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Absorption Kinetics of Carbenicillin Phenyl Sodium and Carbenicillin Indanyl Sodium in Man

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Carbenicillin phenyl sodium (CFPC) and carbenicillin indanyl sodium (CIPC), which are prodrugs of carbenicillin (CBPC), were orally administered to three volunteers at various doses. The absorptions of these prodrugs were compared by means of moment analysis using urinary excretion data. CFPC showed linear absorption kinetics at 0.5, 1.0, and 1.5 g doses. On the other hand, the urinary recovery after oral administration of CIPC decreased and the mean absorption time (MAT) increased as the dose was increased from 0.5 to 1.5 g. Computer simulations by the Runge-Kutta method confirmed the occurrence of capacity-limited absorption of CIPC.

Keywords——carbenicillin; carbenicillin phenyl; carbenicillin indanyl; capacity-limited absorption; moment analysis; mean absorption time

Introduction

Carbenicillin (α-carboxybenzylpenicillin) is a β-lactam antibiotic which has potent activity against *Pseudomonas aeruginosa* and indole-positive *Proteus*.[1-4] Because this penicillin is rapidly deactivated at the pH in stomach and its absorption from the gastrointestinal tract is extremely restricted due to its hydrophilicity,[5,6] clinical use is limited to parenteral administration.[7-9] Carbenicillin phenyl sodium (CFPC)[10] and carbenicillin indanyl sodium (CIPC)[11-14] are prodrugs of carbenicillin for oral use. These prodrugs are acid-stable and their lipophilicity is preferable to absorption from the gastrointestinal tract.[15-17] CFPC and CIPC are absorbed in the form of ester, and the released carbenicillin appears in the blood after rapid hydrolysis of the ester in the intestinal wall and the liver.[10] The ester hydrolysis of CIPC was demonstrated to occur exclusively in the intestinal wall.[18]

Moment analysis, which is a model-independent method, has been recently applied to the evaluation of drug behavior in the body.[19-22] Riegelman and Collier discussed the mean absorption time (MAT) and mean *in vivo* dissolution time (MDT) for the evaluation of the rate of bioavailability.[23] The pharmacokinetic features of several β-lactam antibiotics (cephalexin,[24] cephaloglycin,[25] amino-penicillins,[26,27] oxacillin,[28] cefmetazole,[29] and clavulanic acid[30]) were investigated by moment analysis. The purpose of this article is to clarify the absorption kinetics of CFPC and CIPC in humans by means of moment analysis.

Experimental

Reagents and Materials——CFPC tablets (Uticillin, 500 mg as ester) and CIPC tablets (Geopen-U, 500 mg as ester) were gifts from Beecham Yakuhin Co. (Tokyo, Japan) and Taito Pfizer Co. (Tokyo, Japan), respectively. Carbenicillin used as a standard material was a gift from Fujisawa Pharm. Co. (Osaka, Japan). Methanol and water were purified by distillation and degassed prior to preparation of the mobile phase for liquid chromatography. All other chemicals were of analytical grade, and were used without further purification.

Determination of Carbenicillin in Human Urine——The carbenicillin concentration was determined by reversed phase high performance liquid chromatography. The chromatograph (TRIROTAR, Jasco) was equipped with a UV detector (UVIDEC-100, Jasco) adjusted at 225 nm. The stationary phase was octadecylsilane chemically bonded on totally porous silica gel, packed in a stainless steel column (LiChrosorb RP-18,

4.6 mm i.d. × 25 cm, E. Merck Co.). The mobile phase was water–methanol (2/1, v/v) containing 0.007 M KH_2PO_4, 0.03 M Na_2HPO_4, and 0.003 M tetrabutylammonium bromide. The flow rate was 1.0 ml/min and all operations were carried out at room temperature. D- and L-diastereoisomers of carbenicillin were eluted out at retention times of 11 min and 17 min with complete separation from other urinary components. The combined area of the isomer peaks measured with a digital integrator (CHROMATOPAC C-R1A, Shimadzu, Kyoto, Japan) was used for the quantitation of carbenicillin.

Drug Administration and Sample Preparation——Three healthy male volunteers, 23 to 30 years old, weighing 62 to 75 kg, participated in this study. The subjects were fasted overnight before dosing, and were permitted to eat no food until 2 h after dosing. No other drugs were taken for at least one week prior to and during the study. All the subjects received single oral administrations of 0.5, 1.0, and 1.5 g CFPC and CIPC. Each dosage was separated by at least one week. Urine samples were collected immediately before and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, and 6.0 h after dosing. After the urine volume was measured, a portion was passed through a 0.45 μm pore size membrane filter (Fuji Photo Film Co.), and 5 μl of the filtrate was subjected to liquid chromatographic analysis.

Data Analysis——The statistical moments for the urinary excretion rate-time curve are defined as[20]

$$A_e(\infty) = \int_0^\infty (dA_e/dt)dt \qquad \text{Eq. 1}$$

$$\text{MRT} = \int_0^\infty t(dA_e/dt)dt \Big/ \int_0^\infty (dA_e/dt)dt \qquad \text{Eq. 2}$$

where dA_e/dt is the urinary excretion rate, $A_e(\infty)$ is the total amount of drug excreted in urine to infinite time, and MRT is the mean residence time. These symbols are in accord with the proposal of Rowland and Tucker.[31] $A_e(\infty)$ and MRT were calculated by linear trapezoidal integration with extrapolation of the time course curve to infinite time according to a monoexponential equation. This equation was determined by the least squares method using the last three or four points of the time course data. The fraction of dose absorbed, F, and the mean absorption time, MAT, were obtained from the following equations.

$$F = D_{iv}[A_e(\infty)]_{po}/D_{po}[A_e(\infty)]_{iv} \qquad \text{Eq. 3}$$

$$\text{MAT} = \text{MRT}_{po} - \text{MRT}_{iv} \qquad \text{Eq. 4}$$

where D is dose, and the subscripts po and iv indicate oral and intravenous administrations, respectively. The computations were carried out on a microcomputer (PET 2001, Commodore) programmed in BASIC.

The computer simulations were carried out on FACOM M-200 system in Kyoto University Data Processing Center through a TSS terminal (Silent 700, Texas Instrument). The Runge-Kutta method programmed in FORTRAN was used to numerically solve the simultaneous differential equations.

Results

Carbenicillin Phenyl Sodium

Figure 1 shows the urinary excretion rate–time curves after oral administration of 0.5, 1.0, and 1.5 g of CFPC tablets. Each point indicates the average value of three subjects. The unhydrolyzed ester was not detected in human urine, in accord with the result of Clayton *et al.*[10] The maximum excretion rates increase almost proportionally to dose. Table I lists $A_e(\infty)$, MRT, F, and MAT values at three dose levels. $A_e(\infty)$ and MRT following intravenous injection of 1.0 g carbenicillin were calculated as 0.915 (g) and 1.19 (h), using previously reported data.[32] Höffler *et al.*[3] reported that the plasma half-life of carbenicillin following intravenous administration increased as the dose increased from 1 g to 30 g. This dose-dependency, however, is negligible up to 2 g dose. Thus F and MAT were estimated according to Eqs. 3 and 4, using the average values of $A_e(\infty)$ and MRT. Figure 2 shows the dependencies of F and MAT on dose; the straight lines are the regression lines obtained by the least squares method.

$$F(\%) = 46.6 - 3.4 \cdot D \qquad (r = -0.956)$$

$$\text{MAT (h)} = 1.20 - 0.09 \cdot D \qquad (r = -0.315)$$

where r is the correlation coefficient, and D is dose (g) as CFPC ester. It is found that both F and MAT remain almost constant with respect to dose. Therefore, the absorption of CFPC can be regarded as linear in the present dose range (0.5—1.5 g). The MAT values which express the mean residence time for CFPC in the gastrointestinal tract, are close to MRT_{iv} of

Table I. Statistical Moments after Oral Administration of Carbenicillin Phenyl Sodium to Human Subjects

Dose[a]	(g)	0.397[b]	0.794[b]	1.191[b]
$A_e(\infty)$[a]	(g)	0.162±0.057[c]	0.318±0.067	0.449±0.054
MRT	(h)	2.27±0.14	2.46±0.08	2.18±0.23
F	(%)	44.6	43.8	41.2
MAT	(h)	1.08	1.27	0.99

a) Dose and urinary recovery are given as carbenicillin equivalent.
b) Administered as one, two or three 500-mg tablets of carbenicillin phenyl sodium.
c) Mean ± standard deviation for three subjects.

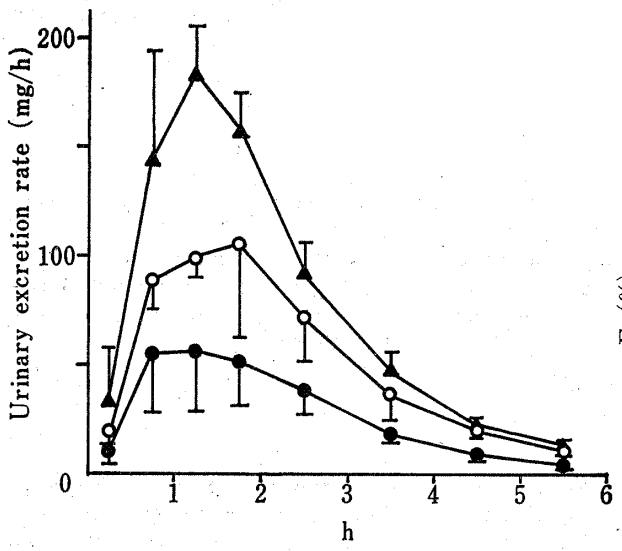

Fig. 1. Urinary Excretion Rate–Time Curves after Oral Administration of Carbenicillin Phenyl Sodium

Points are averages of three subjects and vertical lines represent standard deviations.
●—●, 0.5 g dose; ○—○, 1.0 g dose; ▲—▲, 1.5 g dose.

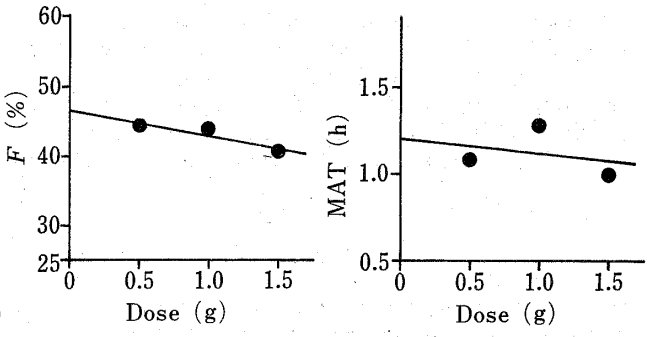

Fig. 2. Dependencies of F and MAT for Carbenicillin Phenyl Sodium on Dose

carbenicillin in the systemic circulation. This suggests that curve fitting using the ordinary one-compartment model with first-order absorption and first-order elimination may give ill-condition in the iterative calculation or erroneously estimated rate constants. It is found that 43% of dose is transferred into the systemic circulation as carbenicillin, and about 40% is recovered in urine.

Carbenicillin Indanyl Sodium

Figure 3 shows the urinary excretion rate-time curves after oral administration of 0.5, 1.0, and 1.5 g of CIPC. These data were obtained from the same three subjects. It should be noted that the maximum excretion rates are not proportional to the dose. Butler *et al.*[11] and Knirsch *et al.*[13] reported that the peak plasma concentrations were not proportional to the dose when 0.5 and 1.0 g CIPC were administered four times a day, suggesting reduced absorption. Since the convolution expression is valid even if the input function represents a nonlinear process,[19] Eqs. 3 and 4 can be applied to the case of capacity-limited absorption. Table II lists $A_e(\infty)$, F, MRT, and MAT values for CIPC, and Fig. 4 shows the dependencies of F and MAT on dose. The regression lines and correlation coefficients are as follows.

$$F(\%) = 77.4-20.4\cdot D \qquad (r=-0.970)$$
$$\text{MAT (h)} = 0.697+0.470\cdot D \qquad (r=0.996)$$

where D is dose (g) as CIPC ester. These results indicate that increase of the dose lowers the extent of bioavailability and prolongs the mean absorption time. Such dose-dependencies demonstrate that the absorption of CIPC is nonlinear, because F and MAT are essentially constant in a linear absorption system. A similar tendency, that is, decrease of the extent of absorption with increase of dose, has been reported for other drugs (amoxicillin,[33] chlorothiazide,[34] riboflavin,[35] and tetracycline[36]). The decrease of absorption rate with increase of dose also coincides with the trend noted in a study of amoxicillin.[33] As the dose increases, the MAT value exceeds the MRT_{iv}. In other words, the absorption of CIPC changes to "flip-flop" as the dose increases.

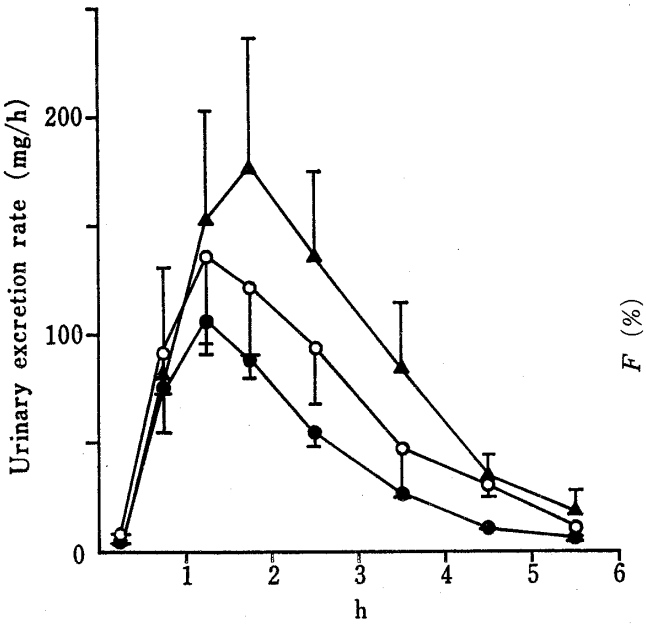

Fig. 3. Urinary Excretion Rate–Time Curves after Oral Administration of Carbenicillin Indanyl Sodium

Symbols are the same as in Fig. 1.

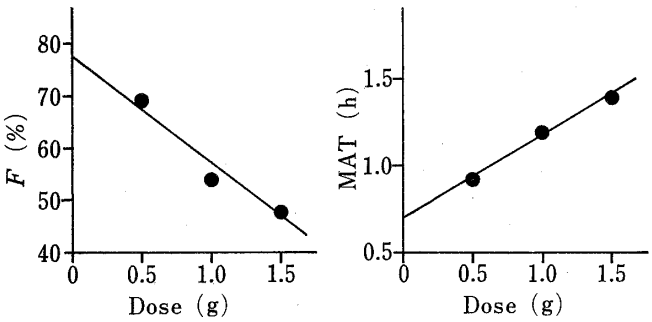

Fig. 4. Dependencies of F and MAT for Carbenicillin Indanyl Sodium on Dose

Table II. Statistical Moments after Oral Administration of Carbenicillin Indanyl Sodium to Human Subjects

Dose[a]	(g)	0.382[b]	0.764[b]	1.146[b]
$A_e(\infty)$[a]	(g)	0.240±0.011[c]	0.378±0.110	0.507±0.152
MRT	(h)	2.11±0.01	2.38±0.17	2.58±0.31
F	(%)	68.7	54.1	48.3
MAT	(h)	0.92	1.19	1.39

a) Dose and urinary recovery are given as carbenicillin equivalent.
b) Administered as one, two or three 500-mg tablets of carbenicillin indanyl sodium.
c) Mean ± standard deviation for three subjects.

Discussion

The difference in absorption behavior between CFPC and CIPC is attributable to their chemical properties. Tsuji *et al.*[15] showed that *in vitro* degradation of CFPC is faster than that of CIPC, and also demonstrated that the partition coefficient of CIPC between octanol and water is greater than that of CFPC.[17] The stability and lipophilicity of CIPC are reflected in the greater absorption of CIPC (48—69%) than CFPC (41—45%).

Ban *et al.*[37] observed linear absorption kinetics following oral administration of 0.5 to 1.0 g CIPC after a meal. The concentration of CIPC in gastrointestinal fluid seems to be lowered by food ingestion. Therefore, it is suggested that the high concentration of CIPC causes nonlinearity in the dissolution process or absorption of released drug when CIPC is administered to fasting subjects.

To qualitatively explain the nonlinear absorption of CIPC, computer simulations were attempted using the simple models shown in Chart 1.

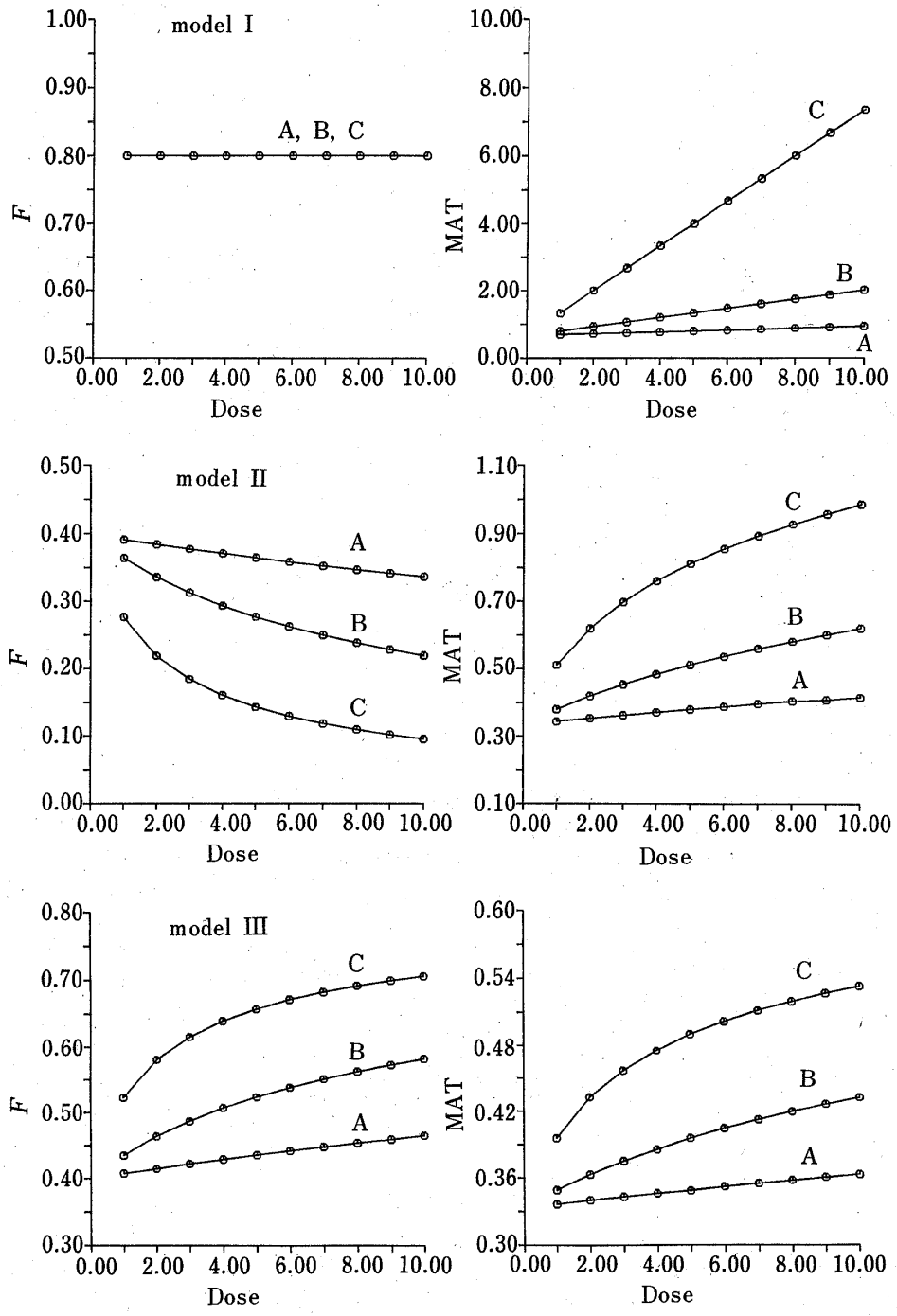

Fig. 5. F and MAT simulated by the Models in Chart 1

A, $K_m=10$, $V_m=15$; B, $K_m=2$, $V_m=3$; C, $K_m=0.4$, $V_m=0.6$. $f=0.8$, $k_d=1.5$, $k_a=1.5$, and $k_e=1.0$.

Model I: Michaelis–Menten absorption and first-order disposition.

Model II: Michaelis–Menten absorption, competitive first-order degradation in the gastrointestinal tract, and first-order disposition.

Mndel III: First-order absorption, competitive Michaelis–Menten degradation in the gastrointestinal tract, and first-order disposition.

Figure 5 shows MAT and F at various dose levels in models I, II, and III. The percent initial saturation values for curves A, B, and C, calculated as $100 \cdot f \cdot D/(K_m + f \cdot D)$, are 7—44%, 29—80%, and 67—95% for the simulated dose range, respectively. In model I, F is constant and MAT increases as the dose increases. In model III, both F and MAT increase as the dose increases. These models are not in accord with the experimental trend of CIPC. In model II, F decreases and MAT increases with increase of the dose. Therefore, the pharmacokinetic behavior of CIPC is consistent with model II as a possible absorption mechanism.

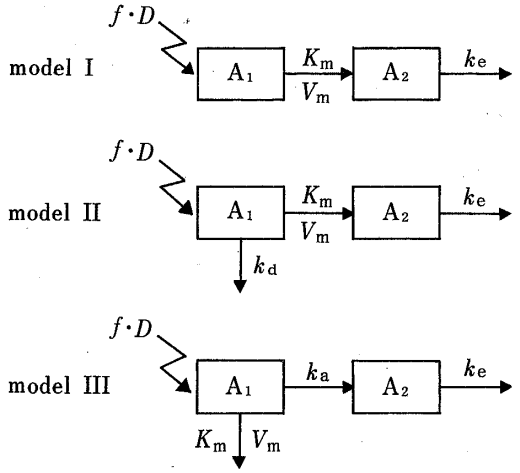

Chart 1. Nonlinear Models for Drug Absorption

Key; A_1, drug amount in absorption site; A_2, drug amount in the body; K_m, V_m, Michaelis–Menten parameters; k_a, first-order absorption rate constant; k_d, first-order degradation rate constant; k_e, first-order elimination rate constant; f, fraction of dose reaching absorption site as an intact form; D, dose.

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