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Pharmacokinetic Studies of the Urinary Excretion of Clavulanic Acid in Man

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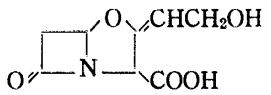
A pharmacokinetic investigation on the urinary excretion of clavulanic acid in man was carried out. Combinations of clavulanic acid (125 mg) and amoxicillin (250 mg) were given orally to human subjects, and the subsequent urinary concentrations of intact clavulanic acid, amoxicillin, and the latter's metabolites (penicilloic acid and penamaldic acid) were determined by the high performance liquid chromatography method. From the urinary excretion rate *vs.* time curves for each species, the values of cumulative excretion amount at infinite time and of mean residence time were estimated by means of the moment analysis method. The results showed that when clavulanic acid was dosed in combination with amoxicillin, 27 to 45% of the dosed amount of clavulanic acid was excreted in the urine as an intact form with a mean residence time of 1.6 to 2.1 h after administration, while the corresponding values for amoxicillin were 56 to 73% (including metabolites) and 2.0 to 2.6 h (for the intact form). These results indicate that clavulanic acid may undergo less absorption (and/or more extensive metabolism and extra-urinary excretion) and faster urinary excretion than amoxicillin. By comparison of the results with those obtained after individual doses of clavulanic acid and amoxicillin to the same subjects, it was found that there was no appreciable kinetic interaction between clavulanic acid and amoxicillin ascribable to the combined dose, although the analysis of variance indicated that the difference in the excreted amounts of penamaldic acid of amoxicillin between combined and individual doses was significant at the 5% level.

Keywords——clavulanic acid; amoxicillin; combined dose; metabolites; high performance liquid chromatography; pharmacokinetics; urinary excretion; moment analysis; mean residence time

Introduction

There are two strategies for overcoming β-lactamase-producing bacteria; one is to develop new drug derivatives which are resistant to β-lactamases and the other is to use β-lactamase inhibitors which can restore the activities of conventional β-lactam antibiotics. Clavulanic acid (I), a novel fused β-lactam produced by *Streptomyces clavuligerus*,[1,2] is a promising candidate of the latter type and shows an intrinsic weak antibacterial activity.



I

It is known from a number of *in vitro* microbiological investigations that clavulanic acid, when used in combination with certain penicillins or cephalosporins, causes marked reduction in their MIC values against various β-lactamase-producing Gram-positive and Gram-negative bacteria.[3] Chemical[4] and kinetic[5] investigations of such inhibitory activities have revealed that the inactivation of β-lactamase involves the irrevesible binding of clavulanic acid to the enzyme protein. Few *in vivo* studies of clavulanic acid have appeared, though doses of clavulanic acid and amoxicillin showed appreciable synergistic effects on patients with bronchopulmonary infection[6] and with urinary tract infection[7] due to β-lactamase-producing bacteria, and the use of penicillin G together with clavulanic acid was effective in

reducing morbidity and bacterial counts in the kidneys of mice infected with a penicillinase-producing *Streptococcus aureus*.[8] A project for a series of clinical and biopharmaceutical investigations on the combined use of clavulanic acid and amoxicillin is now being developed.[9]

Before effective use can be made of these findings for clinical chemotherapy, many problems remain to be solved. One is to determine the pharmacokinetic features of absorption, disposition, metabolism and elimination of clavulanic acid in healthy and diseased humans. The aim of the present work was to investigate the pharmacokinetic features of urinary excretion of clavulanic acid given alone or together with amoxicillin to human subjects.

Experimental

Reagents and Materials——Clavulanic acid (potassium salt) and amoxicillin were gifts from Beecham Yakuhin Co. Ltd. (Tokyo, Japan). These drugs were separately packed in capsules for oral administration, each containing either 125 mg clavulanic acid or 250 mg amoxicillin. Tetrabutylammonium bromide (TBAB) and other chemicals used for HPLC analysis were commercial products of reagent grade. Sodium *n*-heptylsulfonate, used as an ion-pairing agent, was synthesized by means of the Strecker reaction.[10] Methanol and water were purified by distillation and degassed prior to preparation of the mobile phase.

Chromatography——(i) For the assay of clavulanic acid, a high performance liquid chromatograph (LC-3A, Shimadzu Co., Kyoto, Japan) equipped with a variable wavelength UV-detector (SPD-2A, Shimadzu Co.) was used with a stationary phase of LiChrosorb RP-18 (E. Merck Co., West Germany) packed in a stainless steel tube (25 cm × 4.6 mm i.d.) and a mobile phase of aqueous 10 mM TBAB + 0.5 mM Na_2HPO_4 + 0.5 mM NaH_2PO_4 solution mixed with methanol at a volume ratio of 10/1 (pH 7.14), whose flow rate was maintained at 1.5 ml/min (60 kg/cm^2). The effluent was monitored at 220 nm, and all operations were carried out at ambient temperature.

(ii) For the assays of amoxicillin and its metabolites, a high performance liquid chromatograph (TRIROTAR-III, Jasco, Tokyo, Japan) equipped with a variable wavelength UV-detector (UVIDEC-100-III, Jasco) was used with a stationary phase of Cosmosil $5C_{18}$ (Nakarai Chemicals Co., Kyoto, Japan) packed in a stainless steel tube (15 cm × 4.6 mm i.d.) and a mobile phase of water/methanol = 4/1 (v/v) containing 5 mM sodium *n*-heptylsulfonate + 2 mM Na_2HPO_4 (the pH adjusted to 2.67 by addition of 0.5 N HCl), whose flow rate was 0.9 ml/min (90 kg/cm^2). The column temperature was kept at 40°C and the effluent was monitored at 228 nm. A short column (5 cm × 4.6 mm i.d. stainless steel tube) packed with LiChrosorb RP-2 was used to guard the main column in each case.

Urinary Excretion——Three healthy male adults participated in this experiment. Each subject fasted for 12 h and then received a 125 mg clavulanic acid capsule or 250 mg amoxicillin capsule or both simultaneously. Urine was collected at $t = 0$, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, and 8.0 h after administration. After measurements of volume, the urine was passed through a 0.45 μm pore size membrane filter (Fuji Photo Film Co., Tokyo, Japan), and a 5 to 20 μl portion of the filtrate was subjected to HPLC analysis under the conditions described above. The assays of clavulanic acid and amoxicillin were carried out by reference to calibration graphs obtained by plotting peak height (or area) *vs.* known concentrations of the materials dissolved in the urine obtained at $t = 0$. The procedure for the assays of amoxicillin metabolites (penicilloic acid and penamaldic acid) was the same as described in the previous paper.[11]

Estimation of Statistical Moments——Urinary excretion amounts at infinite time (X_u^∞) and mean residence time (MRT) are given by the zero and first normal moments, respectively, defined as

$$X_u^\infty = \int_0^\infty (dX_u/dt)dt$$

$$\mathrm{MRT} = \int_0^\infty t(dX_u/dt)dt / X_u^\infty$$

where dX_u/dt is a function expressing the urinary excretion rate *vs.* time curve. In using the above equations, the moments were calculated by rectangular integration of the time course curve with extrapolation to infinite time on the basis of a monoexponential equation. The equation was determined by the least-squares method using the last four to eight data points on the time course curve. The details of the mathematical operations used to obtain the moments from experimental data were described in the previous paper.[12] The computations were carried out on a microcomputer (PET 2001, Commodore Co., Palo Alto, Ca., U.S.A.) with programming in BASIC.

Results

Fig. 1 depicts the chromatogram of urine collected at 3 h after a combined dose of clavulanic acid and amoxicillin. Intact clavulanic acid gave a peak with a retention time of 27.6 min,

and was completely separated from endogenous urinary components. Since amoxicillin and its metabolites coexisting in this urine specimen were eluted with longer retention times, they were separated under different conditions. The resulting chromatogram of the same urine is shown in Fig. 2.

The time course data for excretion of clavulanic acid, amoxicillin and the metabolites following administration of 125 mg clavulanic acid or 250 mg amoxicillin or both simultaneously are listed in Tables I to IV, where the values for penicilloic acid and penamaldic acid are given as equivalent to amoxicillin. The excretion rate *vs.* time curves obtained after administration of combined doses to three subjects are shown in Figs. 3 to 5. The results for the excretion amount at infinite time (X_u^∞) and mean residence time (MRT) for each species are given in Table V.

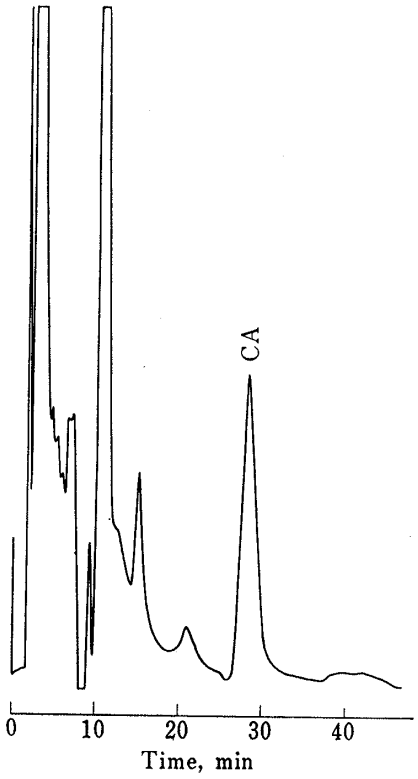


Fig. 1. HPLC Separation of Clavulanic Acid (CA) excreted in Human Urine 3 h after a Combined Oral Dose of 125 mg Clavulanic Acid and 250 mg Amoxicillin

Stationary phase, LiChrosorb RP-18 (25 cm × 4.6 mm i.d.); mobile phase, aqueous 10 mM TBAB + 0.5 mM Na_2HPO_4 + 0.5 mM NaH_2PO_4 solution/MeOH = 10/1 (v/v) (pH 7.14); flow rate, 1.5 ml/min (60 kg/cm²); column temperature, ambient; injection volume, 10 μl of neat urine; detection, UV-220 nm; full scale, 0.04 a.u.; estimated concentration of clavulanic acid, 230 μg/ml.

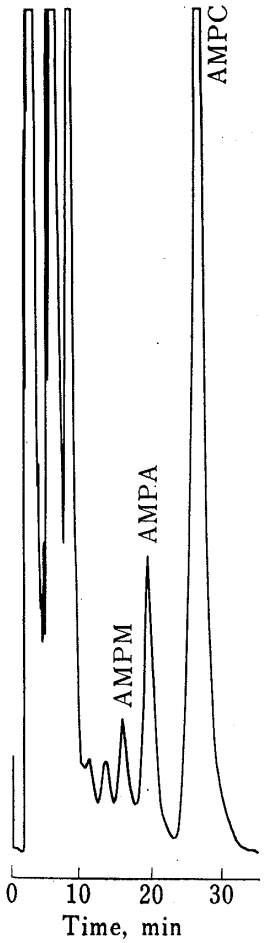


Fig. 2. HLPC Separation of Amoxicillin and Its Metabolites excreted in Human Urine 3 h after a Combined Oral Dose of 125 mg Clavulanic Acid and 250 mg Amoxicillin

AMPC, amoxicillin; AMPA, penicilloic acid of amoxicillin; AMPM, penamaldic acid of amoxicillin.

Stationary phase, Cosmosil $5C_{18}$ (15 cm × 4.6 mm i.d.); mobile phase, MeOH/H_2O = 1/4 (v/v) containing 5 mM sodium *n*-heptylsulfonate + 2 mM Na_2HPO_4 (pH 2.67 adjusted with 0.5 N HCl); flow rate, 0.9 ml/min (90 kg/cm²); column temperature, 40°C; injection volume, 5 μl; detection, UV-228 nm; full scale, 0.08 a.u.; estimated concentration, AMPC = 700 μg/ml, AMPA = 180 μg/ml, AMPM = 50 μg/ml.

TABLE I. Urinary Excretion (mg) of Clavulanic Acid following Administration of a Combined Dose of 125 mg Clavulanic Acid and 250 mg Amoxicillin or an Individual Dose of 125 mg Clavulanic Acid(Given in Parentheses)

Time (h)	J.H.	T.H.	H.M.	Mean	S.D.
0 —0.5	0.49(2.40)	1.86(2.07)	1.56(1.26)	1.30(1.91)	0.59(0.48)
0.5—1.0	14.55(16.50)	11.94(11.35)	8.64(8.79)	11.71(12.21)	2.42(3.21)
1.0—1.5	15.30(12.55)	8.18(9.53)	11.43(8.19)	11.64(10.09)	2.91(1.82)
1.5—2.0	8.92(8.80)	4.53(6.26)	8.54(4.49)	7.33(6.52)	1.99(1.77)
2.0—2.5	5.86(5.86)	2.16(4.03)	5.36(3.70)	4.46(4.53)	1.64(0.95)
2.5—3.0	3.91(3.99)	1.70(2.41)	4.28(2.56)	3.30(2.99)	1.14(0.71)
3.0—4.0	4.38(4.19)	1.98(2.48)	4.99(3.37)	3.78(3.35)	1.30(0.70)
4.0—5.0	1.55(2.13)	0.93(1.35)	2.61(1.36)	1.70(1.61)	0.69(0.37)
5.0—6.0	0.66(0.80)	0.49(0.48)	1.10(0.65)	0.75(0.64)	0.26(0.13)
6.0—8.0	n.d.(1.08)	n.d.(0.73)	n.d.(n.d.)	— (0.60)	(0.45)
Total	55.62(58.30)	33.77(40.69)	48.51(34.37)	45.97(44.45)	9.10(10.13)

TABLE II. Urinary Excretion(mg) of Amoxicillin following Administration of a Combined Dose of 125 mg Clavulanic Acid and 250 mg Amoxicillin or an Individual Dose of 250 mg Amoxicillin (Given in Parentheses)

Time (h)	J.H.	T.H.	H.M.	Mean	S.D.
0 —0.5	0.16(0.14)	6.90(3.90)	2.84(3.08)	3.30(2.37)	2.77(1.16)
0.5—1.0	13.46(11.90)	37.30(26.69)	18.59(18.94)	23.21(19.18)	10.25(6.04)
1.0—1.5	20.35(39.15)	34.31(28.94)	26.59(27.32)	27.08(31.80)	5.71(5.24)
1.5—2.0	17.86(31.13)	22.08(21.69)	17.41(17.83)	19.12(23.55)	2.10(5.59)
2.0—2.5	15.74(18.29)	15.18(15.19)	13.24(13.38)	14.72(15.62)	1.07(2.03)
2.5—3.0	13.37(13.51)	10.45(13.59)	13.18(12.06)	12.33(13.05)	1.63(0.70)
3.0—4.0	14.06(15.89)	10.74(22.27)	18.96(15.65)	14.59(17.94)	3.38(3.07)
4.0—5.0	5.84(4.86)	5.89(10.77)	8.80(8.14)	6.84(7.92)	1.38(2.42)
5.0—6.0	3.41(2.02)	3.95(6.09)	4.64(3.23)	4.00(3.78)	0.50(1.71)
6.0—8.0	4.91(1.52)	4.14(5.26)	4.09(3.31)	4.38(3.36)	0.38(1.53)
Total	109.16(138.41)	150.94(154.39)	128.34(122.94)	129.48(138.58)	17.08(12.84)

TABLE III. Urinary Excretion(mg) of Penicilloic Acid following Administration of a Combined Dose of 125 mg Clavulanic Acid and 250 mg Amoxicillin or an Individual Dose of 250 mg Amoxicillin (Given in Parentheses)

Time (h)	J.H.	T.H.	H.M.	Mean	S.D.
0 —0.5	n.d.(n.d.)	0.20(n.d.)	n.d.(n.d.)	0.07(—)	0.09(—)
0.5—1.0	0.59(0.64)	2.59(1.74)	1.26(2.74)	1.48(1.71)	0.83(0.86)
1.0—1.5	2.08(3.33)	3.56(3.15)	3.85(3.71)	3.16(3.40)	0.78(0.23)
1.5—2.0	2.45(4.12)	4.42(3.24)	3.64(4.07)	3.50(3.81)	0.81(0.40)
2.0—2.5	2.49(3.25)	2.96(2.49)	3.17(3.84)	2.87(3.19)	0.28(0.55)
2.5—3.0	2.46(2.88)	2.62(1.86)	3.38(3.12)	2.82(2.62)	0.40(0.55)
3.0—4.0	3.57(4.80)	2.22(3.32)	6.75(5.71)	4.18(4.61)	1.90(0.98)
4.0—5.0	2.09(3.93)	1.40(1.86)	5.06(2.61)	2.85(2.80)	1.59(0.86)
5.0—6.0	1.65(1.83)	0.74(1.06)	3.52(1.90)	1.97(1.79)	1.16(0.58)
6.0—8.0	1.30(2.08)	0.81(1.35)	1.29(2.49)	1.13(1.97)	0.23(0.47)
Total	18.68(26.86)	21.52(20.07)	31.92(30.19)	24.04(25.71)	5.69(4.21)

Table IV. Urinary Excretion (mg) of Penamaldic Acid following Administration of a Combined Dose of 125 mg Clavulanic Acid and 250 mg Amoxicillin or an Individual Dose of 250 mg Amoxicillin (Given in Parentheses)

Time (h)	J.H.	T.H.	H.M.	Mean	S.D.
0 —0.5	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	— (—)	— (—)
0.5—1.0	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	— (—)	— (—)
1.0—1.5	0.57 (0.38)	0.38 (0.62)	0.32 (0.87)	0.42 (0.62)	0.11 (0.20)
1.5—2.0	0.64 (0.55)	0.53 (0.64)	0.64 (1.41)	0.60 (0.87)	0.05 (0.39)
2.0—2.5	0.73 (0.62)	0.62 (0.92)	0.74 (1.53)	0.70 (1.02)	0.05 (0.38)
2.5—3.0	0.84 (0.71)	0.54 (1.06)	0.97 (1.52)	0.78 (1.10)	0.18 (0.33)
3.0—4.0	1.86 (2.11)	0.92 (1.59)	2.14 (2.89)	1.64 (2.20)	0.52 (0.53)
4.0—5.0	1.18 (1.65)	0.70 (1.25)	1.65 (1.66)	1.18 (1.52)	0.39 (0.19)
5.0—6.0	0.95 (1.36)	0.89 (1.10)	0.83 (0.88)	0.89 (1.11)	0.05 (0.20)
6.0—8.0	1.16 (2.23)	1.14 (1.68)	0.40 (1.53)	0.90 (1.81)	0.35 (0.30)
Total	7.93 (9.61)	5.72 (8.86)	7.69 (12.29)	7.11 (10.25)	0.99 (1.47)

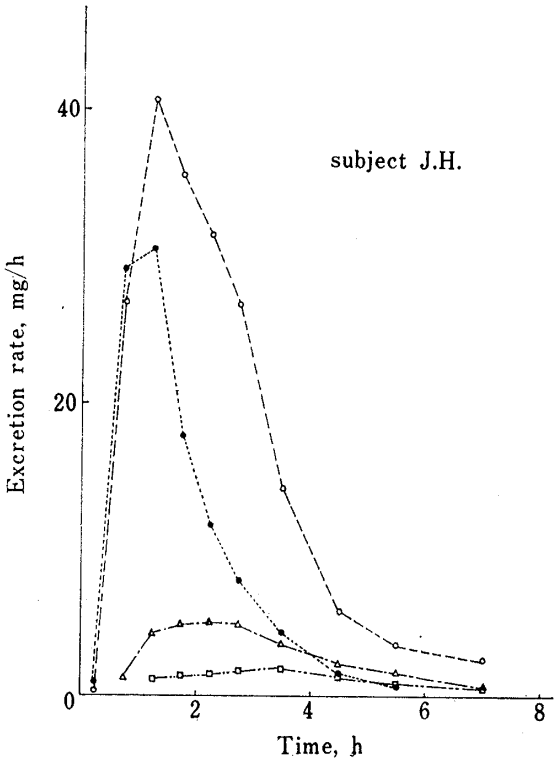


Fig. 3. Time Courses of Urinary Excretion Rates for Clavulanic Acid, Amoxicillin, and Metabolites of Amoxicillin following a Combined Oral Dose of 125 mg Clavulanic Acid and 250 mg Amoxicillin to Human Subject (J.H.)

○----○, AMPC; ●·····●, CA; △——△, AMPA; □——□, AMPM.

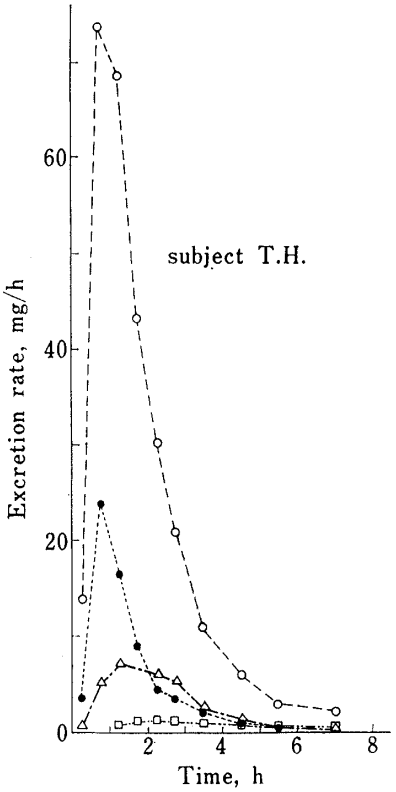


Fig. 4. Time Courses of Urinary Excretion Rates for Clavulanic Acid, Amoxicillin, and Metabolites of Amoxicillin following a Combined Oral Dose of 125 mg Clavulanic Acid and 250 mg Amoxicillin to a Human Subject (T.H.)

Symbols are the same as in Fig. 3.

TABLE V. Statistical Moments for Clavulanic Acid and Amoxicillin following Administration of a Combined Dose of 125 mg Clavulanic Acid and 250 mg Amoxicillin or an Individual Dose of 125 mg Clavulanic Acid or 250 mg Amoxicillin (Given in Parentheses)

	J.H.	T.H.	H.M.	Mean	S.D.
Clavulanic acid					
X_u^∞ (mg)	56.10 (58.57)	34.19 (40.85)	49.71 (35.04)	46.67 (44.82)	9.200(10.01)
MRT (h)	1.765(1.826)	1.580(1.752)	2.094(1.906)	1.813(1.828)	0.213(0.063)
Amoxicillin					
X_u^∞ (mg)	111.1 (138.8)	152.3 (157.5)	130.6 (124.3)	131.4 (140.2)	16.79 (13.59)
MRT (h)	2.583(2.112)	1.985(2.502)	2.498(2.328)	2.355(2.314)	0.264(0.160)
Penicilloic acid					
X_u^∞ (mg)	19.78(28.84)	21.83 (20.99)	33.49 (32.21)	25.03 (27.35)	6.038(4.701)
MRT (h)	3.594(3.715)	2.511(3.162)	3.566 (3.477)	3.224(3.451)	0.504(0.227)
Penamaldic acid					
X_u^∞ (mg)	9.221(14.61)	9.146(12.02)	7.862(13.72)	8.743(13.45)	0.624(1.074)
MRT (h)	4.912(7.555)	8.191(6.355)	3.785(4.369)	5.629(6.093)	1.869(1.314)

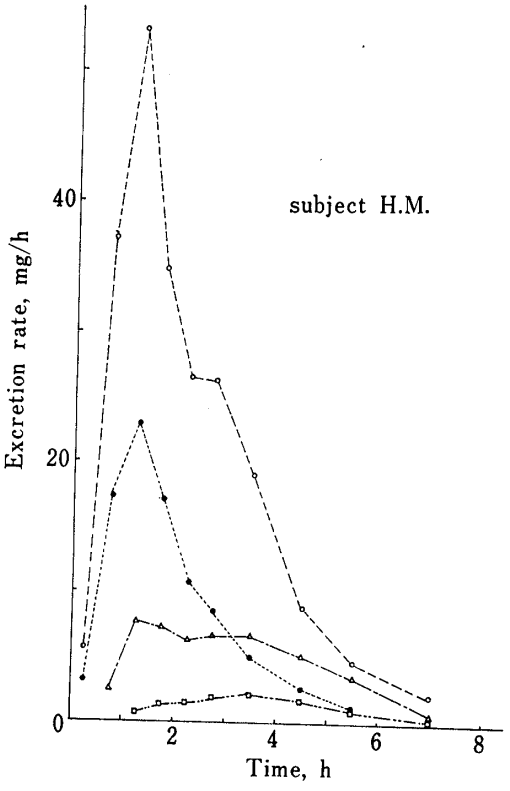


Fig. 5. Time Courses of Urinary Excretion Rates for Clavulanic Acid, Amoxicillin, and Metabolites of Amoxicillin, following a Combined Oral Dose of 125 mg Clavulanic Acid and 250mg Amoxicillin to a Human Subject (H.M.)

Symbols are the same as in Fig. 3.

Discussion

It is sometimes found in pharmacokinetic investigations that inconsistent results arise from differences in analytical methodology. The assays of clavulanic acid so far used have been based on a microbiological method. The method is highly sensitive and allows concentrations of clavulanic acid as low as that in plasma to be determined, but it seems to be less specific and accurate than the HPLC method. No other methods involving chemical and/or spectrometric procedures are available, possibly because clavulanic acid has poor UV and visible absorption above 210 nm,[2] and iodometric and hydroxamate assay methods are unsuitable for quantitative purposes.[4] In the previous paper,[13] we investigated solvent effects on the UV absorption of clavulanic acid and established a HPLC method with UV detection for the determination of clavulanic acid in urine and aqueous solutions. The established method was utilized for the investigation of stability in aqueous solutions[14] and was also employed in the present studies with a slight modification.

The results of moment analysis (Table V) show that the excretion ratio (X_u^∞/D, $D=125$ mg) of clavulanic acid as an intact form ranges from 27 to 45% for the combined dose and from 28 to 47% for the individual dose, and that the mean residence time (MRT), which corresponds to the peak time of urinary excretion rate, ranges from 1.6 to 2.1 h after the combined dose and 1.8 to 1.9 h after the individual dose. The corresponding values for amoxicillin are $X_u^\infty/$

D (D=250 mg)=56 to 73% (including metabolites) for the combined dose and 68 to 76% for the individual dose, and MRT of the intact form=2.0 to 2.6 h for the combined dose and 2.1 to 2.5 h for the individual dose. These results indicate that the weight ratio of clavulanic acid/amoxicillin changes from 1/2 at the time of administration to about 1/3 at the time of excretion during residence in the human body. This suggests that clavulanic acid may be lost more readily than amoxicillin in at least one of the processes of absorption, metabolism, and excretion.

The MRT data show that clavulanic acid is excreted about 30 min faster than amoxicillin on average; the peak times with the combined dose were at 2.3 h for intact amoxicillin and at 1.8 h for clavulanic acid. This may reflect a difference in the peak times of plasma level between clavulanic acid and amoxicillin, because renal clearance of these drugs is expected to be almost constant relative to the dosed amounts. Comparison of the results for moments (Table V) between combined and individual doses indicates that there is no appreciable kinetic interaction between clavulanic acid and amoxicillin used in combination, except that the difference in X_u^∞ value of penamaldic acid between combined and individual doses was significant at the 5% level. It is also apparent from comparison with previous results[13] that there is no difference in pharmacokinetic behavior of clavulanic acid between oral administration of tablets and capsules.

As is clear from the difinition of MRT, the MRT value for a metabolite of amoxicillin given in Table V represents the mean time from administration of amoxicillin to urinary excretion of metabolite, that is, the mean overall time required for the so-called ADME processes. Therefore, in general, the intrinsic MRT value for a metabolite (that is, the mean time period during which a metabolite remains in the human body as its intact form) can be represented by the difference in MRT value between the metabolite and its immediate precursor, because MRT can be additive in linear systems.[12] For instance, since the metabolism of amoxicillin (AMPC) in man is known to involve hydrolysis of the β-lactam ring followed by fission of the C–S bond of the thiazolidine ring,[11] the intrinsic MRT value for penicilloic acid of amoxicillin (AMPA) can be estimated as $MRT_{AMPA}-MRT_{AMPC}$=0.869 h for the combined dose and 1.137 h for the individual dose. The corresponding value for penamaldic acid of amoxicillin (AMPM) is obtained as $MRT_{AMPM}-MRT_{AMPA}$=2.405 h for the combined dose and 2.642 h for the individual dose. These results indicate that penamaldic acid is retained longer in the human body and excreted in less amount than penicilloic acid. The analysis of variance indicated that there are no significant differences in intrinsic MRT values for these two metabolites between the combined and individual doses.

This is the first report on the pharmacokinetic behavior of clavulanic acid in man, though the discussion is limited largely to urinary excretion of the unchanged form. Detailed investigation on the time course of plasma level of clavulanic acid will be carried out.

References and Notes

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