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Capacity-limited elimination of cefmetazole in rat

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Summary

Elimination kinetics of cefmetazole (CMZ) in the rat is studied with changing the dosing amount (D). The normalized area under plasma concentration–time curve (AUC/D) and the mean residence time (MRT) increase with increasing amount of i.v. dose of CMZ. These increases prove the capacity-limited elimination of CMZ without regard to any pharmacokinetic model. The apparent steady-state volume of distribution (V_{ss}^{a}) which is defined by $D \cdot MRT/AUC$ is almost independent of D. The simultaneous multi-lines fitting using time course curves following different doses is also attempted to determine the pharmacokinetic model by the application of the minimum AIC estimation (MAICE). AIC, consequently, shows the minimum for the two-compartment model with the Michaelis-Menten elimination. The V_{ss} estimated from this model is about 450 ml/kg which agrees with V_{ss}^{a} estimated by moment analysis.

Introduction

There has been much experimental evidence and theoretical investigations on the capacity-limited elimination of various drugs. Phenytoin (Jusko et al., 1976) is metabolized according to the Michaelis-Menten kinetics. Salicylate (Levy et al., 1972) is a representative drug that shows the non-linear metabolism in the regular dosing range. Chau (1976) discussed the dependence of AUC on dosing amount in non-linear distribution and elimination. The behavior and the shape of the time course curve which is derived from the one-compartment model with the Michaelis-Menten elimination was considered by Wagner (1973). Lin et al. (1978) proposed the

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method to estimate the volume of distribution when a drug is eliminated according to the Michaelis-Menten kinetics.

Sedman et al. (1974) showed that a one-compartment open-model involving parallel Michaelis-Menten paths is well approximated by the model with a single Michaelis-Menten path (the pooling model). Martis and Levy (1973) proposed a method for determining the extent of bioavailability of drugs which obey simultaneous first-order and capacity-limited elimination kinetics.

Recently the moment analysis which is a model-independent method was proposed to estimate the extent and rate of bioavailability of drugs (Cutler, 1978; Yamaoka et al., 1978; Riegelman et al., 1980). Benet et al. (1979) defined the steady-state volume of distribution (V_{ss}) in terms of statistical moments as

$$V_{ss} = D \cdot MRT/AUC \tag{1}$$

where

$$AUC = \int_0^\infty C_p\,dt \tag{2}$$

and

$$MRT = \int_0^\infty tC_p\,dt \Big/ \int_0^\infty C_p\,dt \tag{3}$$

MRT and AUC are the first central and zero moments of plasma concentration–time curve, $C_p(t)$, respectively.

Wagner implicitly gave Eqn. 1, although he defined V_{ss} by the coefficients and exponents of the poly-exponential equation. Haginaka et al. (1979) evaluated the effect of food ingestion on the extent and rate of bioavailability of cephalexin. Uno et al. (1981) and Murai et al. (1981) applied the moment analysis to evaluation of metabolisms of some amino-penicillins and oxacillin, respectively.

The present article reports the elimination kinetics of cefmetazole (CMZ) which is one of new β-lactam antibiotics (Shindo, 1979). The chemical structure of CMZ is shown in Fig. 1. Moment analysis is applied to detect the saturability in elimination of CMZ in the rat.

Akaike (1973) proposed the minimum AIC estimation (MAICE) to select the best model from several possible models. AIC, the abbreviation of 'an information

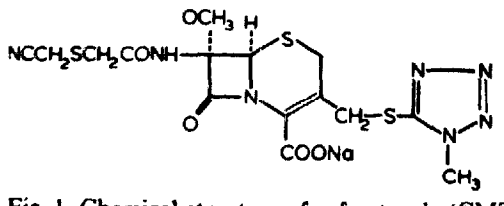

Fig. 1. Chemical structure of cefmetazole (CMZ).

criterion', was derived for model selection in relation to maximum likelihood estimation. MAICE picks out the model with the smaller number of parameters according to the 'principle of parsimony'. MAICE is used in the pharmacokinetic field (Yamaoka et al., 1978; Guentert et al., 1979). In the present study, the model-dependent pharmacokinetics are also attempted, to grasp the elimination kinetics of CMZ by applying MAICE.

The symbols adopted in this report are in accord with the proposal of Rowland and Tucker (1980).

Experimental

Reagents and materials

CMZ for injection was a kind gift from Sankyo (Tokyo). Urethane, acetonitril, tetrahydrofuran, potassium phosphate dibasic and monobasic were of reagent grade. Water was distilled and degassed prior to preparation of mobile phase for liquid chromatography.

Determination of CMZ

A high-performance liquid chromatograph (Twincle, Jasco) equipped with a UV-detector (254 nm, Uvidec 100-III, Jasco) was used in a reverse-phase mode with a stationary phase of LiChrosorb RP-18 (E. Merck) packed in a stainless steel tube (4.6 mm i.d.×25 cm) and a mobile phase of 0.05 M phosphate buffer (pH = 6.4)–tetrahydrofuran (10:1 v/v), whose flow rate was maintained at 1.0 ml/min. A short pre-column (4.6 mm i.d.×5 cm) packed with Lichrosorb RP-2 was used to guard the main column. All operations were done at room temperature. CMZ was eluted in 7 min with complete separation from any interfering peaks. No peak corresponding to the metabolite from CMZ was observed in this chromatographic condition. This coincides with the result of Shindo (1979), who concluded that CMZ is quite stable in animal body.

Animal experiments

Fourteen male Wistar rats, 250–420 g in weight, were used. Each rat received different dosing amount from 148 mg/kg to 2000 mg/kg. Under urethan anesthesia, CMZ dissolved in the physiological saline was rapidly injected through a femoral vein. A blood sample of 0.2 ml was collected from the jugular vein at 5, 10, 15, 20, 30, 40 and 60 min after injection. After centrifugation of blood at 3000 rpm for 5 min, 150 μl of acetonitril was added to 50 μl of the plasma. After the subsequent centrifugation and precipitation of protein, 5 μl of supernatant was injected into the liquid chromatograph. Calibration graph was obtained by using control rat plasma spiked with several known amounts of CMZ. The peak height was used for the quantitation. The sensitivity of CMZ assay in plasma is about 1 μg/ml.

Data analysis

AUC and MRT were calculated by the trapezoidal integration with extrapolation

of time course curves to the infinite time using a monoexponential equation (Yamaoka et al., 1978). The equation was determined by the least-squares method using the last 3 or 4 points on the time course data. The computations were achieved on a microcomputer (PET 2001, Commodore) programmed in BASIC language.

SALS (Nakagawa et al., 1981) was used for curve fitting on FACOM M-200 system in Kyoto University Data Processing Center through a TSS terminal (Silent 700, Texas Instrument). The equations to fit were defined in the form of simultaneous differential equations which were numerically integrated by the Runge-Kutta method.

Residual sum of squares (SS) was calculated by Eqn. 4 for simultaneous multi-lines fitting.

$$SS = \sum_i \sum_j W_{ij} \cdot \left(C_{ij} - \hat{C}_{ij}\right)^2 \quad (4)$$

where C_{ij} is the value of the j^{th} point on the i^{th} time course of plasma concentration, $\hat{C}_{ij}$ is the value estimated from a pharmacokinetic model, and W_{ij} is weight of points. A weight of unity was employed here.

Akaike's information criterion (AIC) was calculated by Eqn. 5

$$AIC = N \cdot \ln(SS) + 2 \cdot M \quad (5)$$

where N is the number of observed total points of data and M is the number of parameters to estimate. The pharmacokinetic model that gives the minimum AIC is supposed best.

Result and discussion

Moment analysis

When a time course of drug is represented by a monoexponential equation, the non-linearity in the system can simply be verified by the dependence of half-life on dosing amount (Höffler et al., 1974). Wagner (1973) discussed the 'hockey-stick' shape of time course curve expressed by the one-compartment model with the Michaelis-Menten elimination. If, however, a disposition process is approximated by the two-compartment model with the Michaelis-Menten elimination, the α-phase can pile up on the 'hockey-stick' phase. This makes it difficult to generally detect the capacity-limited elimination. Moment analysis, in such a case, offers a simple method to detect the non-linear elimination without consideration of pharmacokinetic model. In the non-linear pharmacokinetics, AUC/D and MRT must be dependent on D (Yamaoka et al., 1978).

The results of CMZ elimination in the rat are shown in Figs. 2 and 3 where the normalized AUC and MRT are plotted against dose per kg of rat, respectively. It is found that AUC/D and MRT increase as D increases. The straight lines in the figures are obtained by linear regression analysis. The correlation coefficient is expressed by r in the figures. Increase of D prolongs the mean residence time of

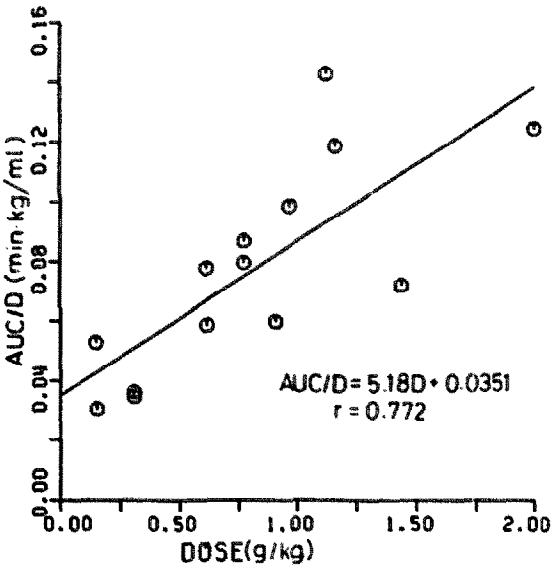

Fig. 2. AUC/D per kg versus dose per kg.

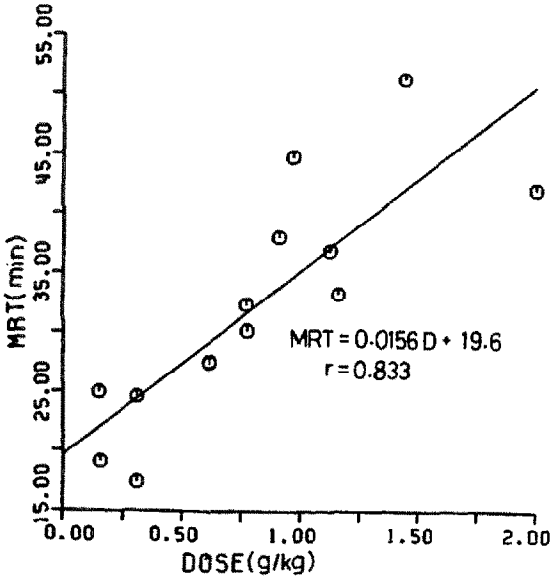

Fig. 3. MRT versus dose per kg.

CMZ in the rat. The total body clearance (Cl_T) is defined by Eqn. 6 (Gibaldi and Perrier, 1975)

$$Cl_T = D/AUC \tag{6}$$

Cl_T is just inverse of the ordinate of Fig. 2. The increase of D, therefore, makes Cl_T decrease; that is, the increase of D impedes the elimination of CMZ.

Non-linear distribution or tissue binding can also cause the dependences of AUC/D and MRT on D. Chau (1976) theoretically demonstrated that AUC/D decreases with the increase of D in the case of the Langmuir-type binding with linear elimination. The present result contradicts the anticipation due to the Langmuir-type binding. It is expected that the capacity-limited elimination is the major factor for the non-linear behavior of CMZ, though the capacity-limited distribution may simultaneously occur.

Fig. 4 shows the apparent steady-state volume of distribution (V_{ss}^a) versus D, which was calculated by Eqn. 1. V_{ss}^a is different from V_{ss}, because Eqn. 1 is derived under the assumptions that: (a) the system must be linear (i.e. first-order); and (b) the elimination of the substance must be directly from the central compartment. Fig. 4 shows that V_{ss}^a is almost independent of D.

Although Eqn. 1 is derived under the restricted assumptions, it includes general statistical meanings. AUC/MRT in Eqn. 1, which has the dimension of concentration, signifies the 'averaged' plasma concentration when the time course curve is transformed to a rectangular shape in condition of constant area under the curve (as explained in Fig. 5). Consequently, V_{ss}^a means the 'averaged' volume of distribution.

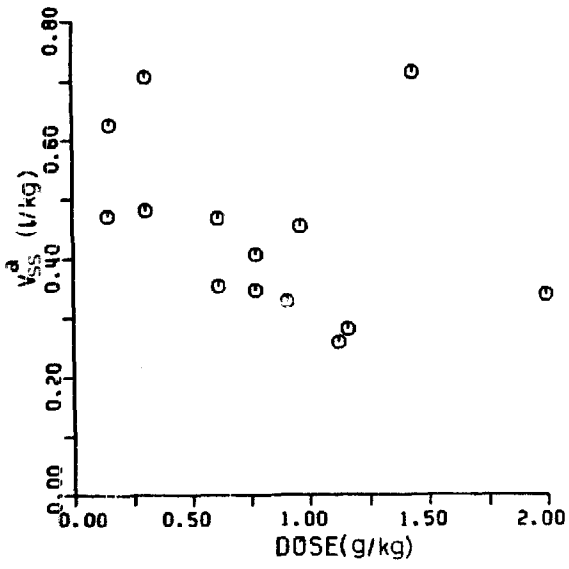

Fig. 4. V_{ss} per kg versus dose per kg.

Combining Eqn. 1 and Eqn. 6,

$$V_{ss}^{a} = Cl_{T}^{a} \cdot MRT \tag{7}$$

where Cl_{T}^{a} indicates the apparent quantity, because the elimination of CMZ is non-linear and the clearance always changes depending on the plasma concentration.

It is noted that Eqn. 7 has a similar form to the classical expression:

$$V_{d} = Q/k_{el} \tag{8}$$

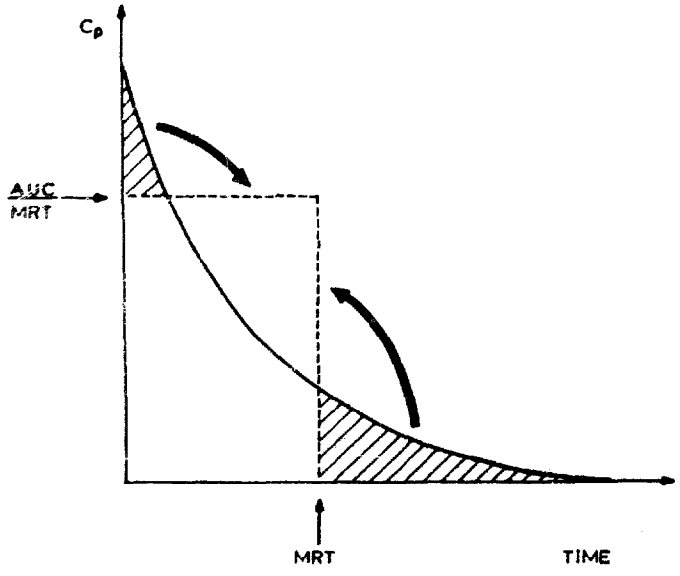

Fig. 5. Explanation of meaning of quantity, AUC/MRT.

which is derived from the one-compartment model with first-order elimination, where Q is clearance, V_d is volume of distribution and k_{el} is the elimination rate constant. V_{ss}^a, calculated from the data in Fig. 4 as 445 ± 141 ml/kg is slightly less than the total body water of approximately 600 ml/kg (Forbes, 1955) which is attained by ethanol (Lin et al., 1978).

Minimum AIC estimation

In order to find the proper model for the elimination of CMZ, the 6 pharmacokinetic models as shown in Fig. 6 were tested by AIC estimation. All the models are supposed as the mammillary model (Benet, 1972), where the elimination exclusively occurs from the central compartment. Table 1 shows AIC values obtained by fitting these models to a total 92 points on 14 time course curves. AIC takes the minimum value for the two-compartment model with the Michaelis-Menten elimination (Model 5). Fig. 7 demonstrates some of CMZ time course data points of different dosing amounts, and the fitted curves using Model 5.

V_{ss} is given for this model (Gibaldi et al., 1975) as:

$$V_{ss} = V_1 \cdot (k_{12} + k_{21})/k_{21} \tag{9}$$

It is noted that 456 ml/kg of V_{ss} which is calculated by putting the parameters in Fig. 7 into Eqn. 9 is very close to 445 ml/kg by moment analysis.

Lin et al. (1978) adopted Model 5 for the elimination of ethoxybenzamide in rat. Lawyer et al. (1980) examined the pharmacokinetic model selection for valproic acid in rat by the *F*-test (Boxenbaum et al., 1974). They concluded from the curve fitting of each single time course that Model 5 gives the best fit for the higher dose, whereas the lower dose is best described by the linear two-compartment model (Model 2). If they had applied the simultaneous multi-lines fittings to all of their time courses, Model 5 would have been found to systematically describe the elimination kinetics of valproic acid over all dosing range.

The model-dependent pharmacokinetics has a disadvantage. In addition to its exhausting calculations, the undeniable fact that there is no 'true' model makes it difficult to accept the model-dependent parameters as the index representing the

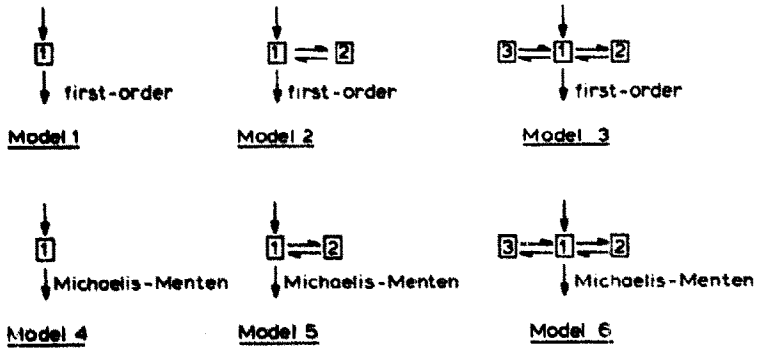

Fig. 6. Several compartment models for AIC estimation.

TABLE I

CONVERGED SS AND AIC VALUES OBTAINED BY FITTING 6 DIFFERENT MODELS TO TIME COURSE CURVES BY SALS

Model	1	2	3	4	5	6
SS	23.5	22.0	22.0	21.0	18.4	18.4
AIC	297	292	296	286	278	282

properties of a drug in body. The 'model' means a simplification or an approximation to seize the main feature or structure of a pharmacokinetic phenomenon. Unfortunately, numerous models that reasonably explain the pharmacokinetic phenomenon can be constructed. For example, Benet (1972) gave a criterion for the determinable number of parameters included in linear mammillary models as:

$$R = 2 \cdot (n - 1) + 1 \tag{10}$$

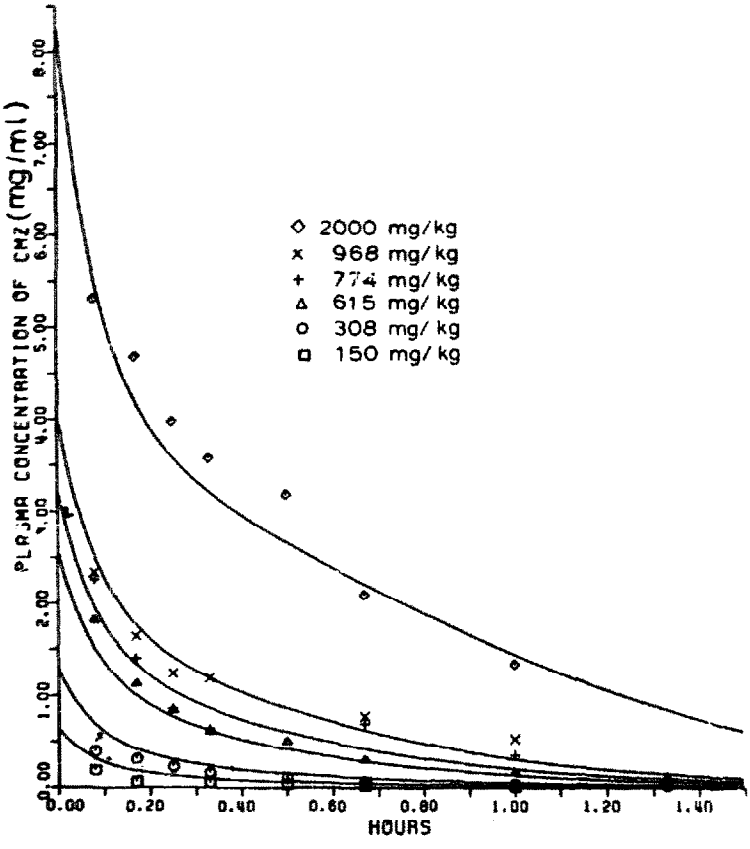

Fig. 7. Some of CMZ time course data points of different dosing amounts, and the fitted curves using the Model 5, where $V_m = 7.50$ mg/ml/h, $K_m = 1.18$ mg/ml, $k_{12} = 5.28$/h, $k_{21} = 5.97$/h and $V_1 = 242$ ml/kg. The time interval for Runge-Kutta integration is 0.005 h.

where R is maximum number of solvable pharmacokinetic parameters and n is number of compartments.

Using Eqn. 10, he proved that it is impossible to ascertain from only the plasma data available whether the model should have elimination from one, two or three compartments.

Several different equations other than the Michaelis-Menten equation can be voluntarily generated to approximate the capacity-limited kinetics. If the diffusion model which is expressed by partial differential equations (Radziuk, 1980) is also taken into account as a pharmacokinetic model, it is easily found that establishing one pharmacokinetic model is essentially arbitrary. It is at least conscientious to adopt a model by considering the amount of information included in the time course data. Since AIC is a statistical criterion for the amount of information, MAICE gives a base for the model selection.

On the other hand, AUC and MRT which are calculated by a simple integration without any pharmacokinetic model, can be an index that reflects the properties of a drug in body. The cooperative use of both the curve fitting which is model-dependent and the moment analysis which is model-independent, offers the effective indication to estimate the behaviour of drugs in body.

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