Veterinary Research Communications, 21 (1997) 273–281

THE EFFECTS OF THE METHOD OF CALCULATION ON THE EVALUATION OF THE PHARMACOKINETIC PARAMETERS OF OXYTETRACYCLINE AFTER INTRAVENOUS ADMINISTRATION TO CALVES

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ABSTRACT

Errecalde, J.O., Mestorino, N. and Mariño, E.L., 1997. The effects of the method of calculation on the evaluation of the pharmacokinetic parameters of oxytetracycline after intravenous administration to calves. *Veterinary Research Communications*, **21 (4)**, 273–281

The aim of this study was to assess the differences in the values of the pharmacokinetic parameters attributable to the use of either linear or nonlinear regression analysis and to find the effect of weighting schemes on these differences. Six calves received 20 mg/kg oxytetracycline i.v. Blood samples were drawn during 72 h. The assay of the drug was performed microbiologically. A bicompartmental pharmacokinetic model was used, kinetic analysis being carried out by linear regression (LR) and by weighted least-squares nonlinear regression (WLSNLR). Statistical analysis included a test for normality, the Kruskall–Wallis test and ANOVA with log transformation.

The A_0, α and B_0 did not show any statistically significant differences attributable to the mathematical method used. On the other hand, the statistically significant differences in the β values found using the Kruskall-Wallis test and ANOVA with log transformation could be attributed to the different methods employed. ANOVA with log transformation determined statistically significant differences between the parameters obtained by linear analysis and those obtained by WLSNLR when the weighting (w) was 1. When weights were $1/x$, $1/x^2$ or $1/\sqrt{x}$, no statistically significant differences were found. The optimal weighting scheme was $w = 1/x^2$ because of a more homogeneous scatter and random distribution of residuals about the abcissa axis in a plot of weighted residuals in the ordinate versus time in the abcissa. It was concluded that the use of these different procedures can give major variations in the apparent value of β, the most important pharmacokinetic parameter. The correct selection of the weighting procedure is therefore fundamental in obtaining the best estimate of this pharmacokinetic parameter in WLSNLR.

Keywords: calculations, calves, intravenous, oxytetracycline, pharmacokinetics, plasma, tissue

Abbreviations: ANOVA, analysis of variance; i.v., intravenous(ly); LR, linear regression; NLR, nonlinear regression; OTC, oxytetracycline; PCK, pharmacokinetic; w, weighting; WLSNLR, weighted least-squares nonlinear regression; x, concentration of drug

INTRODUCTION

During the last few decades much pharmacokinetic (PCK) research has been undertaken in veterinary medicine, where it presents an excellent tool for understanding the behaviour of drugs in different animals.

The methods used in pharmacokinetic calculations have recently evolved from the classical linear regression (LR) analysis, which needs logarithmic transformation of the serum concentrations, to a nonlinear methodology, in which linearization of the function is not required. The pharmacokinetics of oxytetracycline have been studied by both linear and nonlinear regression analysis (Ziv and Sulman, 1974; Toutain and Raynaud, 1983; Nouws *et al.*, 1985).

A drawback inherent in using least-squares nonlinear regression (NLR) to estimate PCK parameters is the assumption of homogeneous variance. Since constant variance is rarely found in PCK data, weighted least-squares nonlinear regression (WLSNLR) analysis may be used. Choice of the weighting in WLSNLR is generally complex. Improper choices may lead to very imprecise estimates being obtained for the PCK parameters. Weightings commonly used include 1, $1/\sqrt{x}$, $1/x$ and $1/x^2$ (Boxenbaum *et al.*, 1974) where x is the measured concentration of the drug.

In general, in veterinary medicine, pharmacokinetic analysis is a technical tool used to obtain pharmacokinetic parameters, of which the half-life of elimination ($t_{1/2\beta}$) and body clearance (Cl_B) are the most important as regards dosage regimens. Each author usually selects his own methodology to identify the various parameters, and uses this to study the kinetics of very different systems. This can result in considerable variability in the results between authors, depending on the methodology selected.

Numerous papers have been written on the pharmacokinetics of oxytetracycline (OTC). Its i.v. pharmacokinetics has been studied by means of bi- and tricompartmental models (Pilloud, 1973; Ziv and Sulman, 1974; Schifferli *et al.*, 1982; Toutain and Raynaud, 1983; Nouws *et al.*, 1983, 1985). This was the reason for selecting OTC for the present study.

The aim of the present paper was not so much to determine the best estimates for the pharmacokinetic parameters obtained after i.v. administration of OTC in cattle and to recommend a method for obtaining these. Rather it was to emphasize the large differences in PCK parameters that may be obtained using LR or WLSNLR analysis, and to determine the effects of some different weighting schemes on those differences.

MATERIALS AND METHODS

Six healthy Hereford female calves weighing 120 ± 14 kg were used.

A solution of 10% oxytetracycline in propylene glycol (Terramycin 100 (r), Pfizer Argentina) was administered at a dose rate of 20 mg/kg i.v. via the left jugular vein. Blood samples were drawn from the right jugular vein at the following times after injection: 2.5, 5. 7.5, 10, 15, 20, 30, 40 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48, 60 and 72 h. The serum was separated and frozen until assayed. The oxytetracycline concentrations in the sera were determined microbiologically using a modified cylinder-plate diffusion method (Grove and Randall, 1955), the test organism being *Bacillus cereus* var. *mycoides* ATCC 11778 and the culture medium antibiotic medium number 2 (Difco Laboratories, Detroit, MI, USA). The detection limit was 0.125 μg/ml.

The pharmacokinetic model that best fitted the oxytetracycline plasma concentration profiles was selected by means of the MAICE (Minimum Akaike Information

Criterion Estimation) test (Yamaoka *et al.*, 1978), which uses the AIC (Akaike Information Criterion) (Akaike, 1976). An open bicompartmental pharmacokinetic model was selected, in which the evolution of the drug plasma concentrations changed according to the following expression:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

where C is the plasma concentration, A and B are intercept terms, e is the base of the natural logarithm, α and β are the slopes of the fast and slow disposition phases, and t is time.

The pharmacokinetic analysis was performed in two ways:

LR analysis: The CSTRIP program was used to obtain polyexponential parameter estimates by an automated stripping technique (Sedman and Wagner, 1976).

WLSNLR analysis: The pharmacokinetic parameters were calculated directly from the serum concentrations without linearization. Nonlinear regression was performed using a program package known as ADAPT (D'Argenio and Schumitzky, 1979), which uses the algorithm called SIMPLEX (Nelder and Mead, 1965). Weightings used for the xth measured drug concentration included 1, $1/\sqrt{x}$, $1/x$ and $1/x^2$ (Boxenbaum *et al.*, 1974). The optimum weighting scheme was selected by inspecting the plots of weighted residuals in the ordinate versus time in the abcissa for the six animals and the four weighting schemes. The scatter and random distribution of residuals about the abcissa axis were the determinants in the final selection.

Statistical analysis was carried out in three steps:

1. A test of the goodness of fit to the normal distribution to ascertain whether the distributions of the samples were normal or not.

2. Kruskall–Wallis test (Kruskall and Wallis, 1952). The hypothesis of normal distribution was rejected for all four PCK parameters so this nonparametric test was used to find whether there was a statistically significant difference among the PCK parameters obtained by the five different approaches used (LR and WLSNLR using the four weighting schemes detailed above).

3. ANOVA with log transformation (Clarke, 1980) to corroborate the results of the Kruskall–Wallis test, to compare pairs of parameters and to find between which of the methodologies employed the difference was occurring.

In both the Kruskall–Wallis test and ANOVA, a level of probability of 0.05 was regarded as statistically significant.

RESULTS

The MAICE test showed that the i.v. plasma profile of oxytetracycline was best described by a bicompartmental model. A representation of the bicompartmental model and a semilogarithmic plot of the mean plasma oxytetracycline concentrations as a function of time are presented in Figure 1.

The linear and nonlinear (with the four weighting schemes) mean PKC parameters obtained are given in Table I.

Application of the Kruskall–Wallis test yielded the results shown in Table II. Statistically significant differences were only found in the values for the slow disposition rate constant (β).

The results of the application of the log-transformed ANOVA coincided with the results of the Kruskall–Wallis test in finding significant differences among the β values obtained by means of the five different methodologies (Table III).

Figure 1. Semilogarithmic plot of averaged oxytetracycline serum concentrations for six calves following intravenous administration of 20 mg/kg body mass. A schematic representation of a bicompartmental model can be seen in the top right

TABLE I
Pharmacokinetic parameters obtained by linear regression analysis and least-squares nonlinear regression analysis applying four different weighting procedures (1, $1/\sqrt{x}$, $1/x$, $1/x^2$) after i.v. administration of 20 mg/kg oxytetracycline to six calves[a]

Parameter	LR	$w = 1$	$w = 1/\sqrt{x}$	$w = 1/x$	$w = 1/x^2$
A	89.09 ± 29.34	94.59 ± 34.22	93.75 ± 33.28	77.88 ± 18.61	57.01 ± 8.45
α	6.20 ± 1.26	8.41 ± 2.74	7.91 ± 2.57	5.57 ± 0.73	3.88 ± 0.69
B	6.96 ± 1.23	16.11 ± 7.47	14.28 ± 6.99	7.82 ± 1.97	5.12 ± 0.90
β	0.085 ± 0.004	0.40 ± 0.18	0.33 ± 0.038	0.14 ± 0.083	0.083 ± 0.008

[a]Values are expressed as mean ± SEM

A and B are y-axis intercepts of regression lines best fitted to the fast and slow disposition data points, respectively; α and β are fast and slow disposition rate constants

TABLE II
Results of the comparison of the pharmacokinetic parameters obtained by linear regression analysis and by weighted least-squares nonlinear regression applying four different weighting procedures (1, $1/\sqrt{x}$, $1/x$, $1/x^2$) by means of the Kruskall–Wallis test

Parameter	d.f.[a]	Kruskall–Wallis (H)	p[b]
A	4	2.611	0.6249
α	4	4.514	0.3409
B	4	7.867	0.0966
β	4	11.380	0.0226*

[a]d.f. = degrees of freedom

[b]p = level of probability

*Statistically significant difference

The results of the application of the log-transformed ANOVA to compare pairs of the β values obtained by the different methods are presented in Table IV. Statistically significant differences were found when comparing LR with WLSNLR ($w = 1$) and when comparing WLSNLR ($w = 1$) with WLSNLR ($w = 1/x^2$). No significant differences were found in the other comparisons.

From the inspection of the plot of weighted residuals in the ordinate versus time in the abcissa it was concluded that the best weighting scheme was $w = 1/x^2$.

TABLE III
Results of the comparison of the pharmacokinetic parameters obtained by linear regression and by weighted least-squares nonlinear regression with four weighting procedures (1, $1/\sqrt{x}$, $1/x$, $1/x^2$) by means of log-transformed ANOVA

Parameter	d.f.[a]	*F* ratio	*p*[b]
A	4/25	0.403	0.8047
α	4/25	1.887	0.1414
B	4/25	1.833	0.1540
β	4/25	3.338	0.0254*

[a]d.f. = degrees of freedom

[b]*p* = level of probability

*Statistically significant difference

TABLE IV
Paired comparisons between elimination rate constants obtained by linear regression (LR) and weighted least-squares nonlinear regression (WLSNLR), with four weighting procedures (1, $1/\sqrt{x}$, $1/x$, $1/x^2$)

Paired methods	d.f.[a]	*F* ratio	*p*[b]
LR-WLSNLR ($w = 1$)	1/10	7.776	0.0192*
LR-WLSNLR ($w = 1/x$)	1/10	2.182	0.1705
LR-WLSNLR ($w = 1/\sqrt{x}$)	1/10	4.357	0.0634
LR-WLSNLR ($w = 1/x^2$)	1/10	0.188	0.6735
WLSNLR ($w = 1$)–WLSNLR ($w = 1/x$)	1/10	2.575	0.1396
WLSNLR ($w = 1$)–WLSNLR ($w = 1/\sqrt{x}$)	1/10	0.176	0.6839
WLSNLR ($w = 1$)–WLSNLR ($w = 1/x^2$)	1/10	7.977	0.0180*
WLSNLR ($w = 1/x$)–WLSNLR ($w = 1/\sqrt{x}$)	1/10	1.105	0.3180
WLSNLR ($w = 1/x$)–WLSNLR ($w = 1/x^2$)	1/10	2.422	0.1507
WLSNLR ($w = 1/\sqrt{x}$)–WLSNLR ($w = 1/x^2$)	1/10	4.611	0.0573

[a]d.f. = degrees of freedom

[b]*p* = level of probability

*Statistically significant difference

TABLE V
Oxytetracycline half-life values in cattle obtained using five different calculation methods in the present paper compared with those previously obtained by different authors

Author	Animal	Age/ condition	Dose (mg/kg)	Route	Breeds	No.	Method	Model	$t_{1/2\beta}$(h)
Present paper	Calves	20 weeks	20	i.v.	Hereford	6	LR	Bi	8.15
	Calves	20 weeks	20	i.v.	Hereford	6	NLR (1)	Bi	1.73
	Calves	20 weeks	20	i.v.	Hereford	6	NLR ($1/x$)	Bi	2.10
	Calves	20 weeks	20	i.v.	Hereford	6	NLR ($1/\sqrt{x}$)	Bi	4.95
	Calves	20 weeks	20	i.v.	Hereford	6	NLR ($1/x^2$)	Bi	8.35
Pilloud (1973)	Cows	4–11 years	2.5	i.v.	NS[a]	5	LR	Bi	9.12
Nouws *et al.* (1983)	Calves	3 weeks	7.54	i.v.	NS	4	LR	Bi	13.50
	Calves	12 weeks	6.88	i.v.	NS	4	LR	Bi	8.80
	Calves	14 weeks	17	i.v.	NS	6	LR	Bi	10.80
	Cows	Lactating	3.32	i.v.	NS	4	LR	Bi	9.70
	Cows	Non-lactating	7.94	i.v.	NS	5	LR	Bi	10.30
Schifferli *et al.* (1982)	Calves	6 weeks	10	i.v.	Red Holstein	8	NLR	Bi	7.16
Toutain and Raynaud (1983)	Calves	Young beef cattle	20	i.v.	French Friesian	6	LR	Bi	9.04
Xia *et al.* (1983)	Cows	Non-lactating	10	i.v.	NS	4	NLR	Tri	6.10
Ziv and Sulman (1974)	Cows	Lactating	20	i.v.	Israeli Friesian	6	LR	Bi	9.24
Bretzlaff *et al.* (1982)	Cows	Non-lactating	22	i.v.	NS	5	LR	Bi	6.52
Burrows *et al.* (1987)	Calves	Newborn	10	i.v.	Holstein	4	NLR	Bi	11.21
	Calves	More than 8 weeks	10	i.v.	Holstein	4	NLR	Bi	
Nouws *et al.* (1985)	Cows	NS	5	i.v.	Friesian Holstein	5	NLR	Tri	9.46

[a]NS = not specified

DISCUSSION

It is clear from Table V that a difference of almost 5-fold in the apparent half-life of OTC depends on the calculation methodology.

As the results of the test for the goodness of fit of the distribution of the variables showed that none of the PCK parameters was normally distributed, the PCK parameters could not be statistically assessed by parametric tests, such as the Student's *t*-test. For that reason, it was necessary to apply a Kruskall–Wallis test. As can be seen in Table II, the only differences shown by the Kruskall–Wallis test were those corresponding to the slow disposition rate constant. Alpha phase values generally exhibit more variability than those for the elimination phase in pharmacokinetic studies and so differences are less likely to show statistical significance.

The slow disposition rate constant is the most important pharmacokinetic parameter, as it is essential in calculating all eliminative data, and therapeutic regimens are also largely dependent on it.

It was necessary to discriminate which of the methodologies employed to calculate β gave rise to the significant difference found by means of the Kruskall–Wallis test. This was done by applying ANOVA with log transformation to compare pairs of results. The use of nonlinear means without adjustment of the data ($w = 1$) yielded the highest differences from the β values by LR. Using a slight adjustment ($w = 1/\sqrt{x}$), important differences remained in the β phase, although the α phase values were similar. When an intermediate weighting procedure ($w = 1/x$) was used, there were no large differences in either the distributive or the eliminative PCK parameters, although none of them fitted perfectly. Finally when a strong adjustment was applied ($w = 1/x^2$), there were still some differences in the distributive phase, but the differences in the eliminative phase PCK parameters were minimal. As this last was the procedure selected by inspection of the plot of weighted residuals vs time, we concluded that WLSNLR ($w = 1/x^2$) was, in the present case, the nonlinear method able to yield the most reliable results.

It is interesting to note from Table V that LR provided a very similar β value to WLSNLR using $1/x^2$ as the weighting factor. In fact, the peeling method, by selecting two phases more or less arbitrarily, is a kind of weighting method.

The differences in the half-life of oxytetracycline reported by different authors (see Table V) and also attributed to differences in formulation, dose, route of administration, age and diet, might be related, at least partially, to the employment of linear or nonlinear regression analysis, and, in the last case, to the use of different weighting procedures in the calculations.

Similar situations have been reported in humans. For paracetamol, Clements and Prescott (1976) reported a 7-fold variation in plasma half-life estimates, depending on the application of various weighting algorithms, while for furosemide, Chennavasin and colleagues (1981) reported that the application of standard weighting procedures to their pharmacokinetic data resulted in a 20-fold variation in the estimated plasma half-life.

We conclude that the correct selection of the weighting procedure is a fundamental choice in seeking the most accurate estimation of PK parameters in WLSNLR.

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(Accepted: 18 October 1996)