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Moment analysis for disposition kinetics of several cephalosporin antibiotics in rats

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Disposition kinetics of cefamandole, cefoperazone, cefotiam, cefmenoxime and cefmetazole following rapid intravenous injection into rats is investigated. The plasma concentrations of the antibiotics are determined by the high performance liquid chromatographic method, and the pharmacokinetic behaviours of the cephalosporins are evaluated by moment analysis which is a model-independent method. Analysis of variance (ANOVA) followed by the paired *t*-test reveals that cefmetazole and cefmenoxime have greater mean residence time (MRT) than cefotiam, cefamandole and cefoperazone, and that cefamandole and cefmetazole show larger steady-state volume of distribution (V_{ss}) than the other cephalosporins. Cefamandole has the greatest total body clearance (40·2 ml kg^{-1} min^{-1}) and cefmenoxime the smallest (7·72 ml kg^{-1} min^{-1}).

The moment analysis which had been developed in the fields of chemical engineering and chromatographic theory (Yamaoka & Nakagawa 1974) was recently applied to the pharmacokinetic field (Cutler 1978; Yamaoka et al 1978; Riegelman & Collier 1980; Johnson et al 1981). The moment analysis which enables a model-independent evaluation for drug behaviour in the body is effective for assessing the main feature of a pharmacokinetic phenomenon. Benet & Galeazzi (1979) defined the steady-state volume of distribution (V_{ss}) in terms of moments of plasma concentration – time curve.

$$V_{ss} = D \cdot MRT/AUC \quad (1)$$

where

$$AUC = \int_0^\infty C_p \, dt \quad (2)$$

and

$$MRT = \int_0^\infty t \, C_p \, dt \Big/ \int_0^\infty C_p \, dt \quad (3)$$

AUC is area under the plasma concentration–time curve, MRT is mean residence time in the systemic circulation and C_p is plasma concentration–time course following a rapid intravenous dose (D). V_{ss} signifies the volume of distribution averaged over the total body (Yamaoka et al 1982). Total body clearance (CL_T) is given by (Gibaldi & Perrier 1975).

$$CL_T = D/AUC \quad (4)$$

From equations (1) and (4), the following expected relationship is derived (Benet & Galeazzi 1979).

$$CL_T = V_{ss}/MRT \quad (5)$$

V_{ss} and MRT are characteristics which extract independent information concerning drug behaviours in the body. The disposition of a drug includes its distribution into the tissues and elimination from the systemic circulation. Equation 5 means that the disposition, represented by CL_T, is separated into the distribution and the elimination processes reflected by V_{ss} and MRT, respectively. Equation 1 is derived from the assumptions (Benet & Galeazzi 1979) that: (a) the disposition process is linear, (b) the elimination of drug is direct from the central compartment. However, Yamaoka et al (1982), have demonstrated that equation 1 can be derived stochastically without a compartment model. The use of CL_T, V_{ss} and MRT offers the advantage of characterizing the disposition properties of a drug without a specific compartment model.

Cefamandole, cefoperazone (T1551), cefotiam (SCE-963), cefmenoxime (SCE-1365) and cefmetazole are classified as cephalosporin antibiotics of the second and third generations (for reviews see Goto & Miyazaki 1980; Neu 1980; Moellering 1981; Gudsoorhar 1981). These compounds, which contain a tetrazole ring, have similar chemical structures (Fig. 1). Therefore, the comparative evaluation of their disposition kinetics offers the prospect of relating these with their chemical structures. We have therefore compared the disposition properties of these cephalosporins in rats in terms of CL_T, V_{ss} and MRT.

MATERIALS AND METHODS

Reagents and materials

Cefotiam and cefmenoxime were gifts from Takeda Chemical Industries. Cefamandole, cefoperazone and cefmetazole were gifts from Shionogi Co,

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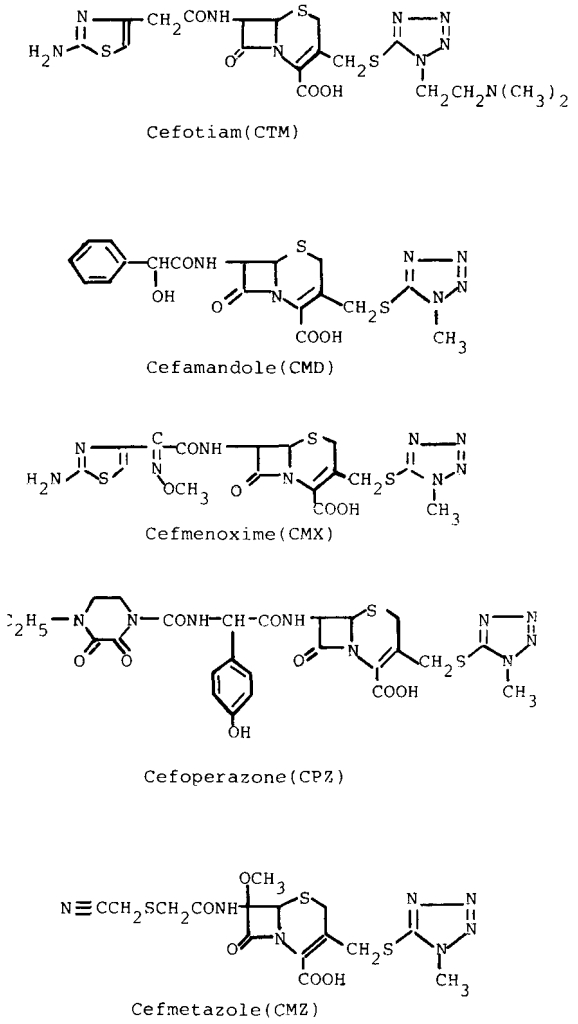

FIG. 1. Chemical structures of five cephalosporins.

Toyama Kagaku Co and Sankyo Co, respectively. Methanol and water were purified by distillation and were degassed before the preparation of mobile phase for liquid chromatography. Sodium pentobarbitone solution (Somnopentyl for animal injection, Pitman Moore Co) was used for the anaesthesia of rats. All other chemicals were of analytical grade and were used without further purification.

Determination of cephalosporins by h.p.l.c.

A high performance liquid chromatograph (ALC/GPC [illegible] Water Assoc.) equipped with a u.v.-detector (254 nm, Model 400, Water Assoc.) was used with a stationary phase of LiChrosorb RP-18 (E. Merck Co) packed in a stainless tube (4·6 mm i.d. × 25 cm). A short precolumn (4·6 mm × 5 cm) packed with LiChrosorb RP-2 was attached to guard the main column. The mobile phase compositions were as follows: cefamandole—phosphate buffer–methanol (3:1); cefoperazone— phosphate buffer–methanol (2:1); cefotiam—phosphate buffer–tetrahydrofuran (10:1); cefmenoxime—phosphate buffer–tetrahydrofuran (20:1); cefmetazole—phosphate buffer–tetrahydrofuran (20:1); where the buffer is 0·05 M potassium buffer (pH 6·6) and the mixing ratio of buffer and solvent is given by volume. The flow rate of the mobile phase was maintained at 1·0 ml min^{-1}. All operations were at room temperature (20 °C). The antibiotics were eluted around 10 min with complete separation from any interfering peaks of plasma.

Rat experiments

Male Wistar rats, 150–350 g, were used (n = 3) for each cephalosporin. Under pentobarbitone anaesthesia antibiotic (50 mg kg^{-1}) dissolved in 0·9% NaCl (saline) was rapidly injected into a femoral vein. A blood sample (0·2 ml) was collected from the jugular vein at 5, 10, 15, 20, 30, 40, and 60 min after injection. EDTA was used as the anticoagulant. After centrifugation of the blood at 3000 rev min^{-1} for 5 min, 150 µl of acetonitrile was added to 50 µl of the plasma. After the subsequent centrifugation and precipitation of protein, 5 µl of the supernatant was injected into the liquid chromatograph. The calibration graph was obtained by using control rat plasma spiked with several known amounts of each cephalosporin. The peak height was used for quantitation. The limit of the assay for all cephalosporins was about 1 µg ml^{-1}. No peak corresponding to the metabolite of cephalosporins was observed under the chromatographic conditions.

Data analysis

Initial plasma concentration (C_o) of cephalosporins were estimated by the back projection method. AUC and MRT were calculated by the standard linear trapezoidal integration with extrapolation to infinite time (Yamaoka et al 1978). V_{ss} and CL_T were calculated according to equations (1) and (4), respectively. The values of CL_T, V_{ss} and MRT thus obtained were compared between the five cephalosporins by means of the one-way analysis of variance (ANOVA) followed by the paired *t*-test.

RESULTS AND DISCUSSION

Fig. 2 shows the semi-logarithmic plot of the time courses (each an average of three rats) following the intravenous doses of the cephalosporins into rats.

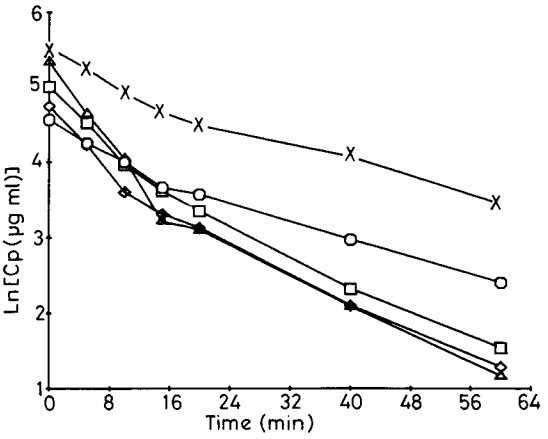

FIG. 2. Semilogarithmic plots of plasma concentrations of five cephalosporins versus time where × cefmenoxime, △ cefotiam, ◇ cefamandole, ○ cefmetazole, □ cefoperazone.

The initial plasma concentrations are the estimated values. The plasma concentration of cefmenoxime was much higher than that of the other cephalosporins, and cefmetazole had an elimination rate similar to cefmenoxime. Table 1 presents the estimated values of C_o, AUC MRT, V_{ss} and CL_T together with the time course data. Cefmenoxime showed the highest initial concentration (249 ± 32 μg ml^{-1}), while cefmetazole had the lowest ($95{\cdot}3 \pm 11{\cdot}7$ μg ml^{-1}). ANOVA indicated that the differences of CL_T, V_{ss} and MRT values among the five cephalosporins were significant at 5% level. The paired *t*-test at the 5% significant level revealed that cefmenoxime and cefmetazole make a group having a greater MRT than the other antibiotics, and that cefamandole and cefmetazole show larger V_{ss} values than the others. Francis et al (1981) and Shindo (1979) demonstrated that cefamandole and cefmetazole extensively distribute into the kidney and liver of rat. Their results are consistent with the large V_{ss} values we found for these compounds. It is well known that cefoperazone is very slowly eliminated from the human (Neuman 1980). Saikawa et al (1980) compared the disposition of cefoperazone in several animals. They found it to be rapidly eliminated from the rat, the amount excreted into the bile reaching 80%, and that its elimination from the other species was slow, the bile excretion being only 17% in rabbit, 8% in dog and 20% in monkey. Their result coincides with the smaller MRT we found for cefoperazone in the present experiments. The greatest CL_T of cefamandole can be explained by the smaller MRT and the larger V_{ss}, while the smallest CL_T of cefmenoxime is attained by the larger MRT and the smaller V_{ss}.

Although CL_T, V_{ss} and MRT are obtained without any specific compartment model, these characteristics are correlated with the classical pharmacokinetic constants when the plasma concentration-time data following intravenous injection can be well approximated by a monoexponential equation, i.e. $C_p = C_o \cdot \exp(-k_{el}t)$.

$$C_o = \text{AUC/MRT} \quad (6)$$

$$k_{el} = 1/\text{MRT} \quad (7)$$

$$t_{1/2} = 0{\cdot}693 \cdot \text{MRT} \quad (8)$$

$$V_d = V_{ss} \quad (9)$$

$$Q\ (=k_{el} \cdot V_d) = CL_T \quad (10)$$

where C_o is initial plasma concentration, k_{el} is elimination rate constant, $t_{1/2}$ is half life in body, and Q and V_d are clearance and volume of distribution in one compartment model, respectively.

Hence, V_{ss}, MRT and CL_T are reasonable extensions of the classical parameters which are based on the one-compartment model.

The multi-compartment models are indispensable for estimating the local parameters such as volume of

Table 1. Plasma concentration, μg ml^{-1} (s.d.), and in-vivo characteristics of five cephalosporins in rats after an intravenous dose of 50 mg kg^{-1}.

Time (min)	Cefamandole	Cefoperazone	Cefotiam	Cefmenoxime	Cefmetazole
5	68·1 (16·9)	90·5 (8·2)	102·2 (16·1)	187·5 (11·7)	69·5 (7·9)
10	36·2 (12·5)	51·8 (14·2)	56·0 (10·5)	134·0 (14·4)	54·0 (11·0)
15	27·2 (11·5)	37·1 (9·72)	24·8 (3·3)	104·7 (9·2)	38·3 (5·9)
20	22·6 (4·6)	28·3 (10·0)	22·0 (5·8)	87·2 (11·3)	35·0 (4·2)
40	8·1 (5·3)	10·1 (3·7)	8·0 (3·7)	57·3 (2·3)	19·6 (2·0)
60	3·6 (2·0)	4·6 (1·1)	3·2 (2·4)	30·5 (4·7)	10·9 (0·9)
C_o (μg ml^{-1})	114·6 (15·4)	148·4 (6·1)	207·0 (46·0)	248·8 (32·0)	95·3 (11·7)
AUC (mg min ml^{-1})	1·46 (0·52)	1·92 (0·37)	2·03 (0·33)	6·54 (0·65)	2·37 (0·25)
MRT (min)	16·6 (4·6)	15·7 (2·8)	13·6 (3·6)	35·0 (4·1)	31·5 (2·5)
V_{ss} (ml kg^{-1})	592 (61)	411 (68)	334 (61)	268 (20·3)	677 (104)
CL_T (ml min^{-1} kg^{-1})	40·2 (16·5)	26·6 (4·5)	25·4 (4·7)	7·72 (0·80)	21·7 (2·1)

a peripheral compartment and for evaluating the precise dosing regimen of a drug using the time course data following a single dose. However, the number of compartments is changed by the route of administration. The concept 'peripheral' is not always associated with the actual animal tissues and organs. In order to estimate the local pharmacokinetic constants, precise attention must be paid to the sampling intervals. It is also possible that the number of exponential terms depends not only on drugs but also on the animals used. In the present study, one rat gave a mono-exponential time course for a cephalosporin and another rat gave a bi-exponential time course for the same cephalosporin. This makes it difficult to compare the in-vivo behaviour of drugs using the same pharmacokinetic parameters.

Though moment analysis gives no information about the microscopic behaviour of a drug in the body, the method averageing over the total body offers some characteristics which allow an understanding of the macroscopic aspects of the disposition process. Since V_{ss} means the total volume of distribution, V_{ss} is given as the sum of local volume, V_i.

$$V_{ss} = \sum_i V_i \quad (11)$$

V_i is not the volume estimated by the compartment model, but the distribution volume of the actual tissue or organ. If a drug distributes into a specific tissue in large amount, it is expected that the contribution of V_i to V_{ss} becomes larger.

If a drug is eliminated from the systemic circulation by several routes, the following relationship is obtained:

$$CL_T = \sum_i CL_i \quad (12)$$

where CL_i is the clearance through a specific route i. Provided that the drug is eliminated exclusively through route i, the following equation holds:

$$CL_i = V_{ss}/MRT_i \quad (13)$$

where MRT_i is the mean residence time when the drug is eliminated exclusively through the route i. Substituting equations (5) and (13) into equation (12):

$$1/MRT = \sum_i 1/MRT_i \quad (14)$$

Equation 14 is intuitively understandable by considering that MRT has the same meaning as the resistance in electric circuits. The contribution of the route i for the elimination of the drug is given as

$$f_i = (1/MRT_i)/(1/MRT) = MRT/MRT_i \quad (15)$$

MRT_i can be evaluated by a surgical or chemical blocking of the other eliminating routes.

The above discussions are based on the assumptions that: (1) The disposition process of a drug can be regarded as linear. (2) The isolation of a tissue from the systemic circulation or the blocking of an elimination route does not appreciably change the physiological condition of the body.

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