Bioavailability of Flumequine After Semisimultaneous Administration to Veal Calves

Bert A. Meijer,[1,2] Kristel G. F. Ceyssens,[1] Wytze Th. de Jong,[1] and Ben I. J. A. C. de Grève[1]

Received February 12, 1993; accepted June 1, 1993

The absolute bioavailability of flumequine after semisimultaneous intramuscular administration as a water-based suspension to veal calves was 92 ± 14%. The semisimultaneous experimental design provided a reliable determination of absorption rate and demonstrated flip-flop pharmacokinetics. No period or sequence effects were detected. Calculated elimination rate, clearance, and volume of distribution after intravenous administration were comparable to values obtained from traditional design studies. The semisimultaneous experimental design proved to be valuable for the assessment of bioavailability and pharmacokinetics of drugs in food-producing animals while preventing violation of basic clearance assumptions.

KEY WORDS: flumequine; bioavailability; semisimultaneous administration; method evaluation.

INTRODUCTION

The traditional method for determining the extent of drug absorption is to divide the area under the plasma concentration–time curve (AUC) after the test administration by the dose-corrected AUC after the reference (commonly intravenous) administration. Complete elimination of the drug after the first administration must be ensured before the second administration takes place, and drug clearance is assumed not to change during the experiment. Food animals are generally selected on the basis of superior growth qualifications; a significant increase in body weight can often be recorded within days. This increase in body weight may result in variations in the pharmacokinetic characteristics of drugs, due to changes in drug elimination mechanisms and extracellular and total body water (1–3), violating the basic assumption of constant clearance. Different correction procedures such as clearance corrections (4) and stable isotope techniques (5) are used, each with its own limitations. Recently, an alternative method for bioavailability assessment was presented that reduces the influence of intraindividual variability (6). In this *semisimultaneous* method, the test and reference doses are not separated by complete elimination, but the second dose is administered after complete distribution of the first dose. The analysis of the resulting data is based on the linearity and time invariance of the kinetic processes. This method was validated by a Monte Carlo study (7) and a comparative study in humans (8). In addition to the advantage of reducing the influence of intraindividual variabilities, this experimental design also reduces the time needed to perform an experiment and decreases the number of plasma samplings needed.

The aim of the present study was to evaluate the semisimultaneous experimental design by establishing the absolute bioavailability of flumequine after i.m. administration to veal calves.

MATERIALS AND METHODS

Animals and Accommodations

The experiment included eight clinically healthy male Frisian–Holstein veal calves (12 weeks old) with immature rumen function. The mean body weight of the animals was 120.0 ± 5.9 kg. The animals were housed in individual calf boxes and were fed liquid milk replacer (Tentego B.V., Mijdrecht, The Netherlands) twice daily. The housing section was well ventilated, and the temperature was regulated by heat-blowers. The section was illuminated by neon lamps during daytime only.

Drugs and Experimental Design

The experiment was conducted in a crossover design; four animals were treated in the order i.v.–i.m., and the four others in the order i.m.–i.v. The calves were individually weighed before administration, to ensure correct dosing. A sterile 1% (w/v) solution of flumequine was administered by intravenous infusion into the vena jugularis at a dose of 10 mg/kg body weight. The duration of the infusion was approximately 60 sec. Intramuscular injections were given into the neck musculature at a dose of 10 mg/kg body weight using Fluject (Dopharma B.V., Raamsdonksveer, The Netherlands), a water-based flumequine suspension. The total dose was divided into two equal portions and injected into the left and right side of the neck. The time interval between the doses (τ) was approximately 12 hr. During a period of 5 days after the first administration, 33 blood samples were drawn from the vena jugularis and collected in 10-mL EDTA vacuum tubes. Plasma was transferred in glass vials and frozen at −20°C until analysis.

Analytical Methods

Plasma samples were deproteinized by mixing 500 μL of plasma with 500 μL of cold 20% trichloroacetic acid in methanol. After mixing (vortex) the samples were placed in an ice bath for 5 min. The test samples were centrifuged for 15 min at 3000*g* (0°C). The supernatant was transferred to glass vials and 50 μL was injected onto the column.

Samples were analyzed by high-performance liquid chromatography. All chromatographic solvents were HPLC grade (LiChrosolv Merck). All other chemicals were analytical reagent grade. A Hewlett Packard 1090M liquid chromatograph equipped with a variable volume injector, an autosampler, a column oven, and a Hewlett Packard 1046A fluorescence detector was used. The detector was operated at an excitation wavelength of 248 nm and an emission wavelength of 381 nm. Analyses were performed on a C8 modified polymer column (PLRP-S; 150 * 4.6-mm I.D.; particle size,

[1] Dopharma Research, Zalmweg 24, 4941 VX Raamsdonksveer, The Netherlands.
[2] To whom correspondence should be addressed.

0724-8741/94/0100-0117$07.00/0

5 μm; Polymer Laboratories, England) at a temperature of 40°C. The mobile phase was prepared by mixing 540 mL of acetonitrile with 460 mL of a 0.02 *M* solution of oxalic acid in water. The mobile phase was filtered and degassed using a vacuum manifold equipped with a 0.45-μm filter. The flow rate was set to 1.00 mL/min.

Calibration curves were prepared by spiking 475 μL of plasma with 25 μL of a flumequine stock solution. Stock solutions were prepared by dissolving known amounts of flumequine in 0.1 *M* NaOH and subsequently diluted with 0.1 *M* NaOH to the desired concentrations. The calibration curves were fitted using least-squares linear regression. Calibration points outside the 95% prediction band were treated as outlier. From the total calibration curve (wide range), the limit of quantification (LOQ) was calculated according to the method described by Wernimont (9). By decreasing the range of the calibration curve, a calibration curve was constructed with a lower LOQ (limited range). Samples with a concentration below the LOQ of the calibration curve with wide range were calculated on the curve with limited range. Samples with a calculated concentration below the LOQ of the curve with limited range were excluded from pharmacokinetic data analysis. The limits of quantification for the calibration curves with a wide range and a limited range were 1.057 and 0.031 mg/L, respectively. The repeatability over a concentration range of 0.218–34.500 mg/L was better then 2.9%. Recovery experiments revealed no random, proportional, or constant errors ($\alpha = 0.05$).

Pharmacokinetics and Statistical Analysis

Plasma concentration–time curves were fitted using the SIPHAR package (SIPHAR pharmacokinetic package Version 4.0, SIMED 1990, Creteil, France) by summarizing the mathematical models used for describing plasma concentrations after intramuscular and intravenous administration [Eq. (1)] (6):

$$C_t = C_{iv} + C_{im} \tag{1}$$

in which

$$C_{iv} = \sum_{i=1}^{n} C_i' e^{-\lambda_i t''}$$

and

$$C_{im} = \frac{D_{iv}}{D_{im}} \cdot F_{fit} \cdot k_a \sum_{i=1}^{n} \left[\frac{C_i'}{(\lambda_i - k_a)} e^{-k_a t'} - \frac{C_i'}{(\lambda_i - k_a)} e^{-\lambda_i t'} \right]$$

t'' is the time after the i.v. dose, C_{iv} is the plasma concentration of the drug at time t'', t' is the time after the i.m. dose, C_{im} is the plasma concentration of the drug at time t', C_i' are the 0 time plasma drug concentration intercepts, λ_i are the first-order disposition rate constants, k_a is the first-order absorption rate constant, and D is the administered dose. F_{fit} is the fitted bioavailability after i.m. administration. Data were fitted according to Eq. (1) with $n = 2$ and with a weighing factor proportional to the inverse of the predicted concentration ($1/y_{calc}$).

The major model-independent pharmacokinetic parameters clearance (CL) and volume of distribution ($V_{d(area)}$) were calculated using the following equations (λ_t always refers to the terminal elimination rate constant):

$$CL = \text{Dose/AUC} \tag{2}$$

$$V_{d(area)} = \frac{\text{Dose}}{\text{AUC} \times \lambda_t} = \frac{Cl}{\lambda_t} \tag{3}$$

To evaluate the fitted F value, the areas under the plasma concentration–time curves were also calculated by the trapezoidal rule using Lotus 1-2-3. AUCs were extrapolated using the fitted model and the individually calculated parameters listed in Table I. F_{trap} was calculated according to Eq. (4):

$$F_{trap} = \frac{AUC_{im}}{AUC_{iv}} \times \frac{Dose_{iv}}{Dose_{im}} \tag{4}$$

Calculated AUCs were normalized to a dose of 10 mg/kg and statistically analyzed by ANOVA on log-transforms, distinguishing effects due to subjects, periods, treatments, treatment*period interaction, and residue, by using the program BIOEQV40 (10). Student's group mean t test was used to compare additionally the AUCs per type and per sequence of administration. Student's paired t test was used to compare the individual values of F_{fit} and F_{trap}. $P < 0.05$ was considered significant.

Table I. Fitted Pharmacokinetic Constants for the Disposition of Flumequine After Semisimultaneous Administration to Calves at a Dose of 10 mg/kg Body Weight

Animal No.	C_1' (mg/L)	C_2' (mg/L)	λ_1 (hr^{-1})	λ_2 (hr^{-1})	$T_{1/2term}$ (hr)	F_{fit}	k_a (hr^{-1})	Order
1715	20	8.3	2.5	0.27	2.6	0.95	0.084	i.v. → i.m.
5495	21	6.1	1.1	0.23	3.0	0.95	0.060	i.v. → i.m.
2873	19	7.8	1.1	0.31	2.2	1.00	0.096	i.v. → i.m.
0924	21	3.1	0.9	0.17	4.1	1.12	0.078	i.v. → i.m.
2363	22	2.1	1.1	0.09	7.7	0.65	0.168	i.m. → i.v.
8780	135	14.2	8.8	0.40	1.8	0.85	0.054	i.m. → i.v.
2400	20	3.0	1.2	0.14	5.0	0.96	0.066	i.m. → i.v.
4572	26	1.6	1.4	0.14	4.8	0.89	0.066	i.m. → i.v.
Mean	36	5.8	2.3	0.22	3.9	0.92	0.084	
SD	40	4.3	2.7	0.10	2.0	0.14	0.037	

RESULTS

During the experiment, no problems were encountered except for the i.v. administration of animal 8780; a part of the dose was administered perivenously. These problems are reflected in exceptional values for the fitted parameters. Probably, perivenous administration resulted in a fast absorption from perivenous tissue instead of a momentary distribution. Pending the times of plasma samplings, this could explain the higher plasma concentrations during the first 30 min after administration.

Figures 1 and 2 represent the plasma concentration–time profiles after i.v.–i.m. and i.m.–i.v. administration, respectively. The fitted parameters are shown in Table I and the resulting pharmacokinetic parameters are given in Table II. The relatively low bioavailability for animal 2363 is attributed to normal biological variation; although the rate of absorption for this animal was high, no correlation was detected between F_{fit} and k_a.

The analyses of variance on log-transformed AUC did not show significant period effects or treatment*period interactions. The *inter*subject variability was significant. The residual coefficient of variation was only 9.5%, indicative of low *intra*subject variability. Effects due to treatments were statistically significant ($P = 0.024$). The mean value of F_{fit} was 92% (Table I). The point estimate of F_{trap} was 88% from the untransformed AUCs (Table II). Student's two-sided paired t test on the individual values of F_{fit} and F_{trap} showed a very significant difference between the two evaluation procedures ($P < 0.001$, df = 7). The mean calculated values of F_{fit} and F_{trap} were, however, not significantly different ($P = 0.526$).

Using the trapezoidal rule, the mean calculated dose-corrected (10 mg/kg) AUC of 45 ± 3 for the animals that received the first administration intravenously was not significantly different from the calculated mean AUC of secondary i.v. administration (45 ± 11; $P = 0.983$). The same conclusion was drawn for mean AUC values after primary (35 ± 8) and secondary (43 ± 5) intramuscular administration ($P = 0.151$).

DISCUSSION

Extrapolation of plasma curves is of the utmost importance to obtain model-independent estimates of AUCs. The sizes of the extrapolated AUC fractions after i.v. administration as listed in Table II are rather small (mean, 2.8%), and their contribution to the total AUC can be considered of little importance. For the i.m. administration, no significant difference in AUC was detected, despite the difference in the size of the extrapolated AUC fractions after primary and secondary intramuscular administration. This justifies the extrapolation performed to calculate AUCs by the trapezoidal rule.

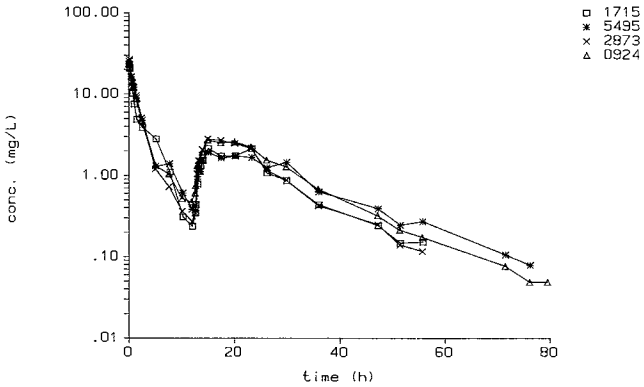

Fig. 1. Plasma concentration–time curves after semisimultaneous administration of flumequine in the order i.v.–i.m. ($n = 4$; dose, 10 mg/kg body weight).

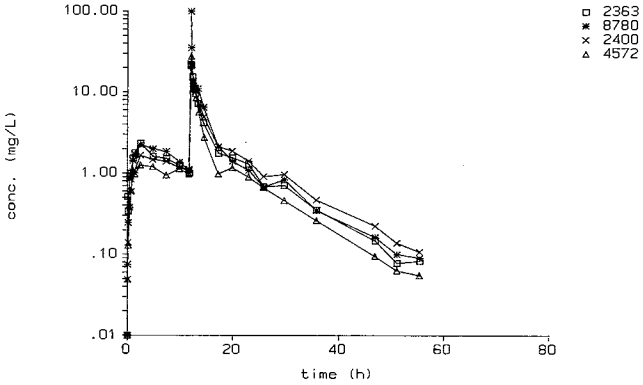

Fig. 2. Plasma concentration–time curves after semisimultaneous administration of flumequine in the order i.m.–i.v. ($n = 4$; dose, 10 mg/kg body weight).

The small but consistent difference in the individual values of F_{fit} and F_{trap} is probably caused by the weighing factor; the use of a weighing factor of $1/y^2_{calc}$ resulted in somewhat lower F_{fit} values; but statistical evaluation of the fits revealed that the observed concentrations were best described by the fitted model using the weighing factor $1/y_{calc}$. Compared to the intersubject variability in the calculated F values, the absolute difference of 4% between F_{fit} and F_{trap} is of minor importance.

In many pharmacokinetic studies concerning food-producing animals, significant period effects are detected. This observation often violates the basic assumption of constant clearance during a bioavailability study. One of the advantages of semisimultaneous administration is the reduction of intraindividual variability. As can be seen from Eq. (1), the semisimultaneous method implicates identical elimination rates of a drug after both i.v. and i.m. administration, based on linearity and time invariance of kinetic processes. This is accomplished by minimizing the time interval between administrations. In this way, the described variations in pharmacokinetic characteristics due to age-dependent changes in drug elimination mechanisms and extracellular and total body water can be eliminated. In consequence, this experimental design should eliminate period effects induced by weight increase in the experimental animals, which is confirmed by ANOVA test on ln-transformed AUC data; no significant period effect was detected ($P > 0.05$). Given the absence of significant period effects and treatment*period interaction in the analyses of variance, a crossover design may not be necessary.

The calculated pharmacokinetic parameters CL and $V_{d(area)}$ after i.v. administration and the mean elimination half-life time of 3.90 ± 1.95 hr are comparable to values

Table II. Calculated Pharmacokinetic Parameters for the Disposition of Flumequine After Semisimultaneous Administration to Calves at a Dose of 10 mg/kg Body Weight[a]

Animal No.	AUC_{iv} [(mg · hr)/L]	AUC_{im} [(mg · hr)/L]	Cl_{iv} [L/(hr · kg)]	Cl_{im} [L/(hr · kg)]	$V_{d_{iv}}$ (L/kg)	F_{trap}	Order
1715	41 (3.0%)	36 (3.6%)	0.24	0.27	0.90	0.88	i.v. → i.m.
5495	48 (3.7%)	44 (2.7%)	0.21	0.23	0.92	0.92	i.v. → i.m.
2873	45 (1.4%)	43 (2.1%)	0.22	0.23	0.72	0.96	i.v. → i.m.
0924	45 (5.7%)	49 (0.7%)	0.22	0.20	1.31	1.09	i.v. → i.m.
2363	47 (1.2%)	29 (39.6%)	0.21	0.34	2.38	0.62	i.m. → i.v.
8780	58 (3.0%)	46 (57.6%)	0.17	0.22	0.43	0.78	i.m. → i.v.
2400	42 (2.5%)	38 (61.4%)	0.24	0.26	1.74	0.91	i.m. → i.v.
4572	32 (2.1%)	27 (56.0%)	0.32	0.37	2.18	0.86	i.m. → i.v.
Mean	45	39	0.23	0.27	1.32	0.88	
SD	7	8	0.04	0.06	0.71	0.14	

[a] The size of the extrapolated AUC fractions is given in parentheses.

reported by Ziv *et al.* (11). Mevius *et al.*, however, described the elimination of flumequine after i.v. administration to calves by a two-compartment elimination model with a $t_{1/2\beta}$ of up to 9.5 hr (12). This more prolonged terminal elimination rate could be due to the graphical estimation of the parameters as performed by Mevius *et al.* It is unlikely that the slowly declining terminal part of our curves (Figs. 1 and 2), with an apparent half-life of approximately 9 hr, originates from elimination only. The individual half-lives of distribution, elimination, and absorption can be obtained by calculating $(\ln 2)/\lambda_1$, $(\ln 2)/\lambda_2$, and $(\ln 2)/k_a$, respectively, excluding those of animal 8780 in Table I. This provides geometric mean values of $t_{1/2(\text{distr})} = 0.6$ hr, $t_{1/2(\text{elim})} = 3.9$ hr, and $t_{1/2(\text{abs})} = 8.3$ hr. The arithmetic mean ordinate intercepts for C_1 and C_2 obtained are 21.2 and 4.6 mg/L, respectively. When using these mean values as the basis for simulations, we found that concentration–time curves like those in Figs. 1 and 2 are obtained only if half-lives of 0.6, 3.9, and 8.3 hr are assigned to distribution, elimination, and absorption, respectively. Any other combination, for example, interchange of absorption and elimination values, resulted in clearly deviating curves. We also found that in semilogarithmic plots of the simulated concentrations for the i.v.–i.m. group, the terminal part of the curve is not linear but slightly convex. Therefore, the slowly declining terminal part of the curves is due predominantly to slow absorption of the drug from the i.m. depot, which is rate limiting since the processes of distribution and elimination are much faster. It also follows that the descending part of the curves cannot be described by a single parameter (flip-flop kinetics). The results of the simulations provide additional support for the correctness of the simultaneously fitted parameters given in Table I.

Flip-flop kinetics explain the discrepancy in the elimination rates after i.v. and i.m. administration in the study by Ziv *et al.* (11). In the present study, flumequine was administered as a suspension, and being a weak acid, it probably dissolves only slowly at the pH in the muscle (13). Due to the relatively slow absorption, a depot of flumequine is formed at the injection site. Because plasma concentrations are limited by the rate of absorption, this could result in a nonlinear relationship between the dose and C_{max} and a shift in T_{max}. This aspect of the i.m. injection of a flumequine suspension is confirmed by the observations of Ziv *et al.* (11). However, they did not report the AUC values, which probably remain linearly related to the dose.

Modeling of plasma concentration–time curves achieved after semisimultaneous administration of flumequine produced accurate estimates of the relative rate and extent of absorption. Values of F_{fit} are comparable to values calculated by trapezoidal rule. Also, the model-independent parameters established after semisimultaneous administration are comparable to those obtained from traditional studies. Because of the absorption characteristics of flumequine, better precision might be achieved by increasing τ or by a two-period design in which all animals are treated in the order i.v.–i.m. (equal handling of the two doses) (7).

ACKNOWLEDGMENTS

The authors thank H. P. Wynand for helpful suggestions and for critical reading of the manuscript. We also thank Hans van der Mee for his fine performance of the animal experiments and technicians Jack Verstegen, Ronald Siemons, and Ronald Kramer for their accurate analysis of the samples in this study.

REFERENCES

1. J. F. M. Nouws, C. A. M. van Ginneken, and G. Ziv. Age-dependent pharmacokinetics of oxytetracycline in ruminants. *J. Vet. Pharmacol. Ther.* 6:59–66 (1983).
2. M. Atef, S. A. Al-Samarrae, and Y. M. Abdel-Hamid. Half life and volume of distribution of trimethoprim and sulfadoxine in sheep and its relation to age and weight. *Zentralbl. Veterinarmed.* 26:31–36 (1979).
3. P. Nielsen and F. Rasmussen. Influence of age on half-life of trimethoprim and sulfadoxine in goats. *Acta Pharmacol. Toxicol.* 38:113–119 (1976).
4. P. S. Collier and S. Riegelman. Estimation of absolute bioavailability assuming steady state apparent volume of distribution remains constant. *J. Pharmacokin. Biopharm.* 11(2):205–215 (1983).
5. R. L. Wolen. The application of stable isotopes to studies of drug bioavailability and bioequivalence. *J. Clin. Pharmacol.* 26:419–424 (1986).
6. M. O. Karlsson and U. Bredberg. Estimation of bioavailability on a single occasion after semisimultaneous drug administration. *Pharm. Res.* 6:817–821 (1989).

7. M. O. Karlsson and U. Bredberg. Bioavailability estimation by semisimultaneous drug administration: A Monte Carlo simulation study. *J. Pharmacokin. Biopharm.* 18(2):103–120 (1990).
8. M. O. Karlsson and A. Lindberg-Freijs. Comparison of methods to calculate cyclosporine A bioavailability from consecutive oral and intravenous doses. *J. Pharmacokin. Biopharm.* 18(4): 293–311 (1990).
9. G. T. Wernimont. *Use of Statistics to Develop and Evaluate Analytical Methods*, AOAC, Arlington, VA, 1986.
10. H. P. Wynand. On the assessment of bioequivalence in a two-period cross-over design. *Comput. Methods Prog. Biomed.* 37:151–157 (1992).
11. G. Ziv, S. Soback, A. Bor, and B. Kurtz. Clinical pharmacokinetics of flumequine in calves. *J. Vet. Pharmacol. Ther.* 9:171–182 (1986).
12. D. J. Mevius, H. J. Breukink, P. J. M. Guelen, T. Jansen, and B. de Greve. Pharmacokinetics, metabolism and renal clearance of flumequine in veal calves. *J. Vet. Pharmacol. Ther.* 13:159–169 (1990).
13. G. M. Dorrestein, H. van Gogh, M. N. Buitelaar, and J. F. M. Nouws. Clinical pharmacology and pharmacokinetics of flumequine after intravenous, intramuscular and oral administration in pigeons (Columba livia). *J. Vet. Pharmacol. Ther.* 6:281–292 (1983).