Distribution of oxytetracycline and doxycycline to normal and diseased lung tissue from several species.

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Finally, I would like to thank Isobel, who started me on the road, and Elspeth, who stopped me going off the track.

If it's been a long road .....

I have met many friends along the way.

Declaration

I, Peter Baxter, declare that the following work has been done by me, and has not been submitted for consideration for another degree or by another University.

The following publication is based on work contained within this thesis.

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List of Tables

List of Figures

Index Page

Index cont'd Page

Summary

A High Performance Liquid Chromatography (HPLC) method was developed for the measurement of oxytetracycline and doxycycline in plasma and lung tissue from various species. All animals were dosed by the intravenous (IV) route at 10mg/kg IV, except rats which were dosed by the intramuscular route (IM) at the same dose rate. Concentrations in normal rat lung were found to be significantly lower than plasma concentrations. Concentrations of oxytetracycline in normal sheep lung were found to be lower in both apical and diaphragmatic lobes compared to plasma, while doxycycline showed significantly higher lung concentrations in both areas compared to plasma.

The concentration of oxytetracycline at 1 hour following IV administration in sheep diseased apical lung lobes was similar to plasma, which was significantly higher than diaphragmatic lung concentrations in the same animals. In cattle oxytetracycline concentration in diseased lung lobes was similar to normal lung, suggesting adequate drug penetration of the diseased tissue at both 1 and 2 hours.

Oxytetracycline appears to distribute into both normal and diseased lungs of various species at slightly lower concentration than that found in plasma. Doxycycline appears to penetrate normal lungs of sheep at higher concentration than that found in plasma.

Distribution of oxytetracycline and doxycycline to normal and diseased lung tissue from several species.

Chapter 1. Introduction

The tetracycline antibiotics resulted from a worldwide search for antibiotic producing micro-organisms obtained from soil. Chlortetracycline was used first in 1948. The various other members of the tetracycline family have been used as antimicrobial agents for several decades. They have been used widely in domestic animals for the treatment of infection because they are cheap, effective and have a wide spectrum of activity against both gram-negative and gram-positive bacteria (Sande and Mandell, 1990).

The structure of the tetracyclines is based on a four membered ring (Figure 1). The different physiochemical properties of the individual agents are caused by small changes on the side chains. They are all amphoteric in nature, and have poor water solubility at pH 7.0. The hydrochloride salt is the formulation used normally for oral and parenteral administration and, with the exception of chlortetracycline, all are reasonably stable (Prescott and Baggot, 1988).

Although resistance to tetracyclines is now widespread in some infective agents, they are still the drug of choice in many cases, especially where a mixed infection is thought to be present. They are particularly useful against *Chlamydia, Mycoplasma* and *Rickettsiae,* which are resistant to drugs such as the beta lactams, whose actions affect cell wall formation. Tetracyclines have been used as both chemotherapeutics and as in-feed growth promotors. Several members of the family, particularly oxytetracycline, have been used for treatment of lower respiratory tract infection in many species.

Chlortetracycline and oxytetracycline are produced from *Streptomyces Spp.*, while the other tetracyclines such as doxycycline are semi-synthetic derivatives. Their action is primarily bacteriostatic, with the site of action being at the 30 Svedberg unit on bacterial ribosomes. The mechanism of action is by reversible binding to inhibit the access of tRNA to the mRNA-ribosome complex, thus inhibiting the addition of amino acids to the growing peptide chain. The action is reversible when the concentration falls below a threshold level. The degree of drug action is thought to be related to concentration of drug penetrating to the site of action in the tissue, thus the lipophilic tetracyclines which acheive higher concentrations may be more active. Each of the tetracyclines, on a comparitive molecular basis, is thought to be similar for activity against the agent of infection which is present. Resistance to one tetracycline usually confers resistance to the other drugs in the family (Prescott and Baggot, 1988; Sande and Mandell, 1990).

The tetracyclines have been studied in relation to their physical and biological properties, particularly in relation to pharmacokinetic behaviour in plasma (Ziv and Sulman, 1974; Baggot, 1980) and lung (Valcke *et al.*, 1990) Mathematical models have been used to assess the concentrations likely to occur in various tissues. Where lung tissue concentrations have been measured most studies were carried out in healthy animals (McKellar, 1989).

Studies carried out in animals with disease have concentrated on the plasma pharmacokinetic profile between normal and disease states in various species. Various drugs have been used in these studies, including penicillin G, oxytetracycline and chlortetracycline in pigs (Kilroy *et al.*, 1990; Pijpers *et al.*, 1990; Zhen-Ling and Ki-Fai,1990), oxytetracycline, chloramphenicol and lincomycin in calves (Burrows *et al.*, 1986), and gentamicin in calves (Hunter *et al.*, 1991).

Various studies have measured drugs in tissue chambers in an attempt to determine peripheral tissue penetration, and the use of these chambers is

well documented (Clarke, 1989). Oxytetracycline has been measured in surgically implanted chambers, and in discrete body fluids which may be easily obtained, usually synovial fluid (Ziv *et al.*, 1982; Bengtsson *et al.*, 1989; Bengtsson *et al.*, 1991).

Tetracycline has been studied in lung using surgically cannulated lymph nodes to measure efferent concentration in lymph (Cohen *et al.*, 1987). Other studies have used bronchial lavage or expectorate collection to measure the penetration of drug into extracellular secretions (Hartnett and Marlin, 1976; Campbell, 1980).

There are very few studies where the concentration of oxytetracycline has been measured in diseased lung, and compared to normal lung tissue and plasma concentrations in the same animal. Where this type of study has been carried out the concentrations have been measured at relatively long time intervals after administration (Ames *et al.*, 1983; Ames and Patterson, 1985). In these studies the drug was measured during the elimination phase, which may not reflect the disposition in tissue during distribution when oxytetracycline is at therapeutic concentrations. Although plasma pharmacokinetic studies yield detailed data, they do not show the initial uptake of the drug into lung tissue, particularly in relation to the diseased or consolidated portions of the lung.

The use of chambers created artificially, usually sub-dermal, may not reflect the drug penetration in other tissues. Based on area/volume ratio the tissue cage model is basically a large interstitial fluid sac, similar to a discrete nodule such as an abscess. The rate of penetration of antibiotic into the cage may not reflect the rate of penetration in tissues where the diffusion area/volume ratio may be much lower (Keen, 1989). The comparison of drug penetration into diseased lung and artificial chambers may depend on the type of infection present, and the effect it has on the structural morphology

of the lung. It is possible that the penetration of drug may also be enhanced by inflammation present in the diseased tissue.

Oxytetracycline is poorly lipophilic in relation to other tetracyclines such as minocycline and doxycycline. The latter has shown high binding to plasma proteins compared to other tetracyclines, including oxytetracycline. These lipophilic properties result in greater concentrations of doxycycline in milk compared to other, more hydrophilic tetracyclines (Ziv and Sulman, 1974). Minocycline is most closely related to doxycycline, in terms of their lipophilic properties, while tetracycline is similar to oxytetracycline, the latter drugs being more hydrophilic than the former. Lower concentrations of minocycline have been found in pulmonary lymph compared to tetracycline at short time periods after administration at the same dose rate (Cohen *et al.*, 1987). Tetracycline concentration was significantly higher than minocycline during, and at 5 minutes post-infusion. It was higher, although not significantly, in all lymph samples up to 24 hours. This suggests a greater concentrating effect for doxycycline and minocycline in lung tissue compared to tetracycline and oxytetracycline, with possible beneficial effects on treatment of infection if higher comparable tissue concentrations can be achieved at lower doses.

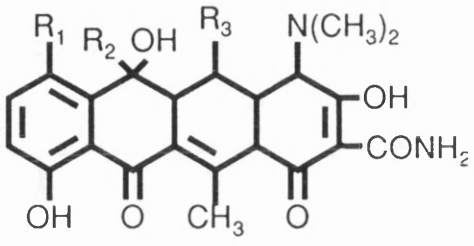
The route of excretion of doxycycline differs from other tetracyclines since it is excreted via the gastro-intestinal route in bile rather than via the kidneys. This may be useful in pyrexic animals where dehydration, and consequent faster drug excretion, may take place, or where renal damage has occured. Use of tetracyclines, including doxycycline is contra-indicated in horses, where the gastro-intestinal tract is apparently more susceptible to antimicrobial associated enterocolitis (Prescott and Baggot, 1988). Doxycycline is at present only licenced in the United Kingdom for use in dogs. In future it may be available for treatment of lower repiratory tract infection in other domestic animals. Doxycycline achieves 20-35% of serum

concentrations in bronchial secretions in man(Hartnett and Marlin, 1976; Campbell, 1980). The lipophilic nature of doxycycline suggests that it ought to accumulate in lung tissue, although the data from the bronchial secretion studies indicates that it does not.

Objectives

The objectives of the study were to determine the concentration of antimicrobial in plasma and lung tissue following intravenous administration of oxytetracycline and doxycycline, and compare drug concentrations achieved between different species. A further objective was to compare drug concentrations achieved in plasma and varying lung areas within species and if lung pathology was observed, a comparison would be made of drug concentrations in normal lung tissue with those animals which showed gross signs of lung pathology.

Figure 1. Basic structure of the tetracycline family of antibiotics



TETRACYCLINE
BACKBONE

Drug	R1	R2	R3
Oxytetracycline	-H	-CH3 -OH	-OH
Doxycycline	-H	-CH3	-OH
Chlortetracycline	-Cl	-CH3	-H
Tetracycline	-H	-CH3 -OH	-H
Minocycline	-N(CH3)2	-H	-H

Chapter 2. Materials and Methods.

The methods published previously for the measurement of tetracyclines were not considered satisfactory for the present studies because a lower limit of detection was sought and a rapid throughput of samples desired. The method used was based on a method devised by Gilbert (1986) using High Performance Liquid Chromatography (HPLC). This method was adapted to facilitate the measurement of tetracyclines in plasma and tissue from a range of species, with reproducible results. Absorption at 354nm was utilised to provide low background readings which were important for the measurement of drug in tissues, and allowed simple extraction procedures and large injection volumes. The method was adapted to suit the drug being measured and the type of material under test. The slight alterations in the method required for each experiment are noted in the relevant chapters.

Standard laboratory glassware was used in the assays. The chemicals were all analytical grade, while the organic solvents used were all of HPLC grade or equivalent.

Specialised equipment used in the analysis of samples included an Ultra-Turrax Tissue Disintegrator (Fisons Ltd, England) and Sep-Pak C18 cartridges (Waters Ltd., England). Chromatographic analysis of samples was carried out on HPLC systems, normally comprising the following units. An Isocratic Altex 110A pump (Burke Electronics, Scotland) connected to either a Negretti and Zambra 342 manual injection valve (Southampton, England) or a Gilson 231/401 Auto-injector system (Scotlab Ltd., Scotland). Separation was achieved using a 160x5mm stainless steel column (Shandon Ltd., England) with C18 packing material (ODS-Hypersil 5μ, Shandon Ltd.). A Specta-Physics 100 or 8450 variable wavelength UV detector with 10mm flow cell (Hemel Hempstead, England) was used. Results were recorded on a Rikadenki chart

recorder (Scotlab Ltd, Scotland) for storage. The solvent used for chromatography varied depending on the drug being measured.

Chemicals and Reagents

All chemicals and organic solvents were anhydrous Analar grade or HPLC Grade, (Fisons Ltd, Scotland) unless stated. The following chemicals were required for the assays. Perchloric acid (70%), trichloroacetic acid, phosphoric acid (60%), potassium dihydrogen orthophosphate, potassium permanganate, 1 heptane-sulphonic acid (Sigma Ltd., England) and acetonitrile, glass-distilled grade (Rathburn Ltd., Scotland). Methanol (Analar, Fisons Ltd.) was redistilled in the laboratory for HPLC use. Water for HPLC was redistilled in the presence of potassium permanganate (0.2%) to remove phenol residues.

Oxytetracycline hydrochloride (88.7% base) was obtained from Sigma Ltd., while the doxycycline hydrochloride was donated by Pfizer Ltd., for the preparation of standards.

Standard solutions for extraction and chromatography were prepared in permanganate-distilled water, unless otherwise stated. Perchloric acid was prepared as a 20% v/v aqueous solution. Trichloroacetic acid was prepared as a 10% w/v aqueous solution. The eluting solution for Sep-paks was prepared by mixing 229ml acetonitrile, 6.12ml phosphoric acid, 2.08g potassium dihydrogen orthophosphate and 0.564g of 1-heptane sulphonic acid per litre of water.

Eluting solution, without acetonitrile, diluted 1:1 with TCA 10%, was used in the preparation of oxytetracycline standards. Doxycycline standards were prepared in 1:1 water : acetonitrile solution.

Differentiation of lung areas

Lungs were divided into four anatomical regions based on the left and right areas on a vertical plane and a theoretical lateral line drawn from the base of the apical lobes (Figure 2). In rat experiments the whole lung was used for sampling. In sheep the total lung area for analysis was macerated, mixed, and a 25g aliquot of the slurry stored. In cattle, given the large lung volume, a randomised selection of 2cm lung slices were selected for maceration, mixed, and a 25g aliquot of the slurry stored.

Preparation of samples for analysis

In all experiments plasma was harvested from blood collected using Monovettes containing lithium heparin as an anticoagulant (Sarstedt Ltd., England). Samples of normal and diseased lung lobes were macerated immediately using a Moulinex Charlotte 3 mincer (Comet Ltd., Scotland). This produced a coarse homogenate containing both liquid and solid material. All samples were stored at -20°C until analysis, which was completed within twelve months of collection.

Analysis of Oxytetracycline in Plasma

Samples of plasma known to be drug free were fortified ("spiked"), with a known quantity of oxytetracycline. The concentrations of oxytetracycline in the spiked plasma varied depending on the samples being measured but were in the range 0.25-25µg/ml of plasma. Stock solutions (500µg/ml) were prepared in eluting solution (without acetonitrile) on the day of analysis, with dilutions for spiking made up in 50 : 50 eluting solution (without acetonitrile) : trichloroacetic acid (10%). Samples were analysed with control spikes to determine the recovery of drug within each assay. The control spikes were fortified with concentrations of drug thought to encompass the range of concentrations to be found in samples determined in the assay.

The samples to be assayed were precipitated with 10% trichloroacetic acid in plastic tubes where sufficient sample was available, or in Eppendorf tubes for low volumes of sample (Sarstedt Ltd., England). The ratio of plasma to trichloroacetic acid was 3:2. For large domestic animals 3ml of plasma was used, while 0.3ml of plasma was used for rat samples. After addition of trichloroacetic acid, samples were vortexed vigorously for 30 seconds. They were then centrifuged at 2000g for 15 minutes at 4°C, the supernatant was removed and a known quantity placed in a sealed vial for injection on the day of extraction.

Samples were chromatographed using a 5μ Hypersil ODS column pumping at 1ml/min. The mobile phase used for oxytetracycline was 30:70 acetonitrile:water containing phosphoric acid (5.1ml), potassium dihydrogen orthophosphate (1.72g) and 1-heptane sulphonic acid (0.564g) per litre of solvent. The mobile phase used for doxycycline was 21:79 acetonitrile:water, with salt concentrations as above. The limit of detection based on twice background noise of the system was 0.1μg/ml or better. Recoveries of drug in plasma for each species are shown in the relevant chapter.

Analysis of Oxytetracycline in Lung Tissue.

Samples of homogenised lung tissue from relevant species, known to be drug free, were fortified ("spiked"), with a known quantity of oxytetracycline. The concentrations in the spiked lung varied depending on the samples being measured but were in the range 0.25-25μg/g. Stock solutions (500μg/ml) were prepared in eluting solution (without acetonitrile) on the day of analysis, with dilutions for spiking made up in 50:50 eluting solution (without acetonitrile) : trichloroacetic acid (10%).

A known quantity of minced lung tissue, usually 5.0(±0.05)g, was weighed into a 50ml ground glass stoppered tube. The lungs obtained from individual rats were usually homogenised whole, giving between 1-2g of

tissue, while lung from larger domestic animals was homogenised with the domestic blender prior to an aliquot of 5g being added to the tube. Drug was added to blank tissue to give spiked samples at this stage. Ten millilitres of perchloric acid was added to each tube and the mixture was homogenised for 30 seconds using the Ultra-Turrax tissue disintegrator. The tube was held in an ice bath to prevent over-heating of the sample. The supernatant was recovered after centrifugation of the sample at 2500g in a refrigerated centrifuge at +4°C. Sep-paks were primed using 5ml of methanol then 10ml of distilled water. Supernatants were loaded onto the Sep-pak at 50µl (one drop) per second. Eluting solution (with acetonitrile) (0.5ml) was used to wash the Sep-pak. This was followed by a further 4ml of eluting solution (with acetonitrile). The 4ml of eluate was collected in 10ml plastic tubes and retained for injection on the HPLC system. Samples collected were stored in stoppered tubes at +4°C until they were injected. Although stable for up to 24 hours, the samples were injected within 12 hours of extraction. The solvent used for analysis of oxytetracycline samples in lung was the same as that used for plasma samples. The limit of detection based on twice background noise of the system was 0.2µg/g or better. Recoveries of drug in lung tissue for each species are shown in the relevant chapter.

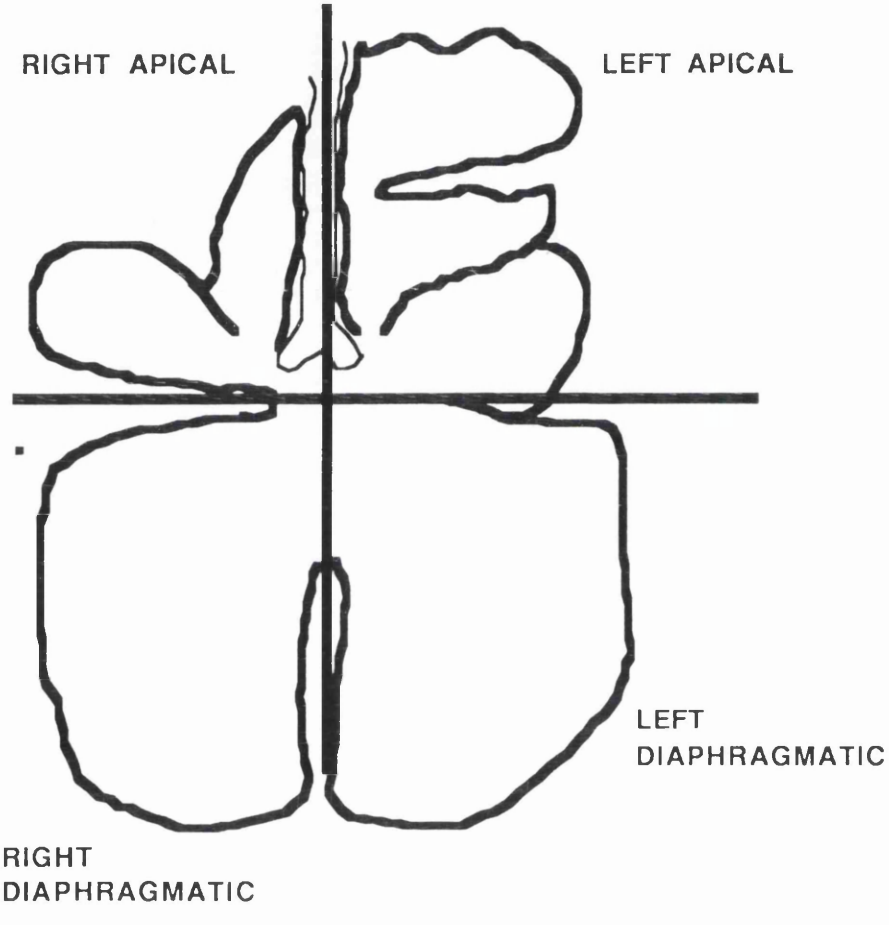
Doxycycline was extracted using the same extraction technique as that described for oxytetracycline.

Analysis of results

Results were analysed using Student's T test for un-paired and paired t-test as indicated in the text. Pharmacokinetic data was derived using a non-linear regression curve-stripping computer programme, CSTRIP (Sedman & Wagner, 1976). Confirmation of the number of exponentials was obtained using Akaike's information criterion (Yamaoka *et al.*, 1978).

Pharmacokinetic data were obtained using standard pharmacokinetic methods (Baggot, 1977)

Figure 2. Section of lung area used to define tissue samples used in sheep (2 left apical lobes) and cattle (3 left apical lobes) experiments.



Chapter 3. Oxytetracycline in rat plasma and lungs.

Introduction

Rodents, especially rats (Rattus rattus), have been used in many models of human disease, and consequently antibiotics have been studied extensively in this species. They have been used in the study of various antibiotics in both normal and disease conditions in mice (Renneberg and Walder, 1988) and rats (Fournet *et al.*, 1989). Rats are particularly suitable for low cost method development and initial protocol development. Where disease conditions, such as respiratory mycoplasmosis, have occurred in rat populations, oxytetracycline has been used as the drug of choice for treatment (Harkness and Wagner, 1977). Although concentrations of oxytetracycline and tetracycline have been measured after oral dosing, few studies using intramuscular or intravenous injection have been carried out in the rat, and where these studies have been undertaken, the concentration of oxytetracycline in lung tissue has not been estimated (Curl *et al.*, 1988).

Both oxytetracycline and tetracycline have been shown to be active against *Mycoplasma pulmonis in vitro* (Gardner *et al.*, 1981), but tetracycline did not acheive therapeutic concentrations after oral administration at dose rates of 300mg/kg by gavage, and between 0.4 and 4 grams per litre administered in drinking water (Porter *et al.*, 1985). Alternative possible routes of administration include intraperitoneal, intravenous or intramuscular injection. The intramuscular dosing route may be chosen as the most convenient method to dose large numbers of animals on multiple occasions, especially in experimental animals which may be receiving other drug treatments by other routes.

In the present study rats were used as a model to determine the concentration of oxytetracycline in lung and its relationship to the corresponding plasma concentration at various time intervals after

intramuscular dosing. An oxytetracycline injectable solution (Terramycin Q50, Pfizer Ltd., England, 50mg/ml) was chosen to give an injection volume which could be measured using a standard, commercially available, plastic syringe, and was considered not too large to cause discomfort or extensive tissue damage. The oxytetracycline concentration was measured in plasma and lung tissue of rats at various times following intramuscular dosing.

Materials and methods.

Thirty-two rats (outbred cfy strain) of known age and weight were dosed with oxytetracycline at 10mg/kg bodyweight (Appendix A). Antibiotic was administered into the Quadriceps muscle of the right rear leg by slow intramuscular injection. The rats were allocated into groups of 4 animals and killed at 1, 2, 4, 8, 24, 48, 72 and 96 hours after dosing. Animals were anaesthetised at the appropriate sample time using Halothane (May and Baker, England) and bled by cardiac puncture. The animals were euthanased by neck dislocation and the lungs removed. No bacteriological examination was carried out prior to, or after dosing. The lungs were assessed for disease condition and blotted on filter paper (Whatman No.1) to remove surplus blood from the tissue. Plasma and lungs were frozen at -20^{o}C until analysis by the standard oxytetracycline HPLC method as defined previously. The lungs from individual animals were frozen whole and not subjected to the mincing step used for the lungs of larger domestic species. Lung weights were approximately 1-2g, which was significantly less than the 5 grams used in the assay of oxytetracycline from other species. Fortified spike concentrations were adjusted accordingly.

Results

Intra/inter-assay variation

Intra- and inter-assay variation of oxytetracycline concentrations in rat plasma were between 70 and 80% and coefficients of variation were 5% or lower (Table 1.). These results are similar to those found in other species using the same method of analysis (see Chapters 4, 5 & 6).

Intra- and inter-assay variation of oxytetracycline concentration in rat lung was between 50 and 60% and coefficients of variation were 10% or lower, except at very low concentrations where inter-assay variation was 12.4% (Table 2.).

Oxytetracycline concentration after intramuscular injection in rats.

Oxytetracycline could be detected in rat plasma and lung up to 24 hours after IM injection (Table 3.). Maximum plasma concentrations (mean = 3.79µg/ml) were found at 2 hours, although the mean 1 hour concentration was only slightly lower (3.71mg/ml).

The concentrations of oxytetracycline which could be detected in plasma of rats within each time group were similar (Table 3.), with a low standard error of mean concentrations within the groups of rats. Concentrations gradually declined until 24 hours, where only 2 of the 4 animals within the last necropsy group had trace concentrations of drug (<0.2µg/ml). No oxytetracycline was detected in plasma or lung at 48, 72 or 96 hours, and the results for these animals are not shown in the tables.

Rat lungs from this experiment did not show consolidation of lung tissue, or signs of disease, in any of the lung areas. The concentrations of oxytetracycline in lung tissue (Table 4.) were more variable than those found in plasma, and comparison of the lung and plasma concentrations showed 30-40% less drug in lung tissue (Figure 3). Lungs contained

significantly less oxytetracycline ($P< 0.05$) in all groups up to 4 hours. At 8 hours lung concentrations were lower but the difference was not significant, probably due to the low concentrations measured. The Area Under Curve (AUC) observed values of Mean oxytetracycline concentration in plasma (28.5μg/ml.h) was significantly different ($P<0.05$) from the AUC(observed values) of Mean oxytetracycline concentration in lung tissue (17.6μg/ml.h).

Discussion

Analytical recoveries were higher in rat lung spikes than in spiked lung tissue from other species using the same method of analysis. This may be due to the lower weight of lung tissue used in the analysis of oxytetracycline concentrations in the rat.

With the exception of one rat in the eight hour group, all concentrations of oxytetracycline measured in lung were lower than those found in plasma. Although the drug concentration in lung tissue was lower in individual animals compared to plasma, the variation between animals within groups was large for concentrations measured in lung tissue. This makes interpretation difficult when using the mean concentration from each group. The concentrations found in plasma were comparable to those found in other studies in rats, where a higher dose rate (60mg/kg), of either short or long-acting formulations of oxytetracycline was used (Curl *et al.*, 1988).

In *in-vitro* studies uptake of drug into lung tissue was measured as an index of penetration, using normal and diseased lung tissue (Fournet *et al.*, 1989). This was carried out by incubating areas of lung tissue in known concentrations of drug under standard conditions. In normal lung tissue, 94% of the concentration found in plasma was measured. This was not demonstrated in sheep *in vivo* given oxytetracycline at 10mg/kg by IM

injection, where the concentration in lung at 4 hours was greater than plasma (McKellar, 1989). The lower concentrations achieved in both lung and plasma of rats compared to sheep are probably due to the much shorter half-life of oxytetracycline seen in animals of lower body mass (Kirkwood and Widdowson, 1990).

The *in vitro* rat lung penetration study (Fournet *et al.*, 1989) also showed that diseased rat lung tissue, both bacterial and cancerous, had a much higher rate of drug uptake (180%) compared to plasma. It is interesting that uptake of tetracycline into lung was higher from buffer than from plasma. This was due, presumably, to protein binding within the plasma giving a lower free drug concentration available for penetration into lung tissue. The concentration of free drug is thought to equilibrate quickly with tissue fluid, therefore antibiotics such as oxytetracycline and tetracycline, which are bound to a lower extent than doxycycline and minocycline, would be expected to diffuse into tissue fluid more quickly (Keen, 1989). This may explain the more rapid accumulation of tetracycline compared to minocycline in the cannulated lymph nodes draining the lungs of sheep (Cohen *et al.*, 1987).

The concentrations of oxytetracycline found in lung tissue, although lower than those in plasma, were above the serum Minimum Inhibitory Concentration (M.I.C.) of tetracycline for *Mycoplasma pulmonis* (0.2μg/ml), *Streptococcus pneumoniae* (0.4μg/ml) and *Corynebacterium spp* (0.8μg/ml) for a period of at least eight hours in both lung and plasma (Porter *et al.*, 1985). As tetracycline and oxytetracycline have similar activities it can be assumed that the MIC data is appropriate for oxytetracycline in these species (Prescott and Baggot, 1988).

Table 1. Intra- and Inter-assay recovery and precision of the HPLC method for concentrations of oxytetracycline in rat plasma (μg/ml). (n=4).

Concentration added (μg/ml)	Recovery (%)		Coefficient of variation(%)	
	Intra	Inter	Intra	Inter
0.5	78.83	80.20	3.21	1.25
1.0	79.47	78.33	0.67	2.08
2.0	75.80	75.75	4.80	2.60
5.0	73.85	73.11	3.53	3.90

Table 2. Intra- and Inter-assay recovery and precision of the HPLC method for concentrations of oxytetracycline in rat normal lung (μg/g, wet wt.). (n=4 for Intra, n=3 for Inter).

Concentration	Recovery (%)		Coefficient of variation (%)	
added (μg/g)	Intra	Inter	Intra	Inter
0.5	54.02	50.78	5.26	12.40
1.0	58.61	56.06	1.70	6.91
2.0	59.22	56.06	1.61	3.59
5.0	56.37	51.48	1.61	8.56

Table 3. Concentration of oxytetracycline (μg/ml) in rat plasma (n=4 rats at each time) after administration of oxytetracycline at a dose rate of 10mg/kg by the intra-muscular route.

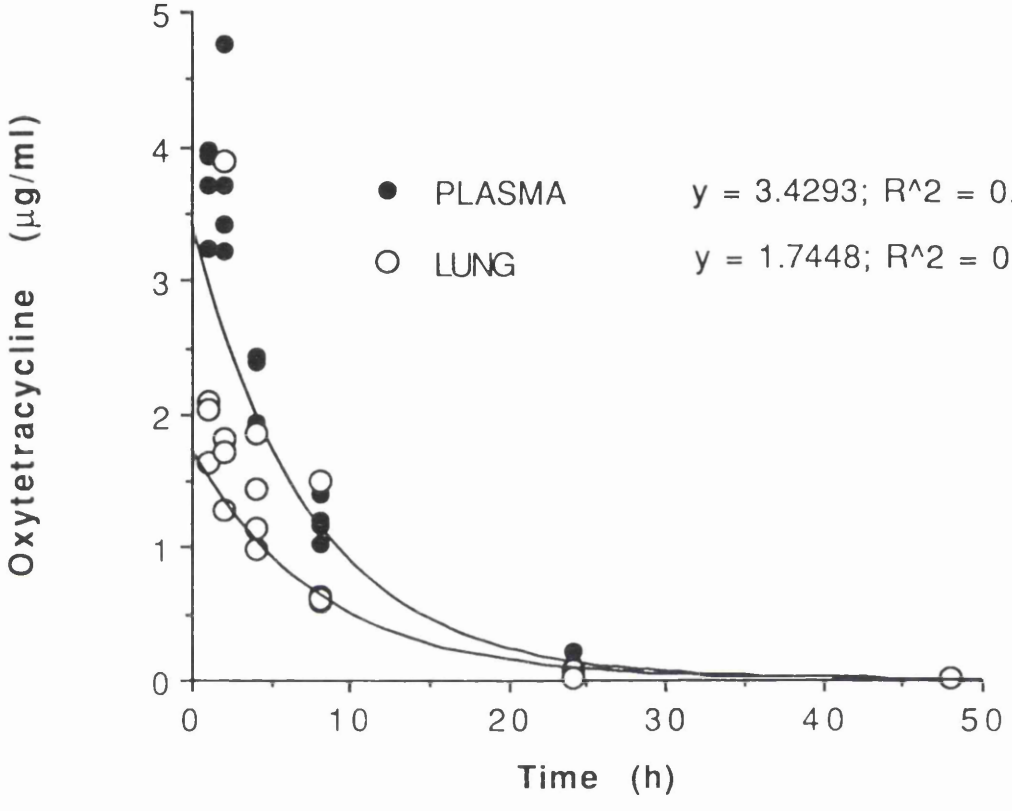
Kill Time (h)	Rat No.	conc.(μg/ml)
1	1	3.72
1	2	3.94
1	3	3.97
1	4	3.24
	Mean±SEM	3.71±0.17
2	5	3.22
2	6	4.77
2	7	3.42
2	8	3.72
	Mean±SEM	3.79±0.34
4	9	1.91
4	10	1.93
4	11	2.44
4	12	2.40
	Mean±SEM	2.17±0.14
8	13	1.41
8	14	1.17
8	15	1.20
8	16	1.03
	Mean±SEM	1.20±0.08
24	17	0.11
24	18	0.21
24	19	0
24	20	0
	Mean±SEM	0.08±0.05

Table 4. Concentration of oxytetracycline(μg/g) in normal rat lung (n=4 rats at each time) after administration of oxytetracycline at a dose rate of 10mg/kg by the intra-muscular route.

Kill Time (h)	Rat No.	conc.(μg/g)
1	1	2.10
1	2	1.64
1	3	2.03
1	4	ns
	Mean±SEM	1.92±0.14
2	5	1.28
2	6	3.88
2	7	1.82
2	8	1.71
	Mean±SEM	2.17±0.58
4	9	0.98
4	10	1.14
4	11	1.85
4	12	1.44
	Mean±SEM	1.36±0.19
8	13	1.50
8	14	0.59
8	15	0.63
8	16	0.61
	Mean±SEM	0.82±0.22
24	17	0.07
24	18	0
24	19	0
24	20	0
	Mean±SEM	0.02±0.02

ns = no sample available

Figure 3. Concentration of oxytetracycline in rat plasma and whole lung after intramuscular injection (10mg/kg) showing extrapolation of the exponential line of best fit.



Chapter 4. Oxytetracycline in sheep plasma and lungs.

Introduction

Oxytetracycline is used frequently in sheep for the treatment of respiratory conditions such as pasteurellosis. There are few sources of information on lung concentrations of oxytetracycline in sheep, although serum and milk concentrations in sheep and other species have been documented previously (Ziv and Sulman, 1974). Tetracycline and minocycline have both been measured in the plasma and pulmonary lymph of sheep following cannulation (Cohen *et al.*, 1987).

Few studies have been carried out to measure the concentration of oxytetracycline in plasma and lung tissue at various time points (McKellar *et al.*, 1988; McKellar, 1989). No studies are available which document the concentration of drug in diseased ovine lung after oxytetracycline administration, although studies have been carried out measuring the concentration in bronchial secretions in humans (MacCulloch *et al.*, 1974; Ruhen and Tanden, 1975).

To provide information on the comparative plasma and lung concentrations, two experiments were undertaken in separate groups of sheep. The first experiment measured the concentration of oxytetracycline in the plasma, and diaphragmatic and apical lobes of clinically normal sheep at various time intervals following drug administration. The second experiment measured oxytetracycline in plasma, normal lung tissue and diseased lung, in individual animals at one hour after intravenous drug administration.

Materials and Methods

The first oxytetracycline study (Experiment 1) was carried out using twenty-four, one year old, clinically-normal female Dorset-cross sheep.

These had been kept indoors from birth and showed no signs of disease before or at post-mortem. Four groups of six sheep were dosed intravenously with oxytetracycline at 10 mg/kg (Terramycin Q100, Pfizer), and killed using a captive bolt pistol at approximately 3, 9, 12 and 24 hours (Appendix B). Blood was taken from the jugular vein, during subsequent exanguination, into lithium heparin tubes. The left apical and diaphragmatic lobes of the lung were removed after bleeding out. Samples were frozen at -20°C, after coarse homogenisation using a domestic mincer as described previously, until analysis by HPLC.

The second oxytetracycline study (Experiment 2) was carried out on ten Dorset-cross sheep, with a history of respiratory disease, manifest by tachypnoea and coughing. The ten sheep were dosed intravenously with oxytetracycline at 10 mg/kg (Terramycin Q100, Pfizer), and killed at approximately one hour after drug administration (Appendix C). Plasma and lung samples were taken as in the first experiment. The lung samples were classified according to the degree of consolidation in the tissue.

The diseased tissue was obtained from the apical lobes, since there were no gross signs of disease in any of the diaphragmatic lobes. The consolidated, diseased tissue was distinct from normal tissue. Tissue obtained from the diaphragmatic lobes which showed no sign of disease was described as normal, although other areas, for instance in the apical lobes, contained consolidated tissue. This differentiation was made solely on the gross morphological appearance, and not on any bacteriological examination. All tissue obtained from diseased lung was from totally consolidated areas with no 'normal' appearance, except in one animal where the apical area was described as partially consolidated as some tissue appeared normal on macroscopic observation.

No bacteriological examination was carried out at post mortem since it was considered results would have been misleading due to the high drug

concentration present in the samples. Oxytetracycline concentrations in sheep were measured using the HPLC techniques described previously.

Results

Intra/inter-assay variation

Intra- and inter-assay extraction recoveries of oxytetracycline in plasma, normal and diseased lung tissue were tested using concentrations from 0.5 to 10.0µg/ml (Tables 5, 6 & 7). Plasma extracted using trichloroacetic acid precipitation gave recoveries around 75%, while tissues extracted using the solid phase extraction technique gave recoveries of approximately 45% for both normal and diseased lung tissue. There was little variation in recovery over the range of concentrations measured in spiked plasma and lung as indicated by the low coefficient of variation. Since there was no variation in recovery between normal and consolidated lung, normal lung was used to create standard curves for the assay of all lung samples.

Experiment 1. Concentration of oxytetracycline in plasma and lung of normal animals.

At necropsy no gross pathological lung lesions were found in any of the animals in the study. The concentration of oxytetracycline was measured in samples of plasma and apical and diaphragmatic lung lobes from animals for up to 24 hours following intravenous administration (Tables 8, 9 & 10). Oxytetracycline concentrations were higher in plasma until 24 hours, when they were similar in plasma and both lung areas (Figure 4). Plasma concentrations were significantly higher than diaphragmatic lobes at 3 and 12 hours, while plasma concentrations were significantly higher than apical lobe concentrations at 3, 9 and 12 hours ($P<0.05$). In lung tissue the oxytetracycline concentration was significantly lower ($P<0.05$) in apical lobes compared to diaphragmatic lobes at 12 hours.

No significant difference ($P<0.05$) was observed at 24 hours in the plasma or lung tissue measured. Plasma concentrations were greater than those found in either area of lung tissue at all times up to 24 hours, except for one animal in the 9 hour group and one animal in the 12 hour group.

Experiment 2. Concentration of oxytetracycline in plasma and lung of diseased animals after one hour.

Concentration of oxytetracycline was measured in the plasma and lungs of ten sheep one hour after intravenous dosing. The lung samples taken from the apical lobes were consolidated in all cases. Mean (±S.E.M.) concentrations were 9.47±1.26 for plasma, 7.03±0.94 for normal diaphragmatic lobes, and 8.09±1.14 for diseased apical lobes (Table 11). Paired t-test showed significantly lower concentrations ($P<0.05$) in the 'normal' diaphragmatic lobes compared to both diseased apical lobes and plasma. There was no significant difference in drug concentration between plasma and diseased apical lobes.

Comparison between consolidated apical and normal-appearing diaphragmatic tissue concentrations of oxytetracycline showed linearity (Correlation Co-efficient = 0.994), suggesting a relationship between the concentration of drug in both areas. Although oxytetracycline concentrations in normal apical and diaphragmatic lung areas over a 24 hour period are less well correlated (Correlation Co-efficient = 0.97), a relationship still exists between drug concentration in the two areas of lung measured.

Discussion

The plasma concentrations of oxytetracycline found in the samples from normal sheep at various intervals up to 24 hours are proportionally higher than those results reported previously in both sheep and cattle

serum (Ziv and Sulman, 1974; Bengtsson *et al.*, 1986) and similar to those reported for cattle at the same dose rate of 10mg/kg (Burrows *et al.*, 1986; Bengtsson *et al.*, 1989).

The significantly higher levels of oxytetracycline found in plasma at almost all time points compared to lung tissue are probably related to the physicochemical properties of oxytetracycline. Greater accumulation of other tetracyclines in milk has been shown to be related to lipophilic properties (Ziv and Sulman, 1974). The similarity between plasma and lung tissues measured at 24 hours in the present study could be due to the low concentrations measured or to equilibration between drug and tissues. The similarity in concentration of oxytetracycline between the 9 hour plasma and diaphragmatic samples may be due to greater accumulation in diaphragmatic lobes compared to apical lobes. As the concentration in all diaphragmatic areas was lower than the corresponding plasma values in individual animals it is probable that larger group numbers would have shown a significant difference. The limited number of animals used does not give a clear statistical indication of differences between the drug concentrations found in blood and lung tissue.

In diseased apical lung tissue the concentration would appear to lie between the concentrations demonstrated in plasma and diaphragmatic lobes, the latter being lower than plasma. This is in contrast to the results found in normal sheep where the concentration found in both lung areas were broadly similar, with the apical lobes lower than diaphragmatic lobes and both areas lower than respective plasma concentrations. Although no measurements were made at one hour in plasma and lung of a control group of normal sheep, the differences seen in the lung areas and plasma would be expected to be similar to those found at later time points such as 3 and 9 hours in Experiment 1. The variations seen in concentration of oxytetracycline between the apical and diaphragmatic lung lobes in normal

sheep may be due to physical characteristics of the lung tissue, where a larger proportion of the tissue may be made up of bronchioles in apical compared to diaphragmatic lobes. Poor perfusion of the cranio-ventral (apical) lobes have been shown in calves infected with bovine respiratory syncytial virus. Where lung lesions were present, these were manifest by chronic catarrhal pneumonia (Verhoeff *et al.*, 1992).

When infection occurs the inflammation may cause an increase in the penetration of oxytetracycline, resulting in the increase in apical oxytetracycline concentration seen after intravenous administration at 1 hour. It is possible that the differences seen are due to changes in blood perfusion levels. Radionuclide imaging has shown a difference in perfusion between diaphragmatic and apical lobes which are normal or have consolidated lesions (Verhoeff *et al.*, 1992).

The suggestion of a relationship between the apical and diaphragmatic areas for tissue oxytetracycline concentration is supported by the correlation co-efficient values (>0.95) obtained from the experiments carried out in normal animals over a 24 hour period.

Table 5. Intra- and Inter-assay recovery and precision of the HPLC method for concentrations of oxytetracycline in plasma of sheep (n=4).

Concentration	Recovery (%)		Coefficient of variation (%)	
added (µg/ml)	Intra	Inter	Intra	Inter
0.5	85.26	72.37	2.67	3.95
1.0	83.02	71.66	2.97	6.01
2.0	79.87	75.15	0.63	4.83
5.0	76.29	74.41	0.39	3.80
10.0	79.10	75.93	0.86	5.65

Table 6. Intra- and Inter-assay recovery and precision of the HPLC method for concentrations of oxytetracycline in normal lung of sheep (n=4).

Concentration	Recovery (%)		Coefficient of variation (%)	
added (µg/ml)	Intra	Inter	Intra	Inter
0.5	46.22	44.21	4.37	4.76
1.0	44.30	45.63	2.17	2.52
2.0	44.46	44.76	1.55	4.08
5.0	40.52	45.98	2.69	7.01
10.0	40.84	47.96	2.23	5.74

Table 7. Intra- and Inter-assay recovery and precision of the HPLC method for concentrations of oxytetracycline in diseased apical lung of sheep (n=4).

Concentration	Recovery (%)		Coefficient of variation (%)	
added (µg/ml)	Intra	Inter	Intra	Inter
0.5	40.02	41.45	8.26	9.17
1.0	45.78	43.82	5.41	5.68
2.0	43.64	46.51	7.65	5.73
5.0	48.33	49.03	3.19	8.99
10.0	43.95	47.18	6.22	6.45

Table 8. Concentration of oxytetracycline in normal ovine plasma after administration of oxytetracycline at a dose rate of 10mg/kg bodyweight by the intravenous route.

Kill Time (h)	Sheep No.	conc. µg/ml
3	1	7.48
3	2	9.50
3	3	7.89
3	4	9.01
3	5	7.75
3	6	10.06
	Mean±SEM	8.61±0.43
9	7	2.91
9	8	3.70
9	9	3.21
9	10	4.06
9	11	3.64
9	12	3.96
	Mean±SEM	3.58±0.18
12	13	3.10
12	14	2.27
12	15	1.55
12	16	2.38
12	17	1.82
12	18	2.55
	Mean±SEM	2.28±0.22
24	19	0.31
24	20	0.39
24	21	0.31
24	22	0.24
24	23	0.41
24	24	0.46
	Mean±SEM	0.35±0.03

Table 9. Concentration of oxytetracycline in the apical lobes of normal ovine lung after administration of oxytetracycline at a dose rate of 10mg/kg bodyweight by the intravenous route.

Kill Time (h)	Sheep No.	conc. µg/g
3	1	6.38
3	2	6.13
3	3	5.64
3	4	7.22
3	5	6.07
3	6	6.40
	Mean±SEM	6.31±0.21
9	7	3.10
9	8	3.55
9	9	2.28
9	10	2.53
9	11	2.30
9	12	3.76
	Mean±SEM	2.92±0.26
12	13	1.81
12	14	1.81
12	15	1.22
12	16	1.76
12	17	1.21
12	18	1.86
	Mean±SEM	1.61±0.13
24	19	0.41
24	20	0.36
24	21	0.36
24	22	0.29
24	23	0.29
24	24	0.29
	Mean±SEM	0.33±0.02

Table 10. Concentration of oxytetracycline in the diaphragmatic lobes of normal ovine lung after administration of oxytetracycline at a dose rate of 10mg/kg bodyweight by the intravenous route.

Kill Time (h)	Sheep No.	conc. µg/g
3	1	6.51
3	2	6.85
3	3	6.27
3	4	6.04
3	5	6.09
3	6	7.09
	Mean±SEM	6.47±0.17
9	7	2.99
9	8	3.41
9	9	2.79
9	10	2.76
9	11	2.39
9	12	3.52
	Mean±SEM	2.98±0.17
12	13	2.17
12	14	1.95
12	15	1.19
12	16	1.79
12	17	1.62
12	18	2.13
	Mean±SEM	1.81±0.15
24	19	0.41
24	20	0.37
24	21	0.40
24	22	0.29
24	23	0.33
24	24	0.29
	Mean±SEM	0.35±0.02

Figure 4. Concentration of oxytetracycline (μg/ml;μg/g) after intravenous injection in sheep plasma, apical and diaphragmatic lung, showing exponential line of best fit.

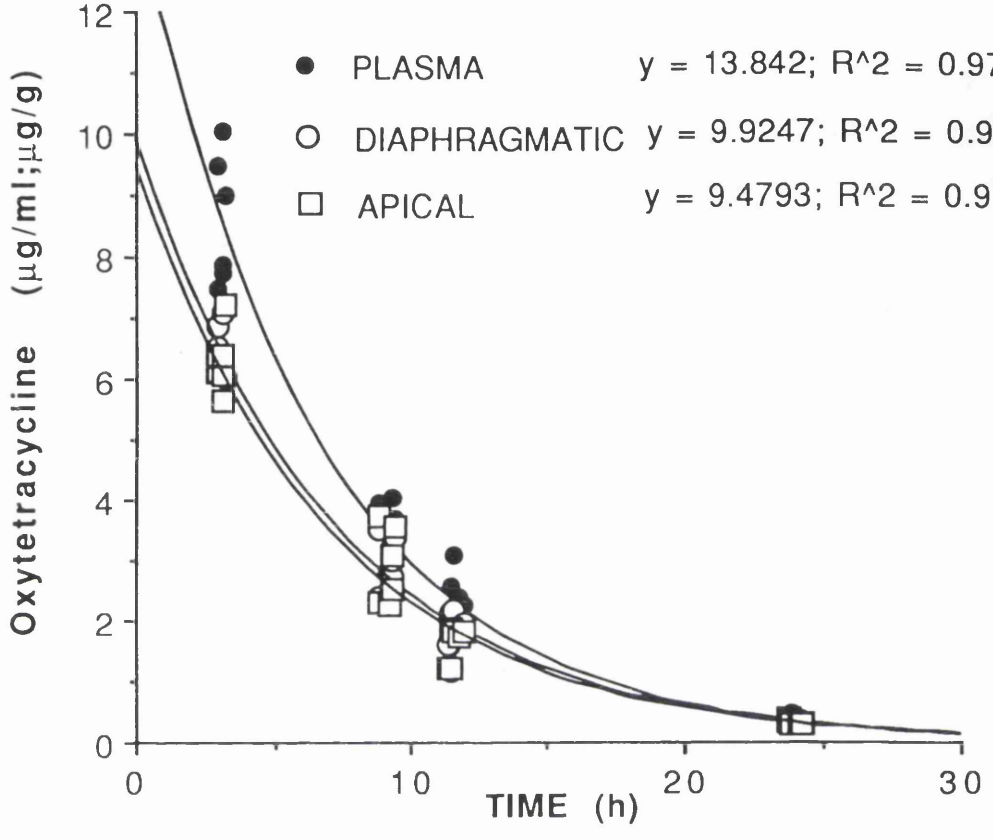


Table 11. Concentration of oxytetracycline in ovine plasma (μg/ml) and normal diaphragmatic and diseased apical lung tissue (μg/g) approximately one hour after administration of a single intravenous dose at 10mg/kg.

Animal No.	Plasma	Normal* Lung	Diseased** Lung	Time (mins)
6	4.34	3.74	4.19	65
7	5.21	2.97	3.01	62
8	13.25	11.37	13.33	66
11	5.60	4.70	5.43	66
13	13.59	10.52	12.87	58
15***	13.81	7.61	9.43	58
16	13.87	9.86	10.86	59
26	7.96	8.50	9.61	66
28	10.68	5.97	6.48	65
31	6.39	5.05	5.66	58
Mean	9.47	7.03	8.09	62.3
S.E.M	1.26	0.94	1.14	1.20

* Diaphragmatic lobes

** Apical lobes

*** partial consolidation, all others complete consolidation.

Chapter 5. Doxycycline studies in sheep.

Introduction

Few studies have been carried out on domestic species using doxycycline, although it has been widely researched and extensively used in man. Doxycycline is now available for use in animals in the United Kingdom, as a veterinary preparation marketed for dogs. The properties of doxycycline, including greater lipid solubility and better absorption than other members of the tetracycline group, suggest that it may be a useful drug in sheep. Based on its physical properties it may achieve higher lung concentrations compared to plasma than oxytetracycline.

Minocycline, which has similar physical properties to doxycycline, has been measured in the plasma and pulmonary lymph of sheep following cannulation (Cohen *et al.*, 1987). The pharmacology and toxicology of doxycycline has been reviewed in relation to man, and the pharmacokinetics and protein binding compared to other tetracyclines (Riond and Riviere, 1988). *In vitro* studies in bovine lung tissue, using an HPLC method, have been carried out to determine recovery from tissues (Riond *et al.*, 1989).

Studies have been carried out measuring the concentration of doxycycline in bronchial secretions in humans (MacCulloch *et al.*, 1974; Ruhen and Tanden, 1975). Serum and milk concentrations in sheep and other species have also been documented previously (Ziv and Sulman, 1974). Concentration ratios between plasma and milk have been used as a measure of lipid solubility of tetracyclines, including doxycycline, which achieved higher concentrations in plasma and accumulation in milk compared with oxytetracycline (Prescott and Baggot, 1988).

To provide information on plasma and lung concentrations doxycycline was administered intravenously to sheep and blood samples collected until

the animals were killed, at which time lung tissue was obtained. The concentration of doxycycline was measured in plasma, apical and diaphragmatic lobes of clinically-normal sheep killed at various time periods following administration.

Materials and Methods

The experiment was carried out on eighteen Dorset-cross sheep kept indoors since weaning. Three groups of six sheep (Groups A, B and C) were dosed intravenously with doxycycline(Vibraneuse 10%, Pfizer Ltd, France) at 3 mg/kg, and killed at approximately 3, 9, and 24 hours (Appendix D). Blood samples were collected from the sheep in the 9 hour and 24 hour groups at 0.083, 0.25, 0.5, 1, 2, 4, 6 , (6.75h for group B), 8 hours and at slaughter to measure plasma doxycycline concentration. Sampling methods for plasma and lung tissue were similar to those used in the sheep oxytetracycline experiments.

Doxycycline was measured using the same extraction procedure as oxytetracycline, although the HPLC solvent contained only 70% of the acetonitrile volume used for oxytetracycline as the HPLC retention time for doxycycline was much shorter at similar acetonitrile concentration.

Results

Intra/inter-assay variation

Intra- and inter-assay extraction recoveries of doxycycline in plasma and both apical and diaphragmatic lung tissue were determined using concentrations from 0.5 to 5.0μg/ml (Tables 12 & 13). Variation in recovery was small in plasma and normal lung over the range of concentrations measured. The variation was from 80 to 90% recovery in plasma and 51 to 58% in lung tissue. Drug recovered from both plasma and lung spikes was

greater (5%), than recoveries obtained in corresponding oxytetracycline spikes.

Concentration of doxycycline in plasma and lung areas of normal animals.

Most sheep showed hyperpnoea for up to 1 hour after administration. This was considered to be a reaction to IV dosage with doxycycline. The two animals showing the most severe signs (sheep 7 and 11) from the 9 hour group) had normal lungs at post-mortem examination. All other sheep had normal lungs on macroscopic examination. Bronchospasm, hypotension and urticaria have been reported in man after IV dosage with doxycycline.

Doxycycline was measured in samples from animals for up to 24 hours in plasma (Tables 14 & 15), and both apical and diaphragmatic lung lobes, following intravenous administration (Table 16). Mean plasma concentrations at various time points in the 24 hour group (C) and the 9 hour group (B) were similar for doxycycline concentrations. The concentration of drug in plasma and lung in the three hour group (A) lay between the concentration of drug found in the plasma of groups B and C at two and four hours.

The samples from lung and plasma were compared using paired t-tests. Concentrations found in both apical and diaphragmatic lung lobes were significantly higher at 3 hours ($P < 0.01$) and 9 hours ($P < 0.05$) than the concentrations found in plasma. In all samples from the animals killed at 24 hours doxycycline concentrations were below the limit of analytical detection, with the exception of one animal which had trace levels in the apical lobe sample (Group C, sheep 13).

Five animals (Group C) were used to calculate pharmacokinetic data of doxycycline in sheep following intravenous administration (Table 17). In Group C one of the sheep (No.18) received a perivenous dose and was not used for the calculation of pharmacokinetic data. Concentrations of drug were

similar in the 5 animals used (Figure 5). The data curves from the five Group C sheep which were used showed best fit for three exponentials using Akaike's formula (Yamaoka *et al.*, 1978).

Discussion

The concentration of doxycycline in sheep plasma was lower than the concentration in lung tissue, while the concentration of oxytetracycline in sheep plasma was higher than the concentration found in lung. The concentrations of doxycycline in apical and diaphragmatic lobes were significantly greater in the 3 hour and 9 hour group than the concentration found in plasma, which contrasts with the results found in sheep dosed with oxytetracycline, where the concentration in plasma was higher compared to lung, and significantly different at 3, 9 & 24 hours in most cases. The increased concentration of antimicrobials, including doxycycline in lung, which penetrate highly lipid compartments such as milk and a wide range of other tissues including lung, have been previously documented (Riond and Riviere, 1988; Ziv & Sulman 1974). The concentration of doxycycline found in the apical lobes was lower than the concentrations found in diaphragmatic lobes from individual animals. This may suggest that this area of lung contains less glycoprotein, which is known to bind tetracyclines to a varying extent. Doxycycline and related tetracyclines have been shown to bind extensively to plasma proteins, glycoproteins, and liver homogenate fractions compared to the more hydrophilic members of this group, such as tetracycline and oxytetracycline (Riond and Riviere, 1990a; Riond and Riviere, 1988; Cohen *et al.*, 1987)

The elimination half life of doxycycline (t1/2ß) of 2.64h found in this study was shorter than the value expected given the documented lipophilic properties of the drug compared to oxytetracycline and others which are poorly lipid soluble. The correlation of the data to a triexponential decline agrees with a recent study in sheep at a similar dose rate of 4mg/kg (Shi *et*

al., 1988), but not with a sheep study where the dose was 20/mkg (Ziv & Sulman, 1974) The half-life is also shorter than the half-life of oxytetracycline in sheep of 220 minutes previously reported (Kirkwood and Widdowson, 1990).

Earlier studies have shown longer elimination half-lives in man (15.6h), cattle (20h), and particularly sheep (24.7h) for doxycycline than those found in the present study (Riond and Riviere, 1988). Recent studies in sheep and related species, detailed below, have demonstrated t1/2ß closer to the mean of 2.64 hours found at a dose rate of 3mg/kg in this study. In sheep dosed at 4mg/kg the Volume of Distribution of the central compartment (Vc) was 0.15L/kg (Shi *et al.*, 1988) compared to 0.23 in this study. In pigs dosed at 20mg/kg the t1/2ß was found to be 3.92 hours, with Volume of Distribution at steady state (Vd(ss)) of 0.47 and total body clearance (Cl) of 100ml.kg/h (Riond and Riviere, 1990a) compared to mean results of 2.64 hours, 0.48ml/kg/h and 171ml.kg/h found in this study. The values for t1/2ß found in dogs and cats were 4.56 and 6.99 respectively (Riond *et al.*, 1990).

Reasons for variation in t1/2ß and other values obtained are not clear, although possible reasons for the difference in measured pharmacokinetic parameters may include the use of varying mathematical models, or the use of different analytical techniques. Biological variation including differences in species, bodyweight and formulation/dose administered may produce varied results. The elimination half-life of doxycycline and oxytetracycline has been shown to vary depending on bodyweight, although high protein binding of doxcycline in cats can influence the results when compared to other species (Riond and Riviere, 1990b; Kirkwood and Widdowson, 1990)

The short period of time during which doxycycline could be measured was unexpected, with the concentration at or below the limit of detection (0.1µg/ml/g) for the assay by 24 hours. Doxycycline, given its more

lipophilic properties, was expected to remain for a longer period than oxytetracycline, dosed at 10mg/kg (Chapter 4), which showed detectable levels of 0.3μg/ml or μg/g in both plasma and lung at 24 hours. The relatively high concentration of doxycycline in lung tissue does however indicate that it may be useful in the treatment of infection in this and other tissues, although possibly at a dose rate higher than the 3mg/kg used in this study.

Table 12. Intra- and Inter-assay recovery and precision of the HPLC method for concentrations of doxycycline in sheep plasma (μg/ml) (n=4).

Concentration	Recovery (%)		Coefficient of variation (%)	
added (μg/ml)	Intra	Inter	Intra	Inter
0.5	89.31	82.85	6.84	6.61
1.0	87.88	83.44	3.20	4.60
2.0	90.70	81.06	4.84	8.26
5.0	89.00	90.93	2.22	3.86
25.0	98.78	103.77	2.83	5.75

Table 13 . Intra- and Inter-assay recovery and precision of the HPLC method for concentrations of doxycycline in sheep lung (μg/g wet wt.) (n=4).

Concentration	Recovery (%)		Coefficient of variation (%)	
added (μg/g)	Intra	Inter	Intra	Inter
0.5	49.19	58.74	8.15	9.72
1.0	49.15	52.62	3.82	8.72
2.0	53.94	51.61	2.78	7.28
5.0	54.55	52.41	0.59	11.98

Table 14. (Group C) Concentration (μg/ml) of doxycycline in sheep plasma up to twenty four hours after administration at a dose rate of 3mg/kg by the intravenous route.

Sheep No.	1	2	3	4	5	6*	Mean	S.E.M.
Time (h)								
0	0	0	0	0	0	0	-	
0.083	10.36	10.05	10.05	9.67	13.70	2.81	10.77	0.66
0.25	7.10	7.05	6.66	6.88	8.93	2.41	7.32	0.36
0.5	5.55	5.59	5.13	5.04	6.91	1.74	5.64	0.30
1.0	3.97	4.40	3.76	3.70	5.41	1.42	4.25	0.28
2.0	2.56	2.72	2.11	2.09	3.83	0.85	2.66	0.28
4.0	1.41	1.42	1.01	0.75	2.15	0.33	1.35	0.21
6.0	0.70	0.64	0.45	0.29	1.22	0.17	0.66	0.14
8.0	0.43	0.36	0.23	0.15	0.76	0.11	0.39	0.09
12	0.15	0.10	0.06	0.05	0.27	0.08	0.13	0.08
24	0.05	0	0	0	0	0.06	0	-

* Sheep 6 dosed perivenously, not used for calculation of means.

Table 15. (Groups A and B) Concentration (μg/ml) of doxycycline in sheep plasma up to three and nine hours after administration at a dose rate of 3mg/kg by the intravenous route.

Group B

Sheep No.	1	2	3	4	5	6	Mean	S.E.M.
Time (h)								
0	0	0	0	0	0	0	-	
0.083	10.47	11.24	10.38	9.79	11.80	12.09	10.96	0.33
0.25	5.59	7.63	8.04	6.85	7.77	7.28	7.19	0.33
0.5	3.90	5.35	6.34	4.96	5.92	5.11	5.26	0.31
1.0	3.48	4.04	4.78	4.28	4.23	3.82	4.11	0.16
2.0	2.52	2.50	2.91	2.08	2.64	2.27	2.49	0.11
4.0	1.13	1.27	1.23	0.92	1.14	1.15	1.14	0.04
6.75	0.36	0.48	0.41	0.28	0.31	0.49	0.39	0.03
8.0	0.29	0.33	0.26	0.18	0.19	0.34	0.26	0.03
9.0	0.16	0.32	0.21	0.15	0.17	0.31	0.22	0.03

Group A

Sheep No.	1	2	3	4	5	6	Mean	S.E.M.
Time (h)								
3.0	1.35	1.65	1.22	1.90	1.83	1.21	1.53	0.11

Table 16 Concentration of doxycycline at 3, 9 and 24 hours in sheep plasma (μg/ml), apical and diaphragmatic lobes (μg/g, wet wt.) of normal lung after administration at a dose rate of 3mg/kg by the intravenous route.

Group A	Animal No.	kill time(h)	Plasma	Apical	Dia
	1	3	1.35	1.52	1.71
	2	3	1.65	2.01	1.94
	3	3	1.22	1.98	2.09
	4	3	1.90	2.20	2.28
	5	3	1.83	2.24	2.22
	6	3	1.21	1.80	1.83
	Mean	-	1.53	1.96	2.01
	S.E.M.	-	0.11	0.11	0.09

Group B	Animal No.	kill time(h)	Plasma	Apical	Dia
	1	9	0.16	0.42	0.35
	2	9	0.32	0.40	0.35
	3	9	0.21	0.25	0.24
	4	9	0.15	0.33	0.31
	5	9	0.17	0.22	0.26
	6	9	0.31	0.62	0.56
	Mean	-	0.22	0.37	0.34
	S.E.M.	-	0.03	0.06	0.04

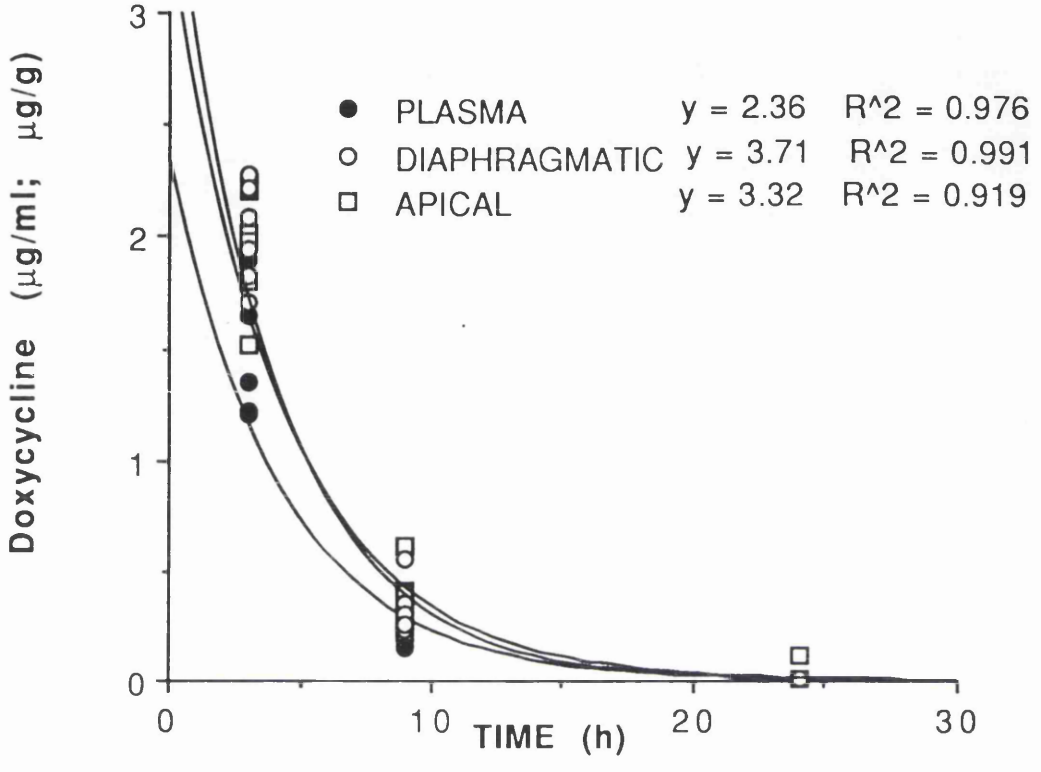
Group C	Animal No.	kill time(h)	Plasma	Apical	Dia
	1	24	0.05	0.12	0
	2	24	0	0	0
	3	24	0	0	0
	4	24	0	0	0
	5	24	0	0	0
	6	24	0.06	0	0
	Mean	-	0	0	0
	S.E.M.	-	-	-	-

Apical = Apical lobes; Dia = Diaphragmatic lobes

Table 17. Pharmacokinetic parameters based on a three compartment model measured after intravenous administration of doxycycline at 3 mg/kg to Group C.

Sheep no.	1	2	3	4	5	Mean	S.E.M.
Parameters							
B1(t1/2ß)h	4.37	2.09	1.94	2.10	2.69	2.64	0.45
B2(t1/2 dist.)h	0.94	0.63	0.53	0.82	0.61	0.71	0.076
B3(t1/2alpha)h	0.08	0.08	0.07	0.10	0.08	0.082	0.005
C'po (µg/ml)	14.2	13.8	14.8	12.4	19.7	14.98	1.24
AUCobs(µg/ml.h)	18.2	17.8	14.2	13.0	26.6	17.96	2.38
Vc(ml/kg)2E	265	233	227	253	161	228	18.0
Vd(area)(ml/kg)	841	474	528	546	399	558	75.2
Vd(ss)(h/ml/kg)	641	441	474	470	384	482	42.8
K31/h	0.29	0.86	0.86	0.50	0.82	0.67	0.12
K13/h	0.38	0.29	0.52	0.20	0.51	0.38	0.06
K21/h	3.27	3.48	4.06	2.30	3.85	3.39	0.30
K12/h	4.53	5.02	5.56	4.20	4.22	4.71	0.26
Kel/L	0.73	0.76	1.00	0.92	0.74	0.83	0.05
Cl.B(ml/kg.h)	155	165	203	222	112	171.4	19.2

Figure 5 Concentration of doxycycline, after intravenous injection, in sheep plasma (μg/ml), and apical and diaphragmatic lung (μg/g), showing exponential line of best fit.



Chapter 6 Oxytetracycline concentrations in bovine lung

Introduction

Oxytetracycline has been studied more extensively in cattle than in other species. Studies carried out have included those where a clinical response to the treatment was measured, where pharmacokinetics in both normal and diseased animals were determined, and in a limited number of studies, where concentrations of oxytetracycline, and other drugs, in the lung tissue of normal and infected animals were measured. Few studies have been undertaken where the uptake of oxytetracycline in lung tissue has been compared in normal control animals and pneumonic animals.

Where studies were carried out previously, comparing oxytetracycline in normal and diseased tissue, the numbers of animals were relatively small and the concentration of drug was measured at the end of pharmacokinetic experiments, when concentrations were low (Ames *et al.*, 1983; Ames and Patterson, 1985). The drug concentrations found in these studies demonstrated accumulation of oxytetracycline after 24 and 48h in the diseased area of the lung, compared to either normal appearing lung from the same animal or compared to control animals which were not infected (Ames *et al.*, 1983; Ames and Patterson, 1985).

After intravenous (IV) administration the concentration of oxytetracycline in lungs from pneumonic animals was significantly different compared to normal, non-infected control animals but not significantly different to the normal-appearing areas of the infected lungs from the same animal, which had a concentration intermediate to the control and diseased lungs (Ames *et al.*, 1983). After intramuscular injection of oxytetracycline the concentration in macroscopically-affected lung was found to be significantly different to that in normal-appearing, lung from

the same animals at both 24 and 48h, but was not significantly different to the concentration in a non-infected control group (Ames and Patterson, 1985).

The reasons for the discrepancies in the two previous studies are unclear. Oxytetracycline may have been accumulating at a different rate in normal and diseased lung tissue, with drug diffusing slowly into and out of the diseased tissue compared to normal tissue. This would produce greater concentrations in diseased tissue in the later elimination phase. If the normal and diseased lung tissue accumulated drug at the same rate after injection, the most probable cause of the increased concentration in the diseased lung was that the conditions present in the pneumonic area of lung prevented release of the drug from the tissues. Analysis in normal and diseased lung tissue at short time periods after oxytetracycline administration were expected to show whether drug accumulated at a different rate in normal or diseased tissues. The following study was designed to determine whether the rate of uptake of oxytetracycline in apparently normal or diseased lung differed, by measuring oxytetracycline concentration at short time periods following IV administration at the manufacturer's recommended dose rate (10mg/kg).

Materials and Methods

Twenty six animals were used in the study (Appendix E), allocated into four groups comprising two control groups and two diseased groups. All animals were treated at a dose rate of 10mg/kg prior to slaughter. Terramycin-LA (200mg/ml) was used to provide a small dose volume for IV administration (Pfizer Ltd, England) Each animal was killed as near as possible to the target time allotted to that animal. If an animal was likely to have a pneumonic condition it was allocated to one of the diseased groups.

The four groups were as follows:

1. Group A. Control animals killed one hour after dosing (n=7).
2. Group B. Control animals killed two hours after dosing(n=7).
3. Group C. Pneumonic animals killed one hour after dosing(n=6).
4. Group D. Pneumonic animals killed two hours after dosing(n=6).

Samples of blood were taken during exsanguination using lithium heparin Monovettes (Sarstedt Ltd, England), for estimation of plasma oxytetracycline concentrations. These were stored at -20°C until analysed. Lungs were removed and allocated to one of the four groups previously described, based on elapsed time from dosing and the macroscopic examination of lung tissue. The lungs were sectioned into four areas and processed as described previously in the general materials and methods section (Chapter 2). Lungs from Groups A (1h control), C (1h diseased) and D (2h diseased) had samples taken from the four areas of lung previously described (Figure 2, Chapter 2). Lungs from Group B (2h control) had samples taken from only two areas of lung, the right apical and right diaphragmatic regions. In the diseased groups (C&D), where a lung area was found to be affected, only diseased tissue was taken for drug analysis.

The method of analysis for both plasma and bovine lung was the same as that used for other species and is descibed previously in the materials and methods section (Chapter 2). Intra- and inter- assay variations were tested using the standard format of 4 replicates at four concentrations of oxytetracycline within, and between assays (Tables 18, 19 & 20). The concentrations used were selected to encompass the range found in the samples of plasma, normal control lung, and diseased tissue analysed, and ranged from 0.5 to 25.0µg/ml.

Results

Of the twenty-six animals used in the experiment twelve were found to have lesions thought to be associated with pneumonic conditions (Appendix E). No bacteriological assessment was carried out after necropsy since it was considered that the results obtained may have been misleading due to the high concentration of antibiotic present.

The animals in the pneumonic groups had chronic broncho- or suppurative pneumonias, comprising necrotic areas of lung with various amounts of pus present. Some animals were found to have other disease conditions present at post-mortem examination (Appendix E). Lungs were divided into four areas, called left and right apical and left and right diaphragmatic, as in previous experiments, with representative portions taken from each. The areas of diseased lung were located in the smaller, apical lung lobes in almost all the pneumonic cases.

Intra/inter-assay variation

Recovery of oxytetracycline from plasma, normal and diseased lung in spiked samples was similar to recoveries recorded in other species. Mean intra-assay recoveries for plasma varied from 74 to 81%, with a Coefficent of Variation of less than 5% over the range of concentrations tested. Mean inter-assay recoveries varied from 73 to 77% with a Coefficent of Variation of less than 8% over the range of concentrations tested. Mean intra-assay recoveries for normal lung varied from 43 to 55%, with a Coefficent of Variation of less than 7% over the range of concentrations tested. Mean inter-assay recoveries varied from 43 to 47% with a Coefficent of Variation of less than 11%. Intra-assay recoveries for diseased lung varied from 42 to 47%, with a Coefficent of Variation of less than 4%. Inter-assay variation was 42 to 48 % with a Coefficent of Variation of less than 5% over the range of concentrations tested.

Concentration of oxytetracycline in plasma and lungs of normal and diseased animals.

(a) Group A (1h control). Mean concentration of oxytetracycline in plasma was 17.17μg/ml, with mean concentrations in the 4 areas of lung of between 14.80 and 15.58μg/g (Table 21). There was no statistically significant difference between concentrations in diaphragmatic lobes and plasma. The apical lobe concentrations were significantly lower ($P<0.05$) than plasma concentrations.

(b) Group B (2h control). The mean concentration of oxytetracycline in plasma was 11.18μg/ml, with mean concentrations in the right apical and diaphragmatic lobes being 14.66 and 14.02μg/g (Table 22) respectively. Plasma concentrations were significantly lower ($P<0.05$) than those in apical and diaphragmatic areas. There was no difference between the 2 hour control group right apical and right diaphragmatic lung concentrations.

(c) Group C (1h diseased). The mean concentration of oxytetracycline in plasma was 16.55μg/ml, and mean concentrations in the apical and diaphragmatic lobes were between 13.44 and 13.93μg/g (Table 23). Plasma concentrations in diseased animals were significantly higher ($P<0.05$) than the concentrations found in the areas of apical diseased lung measured, but not in diaphragmatic areas of lungs with normal appearance obtained from the same animals. Eleven of the 12 apical lobes, and 2 of the 12 diaphragmatic lobes had consolidated tissue in which the drug concentration was measured. There were no differences in oxytetracycline concentration between any of the lung areas measured at 1 hour.

(d) Group D (2h diseased). The mean concentration of oxytetracycline in plasma was 8.14μg/ml, and mean concentrations in the apical and diaphragmatic groups between 8.96 and 9.13μg/g (Table 24). There were no

significant differences in oxytetracycline concentration between plasma and any of the 4 lung areas measured, whether normal or diseased. Eleven of the 12 apical lobes, and 2 of the 12 diaphragmatic lobes had consolidated tissue in which the drug concentration was measured.

Variation in plasma and lung concentration between groups.

Inter-group variation in oxytetracycline concentration at one and two hours and between normal and diseased groups was measured using Student's t-test.

There were no significant differences in plasma drug concentration between Group A (1 h control) and Group C (1 h diseased) or Group B (2 h control) and Group D (2h diseased). There were no significant differences between Group A (1 h control) and Group C (1 h diseased) for any of the 4 lung areas in which drug was measured. There was no significant difference between the 1 hour and 2 hour control groups for any of the 4 lung area drug concentrations. Comparison between the 1 hour and 2 hour diseased groups showed significantly lower oxytetracycline concentrations in 3 of the 4 lung areas ($P<0.05$), while the left apical areas were not significantly different ($P=0.09$). The increased concentration found in the apical and diaphragmatic lobes of the control 2 hour group (Group B) compared to the diseased 2 hour group (Group D) was close to significance ($P=0.05$ for diaphragmatic lobes, $P=0.06$ for apical lobes).

Discussion

The variation in concentration of oxytetracycline within individual animals between lung and plasma samples was not large, while the variation between the animals found in each of the control and diseased groups was considerable. The variation in time of kill after drug administration may have been a contributing factor, and the differences could also be due to

inter-animal variation, especially where other disease conditions may have been present.

In the one hour control group (Group A) the apical lobe areas in the individual control group animals showed significantly lower concentrations than plasma, while the diaphragmatic lobes were not significantly different. This is the opposite to the situation found in sheep lung, where the apical lobe concentration of oxytetracycline was closer to the plasma concentration compared with diaphragmatic lobe concentration. The lack of significant difference between oxytetracycline concentrations in the 1 hour control and diseased groups suggests that oxytetracycline penetrates the diseased lung tissue at a similar rate to normal tissue. The similar concentrations of drug in plasma and lung suggest drug concentration equilibration is complete. Perfusion ratios between both sides of lung have been shown to remain constant, even when disease is present (Verhoeff *et al.*, 1992), although perfusion patterns for different lung areas may vary. A possible cause of the significant difference between plasma and apical lung lobe concentration is reduced uptake or faster clearance of oxytetracycline from the apical lobes. This may be due to the physical structure of the apical areas where more cartilaginous and less parenchymal tissue is present, or apical areas could have lower perfusion ratios compared with diaphragmatic lung lobes.

In Group C (1h diseased), of the 4 areas of diseased lung measured, only the 2 consolidated apical areas showed significantly lower concentrations than those measured in plasma. If the diseased lungs had normal lung perfusion patterns then only the apical lobes would have been expected to be significantly lower. This suggests that the uptake of drug into apical lung tissue is less than uptake into diaphragmatic tissue whether the tissue is diseased or normal.

During the short sampling times used in this study the concentrations of oxytetracycline achieved in diseased lung at 2 hours (Group D) does not suggest drug accumulation to the extent seen in normal, control animal lungs (Group B), or it occurs over a longer time period in diseased lung. Similar concentrations were seen in lung and plasma in the two hour diseased group. However, the concentrations measured in the 2 hour control group showed significantly higher oxytetracycline concentrations compared to plasma. This difference suggests that oxytetracycline is accumulating more rapidly, or is not being depleted to the same extent as in the lung tissue of diseased animals. It is possible that uptake of oxytetracycline may have been slower in diseased lung tissue, with the release of the drug during the elimination phase also slower. This could account for the higher oxytetracycline concentrations seen in diseased lung at later sampling times. In other studies the plasma oxytetracycline concentration in pneumonic calves has been shown to be lower over a 24 hour time period compared to a non-diseased treated group (Ames *et al.*, 1983), and this could result in lower concentrations in the lung tissue of diseased animals.

It has been reported previously that pharmacokinetic parameters of oxytetracycline were altered in calves during experimentally-induced pneumonic pasteurellosis. In one study ß(Beta) was increased, t1/2ß was reduced and K12 (uptake from the central compartment) was reduced, but not significantly (Burrows *et al.*, 1986). An earlier study described a decrease in ß(Beta) for oxytetracycline in diseased animals (Ames *et al.*, 1983) and it is unclear why these differences occurred. In the latter study a third decline phase from 12 hours after administration was used for calculation of elimination kinetics. This phase is usually associated with low concentrations "eliminated from poorly perfused tissue" or compartments where drug is tightly bound, for example, in bone (Burrows *et al.*, 1986). This

may account for the increased concentration demonstrated at 24 and 48 hours in diseased lung tissue (Ames *et al.*, 1983; Ames and Patterson, 1985). An increased Volume of Distribution may explain the lower plasma concentration in diseased calves and higher residues in diseased tissue, caused by an increase in t1/2ß, or elimination of oxytetracycline from the plasma, where Cl(ß), the excretion of the drug, was unaffected. The increased volume of distribution was explained by better penetration of oxytetracycline into diseased lung tissue caused by inflammation of the pneumonic compared to normal lungs, although this is contrary to the findings in this study.

The penetration of oxytetracycline into lung tissue in diseased cattle may not be as rapid as the accumulation of drug in normal tissue due to a chronic rather than acute inflammatory condition, or the presence of discrete pockets of diseased tissue as reservoirs of bacterial infection. This may result in a lag phase before increase in concentration compared to normal lung tissue. Use of inflammation models in calves (Bengtsson *et al.*, 1991) to test the distribution of oxytetracycline have shown earlier oxytetracycline accumulation, higher distribution and faster elimination from tissue cages injected with lipopolysaccharide irritant. Other studies using bacterial innoculates, have shown decreased uptake in infected cages (Bengtsson *et al.*, 1991). Where bacterial infection is present, with the formation of necrotic tissue in tissue cages, there may be delayed uptake due to poor penetration or bacterial degradation of the drug, and this may explain the similar findings in the present study, where oxytetracycline was measured in consolidated tissue.

It is possible that uptake of oxytetracycline into infected, chronically-inflamed lung tissue in cattle is a complex equilibrium, where many factors influence the concentration found at a particular time point after dosing.

Table 18. Intra- and Inter-assay recovery and precision of the HPLC method for concentrations of oxytetracycline in bovine plasma (μg/ml). (n=4).

Concentration	Recovery (%)		Coefficient of variation (%)	
added (μg/ml)	Intra	Inter	Intra	Inter
0.5	81.10	76.36	4.73	1.68
1.0	78.81	77.43	1.97	2.09
2.0	74.23	76.57	3.21	5.43
5.0	76.54	77.49	2.36	2.90
25.0	77.82	72.87	2.20	7.57

Table 19. Intra- and Inter-assay recovery and precision of the HPLC method for concentrations of oxytetracycline in normal bovine lung (μg/g, wet wt.). (n=4).

Concentration	Recovery (%)		Coefficient of variation (%)	
added (μg/g)	Intra	Inter	Intra	Inter
0.5	48.49	44.23	6.47	7.01
1.0	55.01	44.56	1.36	6.69
2.0	42.24	42.93	1.04	1.16
5.0	43.23	44.03	2.67	5.25
25.0	43.59	46.89	0.57	10.83

Table 20 Intra- and Inter-assay recovery and precision of the HPLC method for concentrations of oxytetracycline in diseased bovine lung (μg/g, wet wt.). (n=4).

Concentration added (μg/g)	Recovery (%)		Coefficient of variation (%)	
	Intra	Inter	Intra	Inter
0.5	47.39	46.80	3.42	4.26
1.0	49.02	48.38	2.94	2.85
2.0	46.42	46.64	2.97	1.36
5.0	42.36	42.67	3.99	3.05
25.0	43.18	44.93	2.99	3.56

Table 21. Group A. Concentration of oxytetracycline in normal bovine plasma (μg/ml), apical and diaphragmatic lung lobes (μg/g) approximately one hour after administration of drug at 10mg/kg by the intravenous route.

Case No.	Time (h) *	plasma	Left Ap.	Right Ap.	Left Dia.	Right Dia.
11	1.05	14.78	12.63	13.64	11.92	12.31
13	1.00	15.66	11.32	11.45	11.72	12.48
14	1.00	14.16	10.95	11.24	13.23	12.06
29	1.08	17.40	12.52	16.17	12.66	16.60
30	1.10	19.02	16.46	17.30	13.84	12.42
34	1.00	19.24	20.46	18.07	21.71	21.43
35	1.21	19.93	19.24	17.71	21.76	21.76
n=7						
Mean	1.06	17.17	14.80	15.08	15.26	15.58
±S.E.M	0.03	0.81	1.36	1.03	1.57	1.54

Ap = Apical lobe

Dia = Diaphragmatic lobe

* Time is expressed as a decimal fraction of hours

Table 22. Group B. Concentration of oxytetracycline in normal bovine plasma (μg/ml), apical and diaphragmatic lung lobes (μg/g) approximately two hours after administration of drug at 10mg/kg by the intravenous route.

Case No.	Time (h) *	plasma	Left Ap.	Right Ap.	Left Dia.	Right Dia.
20	2.22	11.22	-	11.65	-	11.28
22	2.40	9.72	-	11.72	-	12.38
23	2.48	9.39	-	10.48	-	11.25
25	1.97	15.58	-	23.01	-	20.72
26	2.02	12.29	-	20.76	-	19.53
27	2.17	6.09	-	6.48	-	7.11
32	1.78	13.99	18.58	18.50	16.07	15.86
n=7						
Mean	2.15	11.18	-	14.66	-	14.02
±S.E.M	0.09	1.19	-	2.30	-	1.85

Ap = Apical lobe

Dia = Diaphragmatic lobe

* Time is expressed as a decimal fraction of hours

Table 23. Group C. Concentration of oxytetracycline in diseased bovine plasma (μg/ml), apical and diaphragmatic lung lobes (μg/g) approximately one hour after administration of drug at 10mg/kg by the intravenous route.

Case No.	Time (h) *	plasma	Left Ap.	Right Ap.	Left Dia.	Right Dia.
6	1.33	8.67	8.41d	8.50d	9.17	10.15d
8	1.00	19.62	16.81d	17.32d	18.38	15.66d
9	1.00	18.86	12.93d	11.68d	13.38	13.20
10	1.00	15.14	12.89d	13.76d	12.91	13.08
12	1.05	14.37	9.46d	13.19	12.76	10.92
15	1.00	22.66	20.44d	16.20d	16.96	19.37
n=6						
Mean	1.06	16.55	13.49	13.44	13.93	13.73
±S.E.M	0.05	1.88	1.84	1.30	1.35	1.38

d denotes concentrations measured in consolidated tissue.

Ap = Apical lobe

Dia = Diaphragmatic lobe

* Time is expressed as a decimal fraction of hours

Table 24. Group D. Concentration of oxytetracycline in diseased bovine plasma (μg/ml), apical and diaphragmatic lung lobes (μg/g) approximately two hours after administration of drug at 10mg/kg by the intravenous route.

Case No.	Time (h) *	plasma	Left Ap.	Right Ap.	Left Dia.	Right Dia.
7	1.92	8.98	11.40d	10.15d	10.86	10.30d
18	1.92	8.80	8.41d	9.09d	12.81	12.30
24	2.33	10.57	13.89d	13.87d	14.52d	12.52
28	2.12	11.56	10.52d	8.57d	7.56	7.00
31	1.97	3.58	3.87d	4.52d	3.46	4.60
33	2.00	5.40	6.67d	7.06	5.60	5.92
n=6						
Mean	2.04	8.14	8.96	8.88	9.13	8.77
±S.E.M	0.06	1.44	1.35	1.28	1.75	1.38

d denotes concentrations measured in consolidated tissue.

Ap = Apical lobe

Dia = Diaphragmatic lobe

* Time is expressed as a decimal fraction of hours

Chapter 7. General Discussion

Concentrations of oxytetracycline were measured in rat, sheep and cattle, while doxycycline was measured only in sheep in this study. Comparison was made between plasma and lung concentrations, with various lung areas differentiated in the larger species.

Using High Performance Liquid Chromatography as the method of analysis, a fast and accurate throughput of samples was achieved, with a limit of detection of 0.1µg/ml in plasma and 0.1µg/g in lung tissue, providing similar or greater sensitivity than microbiological assay (unpublished Data). The recovery of tetracyclines from lung tissue were adequate for this study, but has recently been improved using a Photo Diode Array detection system (Rionde *et al.*, 1989).

Concentrations of oxytetracycline in rat plasma were relatively similar to those found previously (Curl *et al.*, 1988). The concentrations measured in lung were lower than plasma after intravenous administration, and large variations were seen in the concentrations achieved in the tissue, although concentrations were maintained above the Minimum Inhibitory Concentration (MIC) of susceptible bacterial species for at least 8 hours (Prescott and Baggot, 1988). The concentration of oxytetracycline after IM injection was also above the tetracycline serum MIC for three pathogens in rats, *Mycoplasma pulmonis* (0.2µg/ml), *Streptococcus pneumoniae* (0.4µg/ml) and *Corynebacterium* spp. (0.8µg/ml) for a minimum period of 8 hours (Porter *et al.*, 1985). The method of administration was relatively simple, giving adequate concentrations of drug in the target tissue. Intramuscular injection has advantages in that accurate doses can be given, unlike oral feeder systems, where dosing cannot be monitored, and the dose achieved in plasma in most cases is lower than the MIC of common rat

pathogens (Porter *et al.*, 1985). The method is suitable for small animal numbers where acute infection requires immediate response.

Concentrations of oxytetracycline in sheep plasma after intravenous administration were similar to those measured in previous studies in cattle (Burrows *et al.*, 1986; Bengtsson *et al.*, 1989), although they were proportionally higher when compared to sheep given a larger dose rate (Ziv & Sulman, 1974). Concentrations in lung were similar up to 12 hours to those in sheep administered oxytetracycline by the intramuscular route (McKellar *et al.*, 1988; McKellar, 1989), although at 24 hours the concentrations in lung were only 30%, suggesting that a depot preparation given IM would be more suitable than IV administration, for practical purposes.

The higher concentration found in plasma compared to apical areas, but not diaphragmatic lobes, in normal sheep between 3 and 24 hours is probably due to the structure of the apical lobes. This difference is seen in normal cattle at 1 hour, where concentrations of oxytetracycline in the apical lobes are also significantly lower than in plasma. The difference in structure of the upper lobes may be responsible for the fact that these lobes are the most common site of infection based on the higher proportion of diseased tissue in apical compared to diaphragmatic lobes (Dungworth, 1993). The perfusion pattern of the apical lobes would appear to be lower based on radionuclide studies carried out in infected calves although no difference was seen in diseased compared to normal calves (Verhoeff *et al.*, 1992). Perfusion patterns in lung change in disease conditions with lower blood flow to the diseased tissue, which may result in local hypoxia in affected tissues (Clercx *et al.*, 1989; Sheehan *et al.*, 1992).

In sheep, the oxytetracycline concentration in diaphragmatic lobes, which appeared normal, was significantly lower than diseased apical lobes from the same animal, and lower than plasma concentrations of drug. The higher concentration in apical compared to diaphragmatic lobes is the

opposite situation to that found in normal sheep, which suggests that oxytetracycline is accumulating in diseased lung tissue in sheep. This accumulation may be due to acute inflammation caused by the infective agent. However, this situation is different in cattle, where higher concentrations were found in normal tissue, and where the disease condition was thought to be chronic in most, if not all cases.

The concentration of doxycycline in sheep was higher in lung tissue compared to plasma. The concentration of drug in normal animals, although significantly higher in apical and diaphragmatic lobes compared to plasma, showed a similar pattern to the oxytetracycline studies, where the apical lobe concentrations were closer to the plasma concentration than the diaphragmatic lobe concentrations. The pharmacokinetic parameters measured, including t1/2ß, Vc and Cl were similar to recent studies (Shi *et al.*, 1988; Rionde and Riviere, 1990a) but did not reflect the values found in earlier studies (Ziv and Sulman, 1974). The half-life of the drug was found to be shorter than that reported previously although this could be because of poorer sensitivity in the present study which meant that the distribution phase was not completely defined. The concentrations achieved do, however, indicate that the drug would be useful for the treatment of respiratory conditions given at a higher dose rate, possibly as a priming dose of 10mg/kg and a 12 hour maintenance dose of 5mg/kg, to give values which would be double the MIC for at least 6 hours, and more than the MIC for at least half the dosing interval of susceptible respiratory pathogens (Prescott and Baggot, 1988). The concentrations acheived at the present dose rate of 3mg/kg are lower than the recommended MIC values for susceptible species at 9 hours, and a single 24 hour dose would probably not give adequate therapeutic concentrations over that time period.

No difference in oxytetracycline concentration was found in samples of apical or diaphragmatic lung tissue when comparing normal and diseased

cattle. Significant differences did occur when the lungs of both were compared to plasma. The concentration measured in plasma of normal animals was higher than apical but not diaphragmatic lung tissue. However, unlike sheep, the concentration found in the apical lobes was significantly lower than plasma in Group C (1h diseased). This suggests that lung concentrations were not as high in diseased compared to normal lung in cattle. The concentration of oxytetracycline in Group D (2h diseased) is similar in plasma and both apical and diaphragmatic lobes, while the lung concentration is greater than plasma in Group B (2h control). This also suggests that oxytetracycline does not accumulate in diseased lung tissue as quickly as normal tissue. Mean plasma concentrations in Group A (1h control) are similar to those of Group C (1h diseased)(17.17 and 16.55µg/ml respectively), while the plasma concentrations in Group B (2h control) are higher than those of Group D (2h diseased)(11.18 and 8.14µg/ml respectively). The half-life of oxytetracycline has previously been found to be shorter in pneumonic animals (Ames *et al.*, 1983), which may account for the relatively lower concentrations found in cattle diseased lung tissue, if the drug is less available for uptake in lung of diseased compared to normal animals.

The differences seen between diseased lung in sheep and cattle may be explained by the type of pneumonia present in each species. The diseased lung tissue found in sheep was of uniform consistency, typified by a collapsed structure compared to normal lungs, but with no vesicles or pus present. There did not appear, on gross pathological examination, to be changes which would result in changes of lung vascular structure, apart from collapse of the alveolar mass. The pathology of the cattle diseased lung tissue was different in appearance to the above with accumulation of pus and cellular debris, accompanied by discrete vesicles up to 2cm in diameter. The differences seen in the normal lung areas are more difficult to comprehend.

It is possible that changes occur, possibly due to mechanisms, including inflammation, which reduce or delay uptake of oxytetracycline in the normal-appearing lung tissue of diseased cattle in the 2 hour group. The lower blood flow seen when areas of lung are poorly perfused may be responsible for the lower uptake of oxytetracycline in diseased cattle lung at 2 hours.

The plasma and tissue concentrations of oxytetracycline in all species, including those where diseased tissue was measured, are above the MIC required for succeptible pathogens, such as *Actinobacillus* sp (0.25µg/ml), *Haemophilus pleuropneumoniae* (0.8µg/ml), or *Mycoplasma bovis* (0.4µg/ml) for a minimum period of at least twelve hours, although resistant strains in these and other common pathogens have been reported (Prescott & Baggot, 1988). The data suggests that an IM dosage regime, possibly with a loading bolus IV dose, may give more prolonged therapy, and may permit an acceptable 24 hour dosing routine.

Although changes in drug penetration may occur in diseased lung tissues the concentration measured in these tissues is still above the MIC required for treatment of susceptible species. This does not necessarily reflect the concentration found at the site of infection, which may be intracellular. It would be interesting to measure drug concentrations in the intercellular fluid, the usual site of infection (Bazra and Cuchural, 1985).

Conclusions

1. Oxytetracycline accumulates in the lung tissue of rats, sheep and cattle at a lower concentration compared to plasma after IV and IM dosing, but not to a significantly lower level.

2. Doxycycline concentrations in normal lung of sheep after IV dosing are significantly higher in both apical and diaphragmatic lobes compared to plasma, although elimination of the drug appears to be more rapid than previously reported.

3. Oxytetracycline concentration after IV dosing in cattle normal and diseased lung tissue is similar. Although concentration of oxytetracycline in diseased lung may be significantly lower in some cases at short time periods, both normal and diseased lung concentrations were around 10-20% lower than plasma concentrations.

4. In the crude tissue homogenates of lung tissue tested, normal and diseased lung drug concentrations of oxytetracycline in cattle and sheep after IV administration were above the MIC for some common respiratory pathogens for at least eight hours.

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Appendix A. Dosing regime, experimental data and necropsy times for rats administered oxytetracycline (50mg/ml) by the intra-muscular route at 10 mg/kg bodyweight.

Rat No.	Weight(g)	Sex	Dose(ml)	*Kill Time(h)	*Actual Time(h)
1	650	m	0.13	1.00	1.00
2	800	m	0.16	1.00	1.03
3	600	m	0.12	1.00	1.08
4	600	f	0.12	1.00	1.14
5	950	m	0.19	2.00	2.12
6	550	m	0.11	2.00	2.12
7	500	f	0.10	2.00	2.13
8	350	f	0.07	2.00	2.15
9	600	m	0.12	4.00	4.01
10	650	m	0.13	4.00	4.06
11	400	f	0.08	4.00	4.09
12	550	m	0.11	4.00	4.13
13	450	f	0.09	8.00	7.53
14	450	f	0.09	8.00	7.56
15	500	f	0.10	8.00	7.58
16	450	f	0.09	8.00	7.59
17	350	f	0.07	24.00	24.10
18	600	m	0.12	24.00	24.10
19	400	f	0.08	24.00	24.10
20	550	m	0.11	24.00	24.10
21	650	m	0.13	48.00	48.00
22	400	f	0.08	48.00	48.00
23	550	m	0.11	48.00	48.00
24	500	f	0.10	48.00	48.00

*Times are expressed as decimal fractions of hours.

Appendix B. Weight and necropsy times for sheep administered oxytetracycline at 10mg/kg by the intravenous route.

Sample Time(h)	Sheep No.	Tag No.	Weight (kg)	Actual Time* (h)
3	1	16	39	2.88
3	2	17	34	2.87
3	3	25	37	3.12
3	4	26	27	3.22
3	5	27	30	3.12
3	6	28	37	3.12
9	7	21	39	9.32
9	8	22	41	9.40
9	9	23	35	9.27
9	10	24	34	9.28
9	11	2	25	8.80
9	12	3	34	8.80
12	13	4	28	11.54
12	14	5	35	11.93
12	15	6	35	11.45
12	16	7	39	11.75
12	17	8	31	11.40
12	18	9	41	11.47
24	19	10	34	23.82
24	20	11	43	23.83
24	21	12	39	23.80
24	22	13	34	24.20
24	23	14	36	24.38
24	24	15	34	23.88

*Times are expressed as decimal fractions of hours.

Appendix C. Weight, dose and necropsy times for sheep with diseased apical lobes administered oxytetracycline at 10mg/kg by the intravenous route.

Sheep no.	Tag No,	Weight (kg)	Dose (ml)	Actual Kill Time(mins)
1	6	21	2.1	65
2	7	24	2.4	62
3	8	25	2.5	66
4	11	19	1.9	66
5	13	24	2.4	58
6	15	25	2.5	58
7	16	22	2.2	59
8	26	22	2.2	66
9	28	21	2.1	65
10	31	22	2.2	58

Appendix D. Weight, dose and necropsy times for sheep administered doxycycline at 3 mg/kg by the intra-venous route.

Group/ Time (h)	Animal No.	Tag No.	Weight (kg)	Dose (ml)	Actual Kill Time (h)*
A/3	1	786	36	5.4	2.98
A/3	2	788	31	4.6	2.98
A/3	3	789	34	5.1	2.98
A/3	4	797	34	5.1	2.98
A/3	5	798	36	5.4	2.98
A/3	6	799	35	5.2	2.98
B/9	7	785	33	5.0	8.79
B/9	8	790	33	4.9	8.79
B/9	9	792	27	4.1	8.79
B/9	10	800	33	5.0	8.79
B/9	11	999	31	4.7	8.79
B/9	12	1000	25	3.7	8.79
C/24	13	787	31	4.6	23.92
C/24	14	791	37	5.5	23.90
C/24	15	793	20	3.1	23.92
C/24	16	794	33	5.0	23.90
C/24	17	795	35	5.3	23.92
C/24	18	796	33	4.9	23.90

*Times are expressed as decimal fractions of hours.

Appendix E. Clinical pathological diagnosis for cattle dosed with oxytetracycline and killed at short time periods following oxytetracycline administration.

Case No.	Group	Breed	Weight (kg)	Pathological Diagnosis
11	normal 1h	Ayrshire	230	nil
13	normal 1h	Ayrshire	185	nil
14	normal 1h	Ayrshire	195	nil
29	normal 1h	Simmental	230	pericarditis
30	normal 1h	Friesian	290	nephritis
34	normal 1h	Ayrshire	300	nil
35	normal 1h	Ayrshire	320	nil
20	normal 2h	Ayrshire	140	nil
22	normal 2h	Ayrshire	150	nil
23	normal 2h	Ayrshire	155	nil
25	normal 2h	Ayrshire	80	pyelonephritis
26	normal 2h	Ayrshire	150	nil
27	normal 2h	Simmental	42.5	nil
32	normal 2h	LimousinX	320	nil
6	diseased 1h	Friesian	300	chronic bronchopneumonia
8	diseased 1h	Limousin	240	chronic suppurative pneum.
9	diseased 1h	unknown	225	chronic bronchopneumonia?
10	diseased 1h	Ayrshire	135	dictyocauliasis?
12	diseased 1h	Ayrshire	185	chronic bronchopneumonia
15	diseased 1h	Friesian	400	necrotising bronchopneumonia
7	diseased 2h	Ayrshire	140	chronic bronchopneumonia
18	diseased 2h	Limousin	120	chronic suppurative pneum.
24	diseased 2h	Charolais	245	chronic suppurative pneum.
28	diseased 2h	Ayrshire	178	bronchopneumonia?
31	diseased 2h	Ayrshire	260	bronchopneum/emphysema
33	diseased 2h	Highland	100	bronchopneumonia?