2162	0.000459
		20	2.5	623.2142	623.2167	0.000393

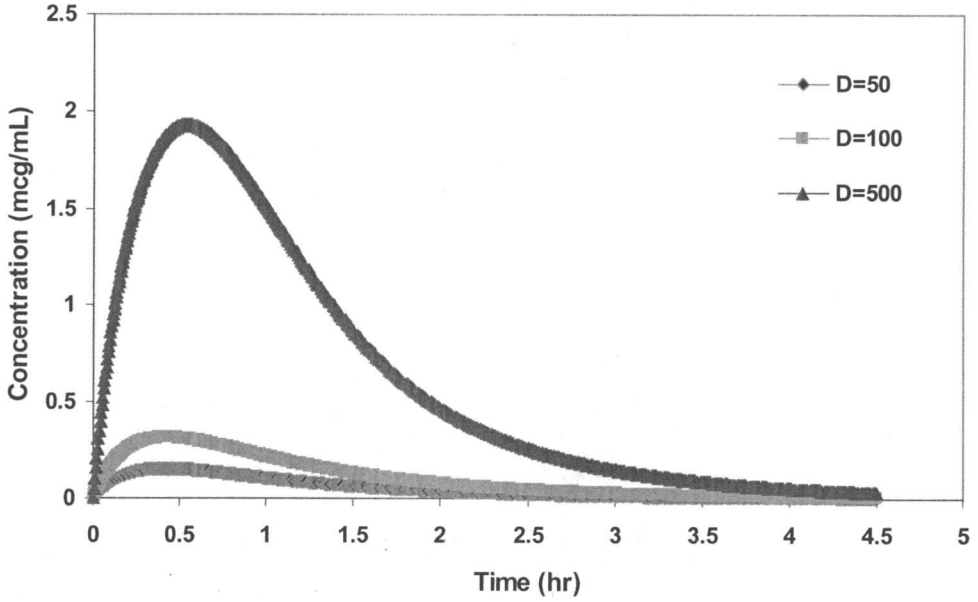


Figure 7.5 Simulated plasma concentration-time profiles for the one-compartment system with first-order absorption using equation (6) when Vm = 20 mg/hr, Km = 4 mg/L, V = 44.8 L, and Dose = 50, 100, and 500 mg.

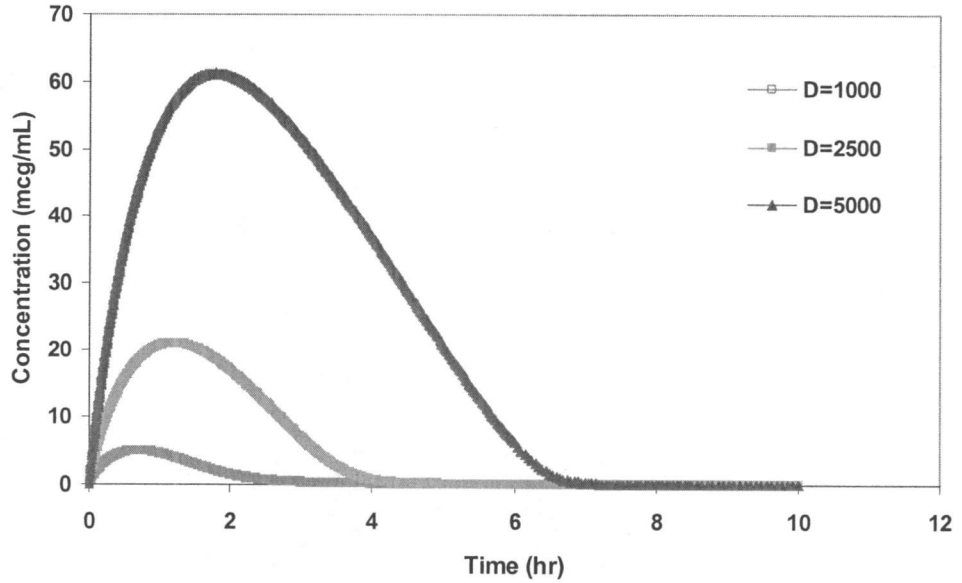


Figure 7.6 Simulated plasma concentration-time profiles for the one-compartment system with first-order absorption using equation (6) when Vm = 20 mg/hr, Km = 4 mg/L, V = 44.8 L, and Dose = 1000, 2500, and 5000 mg.

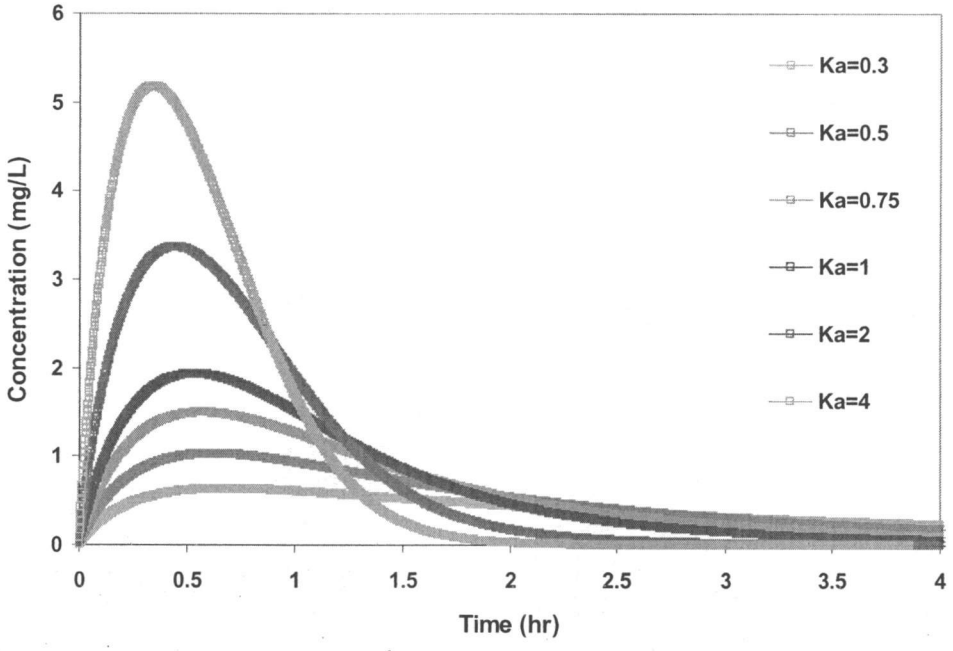


Figure 7.7 Simulated plasma concentration-time profiles for the one-compartment system with first-order absorption using equation (6) when dose = 500 mg, Vm = 20 mg/hr, Km = 4 mg/L, V = 44.8 L, and Ka = 0.3, 0.5, 0.75, 1, 2, and 4 hr^{-1}.

Table 7.2 Comparison of AUC at different Ka and clearance ratios (Vm/Km) of drug for one-compartment Michaelis-Menten system with first-order input at the dose of 50 mg.

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
50	0.3	5	1	5	14.390007	14.491003	-0.696950
			4	1.25	45.142750	45.146466	-0.008230
			10	0.5	105.38713	105.37960	0.0071416
			20	0.25	205.48027	205.45166	0.0139287
50	0.3	10	1	10	6.2770665	6.8345047	-8.156234
			4	2.5	22.315335	22.398016	-0.369143
			10	1	52.590022	52.600571	-0.020055
			20	0.5	102.68827	102.67691	0.0110642
50	0.3	20	1	20	0.5891905	3.1515973	-81.30501
			4	5	10.822862	11.047148	-2.030258
			10	2	26.179464	26.219708	-0.153484
			20	1	51.289320	51.293909	-0.008945
50	0.3	40	1	40	4.3611939	1.4493550	200.90583
			4	10	4.7273851	5.4150593	-12.69929
			10	4	12.942500	13.041965	-0.762649
			20	2	25.583099	25.605850	-0.088849

Table 7.2 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
50	0.75	5	1	5	15.158242	15.105711	0.347757
			4	1.25	45.413180	45.391004	0.048856
			10	0.5	105.50463	105.46293	0.039544
			20	0.25	205.54075	205.46546	0.036650
50	0.75	10	1	10	7.3335208	7.3410860	-0.103052
			4	2.5	22.617838	22.614067	0.0166795
			10	1	52.713419	52.694208	0.0364583
			20	0.5	102.75029	102.71169	0.0375808
50	0.75	20	1	20	3.3490660	3.4911543	-4.069950
			4	5	11.211404	11.231444	-0.178434
			10	2	26.316159	26.311675	0.0170417
			20	1	51.354622	51.336593	0.0351185
50	0.75	40	1	40	1.0503811	1.6194981	-35.141567
			4	10	5.4860072	5.5504728	-1.1614446
			10	4	13.113881	13.122479	-0.0655206
			20	2	25.655869	25.650264	0.02184924

Table 7.2 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
50	1	5	1	5	15.269646	15.216042	0.3522912
		5	4	1.25	45.455906	45.427111	0.0633888
		5	10	0.5	105.52376	105.47035	0.0506355
		5	20	0.25	205.55070	205.44978	0.0491227
50	1	10	1	10	7.4611480	7.4430279	0.2434507
		10	4	2.5	22.662889	22.651328	0.0510380
		10	10	1	52.733002	52.707149	0.0490509
		10	20	0.5	102.76036	102.71068	0.0483717
50	1	20	1	20	3.5220707	3.5750466	-1.4818248
		20	4	5	11.261715	11.266816	-0.0452746
		20	10	2	26.336717	26.326179	0.04002516
		20	20	1	51.364957	51.340864	0.04692834
50	1	40	1	40	1.4383879	1.6822253	-14.494928
		40	4	10	5.5500722	5.6035732	-0.9547662
		40	10	4	13.136623	13.190102	-0.4054460
		40	20	2	25.666747	25.759464	-0.3599334

Table 7.2 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
50	2	5	1	5	15.429209	15.380308	0.3179505
		5	4	1.25	45.518818	45.466809	0.1143888
		5	10	0.5	105.55220	105.44629	0.1004401
		5	20	0.25	205.56557	205.36541	0.0974638
50	2	10	1	10	7.6348234	7.6031493	0.4165920
		10	4	2.5	22.727953	22.701439	0.1167948
		10	10	1	52.761880	52.708894	0.1005268
		10	20	0.5	102.77535	102.67532	0.0974255
50	2	20	1	20	3.7305740	3.7191553	0.3070228
		20	4	5	11.331444	11.319646	0.1042292
		20	10	2	26.366501	26.340378	0.0991726
		20	20	1	51.380184	51.330304	0.0971752
50	2	40	1	40	1.7610353	1.7864068	-1.420250
		40	4	10	5.6308777	5.6304428	0.0073690
		40	10	4	13.168358	13.156510	0.0900542
		40	20	2	25.682478	25.657889	0.0958345

Table 7.2 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
50	4	5	1	5	15.505793	15.449671	0.3632583
		5	4	1.25	45.549756	45.450784	0.2177562
		5	10	0.5	105.56631	105.35090	0.2044682
		5	20	0.25	205.57297	205.17279	0.1950424
50	4	10	1	10	7.7146049	7.6802831	0.4468820
		10	4	2.5	22.759409	22.709215	0.2210273
		10	10	1	52.776100	52.670504	0.2004857
		10	20	0.5	102.78278	102.58266	0.1950853
50	4	20	1	20	3.8174117	3.7967082	0.5453008
		20	4	5	11.363976	11.338662	0.2232567
		20	10	2	26.380940	26.328135	0.2005631
		20	20	1	51.387676	51.287634	0.1950609
50	4	40	1	40	1.8652870	1.8572018	0.4353414
		40	4	10	5.6657223	5.6538282	0.2103725
		40	10	4	13.183250	13.157048	0.1991513
		40	20	2	25.690092	25.640134	0.1948430

Table 7.3 Comparison of AUC at different Ka and clearance ratios (Vm/Km) of drug for one-compartment Michaelis-Menten system with first-order input at the dose of 100 mg.

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
100	0.3	5	1	5	39.418016	38.984149	1.1129329
		5	4	1.25	100.90437	100.84336	0.0604949
		5	10	0.5	221.62125	221.58169	0.0178537
		5	20	0.25	421.94155	421.87793	0.0150810
100	0.3	10	1	10	17.823180	18.059814	-1.3102817
		10	4	2.5	49.647602	49.756289	-0.2184399
		10	10	1	110.43787	110.44682	-0.0081070
		10	20	0.5	210.77426	210.75954	0.0069842
100	0.3	20	1	20	5.8189602	7.9627924	-26.923119
		20	4	5	23.829931	24.304123	-1.9510747
		20	10	2	54.807454	54.919322	-0.2036950
		20	20	1	105.18004	105.20102	-0.0199413
100	0.3	40	1	40	-27.15375	3.4062052	-897.18501
		40	4	10	10.239741	11.722392	-12.648027
		40	10	4	26.893015	27.195046	-1.1106132
		40	20	2	52.358929	52.43940	-0.1534536

Table 7.3 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
100	0.75	5	1	5	41.244139	40.091786	0.7973878
		5	4	1.25	101.77540	101.67726	0.0965229
		5	10	0.5	222.04653	221.94829	0.0442638
		5	20	0.25	422.17101	422.01006	0.0381378
100	0.75	10	1	10	20.028796	19.801321	1.1487850
		10	4	2.5	50.600999	50.549325	0.1022246
		10	10	1	110.88239	110.83389	0.0437606
		10	20	0.5	211.00927	210.92572	0.0396150
100	0.75	20	1	20	9.3206828	9.2983107	0.2406041
		20	4	5	24.991090	25.001205	-0.040460
		20	10	2	55.294916	55.281106	0.0249827
		20	20	1	105.42683	105.39023	0.0347343
100	0.75	40	1	40	3.6632522	4.1664105	-12.07654
		40	4	10	12.131340	12.257053	-1.025638
		40	10	4	27.489350	27.513311	-0.086390
		40	20	2	52.632318	52.623333	0.0170737

Table 7.3 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
100	1	5	1	5	41.249684	41.523094	0.662816
		5	4	1.25	101.81056	101.91440	0.101991
		5	10	0.5	221.99040	222.11589	0.056526
		5	20	0.25	421.99578	422.20880	0.050480
100	1	10	1	10	20.122206	20.332768	1.046414
		10	4	2.5	50.685344	50.746126	0.119196
		10	10	1	110.88694	110.95326	0.059805
		10	20	0.5	210.94566	211.04752	0.048286
100	1	20	1	20	9.5880777	9.6860691	1.022013
		20	4	5	25.131749	25.149774	0.071721
		20	10	2	55.341100	55.368979	0.050377
		20	20	1	105.41406	105.46600	0.049276
100	1	40	1	40	4.3872401	4.2252249	-3.692872
		40	4	10	12.323593	12.372280	-0.393516
		40	10	4	27.570479	27.571921	-0.005230
		40	20	2	52.673434	52.652220	0.0402916

Table 7.3 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
100	2	5	1	5	41.929274	41.735697	0.463815
		5	4	1.25	102.11975	101.97614	0.140828
		5	10	0.5	222.21913	221.98183	0.106898
		5	20	0.25	422.26525	421.84216	0.100297
100	2	10	1	10	20.761547	20.609917	0.735713
		10	4	2.5	50.957200	50.875910	0.159781
		10	10	1	111.05794	110.93726	0.108781
		10	20	0.5	211.10440	210.89739	0.098157
100	2	20	1	20	10.166384	10.053821	1.119599
		20	4	5	25.373063	25.328120	0.177442
		20	10	2	55.476633	55.415694	0.109965
		20	20	1	105.52376	105.41784	0.100472
100	2	40	1	40	4.843034	4.7905520	1.095543
		40	4	10	12.574887	12.558691	0.128958
		40	10	4	27.684489	27.656178	0.102370
		40	20	2	52.733002	52.680731	0.099222

Table 7.3 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
100	4	5	1	5	42.127058	41.936041	0.455497
		5	4	1.25	102.22104	101.97149	0.244725
		5	10	0.5	222.27039	221.80394	0.210299
		5	20	0.25	422.29337	421.44966	0.200193
100	4	10	1	10	20.964637	20.837616	0.609572
		10	4	2.5	51.059876	50.929180	0.256622
		10	10	1	111.10956	110.87502	0.211536
		10	20	0.5	211.13263	210.71547	0.197971
100	4	20	1	20	10.380773	10.290022	0.881934
		20	4	5	25.478600	25.408646	0.275313
		20	10	2	55.528972	55.410766	0.213326
		20	20	1	105.55220	105.34108	0.200408
100	4	40	1	40	5.0831920	5.0325749	1.005789
		40	4	10	12.686531	12.649494	0.292792
		40	10	4	27.738316	27.678982	0.214364
		40	20	2	52.761880	52.656241	0.200619

Table 7.4 Comparison of AUC at different Ka and clearance ratios (Vm/Km) of drug for one-compartment Michaelis-Menten system with first-order input at the dose of 500 mg.

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
500	0.3	5	1	5	640.42539	629.16356	1.789969
		5	4	1.25	943.99786	938.58940	0.576224
		5	10	0.5	1548.0493	1545.6416	0.155777
		5	20	0.25	2551.2928	2550.0066	0.050442
500	0.3	10	1	10	310.83369	300.41228	3.469034
		10	4	2.5	464.61767	459.88562	1.028963
		10	10	1	768.84956	766.91910	0.251717
		10	20	0.5	145.05704	1271.2957	0.070545
500	0.3	20	1	20	224.32510	136.87169	6.368118
		20	4	5	378.95605	377.75210	1.563537
		20	10	2	632.51249	632.03163	0.318716
		20	20	1	59.650943	55.480792	0.076096
500	0.3	40	1	40	102.71624	102.14609	7.156386
		40	4	10	183.33802	183.57956	0.558175
		40	10	4	312.38862	312.59598	-0.131576
		40	20	2	312.38862	312.59598	-0.066332

Table 7.4 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
500	0.75	5	1	5	651.12248	646.08798	0.779228
		5	4	1.25	952.50403	949.76063	0.288852
		5	10	0.5	1554.0836	1552.4233	0.106943
		5	20	0.25	2555.3580	2553.8696	0.058280
500	0.75	10	1	10	322.01791	317.31152	1.483205
		10	4	2.5	473.43079	471.04313	0.506888
		10	10	1	775.03757	773.80245	0.159616
		10	20	0.5	1276.3272	1275.4343	0.070008
500	0.75	20	1	20	157.32884	152.96610	2.852099
		20	4	5	233.80770	231.73324	0.895195
		20	10	2	385.47104	384.52259	0.246656
		20	20	1	636.79210	636.22346	0.089376
500	0.75	40	1	40	74.684799	70.892783	5.348944
		40	4	10	113.81032	112.18353	1.450118
		40	10	4	190.59622	189.94643	0.342092
		40	20	2	316.98368	316.64943	0.105556

Table 7.4 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
500	1	5	1	5	652.86680	648.95374	0.602979
		5	4	1.25	953.89721	951.67832	0.233154
		5	10	0.5	1555.0768	1553.6194	0.093808
		5	20	0.25	2556.0298	2554.6053	0.055762
500	1	10	1	10	323.80061	320.16981	1.134024
		10	4	2.5	474.84843	472.95237	0.400898
		10	10	1	776.04325	774.99729	0.134962
		10	20	0.5	1277.0047	1276.1767	0.064877
500	1	20	1	20	159.19227	155.80106	2.176629
		20	4	5	235.27625	233.61617	0.710600
		20	10	2	386.50227	385.70285	0.207261
		20	20	1	637.48118	636.96235	0.081453
500	1	40	1	40	76.727169	73.668895	4.151376
		40	4	10	115.38935	114.00557	1.213783
		40	10	4	191.68158	191.09109	0.309008
		40	20	2	317.69681	317.37241	0.102212

Table 7.4 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
500	2	5	1	5	655.46317	653.27203	0.335410
		5	4	1.25	955.97410	954.58021	0.146021
		5	10	0.5	1556.5602	1555.4455	0.071663
		5	20	0.25	2557.0345	2555.7127	0.051719
500	2	10	1	10	326.43340	324.47660	0.603061
		10	4	2.5	476.94860	475.83969	0.233042
		10	10	1	777.53844	776.81151	0.093578
		10	20	0.5	1278.0149	1277.3144	0.054839
500	2	20	1	20	161.90030	160.08475	1.134121
		20	4	5	237.42412	236.47619	0.400893
		20	10	2	388.02162	387.49880	0.134920
		20	20	1	638.50237	638.08946	0.064709
500	2	40	1	40	79.596138	77.900756	2.176335
		40	4	10	117.63812	116.80825	0.710458
		40	10	4	193.25113	192.85141	0.207266
		40	20	2	318.74059	318.48117	0.081453

Table 7.4 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
500	4	5	1	5	656.75240	655.44633	0.199263
		5	4	1.25	957.00681	956.04816	0.100271
		5	10	0.5	1557.2989	1556.3771	0.059228
		5	20	0.25	327.73158	2556.3494	0.046400
500	4	10	1	10	477.98705	326.64213	0.000353
		10	4	2.5	778.28010	477.29770	0.144426
		10	10	1	1278.5172	777.73315	0.070326
		10	20	0.5	163.21670	1277.8990	0.048378
500	4	20	1	20	238.47430	162.24105	0.601357
		20	4	5	388.76922	237.92347	0.231515
		20	10	2	639.00745	388.41115	0.092186
		20	20	1	639.00745	638.66540	0.053556
500	4	40	1	40	80.950154	80.043753	1.132382
		40	4	10	118.71210	118.23983	0.399423
		40	10	4	194.01081	193.75194	0.133607
		40	20	2	319.25118	319.04873	0.063454

Table 7.5 Comparison of AUC at different Ka and clearance ratios (Vm/Km) of drug for one-compartment Michaelis-Menten system with first-order input at the dose of 1000 mg.

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
1000	0.3	5	1	5	2395.9584	2367.7032	1.193358
		5	4	1.25	3000.1417	2982.3200	0.597578
		5	10	0.5	4206.1514	4196.1200	0.239063
		5	20	0.25	6212.3473	6206.6408	0.091941
1000	0.3	10	1	10	1179.2907	1152.0877	2.361192
		10	4	2.5	1483.6048	2080.6971	1.143845
		10	10	1	2089.77.37	3091.3049	0.436232
		10	20	0.5	3096.0987	544.48440	0.155073
1000	0.3	20	1	20	570.00165	709.40795	4.686491
		20	4	5	724.59637	1023.2798	2.140998
		20	10	2	1031.1034	1533.8339	0.764568
		20	20	1	1537.6991	241.20179	0.251998
1000	0.3	40	1	40	263.20190	331.44699	9.121039
		40	4	10	343.44699	495.19403	3.620486
		40	10	4	500.71990	755.50208	1.115898
		40	20	2	757.91211	755.50208	0.318997

Table 7.5 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
1000	0.75	5	1	5	2417.8085	2405.7634	0.500676
		5	4	1.25	3019.4515	3011.5172	0.263465
		5	10	0.5	4221.8184	4216.8917	0.116831
		5	20	0.25	6224.2665	6220.8642	0.054691
1000	0.75	10	1	10	1201.6444	1190.1324	0.967286
		10	4	2.5	1503.3075	1495.9522	0.491681
		10	10	1	2105.6990	2101.4331	0.202997
		10	20	0.5	3108.1672	3105.5796	0.083320
1000	0.75	20	1	20	593.41979	582.34651	1.901493
		20	4	5	745.12419	738.21906	0.935376
		20	10	2	1047.5660	1043.7494	0.365653
		20	20	1	1550.0751	1547.9683	0.136099
1000	0.75	40	1	40	289.80052	278.51601	3.767698
		40	4	10	365.80052	359.45450	1.765457
		40	10	4	518.34795	515.00063	0.649965
		40	20	2	770.94208	769.22581	0.223116

Table 7.5 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
1000	1	5	1	5	2421.4093	2412.1444	0.384093
		5	4	1.25	3022.6379	3016.4305	0.205784
		5	10	0.5	4224.4084	4220.4075	0.094798
		5	20	0.25	6226.2408	6223.2975	0.047294
1000	1	10	1	10	1205.2860	1196.5051	0.733876
		10	4	2.5	1506.5258	1500.8516	0.378061
		10	10	1	2108.3101	2104.9360	0.160295
		10	20	0.5	3110.1537	3108.0039	0.069169
1000	1	20	1	20	597.14508	588.70186	1.434209
		20	4	5	748.40776	743.08986	0.715647
		20	10	2	1050.2200	1047.2258	0.285919
		20	20	1	1552.0865	1550.3744	0.110430
1000	1	40	1	40	292.91045	284.83388	2.835538
		40	4	10	369.22088	364.26489	1.360544
		40	10	4	521.09121	518.42247	0.514780
		40	20	2	773.00454	771.59488	0.182693

Table 7.5 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
1000	2	5	1	5	2426.7889	2421.7406	0.208459360
		5	4	1.25	3027.4004	3023.8306	0.118055
		5	10	0.5	4228.2823	4225.7163	0.060724
		5	20	0.25	6229.1957	6226.9891	0.035436
1000	2	10	1	10	1210.7046	1206.0833	0.383165
		10	4	2.5	1511.3189	1508.2272	0.204992
		10	10	1	2112.2042	2110.2184	0.094105
		10	20	0.5	3113.1204	3111.6685	0.046658
1000	2	20	1	20	602.64302	598.25866	0.046658
		20	4	5	753.26291	750.43257	0.377161
		20	10	2	1054.1550	1052.4759	0.159544
		20	20	1	1555.0768	1554.0123	0.068503
1000	2	40	1	40	298.57254	294.35378	1.433228
		40	4	10	374.20388	371.54851	0.714675
		40	10	4	525.11004	523.61736	0.285071
		40	20	2	776.04325	775.19274	0.109715

Table 7.5 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
1000	4	5	1	5	2429.4691	2426.5081	0.122027
		5	4	1.25	3029.7741	3027.4973	0.075206
		5	10	0.5	4230.2142	4228.3257	0.044662
		5	20	0.25	6230.6702	6228.7777	0.030383
1000	4	10	1	10	1213.3944	1210.8601	0.209299
		10	4	2.5	1513.7002	1511.9032	0.118854
		10	10	1	2114.1411	2112.8432	0.061428
		10	20	0.5	3114.5978	3113.4756	0.036043
1000	4	20	1	20	605.35233	603.03708	0.383931
		20	4	5	755.65947	754.10832	0.205693
		20	10	2	1056.1021	1055.1023	0.094757
		20	20	1	1556.5602	1555.8253	0.047235
1000	4	40	1	40	301.32151	299.12680	0.733703
		40	4	10	376.63145	375.21314	0.378002
		40	10	4	527.07753	526.23498	0.160109
		40	20	2	777.53844	777.00201	0.069038

Table 7.6 Comparison of AUC at different Ka and clearance ratios (Vm/Km) of drug for one-compartment Michaelis-Menten system with first-order input at the dose of 2500 mg.

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
2500	0.3	5	1	5	14358.921	14276.588	0.576700
		5	4	1.25	15863.564	15798.970	0.408850
		5	10	0.5	18871.572	18825.705	0.243637
		5	20	0.25	23882.087	23851.373	0.128774
2500	0.3	10	1	10	7132.8641	7052.1533	1.144497
		10	4	2.5	7887.5678	7824.9969	0.799629
		10	10	1	9395.6723	9351.4764	0.472608
		10	20	0.5	11906.300	11877.317	0.244018
2500	0.3	20	1	20	3518.8965	3439.9569	2.294786
		20	4	5	3898.7234	3837.8619	1.585817
		20	10	2	4657.0262	4614.7579	0.915937
		20	20	1	5917.8849	5890.7664	0.460356
2500	0.3	40	1	40	1709.9410	1634.1495	4.637981
		40	4	10	1902.5312	1844.9213	3.122622
		40	10	4	2286.2532	2247.0195	1.746034
		40	20	2	2922.5963	2897.9661	0.849913

Table 7.6 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
2500	0.75	5	1	5	14414.249	14380.126	0.237288
		5	4	1.25	15916.092	15888.885	0.171228
		5	10	0.5	18919.272	18899.545	0.104377
		5	20	0.25	23923.452	23909.612	0.104377
2500	0.75	10	1	10	7188.7064	7155.5951	0.057881
		10	4	2.5	7940.5587	7914.7455	0.462733
		10	10	1	9443.7542	9425.1078	0.326140
		10	20	0.5	11947.951	11935.343	0.197837
2500	0.75	20	1	20	3575.7887	3543.2626	0.105636
		20	4	5	3952.6601	3927.3317	0.917969
		20	10	2	4705.8863	4688.0235	0.644926
		20	20	1	5960.1195	5948.4271	0.381031
2500	0.75	40	1	40	1769.0318	1737.1909	0.196562
		40	4	10	1958.4424	1933.8221	1.832894
		40	10	4	2336.7314	2319.5535	0.740569
		40	20	2	2966.0377	2954.9203	0.376232

Table 7.6 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
2500	1	5	1	5	14423.428	14397.252	0.181808
		5	4	1.25	15924.808	15903.739	0.132475
		5	10	0.5	18927.191	18911.709	0.081862
		5	20	0.25	23930.322	23919.152	0.046697
2500	1	10	1	10	7197.9277	7172.7775	0.350634
		10	4	2.5	7949.3133	7929.6537	0.247925
		10	10	1	9451.7042	9437.6291	0.149138
		10	20	0.5	11954.845	11945.294	0.079959
2500	1	20	1	20	355.09549	3560.4664	0.691736
		20	4	5	3961.4918	3942.2513	0.488057
		20	10	2	4713.8999	4700.2524	0.290356
		20	20	1	5967.0613	5958.0484	0.151272
2500	1	40	1	40	1778.5132	1754.4014	1.374360
		40	4	10	1967.4314	1948.7147	0.960463
		40	10	4	2344.8745	2331.7435	0.563137
		40	20	2	2973.0766	2964.5063	0.289095

Table 7.6 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
2500	2	5	1	5	14437.174	14423.060	0.097646
		5	4	1.25	15937.862	15926.180	0.073352
		5	10	0.5	18939.052	18930.132	0.047119
		5	20	0.25	23940.615	23933.665	0.029037
2500	2	10	1	10	721.71415	7198.6290	0.181771
		10	4	2.5	7962.4043	7952.1040	0.129528
		10	10	1	9463.5955	9456.0600	0.079688
		10	20	0.5	11965.161	11959.818	0.044672
2500	2	20	1	20	3598.9638	3586.3185	0.352597
		20	4	5	3974.6566	3964.6916	0.251343
		20	10	2	4725.8521	4718.6678	0.152251
		20	20	1	5977.5477	5972.5585	0.081442
2500	2	40	1	40	1792.5477	1780.2285	0.692000
		40	4	10	1980.7459	1971.1190	0.488397
		40	10	4	2356.9599	2350.1104	0.291029
		40	20	2	2983.5306	2978.9696	0.153107

Table 7.6 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
2500	4	5	1	5	14444.036	14436.045	0.055357
		5	4	1.25	15944.380	15937.443	0.043529
		5	10	0.5	18944.974	18939.390	0.029484
		5	20	0.25	23945.755	23940.975	0.019967
2500	4	10	1	10	7968.9314	7211.5729	0.097262
		10	4	2.5	9469.5260	7963.3539	0.700390
		10	10	1	11970.307	9465.3040	0.044604
		10	20	0.5	3605.8570	11967.112	0.026700
2500	4	20	1	20	3981.2021	3599.2552	0.183421
		20	4	5	4731.7977	3975.9317	0.132558
		20	10	2	5982.5805	4727.8997	0.082446
		20	20	1	5982.5805	5979.8394	0.045839
2500	4	40	1	40	1799.4819	1793.1574	0.352700
		40	4	10	1987.3283	1982.3464	0.251310
		40	10	4	2362.9260	2359.3262	0.152576
		40	20	2	2988.7113	2986.2342	0.082952

Table 7.7 Comparison of AUC at different Ka and clearance ratios (Vm/Km) of drug for one-compartment Michaelis-Menten system with first-order input at the dose of 5000 mg.

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
5000	0.3	5	1	5	56618.600	56444.129	0.309103
		5	4	1.25	59623.415	59473.125	0.252703
		5	10	0.5	65632.331	65510.569	0.185867
		5	20	0.25	75645.380	75552.561	0.122852
5000	0.3	10	1	10	28216.199	28043.557	0.615619
		10	4	2.5	29721.046	29572.736	0.501510
		10	10	1	32730.019	32611.165	0.364455
		10	20	0.5	37743.144	37653.384	0.238386
5000	0.3	20	1	20	14014.063	13843.779	1.230042
		20	4	5	14768.975	14623.108	0.997510
		20	10	2	16278.062	16161.708	0.719931
		20	20	1	18791.344	18704.085	0.466523
5000	0.3	40	1	40	6911.0815	6744.0141	2.477268
		40	4	10	7291.1253	7148.6171	1.993507
		40	10	4	8050.4477	7937.4881	1.423115
		40	20	2	9314.0524	9230.0462	0.910138

Table 7.7 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
5000	0.75	5	1	5	56729.729	56657.934	0.126716
		5	4	1.25	59731.648	59669.417	0.104293
		5	10	0.5	65735.201	65683.767	0.078305
		5	20	0.25	75740.402	75700.340	0.052921
5000	0.75	10	1	10	28327.846	28257.289	0.249695
		10	4	2.5	29829.769	29768.900	0.204472
		10	10	1	32833.331	32784.181	0.149921
		10	20	0.5	37838.545	37800.947	0.099463
5000	0.75	20	1	20	14126.756	14057.419	0.493244
		20	4	5	14878.690	14819.074	0.402296
		20	10	2	16382.270	16334.425	0.292912
		20	20	1	18887.508	18851.290	0.192125
5000	0.75	40	1	40	7025.9144	6957.4978	0.983351
		40	4	10	7402.8686	7344.2069	0.798748
		40	10	4	8156.4850	8109.6237	0.577847
		40	20	2	9411.7727	9376.5557	0.375586

Table 7.7 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
5000	1	5	1	5	56748.208	56693.358	0.096748
		5	4	1.25	59749.646	59701.920	0.079940
		5	10	0.5	65752.309	65712.416	0.060708
		5	20	0.25	75756.208	75724.422	0.041975
5000	1	10	1	10	28346.367	28292.810	0.189296
		10	4	2.5	29847.808	29801.500	0.155389
		10	10	1	32850.476	32812.925	0.114441
		10	20	0.5	37854.382	37825.444	0.076503
5000	1	20	1	20	14145.364	14092.984	0.371672
		20	4	5	14896.811	14851.710	0.303673
		20	10	2	16399.489	16363.200	0.221773
		20	20	1	18903.408	18875.818	0.146166
5000	1	40	1	40	7044.6963	6993.0763	0.738159
		40	4	10	7421.1543	7376.8393	0.600731
		40	10	4	8173.8528	9138.3797	0.435874
		40	20	2	9427.7999	9401.0572	0.284464

Table 7.7 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
5000	2	5	1	5	56775.903	56746.827	0.051238
		5	4	1.25	59776.622	59751.026	0.042837
		5	10	0.5	65777.952	65755.761	0.033747
		5	20	0.25	75779.899	75761.732	0.023979
5000	2	10	1	10	28374.104	28346.258	0.098234
		10	4	2.5	29874.823	29850.580	0.081214
		10	10	1	32876.154	32856.235	0.060626
		10	20	0.5	378778.10	37862.401	0.041471
5000	2	20	1	20	14173.183	14146.417	0.189211
		20	4	5	14923.904	14900.766	0.155277
		20	10	2	16425.238	16406.478	0.114346
		20	20	1	189273.19	18912.738	0.076418
5000	2	40	1	40	7072.6823	7046.4913	0.371688
		40	4	10	7448.4056	7425.8625	0.303575
		40	10	4	8199.7447	8181.6083	0.221672
		40	20	2	9451.7042	9437.9181	0.146071

Table 7.7 (Continued)

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 10) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
5000	4	5	1	5	56789.741	56773.659	0.028325
		5	4	1.25	59790.099	59775.670	0.024138
		5	10	0.5	65790.764	65777.529	0.020121
		5	20	0.25	75791.738	75780.324	0.015647
5000	4	10	1	10	28387.951	28373.036	0.052567
		10	4	2.5	29888.311	29875.168	0.043991
		10	10	1	32888.976	32877.942	0.033558
		10	20	0.5	37889.949	37880.937	0.023790
5000	4	20	1	20	14187.052	14173.168	0.097959
		20	4	5	14937.411	14925.324	0.080987
		20	10	2	16438.077	16428.151	0.060421
		20	20	1	18939.052	18931.236	0.041282
5000	4	40	1	40	7086.5919	7073.2274	0.188945
		40	4	10	7461.9521	7450.3990	0.155067
		40	10	4	8212.6192	8203.2560	0.114139
		40	20	2	9463.5955	9456.3876	0.076221

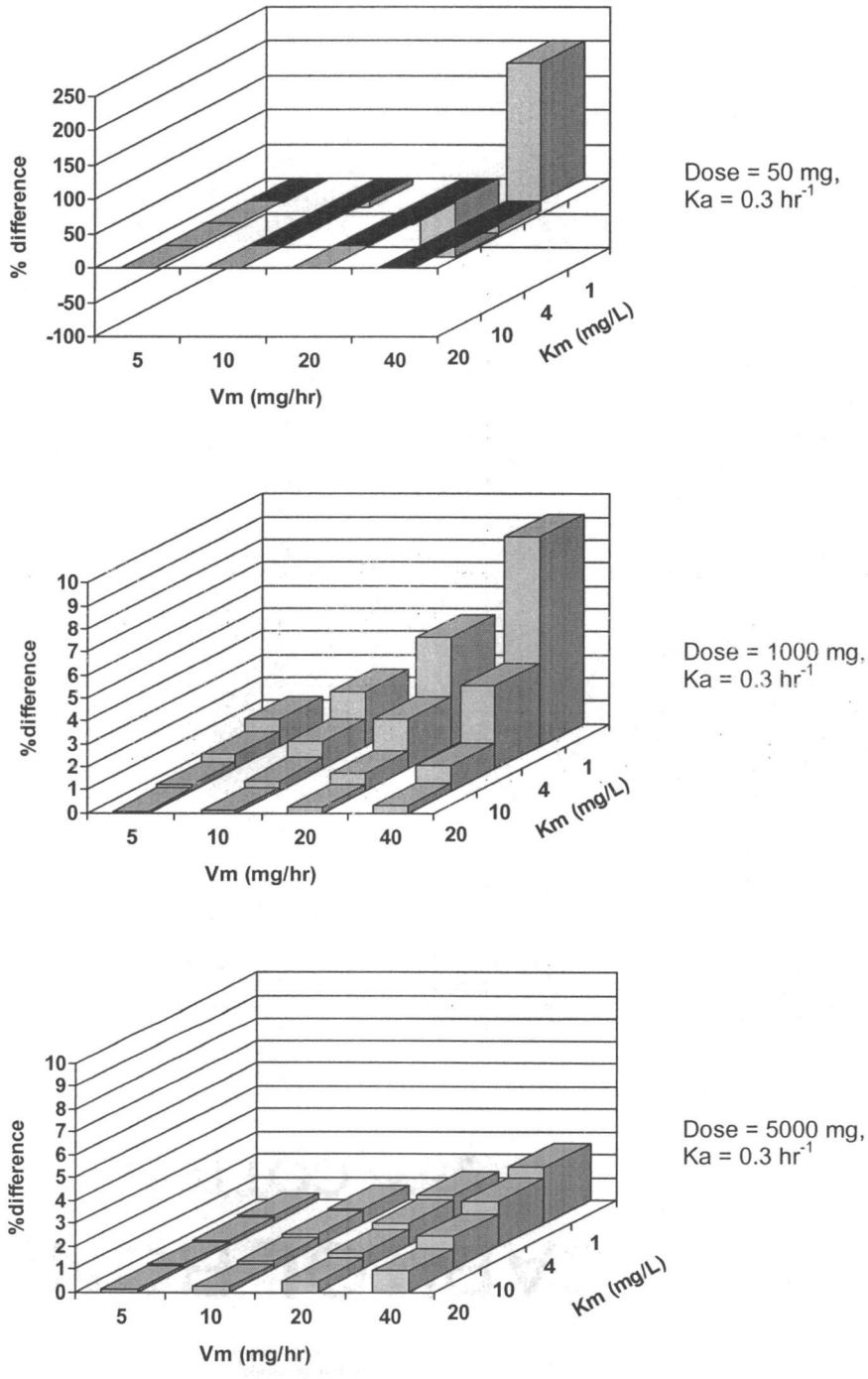
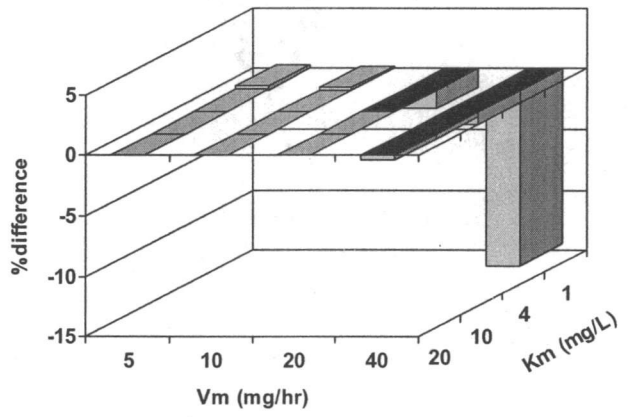
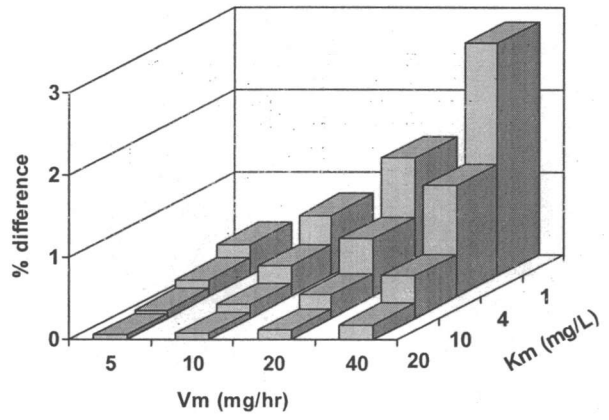


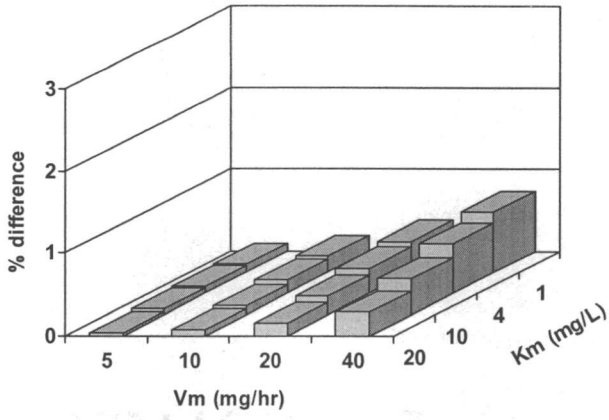
Figure 7.8 Plot of % difference in AUC versus Vm and Km at three different doses (50, 1000, and 5000 mg) when Ka = 0.3 hr^{-1}.



Dose = 50 mg,
Ka = 1 hr^{-1}



Dose = 1000 mg,
Ka = 1 hr^{-1}



Dose = 5000 mg,
Ka = 1 hr^{-1}

Figure 7.9 Plot of % difference in AUC versus Vm and Km at three different doses (50, 1000, and 5000 mg) when Ka = 1 hr^{-1}.

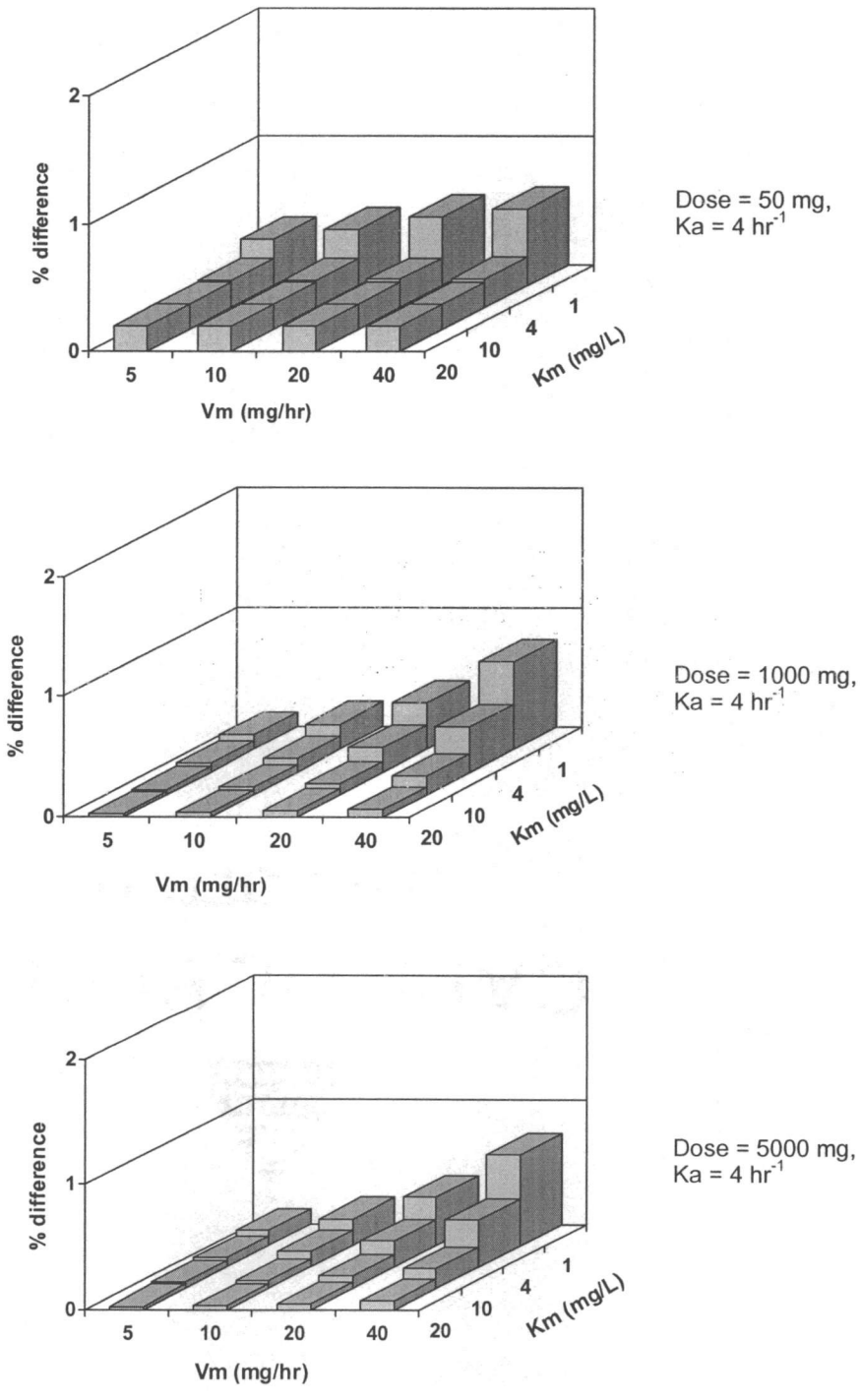


Figure 7.10 Plot of % difference in AUC versus Vm and Km at three different doses (50, 1000, and 5000 mg) when Ka = 4 hr^{-1}.

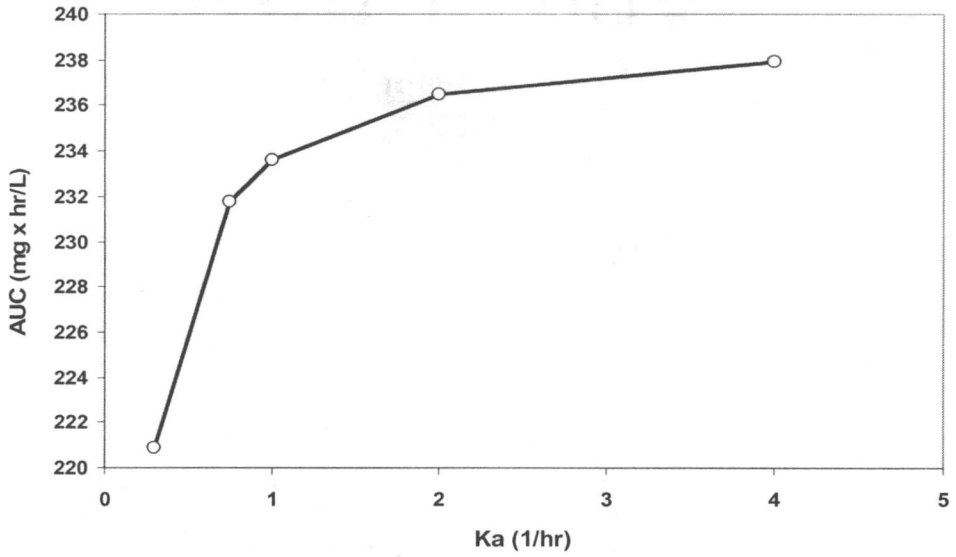


Figure 7.11 Effect of absorption rate constant on AUC (Vm = 20 mg/hr, Km = 4 mg/L, V = 44.8 L, Dose = 500 mg).

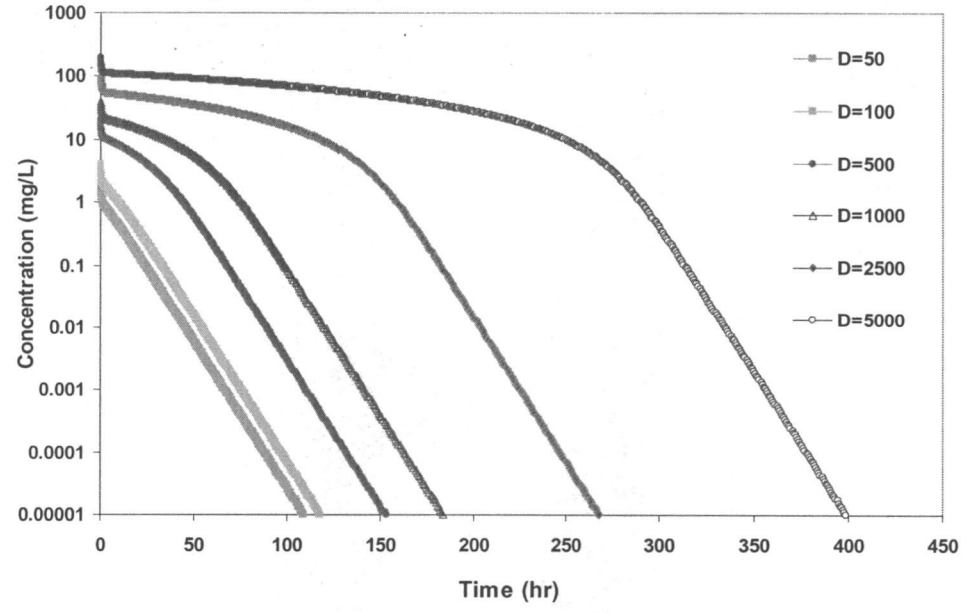


Figure 7.12 Simulated plasma concentration-time profiles for the two-compartment model following IV bolus administration using equation (8) and (9) when Vm = 20 mg/hr, Km = 4 mg/L, CL_D = 28.7 L/hr, V_C = 25.6 L, V_T = 19.2 L, R = 1, and D = 50, 100, 500, 1000, 2500, and 5000 mg.

Table 7.8 Comparison of AUC at different doses and clearance ratios (Vm/Km) of drug for two-compartment Michaelis-Menten system after IV bolus administration.

Dose (mg)	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 13) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
50	5	1	5	15.580357	15.668100	0.560015
		4	1.25	45.580357	45.630121	0.109059
		10	0.5	105.58035	105.96129	0.030840
		20	0.25	205.58035	205.59465	0.006956
50	10	1	10	7.7901785	7.8779932	1.114682
		4	2.5	22.790178	22.839896	0.217678
		10	1	52.790178	52.814780	0.046581
		20	0.5	102.79017	102.80314	0.012608
50	20	1	20	3.8950892	3.9826224	2.197878
		4	5	11.395089	11.443963	0.427075
		10	2	26.395089	26.420398	0.095794
		20	1	51.395089	51.409259	0.027563
50	40	1	40	1.9475446	2.0330439	4.205484
		4	10	5.6975446	5.7447506	0.821724
		10	4	13.197544	13.222317	0.187357
		20	2	25.697544	25.711384	0.053828

Table 7.8 (Continued)

Dose (mg)	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 13) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
50	50	1	50	1.5580357	1.6419682	5.111704
		4	12.5	4.5580357	4.6044203	1.007392
		10	5	10.558035	10.582546	0.231616
		20	2.5	20.558035	20.571786	0.066844
100	5	1	5	42.321428	42.500609	0.421595
		4	1.25	102.32142	102.46300	0.138176
		10	0.5	222.32142	222.40910	0.039420
		20	0.25	422.32142	422.37064	0.011651
100	10	1	10	21.160714	21.342078	0.849797
		4	2.5	51.160714	51.304476	0.280214
		10	1	111.16071	111.24746	0.077976
		20	0.5	211.16071	211.21314	0.024821
100	20	1	20	10.580357	10.768698	1.721596
		4	5	25.580357	25.723010	0.554575
		10	2	55.580357	55.666389	0.154549
		20	1	105.58035	105.63158	0.048494

Table 7.8 (Continued)

Dose (mg)	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 13) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
100	40	1	40	5.2901785	5.4815651	3.491458
		4	10	12.790178	12.930462	1.084912
		10	4	27.790178	27.874857	0.303784
		20	2	52.790178	52.840795	0.095792
100	50	1	50	4.2321428	4.4254575	4.368242
		4	12.5	10.232142	10.371168	1.340505
		10	5	22.232142	22.316152	0.376451
		20	2.5	42.232142	42.282492	0.119079
500	5	1	5	658.03571	658.56240	0.079975
		4	1.25	958.03571	958.89825	0.089951
		10	0.5	1558.0357	1558.9150	0.056405
		20	0.25	2558.0357	2558.7744	0.028871
500	10	1	10	329.01785	329.55032	0.161574
		4	2.5	479.01785	479.88135	0.179939
		10	1	779.01785	779.89585	0.112578
		20	0.5	1279.0178	1279.7434	0.056695

Table 7.8 (Continued)

Dose (mg)	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 13) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
500	20	1	20	164.50892	165.05765	0.332443
		4	5	239.50892	240.37785	0.361482
		10	2	389.50892	390.38578	0.224613
		20	1	639.50892	640.23247	0.113012
500	40	1	40	82.254464	82.836913	0.703127
		4	10	119.75446	120.63567	0.730474
		10	4	194.75446	195.63164	0.448382
		10	4	319.75446	320.47464	0.224742
500	50	1	50	65.803571	66.403252	0.903089
		4	12.5	95.803571	96.690819	0.917613
		10	5	155.80357	156.68103	0.560034
		20	2.5	255.80357	256.52251	0.280264
1000	5	1	5	2432.1428	2432.8559	0.029312
		4	1.25	3032.1428	3033.5657	0.046903
		10	0.5	4232.1428	4233.5657	0.042285
		20	0.25	6232.1428	6233.9223	0.028545

Table 7.8 (Continued)

Dose (mg)	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 13) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
1000	10	1	10	1216.0714	1216.7903	0.059086
		4	2.5	1516.0714	1517.4945	0.093780
		10	1	2116.0714	2117.8507	0.084015
		20	0.5	3116.0714	3117.8337	0.056524
1000	20	1	20	608.03571	608.77085	0.120758
		4	5	758.03571	759.47036	0.188901
		10	2	1058.0357	1059.8165	0.168033
		20	1	1558.0357	1559.7918	0.112590
1000	40	1	40	304.01785	304.79202	0.253998
		4	10	379.01785	380.47360	0.382614
		10	4	529.01785	530.80596	0.336866
		10	4	779.01785	780.77249	0.224730
1000	50	1	50	243.21428	244.00880	0.325611
		4	12.5	303.24128	304.68175	0.481640
		10	5	423.21428	425.00640	0.421667
		20	2.5	623.21428	624.96879	0.280736

Table 7.8 (Continued)

Dose (mg)	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 13) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
2500	5	1	5	14450.892	14451.858	0.006684
		4	1.25	15950.892	15953.200	0.014461
		10	0.5	18950.892	18954.499	0.019028
		20	0.25	23950.892	23952.962	0.008639
2500	10	1	10	7225.4464	7226.4244	0.013534
		4	2.5	7975.4464	7977.7655	0.029070
		10	1	9475.4464	9478.9886	0.037369
		20	0.5	11975.446	11979.743	0.035870
2500	20	1	20	3612.7232	3613.7295	0.027847
		4	5	3987.7232	3990.0630	0.058640
		10	2	4737.7232	4741.2799	0.075016
		20	1	5987.7232	5992.0272	0.071828
2500	40	1	40	1806.3616	1807.3981	0.057351
		4	10	1993.8616	1996.2243	0.118363
		10	4	2368.8616	2372.4333	0.150552
		20	2	2993.8616	2998.1752	0.143875

Table 7.8 (Continued)

Dose (mg)	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 13) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
2500	50	1	50	1445.0892	1446.1491	0.073286
		4	12.5	1595.0892	1597.4700	0.149035
		10	5	1895.0892	1898.6722	0.188707
		20	2.5	2395.0892	2399.4064	0.179927
5000	5	1	5	56803.571	56804.943	0.002415
		4	1.25	59803.571	59806.807	0.005410
		10	0.5	65803.571	65808.420	0.007368
		20	0.25	75803.571	75810.787	0.009518
5000	10	1	10	28401.785	28403.221	0.005054
		4	2.5	29901.785	29905.072	0.010991
		10	1	32901.785	32907.165	0.016347
		20	0.5	37901.785	37908.997	0.019023
5000	20	1	20	14200.892	14202.100	0.008503
		4	5	14950.892	14953.986	0.020685
		10	2	16450.892	16456.133	0.031848
		20	1	18950.892	18958.032	0.037658

Table 7.8 (Continued)

Dose (mg)	Vm (mg/hr)	Km (mg/L)	Vm/Km (L/hr)	AUC (equation 13) (mg•hr/L)	AUC (trapezoidal) (mg•hr/L)	% difference in AUC estimation
5000	40	1	40	7100.4464	7101.7133	0.017840
		4	10	7475.4464	7478.5827	0.041936
		10	4	8225.4464	8230.7097	0.063946
		20	2	9475.4464	9482.5721	0.075145
50	50	1	50	5680.3571	5681.6263	0.022338
		4	12.5	5980.3571	5983.4978	0.052488
		10	5	6580.3571	6585.6240	0.079975
		20	2.5	7580.3571	7587.4885	0.093989

Table 7.9 Comparison of AUC using equation (10), linear pharmacokinetics, and trapezoidal rule method for drugs following one-compartment system with first-order absorption and Michaelis-Menten elimination kinetics at low dose and low Ka.

Dose (mg)	Ka (hr^{-1})	Vm (mg/hr)	Km (mg/L)	Cmax (mg/L)	AUC (trapezoidal) (mg•hr/L)	AUC (linear) (mg•hr/L)	AUC (equation 10) (mg•hr/L)
50	0.3	20	1	0.3884	3.15159	2.5000	0.589190
50	0.3	40	1	0.2458	1.44935	1.2500	4.361193
50	0.3	40	4	0.4941	5.41505	5.0000	4.727385
100	0.3	40	1	0.5572	3.40620	2.5000	-27.15375

DISCUSSIONS AND CONCLUSION

In linear pharmacokinetics, clearance is dose independent, but it becomes concentration and consequently dose dependent in pharmacokinetic systems with nonlinear elimination. Therefore the AUC calculation based on linear pharmacokinetics can underestimate the AUC of drugs having capacity-limited elimination process. The degree of saturation of Michaelis-Menten elimination kinetics depends upon the concentration produced after drug administration. Drugs given intravenously usually produce higher concentrations than administered orally drug except when the rate of absorption from the given dose is very rapid.

For drugs obeying one-compartment open model when elimination is in a nonlinear fashion after intravenous administration, the AUC equation can be derived as shown in equation (7). The percentage of the differences in AUC estimation obtained directly from equation (7) and trapezoidal rule method demonstrated in Table 7.1 is very small and such error can be explained by the truncation error of the procedure. Also, the results in Table 7.1 verify that the mathematical expression of AUC for one-compartment system after intravenous administration having Michaelis-Menten elimination kinetics works for any values of dose, Vm, and Km.

For drugs following a one-compartment open model system with first-order input and Michaelis-Menten elimination kinetics, the AUC equation cannot be derived. Al-Ghazawi (1998) proposed a preliminary equation for AUC prediction for drugs following nonlinear pharmacokinetic following this model and showed that this equation works well when the rate of absorption is very fast. When the given dose is absorbed rapidly, instantaneous input can be assumed as in the case of IV administration, thus equation (7) can be used to approximate the maximum AUC obtained from that dose. The more rapidly a given dose is absorbed, the more closely will the AUC approach that calculated by equation (7). When the absorption rate is very slow, the maximum concentration produced by the administered dose would be sufficiently lower than Km which will not saturate the elimination process. Under the condition of low Ka with high clearance ratio, the behavior of drugs tend to follow linear pharmacokinetics, thus AUC calculated based on linear pharmacokinetics will give a better approximation when compared to that calculated from

equation (10), as shown in Table 7.9.

For drugs obeying two-compartment open model eliminated in nonlinear fashion from the central compartment after intravenous administration, there is no simple mathematical expression for the AUC equation since the differential equation explaining this model cannot be integrated. Equation (13) proposed by Cheng and Jusko (1989) gives a very good prediction with the percentage of the differences in AUC estimation less than one percent in most of the cases of dose, Vm, and Km. In the case of low dose (50 and 100 mg) with clearance ratio higher than 10, the percentage of the differences in AUC estimation is greater than one but still in the range of one to five percent error.

Since the differential equations explaining one-compartment open model system with first-order input and two-compartment open model system after IV administration with nonlinear elimination kinetics cannot be solved, the proposed preliminary AUC equations by Al-Ghazawi (1998) and Cheng and Jusko (1989) can only give an approximation of AUC for drugs following these systems. Although these two equations cannot be applied to all cases of dose, Ka, and clearance ratio, it is a step forward in nonlinear pharmacokinetics which is highly unexplored.

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CHAPTER 8

CONCLUSION

Oxytetracycline is a widely used broad spectrum antibiotic for the treatment of various infections in veterinary medicine. According to the pharmacokinetic parameters obtained, administering oxytetracycline 8 mg/kg intravenously every 12 hours and an initial loading dose of 20 mg/kg and a maintenance dose of 10 mg/kg every 48 hours with LA-IM formulation to alpacas will achieve a steady state trough concentration of 2.26 and 3.04 μg/mL, respectively. These dosing schedules will allow plasma concentrations above the MIC of 2 μg/mL for most microorganisms.

In order to maintain florfenicol plasma level greater than 0.25μg/mL, the MIC for most pathogenic bacteria, 8 mg/kg body weight of florfenicol should be given to alpacas every 12 hours which will achieve a steady state trough concentration of 0.36 μg/mL.

The pharmacokinetics of oxytetracycline and florfenicol in alpacas were compared to the results previously obtained in llamas. The disposition of oxytetracycline in these two animals was significantly different whereas the disposition of florfenicol in llamas and alpacas was similar.

The pharmacokinetics of clorsulon was investigated in llamas following oral administration at a single dose of 14 mg/kg. The plasma concentrations of clorsulon produced by this dose in llamas is lower than the values reported for the clorsulon pharmacokinetic studies in sheep and goats following oral administration at a single dose of 7 mg/kg. This suggests the entire dose of clorsulon is not absorbed in llamas.

The results of the theoretical study in chapter 7 showed that the proposed AUC equations for drug following one-compartment system with first-order input and two-compartment system after IV administration with nonlinear elimination kinetics has limitations for use to predict the AUC of drugs following these models. The proposed AUC equations will not give a good prediction for drugs with low dose, low Ka, and high clearance ratio.

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