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Report

Estimation of Bioavailability on a Single Occasion After Semisimultaneous Drug Administration

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A method for determination of absorption rate and bioavailability was developed to reduce the influence of intraindividual variability and applied to the absolute intraperitoneal availability of lithium in the rat. In this method the test and the reference doses are given with a few hours' interval, and the resulting concentration–time data are analyzed by nonlinear regression. The bioavailability estimation by this approach was compared to that of the traditional method, with test and reference doses given on separate days. The mean estimates of availability were 1.035 ± 0.109 (N = 7) and 0.984 ± 0.052 (N = 11) for the traditional and the alternative method, respectively. Thus, the precision was better in the latter. Major influences of dose- or time-dependent kinetics of lithium on the availability estimates were excluded by the design used. The estimation of bioavailability was robust with respect to the choice of absorption and disposition model and the duration of sampling. The plasma clearance of lithium was 169 ± 15 ml/hr/kg, with a terminal half-life of 5.0 ± 0.5 hr (N = 5).

KEY WORDS: bioavailability evaluation, absorption, intraindividual variability, bioavailability, intraperitoneal; lithium pharmacokinetics in rats.

INTRODUCTION

The extent of bioavailability is frequently determined by dividing the area under the concentration–time curve (AUC) of a test dose by the dose-corrected AUC of a separate reference dose. In this method, referred to here as the "traditional" method, the time interval between the two doses is chosen to ensure complete drug elimination of the first dose before the second dose is given. However, drug disposition may change markedly between the dosing occasions, thereby violating one of the basic assumptions of the method. Although changes in the disposition kinetics between dosing occasions can be corrected for (1), intraindividual variability remains a major problem in bioavailability assessment (2). Such problems are minimized with the stable isotope technique, where the test and labeled reference dose are coadministered (3). Although the performance of the stable isotope technique generally is excellent, it requires extensive analytical facilities, and it suffers from the potential kinetic isotope effects (4).

We present an alternative method for bioavailability assessment that reduces the influence of intraindividual variability without the use of isotope labeling. In this "semisimultaneous" method, the test and reference doses are separated by a few hours, rather than by days or weeks as in the traditional method. The analysis of the resulting data is based on linearity and time invariance of the kinetic processes. An additional prerequisite is the ability to estimate the drug concentration–time profile can be with a mathematical model. Bioavailability and absorption rate are estimated by nonlinear regression.

The semisimultaneous method was tested with lithium administrations to rats. Lithium was chosen because the sensitive assay allowed multiple blood sampling without blood depletion of the rats. Intraperitoneal (ip) drug doses have been used as reference, assuming full availability (5). In the present study the absorption and the disposition kinetics of lithium in the rat were evaluated following traditional and semisimultaneous administrations.

METHODS

Animals

Male Sprague–Dawley rats weighing 240–290 g were used. The animals were housed in groups under a controlled lighting cycle (dark period, 6 PM to 6 AM), with free access to food and water throughout all parts of the study. The day before the first administration of lithium chloride, intravenous (jugular vein) and intraarterial (carotid artery) catheters were implanted under ether anesthesia. The catheters were passed under the skin and exteriorized by the nape of the neck. The rats were not restrained or anesthetized during the experiments, which always were commenced between 6 and 7 AM.

Semisimultaneous Method

A total of 4 mmol lithium chloride/kg body weight, divided in two doses, was given to each rat. The first dose of

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1.33 mmol/kg, given either ip or iv, was followed 2 hr later by a twofold larger dose given by the other route of administration. The two doses were administered in the order iv–ip (N = 5) or ip–iv (N = 6). From each rat a total of 24 arterial blood samples (100 μl, replaced by an equal volume of saline) was collected and centrifuged. Fifty microliters of plasma was separated and diluted with heparinized distilled water. The sampling schemes for the two order of doses were identical: 2, 5, 10, 20, 30, 45, 60, 90, 115, 122, 125, 130, 140, 150, 165, 180, 210, 240, 300, 360, 420, 480, 600, and 720 min.

Traditional Method

The total dose of 4 mmol lithium/kg was divided in two equal doses given at the first and fourth day after the surgery, the order of dosing being either ip–iv or iv–ip [N = 7; (2 + 5)]. The same sampling scheme, 2, 5, 10, 20, 30, 60, 120, 180, 300, 420, 570, and 720 min, was used for both doses and on both occasions. On the second occasion an additional sample was obtained before dosing, giving a total of 25 samples.

Lithium Analyis

Lithium was measured with a Perkin Elmer 373 atom absorption spectrophotometer, with a detection limit corresponding to a plasma lithium concentration of 0.03 mM.

Data Analysis

The following models were fitted to the individual data sets using the nonlinear regression program PCNONLIN (6).

Model 1. Biexponential Disposition with First-Order Input. Equation (3), which was fitted to the semisimultaneous data, summarizes Eqs. (1) and (2) (N = 2). The fitted parameters were the initial dilution volume (V_1), rate constants (λ_i), fractional intercept (C_i'), absorption rate constant (k_a), and bioavailability (F). For the ip–iv dosing t' and t'' denote t and t minus 2 hr, respectively, and vice versa for the iv–ip dosing.

$$C_{ip} = \frac{F \cdot D_{ip} \cdot k_a}{V_1} \sum_{i=1}^{n} \left[\frac{C_i'}{(\lambda_i - k_a)} e^{-k_a t'} + \frac{C_i'}{(\lambda_i - k_a)} e^{-\lambda_i t'} \right] \quad (1)$$

$$C_{iv} = \frac{D_{iv}}{V_1} \sum_{i=1}^{n} C_i' e^{-\lambda_i t''} \quad (2)$$

$$C = C_{ip} + C_{iv} \quad (3)$$

In the data obtained after the traditional dosings, the ip and iv doses were analyzed separately according to Eqs. (1) and (2) (with $t' = t'' = t$). In the former, F/V_1 was fitted as a single parameter, instead of two parameters.

Model 2. Three-Exponential Disposition with First-Order Input. This was as model 1, with N = 3.

Model 3. Biexponential Disposition with Zero-Order Input. This was as model 1, except for C_{ip}, which is described by Eq. (4), in which ϕ represents unity and zero during and after absorption, respectively, T_{abs} is the parameter describing the duration of absorption.

$$C_{ip} = \frac{F \cdot D_{ip}}{T_{abs} \cdot V_1} \sum_{i=1}^{2} \frac{C_i'(1 - e^{-\lambda_i T_{abs}})(\phi - e^{-\lambda_i t'})}{\lambda_i(\phi - e^{-\lambda_i T_{abs}})} \quad (4)$$

Models 2 and 3 were used in the analysis of the data from the semisimultaneous method only. Weights for the regression procedure were proportional to the inverse of the squared predicted concentrations.

The data from the traditional design were also analyzed by the log-linear trapezoidal rule with extrapolation to infinity for determination of AUC. The extrapolated area was calculated as the predicted concentration of the last sample divided by the terminal slope, obtained through linear regression on the last three to five observations. Student's t test was used to check for differences in AUCs due to different dose order or day of experiment.

RESULTS

Representative plasma concentration–time profiles of the semisimultaneous lithium administrations are shown in Figs. 1 and 2. In the disposition of lithium a distribution phase is evident after both the iv and the ip dose. The absorption of lithium from the intraperitoneal space was rapid, with the maximal concentration usually observed in the 5-min sample. The concentration–time profiles were adequately described by a biexponential disposition model with first-order absorption, although minor improvements in the fit were attained in some data sets by the other models tested. For the semisimultaneous method the bioavailability was close to unity in all rats. The precision in the bioavailability estimation was good, and like the accuracy, it was practically independent of both dose order (ip–iv/iv–ip) and chosen pharmacokinetic model (Table I).

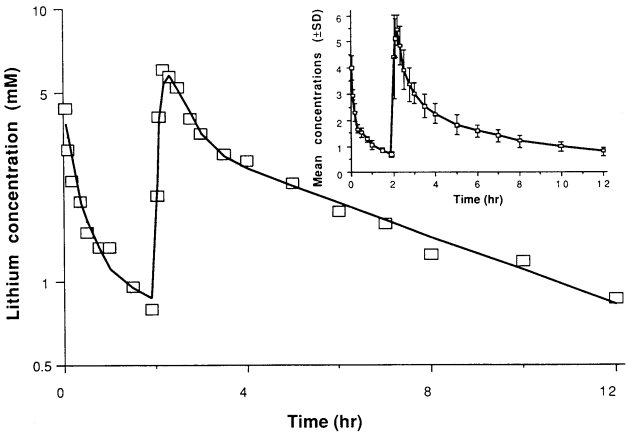

Fig. 1. Example of plasma lithium concentrations following iv–ip semisimultaneous administration. Open squares denote observed concentrations and the solid line shows the fit of the biexponential model with first-order absorption. Parameter estimates (mean ± SE) from this fit were V_1 = 323 ± 26 ml/kg, λ_1 = 3.21 ± 0.16 hr^{-1}, λ_2 = 0.142 ± 0.012 hr^{-1}, C_1' = 0.715 ± 0.026, k_a = 5.95 ± 0.88 hr^{-1}, and F = 1.076 ± 0.072. The inset shows mean concentrations (±SD) for all rats receiving iv–ip dosing.

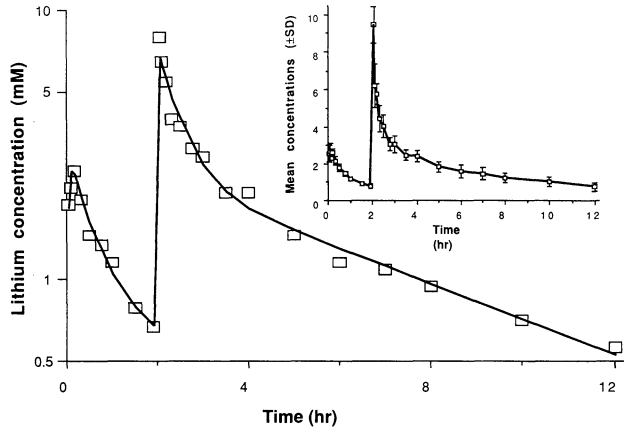

Fig. 2. Example of plasma lithium concentrations following ip–iv semisimultaneous administration. Open squares denote observed concentrations and the solid line shows the fit of the biexponential model with first-order absorption. Parameter estimates (mean ± SE) from this fit were V_1 = 417 ± 24 ml/kg, λ_1 = 1.93 ± 0.27 hr^{-1}, λ_2 = 0.149 ± 0.012 hr^{-1}, C_1' = 0.725 ± 0.024, k_a = 29.8 ± 5.7 hr^{-1}, and F = 0.94 ± 0.052. The inset shows mean concentrations (±SD) for all rats receiving ip–iv dosing.

To test the utility of the semisimultaneous method for drugs with a half-life longer than that of lithium, bioavailability was computed from data sets where observations beyond 4 hr were omitted, thereby increasing the extrapolated area from 20 to 65% of the total AUC (calculated from parameters in Table II). Despite the poorly described terminal phase, neither the accuracy nor the precision of the bioavailability was markedly affected (Table I).

The plasma concentration–time profiles from the traditional design are depicted in Fig. 3. In the bioavailability determinations by the traditional method a correction for a change in clearance according to Eq. (5) was applied, because of marked decreases in AUC between the first and the second dosing occasions.

$$\text{bioavailability} = \frac{AUC_{(ip,\ 1st\ dosing)}}{AUC_{(iv,\ 2nd\ dosing)}} \cdot C \qquad (5a)$$

$$\text{bioavailability} = \frac{AUC_{(ip,\ 2nd\ dosing)}}{AUC_{(iv,\ 1st\ dosing)}} \cdot \frac{1}{C} \qquad (5b)$$

The correction factor, C, was calculated as the ratio of the mean AUCs between the second and the first dosing occasion. Equation (5a) was used for the ip–iv dosing, and Eq. (5b) for the reverse order of administration. The decrease in AUC was independent of the order of dosing. The mean decrease in AUC ± SD in the model-dependent analysis was 23.1 ± 10.6% in the individual rats, from 12.1 ± 0.76 to 9.3 ± 1.1 mM · hr (N = 7, $P < 0.001$). The corresponding values for the model independent analysis were 28.8 ± 8.2%, 12.8 ± 1.2 mM · hr, and 9.1 ± 0.99 mM · hr ($P < 0.001$). Thus, the correction factors used were 0.766 and 0.711 for the model-dependent and model-independent analysis. The resulting bioavailability estimates were near unity, irrespective of whether they were calculated from fitted parameters or by the trapezoidal rule (Table I). The interindividual variability in the bioavailability estimates was greater with the traditional method than with the semisimultaneous method.

The high absorption rate resulted in a rather high variability in the estimated absorption rate constant (Table II). Precision of the k_a determination was lower with the traditional than the semisimultaneous method. Similar mean values of the disposition parameters were predicted from both methods, but the variability was higher for the semisimultaneous dosing than for the single iv bolus doses given on the first day after surgery. The differences of the parameter estimates between the semisimultaneous ip–iv and iv–ip dosings were generally small. Similar individual standard errors of the disposition parameters were obtained for the two methods (e.g., figure legends).

DISCUSSION

The results presented provide evidence that bioavailability and rate of absorption of lithium can be determined by the semisimultaneous method with a better precision than by the traditional method. The observed concentrations were well described by the chosen models, as assessed by visual inspection of the fits, residual analyses and standard errors of the estimates. Further, as the estimates of the bioavailability were similar for the ip–iv and iv–ip dosing, major influences of dose- and time-dependent lithium kinetics were ruled out (7). The semisimultaneous method was developed to reduce the impact of changes in elimination with time.

Table I. Bioavailability of Lithium in the Rat

Model		Method		
		Semisimultaneous		
Disposition	Absorption	iv–ip (N = 5)	ip–iv (N = 6)	Traditional[a] (N = 7)
Biexponential	First order	0.990[b] (6.4)	0.974 (4.2)	1.031 (13.3)
		0.959[c] (6.0)	0.994[c] (4.1)	—
Biexponential	Zero order	0.964 (6.5)	0.962 (4.5)	—
Triexponential	First order	1.035 (9.0)	0.989 (4.7)	—
Model independent		—	—	1.035 (10.9)

[a] Correction according to Eq. (5).
[b] Results are given as the mean [coefficient of interindividual variation (%)].
[c] Models fitted to reduced data sets (0- to 4-hr samples only).

Table II. Pharmacokinetic Parameters of Lithium in the Rat

Parameter	Semisimultaneous method, model dependent[a] iv–ip (N = 5)	Semisimultaneous method, model dependent[a] ip–iv (N = 6)	Traditional method (iv, 1st dosing occasion) (N = 5) Model dependent[a]	Traditional method (iv, 1st dosing occasion) (N = 5) Model independent
V_1 (ml/kg)	363[b] (11.1)	365 (10.3)	312 (4.5)	—
λ_1 (hr^{-1})	3.06 (17.1)	2.58 (22.6)	3.17 (13.2)	—
λ_2 (hr^{-1})	0.132 (11.5)	0.144 (16.6)	0.138 (9.9)	0.108 (11.5)
C_1'	0.726 (2.9)	0.710 (3.3)	0.786 (2.0)	—
k_a (hr^{-1})	27.5 (89.4)	38.8 (28.1)	176[c] (139)	—
$AUC_{12\ hr-\infty}$ (%)[d]	21 (27)	20 (39)	19 (15)	24 (13)
CL (ml/hr/kg)	157 (19.1)	158 (18.5)	169 (8.7)	162 (5.3)

[a] A biexponential disposition model with first-order absorption of the ip dose.
[b] Results are given as the mean [coefficient of interindividual variation (%)].
[c] Determined from all separate ip doses (N = 7).
[d] Ratio of the extrapolated AUC to the total AUC.

Errors in the bioavailability estimates may result mainly from two factors: the magnitude of the change in clearance and the fraction of drug eliminated during the period between the two dose occasions.

The different absorption and disposition models fitted to the lithium data produced similar bioavailability estimates. Insensitivity to the pharmacokinetic model applied is characteristic of the semisimultaneous method. With the use of identical values for the kinetic parameters following test and reference doses, errors of the disposition estimates cancel each other out. For example, failure to consider a terminal drug elimination phase results in proportional underestimates of the AUCs following the two doses. Therefore, drug disposition does not need to be fully characterized, and bioavailability can be assessed in experiments lasting less than one half-life of the drug studied. Different absorption models can be expected to yield similar results if the models give similar AUC estimates when fitted to the data.

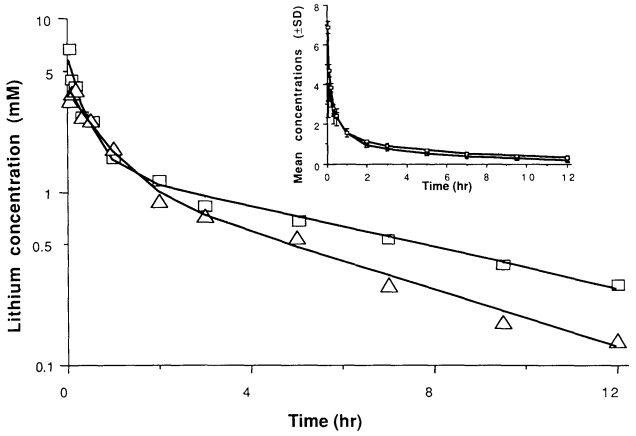

Fig. 3. Examples of plasma lithium concentrations following traditional administration. Open squares and open triangles denote observed concentrations after the iv (day 1) and the ip (day 4) doses to the same rat, respectively. The solid lines show the fit of the equations of model 1 to the two data sets. Parameter estimates (mean ± SE) for the iv dose were V_1 = 327 ± 25 ml/kg, λ_1 = 2.76 ± 0.43 hr^{-1}, λ_2 = 0.136 ± 0.012 hr^{-1}, and C_1' = 0.768 ± 0.024. Parameter estimates for the ip dose were V_1/F = 493 ± 106 ml/kg, λ_1 = 1.41 ± 0.36 hr^{-1}, λ_2 = 0.110 ± 0.021 hr^{-1}, C_1' = 0.700 ± 0.074, and k_a = 244 ± 14,500 hr^{-1}. The inset shows mean concentrations (±SD) for all rats receiving the iv and ip doses at day 1 and day 4, respectively.

Compared to traditional approaches, the semisimultaneous method has advantages for estimating the absorption rate. In the computation of the absorption rate from a separately administered extravascular dose, it may not be possible to resolve a distribution phase, evident from iv data (1). In such cases, the use of a simplified disposition may lead to incorrect estimates of absorption rate. Our results on the absorption rate of lithium suggest superiority of the semisimultaneous method over the fitting of a single extravascular concentration profile.

The increase in clearance from the first to the second dose, found in the traditional study, may be due to autoinduction of renal lithium excretion. Such an effect has been observed in rats treated with lithium for several consecutive days, but only in markedly higher doses than the present study (8). Incomplete recovery from surgery might also account for the lower elimination rate during the first dose. A depression of the renal function 2 hr after surgery performed under ether anesthesia has been described (9). The AUC correction employed for the bioavailability calculation in the traditional method assumes full availability of the ip dose. As the AUC decreased similarly in all rats, we included corresponding corrections to avoid bias against the traditional design.

Lithium kinetics have previously been studied in the rat following oral (8,10,11), subcutaneous (10,11), and intraperitoneal administration (5,10,11). The methods of blood sampling in these studies were cardiac puncture (11), cut tail (8,10,12), and neck wound of decapitated rats (5), allowing only a single or a few blood samples to be obtained from each rat. Frequent sampling in the present study allowed precise assessment of the absorption, distribution, and elimination processes of lithium in the individual rats. The results agree with reported values on terminal half-life and clearance (5,12), whereas the interindividual variability was considerably lower than previously found (5,11,12).

The proposed method may have several advantages in

bioavailability studies: reduced intraindividual variability, ability to detect nonlinear kinetics with administration of both possible dose orders, a good precision in spite of large extrapolated areas, and convenience of the experimental schedule.

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