

Research Summaries

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Oxytetracycline Levels in Healthy and Diseased Calves

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Introduction

Currently accepted dosage regimens in antimicrobial therapy are derived from experimental work in healthy animals and sensitivity tests for bacteria using in vitro assays.¹ The necessary dosage and dosing interval required to maintain specific blood levels for a drug is determined from pharmacokinetic testing in clinically normal animals.²⁻⁴ This information is then combined with knowledge of the minimum inhibitory concentration of the organism being treated and the dosage regimens can be determined.¹

There have been several broad assumptions made regarding the relationship between blood and tissue concentrations of agents used in antimicrobial therapy although sometimes this therapy is based primarily on clinical response. While blood concentrations have been used as rough indications of tissue levels, factors such as protein binding and lipid solubility can affect these tissue or organ concentrations. In addition, pharmacokinetic parameters such as half-life and distribution which are used to predict blood levels can be altered by the disease state. For instance, the half-life of certain drugs has been shown to be increased in certain disease conditions.⁵ The data derived from normal animals must be carefully applied when dealing with the disease state. Measurement of tissue concentrations, their relation to serum concentrations and the effect of disease has been restricted to a few studies.⁶⁻⁸ This project was designed to investigate the effects of disease on the tissue concentrations, serum concentrations, and pharmacokinetics of oxytetracycline (OTC) in pneumonic calves.

Materials and Methods

Fifteen 1 to 2 month old Holstein Friesian bull calves were used. They were randomly divided into three groups of five animals each: group A—healthy treated controls, group B—

diseased nontreated controls, and group C—diseased treated. Group B was essential to determine if the non-specific bacterial inhibitors which are present in inflammatory tissue could affect the bioassay results. Group A represents normal animals that have been treated to determine the tissue and serum concentrations achieved with antibiotic therapy. Group C represents the test group that was used to determine what alterations occurred in tissues and serum concentrations of diseased animals.

Pneumonia in the calves in groups B and C was experimentally induced. This was done by cold stressing them and then inoculating them intratracheally with bovine viral diarrhea virus and *Pasteurella hemolytica*. The calves were then monitored over the following week for the desired disease criteria (temperature greater than 39.5°C and moist rales bilaterally). The treatment was started when these criteria were met; usually 2 to 5 days after the inoculation with *P. hemolytica*.

The treatment group calves (groups A and C) were given one injection of OTC at a dose of 11 mg/kg of body weight IV. Blood was collected over a 24 hour period after which time the calves were euthanized. A blood sample was obtained from the nontreated calves (group B) just before euthanasia so they could be killed at approximately the same time as the other groups.

The standard F.D.A. cylinder plate bioassay⁹ was used to determine OTC concentrations of serum and tissues. The tissues included three sections of healthy appearing lung and pneumonic lung from each side of each calf. In group A, only healthy lung was taken (3 samples from each side). Four samples of liver and 4 samples of kidney were taken from each animal from all groups.

Gross visual examination of the lungs was done to establish the degree of pneumonic involvement and the type

of lesion present. Histologic examination of both normal appearing and pneumonic lung was also done to confirm the gross findings and ensure that healthy appearing lung of group A was unaffected. Viral isolation and bacterial culture of the pneumonic lungs was also done.

The tissue concentrations of OTC in each organ of the 2 treatment groups were compared for any significant difference. The healthy appearing lung of group C was handled as a third treatment group in the statistical analysis. The pharmacokinetic parameters and the plasma curve for treated groups A and C were determined with the aid of a digital computer program for non-linear regression analysis.¹⁰ The parameters included all those classically examined in pharmacokinetic analysis. The group values of these parameters were compared for statistical difference. The ratio and correlation between tissue and serum concentrations of OTC were determined for each organ.

Results

Pneumonia was successfully induced in all ten calves of groups B and C. All calves met the established disease criteria before they were euthanized. On gross pathologic

examination, congestion, consolidation, fibrinous pneumonia, and pleuritis were observed in varying degrees in all animals. The percent involvement on a visual basis ranged from 25% to 70%. Histologic examination of the pneumonic areas confirmed what was observed grossly, with fluid in the alveoli and mild congestion in some sections and severe necrosis in others. The normal appearing lung in some of group B and C animals showed mild congestion and small amounts of fluid in the alveoli while the lungs of the group A (healthy) animals appeared normal.

Pasteurella hemolytica was isolated in all animals of group C and 4 animals of group B. Virus isolation yielded BVD virus in 4 animals of group C and 2 animals in group B.

The OTC tissue concentrations at 24 hours after administration were determined in all calves of all groups (Table 1). The only values which differ significantly among treatment groups were in the lungs. The OTC concentrations (1.48 ± 0.29 ug/ml) in the diseased lung of group C differed significantly ($P = 0.025$) from the levels (1.09 ± 0.09 ug/g) of the healthy treatment group A. The healthy appearing section of lung from the diseased animals of group C had OTC concentrations that were intermediate between the diseased sections of lungs from group C and the lung

TABLE 1. Oxytetracycline Activity (ug/ml or ug/g) at 24 hours after administration.

Tissue	Group A (healthy treated)	Group B (diseased nontreated)	Group C (diseased treated)
Serum	0.51 ± 0.10	0	0.5 ± 0.13
Liver	1.50 ± 0.50	0	1.27 ± 0.35
Liver/Serum	2.94	0	2.49
Kidneys	5.07 ± 0.84	0.22 ± 0.24	4.22 ± 1.43
Kidneys/Serum	9.94	0	8.27
Healthy Appearing Lungs	$1.09 \pm 0.09^*$	0	1.24 ± 0.11
Healthy Appearing Lungs/Serum	2.13	0	2.48
Diseased Lungs	-	0	$1.48 \pm 0.29^*$
Diseased Lungs Serum	-	0	2.96

*Significant difference between Group A and C means ($P = 0.025$)

Data expressed as mean \pm SD.

tissue concentrations of group A (healthy treated).

The 24 hour OTC concentrations in serum, liver and kidney did not differ significantly among treatment groups. Diseased nontreated group B had no bioactivity in any tissue except the kidney.

The tissue concentrations of each organ for each calf were correlated with the 24 hour serum concentrations. The correlation coefficients were very low (0.0101 for liver, 0.2652 for kidneys, 0.1442 for lungs). Whereas the ratios between tissue and serum concentration varied for liver, kidney and lung it should be noted that the ratio between lung and serum concentration was significantly higher for diseased lungs as compared with that in the healthy lungs (Table 1).

From the calves of groups A and C the predicted values from the computer analysis were used to construct the plasma curves (Figure 1). The pharmacokinetic parameters for each group are listed in Table 2. The only parameters which differed significantly between the treatment groups were steady state volume of distribution ($VD_{(ss)}$), volume of distribution calculated from the area under the curve ($VD_{(area)}$), y-intercept of elimination phase (B) and half-life ($T_{1/2(\beta)}$).

Discussion

Pneumonia was successfully produced in all calves of diseased groups B and C. The experimental method utilizing BVD virus, *P. hemolytica*, and environmental stress produced clinical signs consistent with severe pneumonia in all calves. All calves had severe lesions involving large portions of the lungs. The infectious agents used to induce the pneumonia were both isolated at postmortem from a high percentage of the calves. This suggests that these agents were actively replicating and probably were responsible for the lesions observed. It can be concluded that this experimental model was effective in producing the desired systemic disease.

Tissue samples analyzed for OTC activity revealed significantly elevated concentrations in the diseased lungs as compared with that in the control lungs. The OTC activity of the livers and kidneys of the diseased animals (group C) was essentially the same as that found in the livers and kidneys of the healthy calves (group A). The livers and kidneys of the diseased animals were not affected by the disease process. The OTC concentration of the grossly normal appearing sections taken from the pneumonic lungs did not differ significantly from the healthy controls or the inflamed sections of lung. These lung concentrations were intermediate between the 2 others. It is possible that the subtle histopathologic changes found in these lung sections were associated with slightly increased lung concentrations of OTC. This suggests that the degree of inflammation affected the degree of drug penetration.

The nontreated pneumonic group was included in this study to determine if the disease process produced

Figure 1. Plasma concentration of oxytetracycline vs. time graph for healthy treated group A and diseased tested group C.

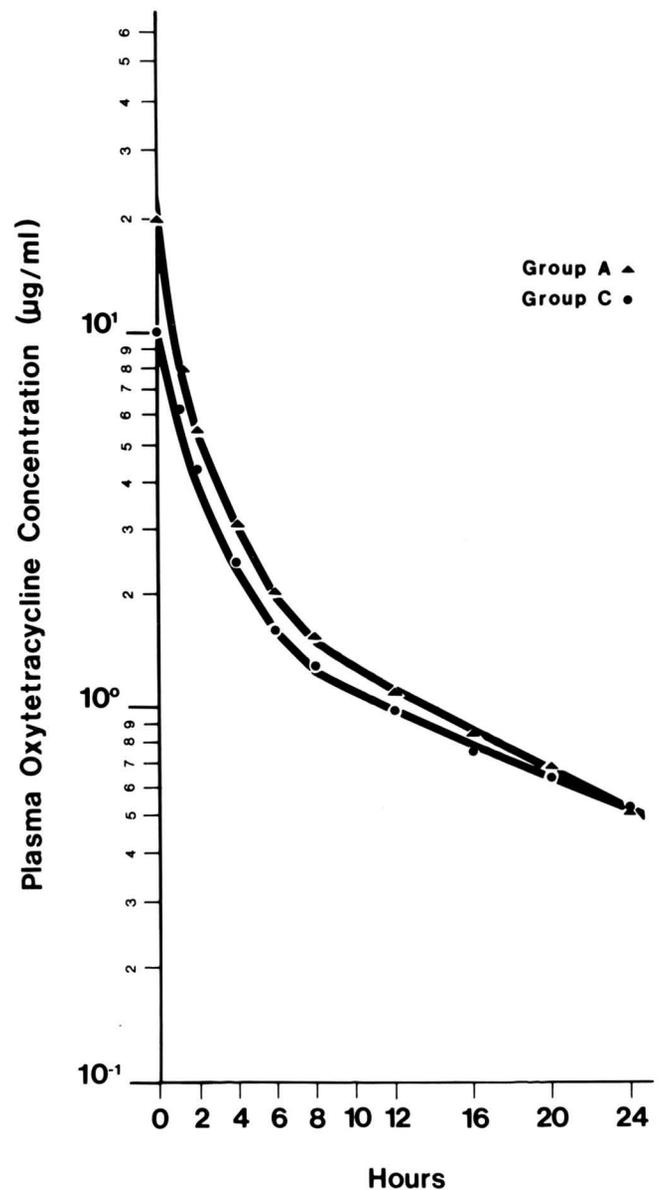


TABLE 2. Pharmacokinetic parameters derived from computer analysis.

Parameter	Group A	Group C
CPo (ug/ml)	12.25 ± 3.32	9.13 ± 2.72
A (ug/ml)	9.50 ± 3.34	7.57 ± 2.80
a (hour ⁻¹)	0.411 ± 0.245	0.563 ± 0.116
B (ug/ml)*	2.15 ± 0.13	1.56 ± 0.32
B (hour ⁻¹)	0.06 ± 0.01	0.048 ± 0.009
T _{1/2} (β) (hour ⁻¹)+	11.84 ± 1.7	14.79 ± 2.79
K ₁₂ (hour ⁻¹)	0.21 ± 0.093	0.25 ± 0.067
K ₂₁ (hour ⁻¹)	0.138 ± 0.019	0.145 ± 0.072
K _{e1} (hour ⁻¹)	0.212 ± 0.38	0.19 ± 0.03
VC (L/kg)	0.969 ± 0.251	1.304 ± 0.378
VD _(area) (L/kg)*	3.339 ± 0.325	4.991 ± 0.641
VD _(ss) (L/kg)*	2.327 ± 0.291	3.602 ± 0.592
Cl _B (L/kg/hour)	0.201 ± 0.024	0.241 ± 0.062

*Significant difference between group means (P < 0.05).

+Significant difference between group means (P = 0.07).

Data expressed as mean ± SD.

antibacterial substances that would affect the OTC assay. The liver, normal appearing lung, pneumonic lung and 24 hour serum failed to reveal any antibacterial activity. However, kidney sections showed low activity of antibacterial substances in 3 of 5 calves. Although the source of these concentrations is not known, they may possibly be due to medicated milk replacers used to feed these calves before their purchase for this study. It can be concluded that the inflammatory process does not produce antibacterial substances that affect the assay and that the changes in antibacterial activity observed in the inflamed lungs were due to increased OTC concentrations present in the tissue. This presence of elevated concentrations of antibiotic in the affected organs of diseased animals agrees with reports of other research workers.^{6-8, 11}

The plasma-time curves for the healthy, treated calves and the diseased, treated calves showed that plasma levels were higher initially in the healthy calves and then decreased with a steeper slope than did the plasma levels of the diseased calves. However, there was no significant difference between the two groups for any given point in time. Initially the levels were lower in the diseased animals suggesting a larger volume of distribution. The serum levels during the elimination phase did not fall as rapidly in the calves with pneumonia as in the healthy calves, reflecting a larger volume of distribution or a slower excretory rate.

The pharmacokinetic parameters support the possibility of an increased volume of distribution in the diseased state. Although the volume of distribution is not an actual definitive volume, but rather a conceptual space, it is a useful parameter for evaluating changes in drug behavior. Both the volume of distribution calculated from the area under the curve ($VD_{(area)}$) and the steady state volume of distribution ($VD_{(ss)}$) were significantly increased in the diseased cattle. The $VD_{(ss)}$ is more useful in the comparison of diseased and healthy animals because it is independent of the elimination process.⁵ The $VD_{(ss)}$ can be thought of as the sum of the volume of the central compartment (V_c) and the volume of the peripheral compartment. The V_c was not significantly different between the pneumonic and the healthy calves. It can be inferred that the increase in $VD_{(ss)}$ occurred primarily as a result of an increase in the volume of the peripheral compartment.

The excretion of a drug can be examined by ($T_{1/2(\beta)}$) or body clearance (Cl_B). The $T_{1/2(\beta)}$ is a hybrid term dependent on all the rate constants such as K_{21} , K_{12} , and K_{el} .¹² As a result it is affected by both elimination and distribution.¹¹ The Cl_B is a more useful parameter to evaluate changes in elimination rate as it is directly proportional to K_{el} .⁵ The present study revealed a significant difference in $T_{1/2(\beta)}$ but not in Cl_B . It might be inferred that the elimination of the drug was not affected by the disease state, and that the change in $T_{1/2(\beta)}$ reflected a change in the volume of distribution.

The exact mechanism involved in causing the increased volume of distribution and increased OTC activity in the inflamed lungs were beyond the scope of this study. The in-

creased volume of distribution may be attributed to increased penetration and retention by the inflamed lungs. This statement is based on the observation that increased concentrations were not observed in any other organ of the diseased animals. Increased OTC penetration into the inflamed lungs may have resulted from an alteration in any of the factors governing tissue concentrations. These would include alterations of blood vessel permeability allowing passage of more protein bound OTC into the tissue space, changes in tissue pH acting to trap ionized OTC in the tissues, or increased active transport of OTC into the tissues.

In reviewing the results of this study it is apparent that there was a poor correlation between serum concentrations and tissue concentrations at 24 hours at this dose (Table I). If the tissue:serum concentration ratios are examined, it is obvious that the serum concentrations were poor predictors of tissue concentrations at this time. The tissue levels varied from 2 to 10 times greater than the concurrent serum concentration. If the ratios for the diseased lung are compared to those of the healthy lung it can be seen that the serum concentrations as predictors of tissue concentrations was even more variable in diseased animals. The 24 hour tissue concentrations were considerably higher (2.96 times) than the 24 hour serum concentrations. This raises a question regarding redosing of OTC at 12 or 24 hours based on declining serum concentrations.

These findings indicate that the diseased state can affect both tissue levels and pharmacokinetic behavior of OTC. They also show that some concepts of tissue levels, serum levels, and the dosage intervals may need reevaluation.

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