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# SOME PHARMACOKINETIC PARAMETERS OF DOXYCYCLINE IN EAST AFRICAN GOATS AFTER INTRAMUSCULAR ADMINISTRATION OF A LONG-ACTING FORMULATION

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ABSTRACT

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A compartmental and non-compartmental study was carried out on five adult goats following intramuscular administration of doxycycline at 20 mg/kg bodyweight. The concentration of the drug in serum was determined by a microbiological assay employing *Bacillus cereus* var *mycoides* (ATCC 11778) as the test organism. The mean serum concentration ($C_{max}$) and the time of maximum concentration ($T_{max}$) were 1.87 $\mu$g/ml and 0.85 h, respectively. Using compartmental analysis, the plasma concentration–time curve of doxycycline best fitted a three-compartment open model with first-order absorption. A three-phase disposition of doxycycline was found, the terminal elimination half-life being approximately 40 h.

The statistical moment theory was mainly used for non-compartmental analysis. The value obtained for the mean residence time (MRT) was 16.41 h. The mean values for the volume of distribution at steady state ($V_{dss}$), determined by compartmental and non-compartmental analyses, were 8.73 and 13.19 L/kg, respectively. There were no statistically significant differences when the major pharmacokinetic parameters were compared.

It was concluded that the pharmacokinetic behaviour of doxycycline in goats after intramuscular administration is characterized by a three-compartment model with a slow terminal elimination phase. Based on current knowledge, this could be due to enterohepatic recycling and/or flip-flop kinetics. The study indicated that a single intramuscular administration of 20 mg/kg of doxycycline may only provide therapeutic concentrations for up to 24 h owing to slow absorption at the injection site.

*Keywords:* doxycycline, goat, intramuscular, pharmacokinetics

*Abbreviations:* ATCC, American Type Culture Collection; $AVC_\infty$, total area under the plasma concentration–time curve; AUMC, area under the curve of the product from time zero to infinity; Cl, total body clearance; i.m., intramuscular; i.v., intravenous; MRT, mean residence time; MIC, minimum inhibitory concentration; PVP, polyvinyl pyrolidone; $V_{d\beta}$, volume of distribution; $V_{dss}$, volume of distribution at steady state

## INTRODUCTION

Doxycycline is a more recently developed derivative of tetracycline with broad-spectrum antibacterial activity in both domestic animals and humans (Aronson, 1980). In addition to its antibacterial activity, doxycycline also has antiprotozoal properties (Kutler and Simpson, 1978; Van Heerden and Immelman, 1979; Immelman and Dreyer, 1982; Chang *et al.*, 1990). Doxycycline is widely used in humans and domestic animals and pharmacokinetic studies in these species have been published (Raghuram

and Krishnaswamy, 1982; Wilson *et al.*, 1988; Riond *et al.*, 1989, 1990; Riond and Riviere, 1990). However, no published information is available on the pharmacokinetics of doxycline in goats, despite the fact that long-acting formulations are now available. The information available from other species cannot simply be applied to goats owing to well-documented species differences in the disposition of this drug.

Although most pharmacokinetic studies are carried out by the intravenous route, the intramuscular route was used in this study because of its clinical relevance, as it is the only practical route of administration for long-acting formulations. The objective of the study was to establish some pharmacokinetic parameters for doxycycline in indigenous African goats, a target species for this drug for which there are no data available at present.

## MATERIALS AND METHODS

### *Animals*

Experiments were carried out on 5 conventionally reared, clinically healthy, small East African goats, with a bodyweight range of 20–26 kg. The goats were kept in pens for 14 days prior to the experiment to acclimatize them to their new environment. They had access to hay and drinking water *ad libitum* throughout the study.

### *Drugs*

Doxycycline monohydrate (Doxycen retard, Cenevisa, s.a Reus, Spain) containing 200 mg/ml active ingredient was injected intramuscularly at a dose rate of 20 mg/kg bodyweight.

### *Experimental protocol*

Following a single deep intramuscular injection into the gluteal muscle, 10-ml blood samples were collected by jugular venepuncture using Vacutainer tubes (Becton Dickinson, NJ, USA) without anticoagulant at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 96 and 144 h after administration. The blood samples were allowed to clot, they were centrifuged at 3000*g* for 15 min and the serum was harvested and stored at −20°C pending assay.

### *Assay*

The serum concentrations of doxycycline were measured by bioassay using an agar-gel diffusion method employing *Bacillus cereus* var *mycoides* ATCC 11778 as the test organism. The limit of detection was 0.05 $\mu$g/ml. Standard solutions were prepared in antibiotic-free goat serum in appropriate serial dilutions. The experimental samples and standards were analysed in triplicate. The measured widths of the zones of

inhibition were converted to concentrations using standard curves developed for each plate. Standard curves were prepared as described by Dornbush and Abbey (1972). The calibration graphs obtained for doxycycline in goat serum were linear within the range 0.05–0.80 $\mu$g/ml; $Y = 14.2 + 17.5x$.

*Pharmacokinetic analysis*

Individual and mean serum concentration–time data for doxycycline were analysed using both compartmental and non-compartmental procedures. For compartmental analysis, initial estimates of the primary parameters were obtained by a linear curve-stripping programme, JANA (Dunne, 1985), and the parameters were determined by iterative weighted non-linear regression analysis (Metzler and Weiner, 1986). Goodness of fit was determined by residual sum of squares (SS) and minimal Akaike's information criterion. For non-compartmental analysis, several pharmacokinetic parameters were calculated using statistical moment theory (Yamaoka *et al.*, 1978; Benet and Gelaizi, 1979). The linear terminal slope ($\beta$) was calculated using the methods of least squares. The half-life ($t_{½\beta}$) was calculated for the quotient $0.693/\beta$. The total area under the serum concentration–time curve ($AUC_{\infty}$) was calculated by the trapezoidal rule with extrapolation to infinity. Similarly, the area under the curve of the product from time zero to infinity (AUMC) was calculated using the linear trapezoidal rule with extrapolation to infinity. The mean residence time (MRT) was determined by the equation MRT = AUMC/AUC.

The total body clearance (Cl) was calculated as the quotient of the dose ($D$) and AUC. The terminal volume of distribution ($V_{d\beta}$) was calculated from the ratio of the total body clearance (Cl) and terminal slope ($\beta$). The volume of distribution at steady state was determined from the equation $V_{dss} = D \times AUMC/(AUC)^2$.

*Statistical analysis*

The pharmacokinetic parameters determined by compartmental and non-compartmental analysis were compared using the Wilcoxon sign-rank test. Data are given as mean ± SD. Significance was accepted at $p \leqslant 0.05$.

## RESULTS

The mean serum concentration–time profile and the best fitting curve are shown in Figure 1. Table I shows the mean ± SD concentrations of doxycycline at various times. The values of the pharmacokinetic parameters determined by compartmental analysis are summarized in Table II, while those calculated by non-compartmental analysis are given in Table III.

After intramuscular administration, the serum concentration–time curves were best described by a three-compartment model with first-order absorption. Absorption of doxycycline from the injection site was very rapid with a $t_{½abs}$ of 0.27 ± 0.04 h. The maximum serum level of 1.87 ± 0.14 $\mu$g/ml was attained at 0.85 ± 0.33 h after administration. Three disposition phases were identified: a fast distribution phase with

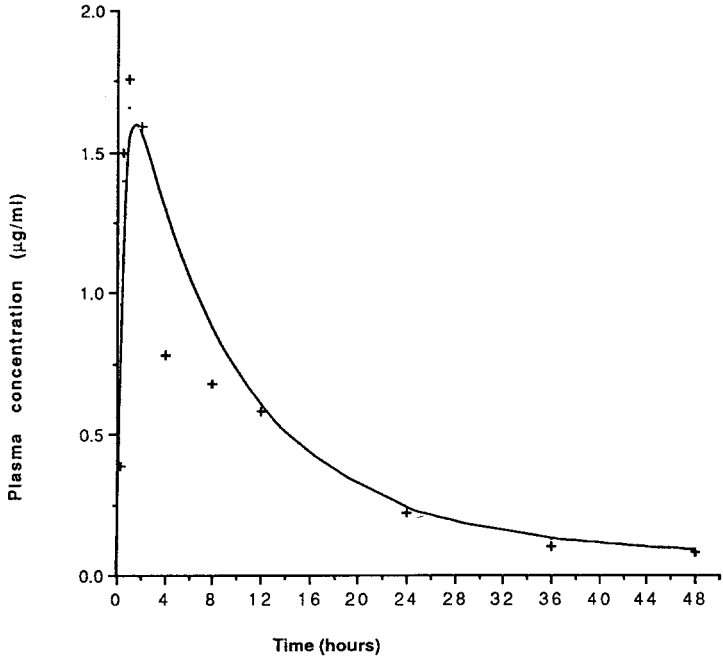


Figure 1. Mean serum concentrations in five goats after intramuscular administration of doxycycline at 20 mg/kg body weight. The continuous line was fitted by the PCNONLIN programme

TABLE I
Doxycycline concentration (mean ± SD; mg/kg) in the serum of five goats after intramuscular administration of doxycycline at 20 mg/kg body weight

| Time (h) | Doxycycline concentration (mean ± SD; mg/kg) |
|---|---|
| 0 | 0 |
| ¼ | 0.86 ± 0.36 |
| ½ | 1.29 ± 0.51 |
| 1 | 1.55 ± 0.55 |
| 2 | 1.49 ± 0.43 |
| 4 | 1.23 ± 0.32 |
| 8 | 0.83 ± 0.22 |
| 12 | 0.59 ± 0.16 |
| 24 | 0.25 ± 0.07 |
| 36 | 0.15 ± 0.04 |
| 48 | 0.09 ± 0.03 |

TABLE II
Pharmacokinetic parameters obtained by compartmental analysis in five goats after intramuscular administration of doxycycline at 20 mg/kg body weight

| Parameter | Mean ± SD |
|---|---|
| $C_{max}$ ($\mu$g/ml) | 1.87 ± 0.14 |
| $t_{max}$ (h) | 0.85 ± 0.33 |
| $A$ ($\mu$g/ml) | 0.96 ± 0.38 |
| $B$ ($\mu$g/ml) | 1.25 ± 0.39 |
| $C$ ($\mu$g/ml) | 0.27 ± 0.17 |
| $\beta$ ($h^{-1}$) | 0.086 ± 0.041 |
| $t_{½\alpha}$ (h) | 1.95 ± 0.84 |
| $t_{½\beta}$ (h) | 8.72 ± 3.55 |
| $t_{½\gamma}$ (h) | 38.31 ± 2.81 |
| $AUC_{\infty}$ ($\mu$g h/ml) | 35.93 ± 6.11 |
| Cl (ml/kg/h) | 0.572 ± 0.123 |
| $V_{dss}$ (L/kg) | 8.73 ± 3.12 |
| $V_{d\beta}$ (L/kg) | 11.22 ± 1.86 |
| $V_{d(area)}$ (L/kg) | 31.586 ± 6.67 |

a $t_{½\alpha}$ of 1.95 ± 0.84 h; an initial elimination phase with a $t_{½\beta}$ of 8.72 ± 3.55 h and a terminal elimination phase with a $t_{½\gamma}$ of 38.31 ± 2.81 h.

The contribution of the slow terminal elimination phase to $AUC_{\infty}$ was 15.52 ± 3.83%.

When the major pharmacokinetic parameters obtained by compartmental and non-compartmental analysis were compared, the Wilcoxon sign-rank test indicated that there were no significant differences in AUC, Cl, $V_{d\beta}$ or $V_{dss}$, although considerable individual variations were observed.

## DISCUSSION

Doxycycline showed a three-compartment disposition in goats. This was also reported by Garcia-Ovando and colleagues (1991) for calves. However, Riond and colleagues (1989) found that this drug followed a two-compartment open model after intravenous administration in veal calves, dogs and cats. It is known that the number of compartments demonstrated often depends on the frequency of the blood sampling schedule (Xia *et al.*, 1983). Garcia-Orvando and colleagues (1991) introduced a $\pi$-phase for the fast distribution before the $\alpha$-phase. In the present study, a $\gamma$-phase, describing slower terminal elimination of doxycycline, was found after the $\beta$-phase.

TABLE III
Pharmacokinetic parameters obtained by non-compartmental analysis in five goats after intramuscular administration of doxycycline at 20 mg/kg body weight

| Parameter | Mean ± SD |
|---|---|
| $\beta$ ($h^{-1}$) | 0.0545 ± 0.0183* |
| $t_{½\beta}$ (h) | 12.93 ± 2.02*,** |
| $AUC_{\infty}$ ($\mu g\ h/ml$) | 27.88 ± 6.35* |
| AUMC ($\mu g\ h^2/ml$) | 358.99 ± 74.25 |
| MRT (h) | 16.41 ± 1.06 |
| Cl (ml/kg/h) | 0.866 ± 0.156* |
| $V_{d\beta}$ (L/kg) | 20.33 ± 10.78* |
| $V_{ss}$ (L/kg) | 13.19 ± 3.21*,** |

*Not significantly different ($p \geq 0.05$) from the values found in Table I; **$p < 0.07$ from the values found in Table I

Doxycycline absorption from the injection site was slow and maximum levels of the drug in serum were observed 3 h after injection. This slow absorption from the injection site may be due to the retard formulation of the drug and/or to the high level of binding of the drug to plasma protein. Although the extent of binding by doxycycline in goats is not known, it is highly bound to protein (>90%) in most domestic animals (Riond *et al.*, 1990). This high level of binding of the drug to immunoglobulins may account for the small amount of free drug in the serum and hence the relatively low levels therapeutically available by 24 h following administration.

Following the peak concentration, the disposition of doxycycline was characterized by rapid distribution and slow elimination. This is in accordance with previous reports in humans (Raghuram and Krishnaswamy, 1982), dogs (Wilson *et al.*, 1988), calves (Riond *et al.*, 1989), pigs (Riond and Riviere, 1990) and cats (Riond *et al.*, 1990). The estimate for $t_{½\beta}$ (8.72 ± 3.55 h) obtained in healthy goats in this study is considerably lower than the values of 14.9 ± 0.9 h previously reported in calves with mature rumen function (Riond *et al.*, 1989), 16.3 ± 1.53 h reported in man (Raghuram and Krishnaswamy, 1982) and 24.75 ± 10.6 h reported for ewes after intravenous administration (Ziv and Sulman, 1974). Following single intravenous administration, a $t_{½\beta}$ of 10.3 h was obtained by Wilson and colleagues (1988) in dogs, while Michel and colleagues (1979) reported a $t_{½\beta}$ value in dogs of 11.8 h after single oral administration. Conversely, the $t_{½\beta}$ recorded for the goats in this study was somewhat longer than the previously reported values of 4.04 ± 0.58 h in pigs (Riond and Riviere, 1990) and of 6.99 ± 1.09 h and 4.56 ± 0.68 h (±SEM) in dogs and cats, respectively (Riond *et al.*, 1990). Such apparent intra- and interspecies variations in the estimation

of elimination half-life may be attributed as much to differences in the routes of administration, drug formulation, pharmacokinetic curve-fitting routines and analytical techniques used as to species differences.

The apparent volume of distribution in the steady state, $V_{dss}$, of 8.73 ± 0.2 L/kg obtained in this study is considerably larger than the 0.93 ± 0.10 L/kg and 0.34 ± 0.03 L/kg reported for dogs and cats, respectively (Riond *et al.*, 1990), 1.81 ± 0.11 L/kg in Holstein calves (Riond *et al.*, 1989) and 0.53 ± 0.04 L/kg for young pigs (Riond and Riviere, 1990). The large volume of distribution and the subsequent longer elimination half-life for doxycycline observed in goats suggest extensive tissue binding and/or intracellular penetration in this species. Previous studies have suggested that pharmacokinetic parameters differ among species owing to differences in the extent of binding to plasma proteins (Welling, 1986).

Greater disparities in pharmacokinetic behaviour are known to occur following intramuscular administration of tetracyclines (Maritim *et al.*, 1986). Thus, the prolonged effect may also be attributed to persistence of the drug at the injection site due to tissue irritation. However, the vehicle in the formulation used contained polyvinyl pyrolidone (PVP), which tends to prevent tissue irritation (Nouws *et al.*, 1990). It is possible that the observed $\gamma$-phase was the result of a flip-flop phenomenon in which the back diffusion rate from deeper tissues was only just slower than the elimination rate. Moreover, the extended terminal elimination phase could have been the result of doxycycline delivery from bone. The deposition of tetracycline in bones has been described (Owen, 1965), but quantitative kinetic studies on bone have not been done. However, the mean contribution of the terminal phase to total $AUC_{\infty}$ in this study was shown to be over 15%. It is doubtful whether this amount could be stored in bone within such a short time. Finally, the pharmacokinetic behaviour of doxycycline could also be explained by enterohepatic recycling. Riond and Riviere (1990) observed secondary peaks in the plasma concentration–time curves of doxycycline in pigs. Doxycycline may also undergo enterohepatic recycling in goats.

It is not possible to give an exact therapeutically active plasma concentration for doxycycline but, based on *Pasteurella haemolytica*, where MICs of 0.2–0.5 $\mu$g/ml have been reported (Ole-Mapenay, 1993), concentrations above 0.5 $\mu$g/ml seem a reasonable suggestion. On this basis, a single intramuscular administration of 20 mg/kg doxycycline should provide therapeutically active concentrations for up to 12 h during treatment of respiratory infections in goats.

The present findings indicate that prediction of tissue residue levels based on pharmacokinetic data will be erroneous unless consideration is given to the terminal elimination phase. Thus, longer withdrawal times might be necessary in food-producing animals to guarantee that the tissue concentrations are below mandatory maximal residue levels.

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