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High Performance Liquid Chromatographic Determination and Pharmacokinetic Investigation of Amino-penicillins and Their Metabolites in Man

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The metabolism and pharmacokinetics of amino-penicillins such as ampicillin (AB-PC), hetacillin (IPAB-PC), talampicillin (TA-PC), amoxycillin (AM-PC) and cyclacillin (AC-PC) in man were investigated. An ion-pair reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of parent penicillins and their metabolites in human urine. The time courses of urinary excretions of unchanged penicillins and metabolites (penicilloic acid (PA) and penamaldic acid (PM)) up to 8 hours after oral administration were determined by using the HPLC method. The moment analysis of urinary excretion rate–time curves gave mean residence times and excretion amounts of the various species. The ratios of excreted amounts to dose (500 mg) at infinite time were estimated to be as follows: (1) AB-PC: 29.4% for unchanged AB-PC, 4.3% for AB-PA and 2.0% for AB-PM (total 35.6%); (2) IPAB-PC: 51.8% for AB-PC, 8.7% for AB-PA and 3.9% for AB-PM (total 63.9%); (3) TA-PC: 68.8% for AB-PC, 10.2% for AB-PA and 4.5% for AB-PM (total 83.5%); (4) AM-PC: 58.9% for unchanged AM-PC, 15.6% for AM-PA and 6.9% for AM-PM (total 81.4%); and (5) AC-PC: 76.6% for unchanged AC-PC and 13.4% for AC-PA (total 90.0%). AC-PC gave no detectable amount of penamaldic acid. The rate constants for absorption, metabolism, and urinary excretion were estimated from the moments of the time course curves by using a one-compartment open model. The pharmacokinetic features of amino-penicillins and the prodrugs, are compared and discussed.

Keywords——ampicillin; hetacillin; talampicillin; amoxycillin; cyclacillin; metabolites; high performance liquid chromatography; pharmacokinetics; mean residence time; rate constants

Introduction

Amino-penicillins such as ampicillin (AB–PC), hetacillin (IPAB–PC), talampicillin (TA–PC), amoxycillin (AM–PC) and cyclacillin (AC–PC) are β-lactam antibiotics widely used in clinical chemotherapy. IPAB–PC and TA–PC are prodrugs which are rapidly transformed to AB–PC during the absorption process.

A literature survey of the extensive investigations on these antibiotics shows that the analytical methods so far employed include microbioassay,[1] chemical assay,[2] spectrophotometry (often combined with chemical procedures)[3–8] and chromatography.[9–16] Among these methods, high performance liquid chromatography (HPLC) has the advantages of high specificity, sensitivity, accuracy and reproducibility in the separation and determination of drugs and their metabolites present in body fluids. Blaha[11] and Tsuji[12] developed the HPLC method for the analysis of penicillins and relevant compounds, but did not apply their method to the analysis of body fluids. Vree[13] reported the reversed phase HPLC analysis of AB–PC and AM–PC in human body fluids, but did not refer to the metabolites. Recently, Lee[14] developed an HPLC method with fluorometric detection for the simultaneous determination of AM–PC and its penicilloic acid in human urine, and discussed some pharmacokinetic considerations. As for the metabolism of amino-penicillins, penicilloic acid is known to be a major metabolite in man. Metabolism of this type, in contrast to side chain biotransformation, is common to most penicillins, because it arises from the degradation of the β-lactam ring. In the previous paper, we found that another skeletal degradation is also involved in the

metabolism of some amino-penicillins in man, that is, cleavage of the C–S bond of the thiazolidine ring occurs in the healthy human body to give penamaldic acid (PM) as a new urinary metabolite.[15,16] Further investigation of the excretion of penamaldic acid showed that its excretion generally accounted for one-third to half of that of penicilloic acid. These preliminary findings prompted us to re-consider the pharmacokinetic profile of amino-penicillins.

The present paper describes an HPLC method for simultaneous assays of the unchanged amino-penicillin, penicilloic acid and penamaldic acid in human urine, and discusses the comparative pharmacokinetic profiles of amino-penicillins and their prodrugs.

Experimental

The structures and abbreviations for the compounds used in this work are presented in Fig. 1.

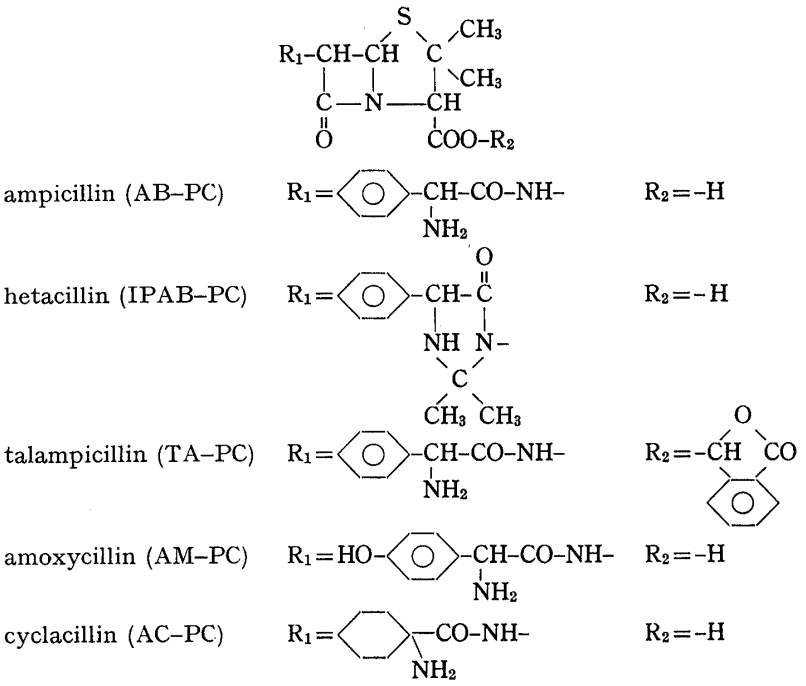

Fig. 1. Structures and Abbreviations of Compounds used in This Work

1. Materials——Anhydrous AB-PC[17] (potency: 983 μg/mg), potassium IPAB-PC[18] (potency: 821 μg/mg), anhydrous TA-PC[19] (potency: 653 μg/mg), trihydrous AM-PC[20] (potency: 845 μg/mg) and anhydrous AC-PC[17] (potency: 1000 μg/mg) were used as standard materials, and AB-PC capsule[17] (Solucillin® 250 mg as potency), IPAB-PC capsule[18] (Natacillin® 250 mg as potency), TA-PC capsule[19] (Yamacillin® 250 mg as potency), AM-PC capsule[20] (Sawacillin® 250 mg as potency) and AC-PC capsule[17] (Vastcillin® 250 mg as potency) were administered to volunteers.

Penicilloic acids were synthesized[16] by hydrolysis of penicillins in alkaline solution according to the following procedures. (1) AB-PA: Anhydrous AB-PC (10 mg) was dissolved in 10 ml of 0.02 N NaOH and the solution was allowed to stand at 37°C for 45 min, then neutralized with 10 ml of 0.02 N HCl. (2) AM-PA: Trihydrous AM-PC (10 mg) was dissolved in 10 ml of 0.04 N NaOH and the solution was left to stand at 20°C for 40 min, then neutralized with 10 ml of 0.04 N HCl. (3) AC-PA: Anhydrous AC-PC (10 mg) was dissolved in 10 ml of 0.025 N NaOH and the solution was left to stand at 20°C for 30 min, then neutralized with 10 ml of 0.025 N HCl. HPLC analysis of these hydrolyzed products gave a single peak in each case, indicating the absence of unreacted penicillin or other degradation products, and the retention time was consistent with that of the corresponding hydrolyzed product obtained by penicillinase treatment (Tokyo Chemical Ind. Co. Ltd., Tokyo, Japan). It was found, therefore, that penicillins were quantitatively (100%) converted to penicilloic acids by these procedures. The penicilloic acids thus obtained remained unchanged for at least several days when kept frozen at −20°C.

The penamaldic acids were synthesized[16] by degradation of penicilloic acid in aqueous $HgCl_2$ solution. A 1 ml portion of the penicilloic acid solution obtained above was mixed with 1 ml aqueous 0.00125% $HgCl_2$ solution, and the mixture was kept standing at room temperature for 3 min. HPLC analysis of the

reaction solution showed no peaks other than those of penamaldic acid and a trace of unreacted penicilloic acid.

Sodium *n*-heptylsulfonate, used as an ion-pairing agent for HPLC, was synthesized by means of the Strecker reaction.[21]

Other chemicals used were of analytical reagent grade.

2. Measurements——A high performance liquid chromatograph (TRI ROTAR, JASCO, Tokyo, Japan) equipped with a variable wavelength UV detector (UVIDEC-100, JASCO) was used in a reversed phase mode with a stationary phase of Nucleosil $10C_{18}$ (M. Nagel, West Germany) packed in a 25 cm × 4.6 mm i.d. stainless steal tubing and operated at ambient temperature. A short pre-column (5 cm × 1.5 mm i.d.) filled with LiChrosorb RP-2 (E. Merck, West Germany) was used to guard the main column. The mobile phase conditions were, (1) AB-PC, IPAB-PC and TA-PC; a mixture of methanol/water (5/8, v/v) containing 0.01 M sodium *n*-heptylsulfonate, 0.005 M NaH_2PO_4 and 1.3% (v/v) of 0.5 N HCl (pH 2.7) was used at a flow rate of 0.8 ml/min and the effluent was monitored at 218 nm, (2) AM-PC; a mixture of methanol/water (2/5, v/v) containing 0.0085 sodium *n*-heptylsulfonate, 0.001 M NaH_2PO_4 and 1.3% (v/v) of 0.5 N HCl (pH 2.8) was used at a flow rate of 0.8 ml/min and the effluent was monitored at 228 nm, and (3) AC-PC; a mixture of methanol/water (295/500, v/v) containing 0.01 M sodium *n*-heptylsulfonate, 0.001 M NaH_2PO_4 and 1.3% (v/v) of 0.5 N HCl (pH 2.8) was used at a flow rate of 0.8 ml/min and the effluent was monitored at 210 nm.

These mobile phases were prepared by micropore filtration (0.45 μm) (Fuji Photo Film Co., Tokyo, Japan) and by degassing a mixture of glass-distilled water and methanol.

3. Administration and Pretreatment——Three healthy male volunteers, 23—24 years old, weighing 58 to 60 kg, participated in this study. The subjects had no past histories of allergic reaction to penicillins and took no drugs other than amino-penicillins during the study. The subjects received two 250 mg capsules (AB-PC, IPAB-PC, TA-PC, AM-PC and AC-PC) together with 100 ml of water after fasting for 12 h and were not permitted to eat until 3 h after dosing. The dosing experiments were conducted in such a way that the three subjects received the same drug at the same time and the same three subjects received different drugs at least two weeks apart.

Urine specimens were collected just before and 0.5, 0.83, 1.25, 1.75, 2.5, 3.5, 4.5, 6.0, 8.0 hours after administration. The volume was measured, then 1 ml portions of urine specimens were each diluted with 1 ml of water, and passed through a 0.45 μm pore-size membrane filter (Fuji Photo Film Co.). A measured amount (5—40 μl) of the filtrate was subjected to HPLC.

4. Calibration Graph——The standard penicillins were dissolved in control urine to make seven different concentrations between 5 and 1000 μg/ml. The standard solutions of penicilloic acids were prepared by diluting the hydrolysis solutions of parent penicillins with control urine to make seven different concentrations between 5 and 500 μg/ml (equivalent to parent penicillin concentration). The calibration graphs (peak hight *vs.* concentration) each showed good linearity and passed through the origin (correlation coefficient, 0.999). The present method quantitates penicillins and penicilloic acids in human urine with almost 100% recovery. The determination of penamaldic acid was achieved by referring to the calibration graph of penicilloic acid, because the peak height of penamaldic acid was proportional to the concentration of penicilloic acid degraded in the $HgCl_2$ solution. The proportionality coefficient, given as the peak height ratio of penamaldic acid to penicilloic acid for a known concentration of penicilloic acid, was 0.882 for AB-PM and 0.904 for AM-PM. Thus, the concentration of penamaldic acid was presented as equivalent to parent penicilloic acid (which is also equivalent to parent penicillin).

Results

1. Time Courses of Urinary Excretion

Figure 2 shows chromatograms of human urine collected after oral administration of amino-penicillins; the broken line shows the background chromatogram due to control urine taken just before the administration. It was found that penamaldic acids (peak (c)) are clearly separated from parent penicillins (peak (a)), penicilloic acids (peak (b)) and endogenous urinary components. The excretion of penamaldic acid as a new metabolite has already been described in our previous paper,[15,16] where the metabolic pathway of amino-penicillin was suggested to be as depicted in Fig. 3.

In order to carry out pharmacokinetic investigations, we determined the time courses of urinary excretion of the various species. Tables I—V show the urinary excretion amounts and excretion rates of unchanged penicillin and metabolites (penicilloic acid and penamaldic acid) after oral administration of AB–PC, IPAB–PC, TA–PC, AM–PC and AC–PC to each of three subjects. The values for the metabolites are given as parent penicillin equivalent.

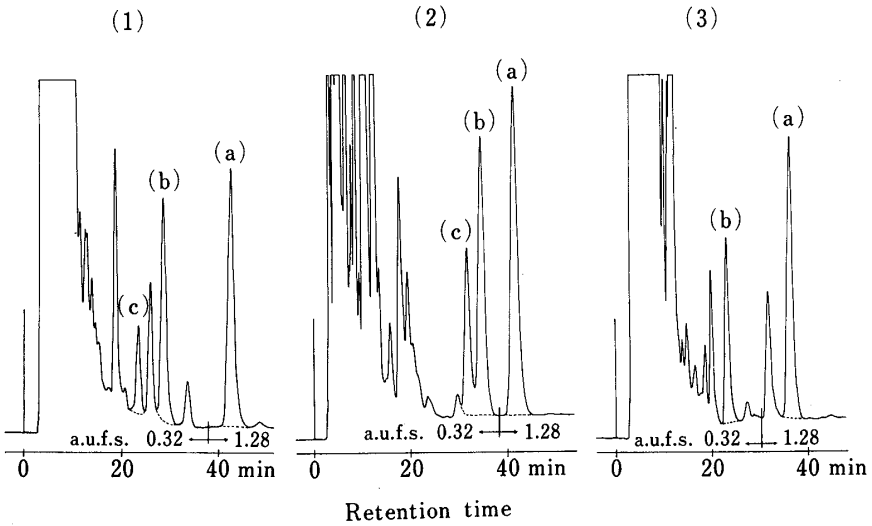

Fig. 2. Chromatogram of Human Urine Specimens after Oral Administration of 500 mg of Amino-penicillins

(1) AB-PC and prodrugs, (2) AM-PC, and (3) AC-PC). Control urine was obtained before drug administration.
Peak: (a) unchanged penicillin, (b) penicilloic acid, (c) penamaldic acid
Conditions: see the text.

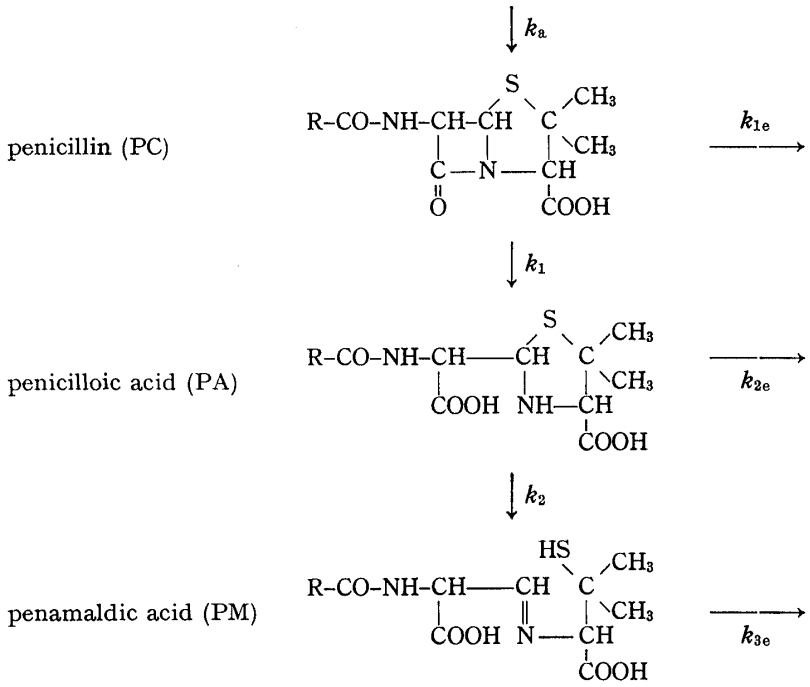

Fig. 3. Metabolic Pathways of Amino-penicillins in Man

The penamaldic acid of cyclacillin (AC-PM) was not detected.

Figures 4—6 indicate the time course curves for excretion rate and cumulative excretion amount by subject J.H.

It was found that the excretion of unchanged penicillin was almost completed at 6 hours after dosing, while a very slight increase in the excretion of penamaldic acid continued. The peak time for the excretion of penicilloic acid was about one hour later than that for unchanged penicillin.

TABLE I. Urinary Excretion Amounts and Rates of AB–PC and Metabolites following a Single Oral Administration (500 mg Capsule)

h		PC		PA		PM	
		mg	mg/h	mg	mg/h	mg	mg/h
0.5	J.H.	0.94	1.87	—	—	—	—
(0.25)	Y.K.	2.74	5.48	—	—	—	—
	Y.M.	0.14	0.27	—	—	—	—
0.83		7.12	21.38	0.43	1.28	—	—
(0.66)		12.01	36.06	0.79	2.36	0.16	0.49
		2.75	8.25	—	—	—	—
1.25		15.87	38.06	1.03	2.47	—	—
(1.04)		24.83	59.56	1.45	3.49	0.39	0.95
		7.69	18.45	0.36	0.84	0.09	0.21
1.75		23.14	46.29	1.73	3.46	0.46	0.92
(1.5)		24.61	49.23	2.24	4.48	0.58	1.16
		16.65	33.30	1.00	2.00	0.25	0.50
2.5		22.73	30.31	3.67	4.90	0.77	1.03
(2.12)		26.14	34.86	3.24	4.32	1.07	1.43
		36.54	48.72	2.38	3.17	0.39	0.52
3.5		24.49	24.49	4.43	4.43	1.42	1.42
(3.0)		21.09	21.09	3.44	3.44	1.64	1.64
		33.61	33.61	3.46	3.46	0.86	0.86
4.5		14.71	14.71	3.59	3.59	1.64	1.64
(4.0)		11.53	11.53	2.81	2.81	1.61	1.61
		22.26	22.26	3.93	3.93	1.07	1.07
6.0		11.13	7.42	3.21	2.14	1.72	1.15
(5.25)		7.15	4.77	2.47	1.64	1.56	1.04
		21.40	14.27	3.73	2.48	1.37	0.92
8.0		5.73	2.87	2.45	1.22	1.55	0.78
(7.0)		3.82	1.91	1.88	0.94	1.17	0.58
		13.87	6.93	2.01	1.01	1.09	0.54
Total		125.87		20.53		7.57	
		133.92		18.32		8.19	
		154.91		16.87		5.13	
		153.97					
		160.43					
		176.91					

TABLE II. Urinary Excretion Amounts and Rates of IPAB–PC and Metabolites following a Single Oral Administration (500 mg Capsule)

h		PC		PA		PM	
		mg	mg/h	mg	mg/h	mg	mg/h
0.50	J.H.	1.57	3.14	—	—	—	—
(0.25)	Y.K.	1.06	2.11	—	—	—	—
	Y.M.	0.20	0.41	—	—	—	—
0.83		13.23	39.73	0.82	2.46	0.18	0.53
(0.66)		12.00	36.02	0.66	1.98	0.08	0.23
		3.25	9.75	0.26	0.78	0.11	0.33
1.25		31.66	75.93	2.78	6.66	0.46	1.09
(1.04)		39.65	95.09	2.37	5.69	0.35	0.83
		14.22	34.09	0.72	1.72	0.13	0.30
1.75		43.40	86.80	4.27	8.54	0.74	1.49
(1.5)		43.79	87.57	4.15	8.31	0.70	1.39
		35.02	70.05	1.51	3.02	0.24	0.48
2.5		50.55	67.40	7.48	9.97	1.36	1.18
(2.12)		43.22	57.62	6.91	9.21	2.09	2.78
		59.02	78.70	4.78	6.37	0.77	1.03

h	PC		PA		PM	
	mg	mg/h	mg	mg/h	mg	mg/h
3.5	49.81	49.81	8.02	8.02	3.16	3.16
(3.0)	41.78	41.78	8.13	8.13	4.13	4.13
	55.62	55.62	9.59	9.59	2.47	2.47
4.5	27.10	27.10	5.61	5.61	2.85	2.85
(4.0)	21.60	21.60	5.74	5.74	2.44	2.44
	33.96	33.96	8.47	8.47	2.13	2.13
6.0	20.36	12.80	4.99	3.33	2.88	1.92
(5.25)	19.41	12.94	4.82	3.21	2.36	1.57
	27.74	18.49	6.44	4.29	2.37	1.58
8.0	12.38	6.19	4.17	2.08	2.55	1.27
(7.0)	10.70	5.35	3.83	1.92	2.08	1.04
	18.72	9.36	4.95	2.48	1.86	0.93
Total	248.90		38.14		14.17	
	233.20		36.61		14.21	
	247.76		36.73		10.07	
	301.21					
	284.02					
	294.56					

Table III. Urinary Excretion Amounts and Rates of TA–PC and Metabolites following a Single Oral Administration (500 mg Capsule)

h		PC		PA		PM	
		mg	mg/h	mg	mg/h	mg	mg/h
0.5	J.H.	0.22	0.44	—	—	—	—
(0.25)	Y.K.	9.49	18.98	—	—	—	—
	Y.M.	0.68	1.38	—	—	—	—
0.83		21.05	63.22	0.40	1.19	—	—
(0.66)		43.02	129.18	1.24	3.73	0.46	1.37
		7.44	22.33	0.63	1.90	—	—
1.25		51.25	123.55	2.58	6.18	0.22	0.52
(1.04)		60.01	143.90	2.75	6.58	0.82	1.97
		37.26	89.35	1.50	3.60	0.48	1.16
1.75		63.47	126.94	5.11	10.23	0.43	0.85
(1.5)		50.38	100.77	4.76	9.52	1.46	2.93
		63.45	126.90	2.82	5.64	0.76	1.51
2.5		59.56	79.41	9.50	12.67	1.49	1.98
(2.12)		54.31	72.41	8.51	11.34	2.58	3.44
		72.98	97.31	7.80	10.39	1.86	2.48
3.5		57.73	57.73	11.74	11.74	4.04	4.04
(3.0)		45.82	45.82	9.79	9.79	3.74	3.74
		68.45	68.45	12.07	12.07	3.35	3.35
4.5		32.73	32.73	8.90	8.90	3.79	3.79
(4.0)		21.11	21.11	6.42	6.42	2.50	2.50
		43.22	43.22	9.54	9.54	4.27	4.27
6.0		25.09	16.73	6.23	4.15	4.26	2.84
(5.25)		12.80	8.53	5.02	3.34	2.87	1.91
		40.34	26.89	7.32	4.88	4.57	3.05
8.0		13.41	6.70	4.38	2.19	2.77	1.38
(7.0)		7.00	3.50	3.79	1.90	2.46	1.23
		24.05	12.02	4.15	2.08	3.26	1.63
Total		324.76		48.84		16.98	
		303.94		42.27		16.89	
		357.86		45.83		18.55	
		390.58					
		363.10					
		422.24					

Table IV. Urinary Excretion Amounts and Rates of AM–PC and Metabolites following a Single Oral Administration (500 mg Capsule)

h		PC		PA		PM	
		mg	mg/h	mg	mg/h	mg	mg/h
0.5	J.H.	2.30	4.59	—	—	—	—
(0.25)	Y.K.	3.75	7.50	0.27	0.53	—	—
	Y.M.	1.72	3.43	—	—	—	—
0.83		20.38	61.19	2.12	6.37	0.06	0.18
(0.66)		33.14	99.51	3.20	9.61	0.47	1.41
		6.75	20.28	0.70	2.11	—	—
1.25		42.69	102.37	5.51	13.20	0.78	1.86
(1.04)		45.86	109.97	6.25	14.99	1.09	2.61
		16.98	40.71	2.46	5.91	0.11	0.26
1.75		54.15	108.31	7.97	15.95	1.53	3.05
(1.5)		46.06	92.12	8.54	17.07	2.11	4.22
		34.58	69.16	5.22	10.44	0.31	0.62
2.5		54.46	72.61	14.11	18.81	3.32	4.42
(2.12)		43.90	58.53	11.07	14.76	3.63	4.80
		66.50	88.67	12.35	16.47	1.48	1.98
3.5		51.86	51.86	15.52	15.52	7.67	7.67
(3.0)		35.89	35.89	9.30	9.30	5.94	5.94
		69.20	69.20	18.70	18.70	4.21	4.21
4.5		27.78	27.78	12.30	12.30	5.93	5.93
(4.0)		14.57	14.57	7.01	7.01	4.84	4.84
		43.49	43.49	16.73	16.73	5.59	5.59
6.0		20.21	13.48	9.19	6.13	6.66	4.44
(5.25)		9.34	6.23	6.15	4.10	3.88	2.59
		37.59	25.06	15.21	10.14	5.89	3.92
8.0		11.83	5.91	5.74	2.87	5.65	2.83
(7.0)		6.96	3.48	3.20	1.60	2.24	1.12
		28.62	14.31	9.88	4.94	4.87	2.44
Total		285.66		72.57		31.60	
		239.47		54.99		24.21	
		305.42		81.26		22.45	
		389.83					
		318.67					
		409.13					

Table V. Urinary Excretion Amounts and Rates of AC–PC and Metabolites following a Single Oral Administration (500 mg Capsule)

h		PC		PA	
		mg	mg/h	mg	mg/h
0.5	J.H.	9.30	18.59	1.42	2.84
(0.25)	Y.K.	98.31	196.62	6.06	12.11
	Y.M.	9.15	18.31	—	—
0.83		99.81	299.73	2.51	7.54
(0.66)		91.43	274.55	8.18	24.56
		36.08	108.35	3.85	11.57
1.25		130.35	312.60	8.58	20.58
(1.04)		89.07	213.60	12.12	29.05
		83.91	201.22	5.09	12.22
1.75		57.04	114.08	14.12	28.23
(1.5)		41.60	83.21	11.30	22.60
		97.41	194.83	8.67	17.34
2.5		61.00	81.34	11.69	15.59
(2.12)		20.07	26.76	10.91	14.54
		90.90	121.20	17.85	23.81

h	PC		PA	
	mg	mg/h	mg	mg/h
3.5	26.02	26.02	11.05	11.05
(3.0)	9.65	9.65	8.96	8.96
	43.32	43.32	17.71	17.71
4.5	8.45	8.45	6.85	6.85
(4.0)	3.06	3.06	4.44	4.44
	16.40	16.40	12.60	12.60
6.0	4.16	2.77	2.84	1.89
(5.25)	1.59	1.06	2.43	1.62
	9.10	6.07	5.24	3.50
8.0	2.45	1.22	0.90	0.45
(7.0)	0.56	0.28	0.99	0.50
	5.33	2.67	1.90	0.95
Total	398.59		59.96	
	355.34		65.38	
	391.61		72.93	
	458.55			
	420.72			
	464.54			

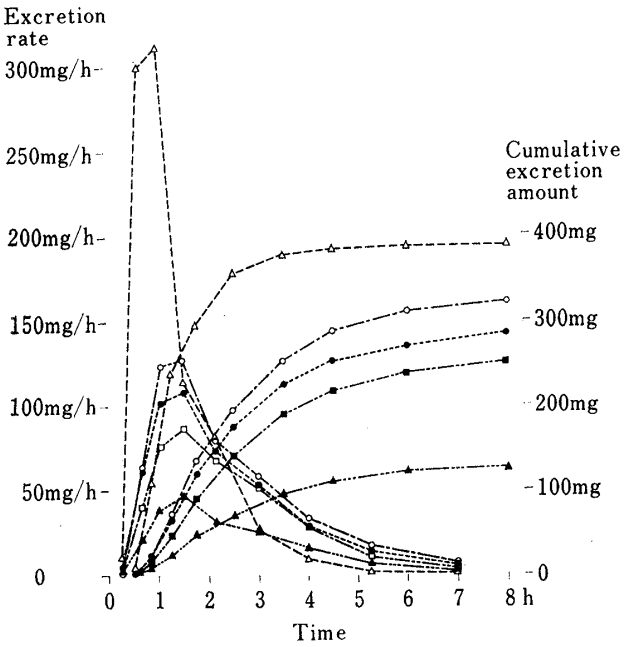

Fig. 4. Time Course Curves for Cumulative Urinary Excretion and Urinary Excretion Rate of Unchanged Penicillin after an Oral Dose of 500 mg of AB–PC (-----▲—), IPAB–PC (—■—), TA–PC (——○—), AM–PC (·····●··) or AC–PC (---△-) to Subject J.H.

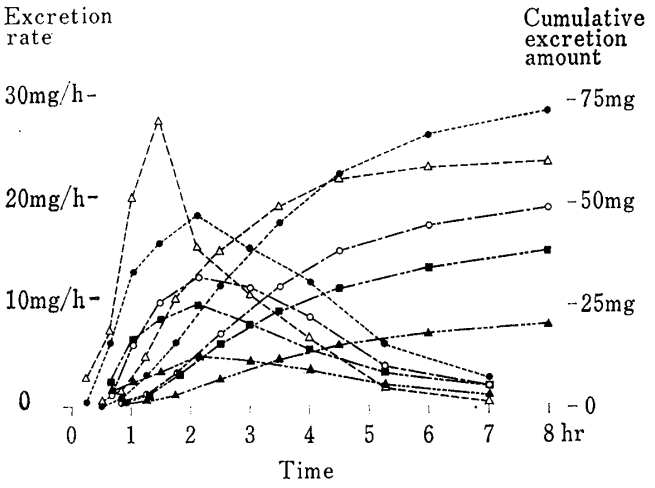

Fig. 5. Time Course Curves for Cumulative Urinary Excretion and Urinary Excretion Rate of Penicilloic Acid after an Oral Dose of 500 mg of AB–PC (-----▲—), IPAB–PC (—■—), TA–PC (——○—), AM–PC (···●···) or AC–PC (---△---) to Subject J.H.

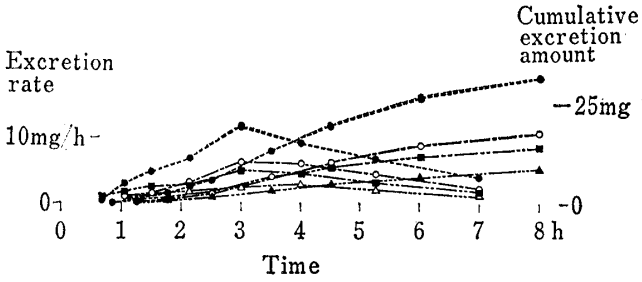

Fig. 6. Time Course Curves for Cumulative Urinary Excretion and Urinary Excretion Rate of Penamaldic Acid after an Oral Dose of 500 mg of AB–PC (-----▲—), IPAB–PC (—■—), TA–PC (——○—) or AM–PC (···●···) to Subject J.H.

2. Pharmacokinetic Calculations

The evaluation of pharmacokinetic parameters was achieved by moment analysis of the time course data. The statistical moments for the urinary excretion rate–time curve, dx_u/dt, have been defined as follows[22]:

$$X_u^\infty = \int_0^\infty (dX_u/dt)dt \quad (1)$$

$$MRT_u = \int_0^\infty t(dX_u/dt)dt / X_u^\infty \quad (2)$$

where X_u^∞ is the zero moment representing area under the urinary excretion rate–time curve, and MRT_u is the first normal moment representing mean residence time of the urinary excretion rate–time curve. The significance and calculation of these moments were described in the previous paper.[22] Provided that amino-penicillins are biotransformed according to the metabolic pathways given in Fig. 3, where all the steps, inclusive of absorption and excretion, can be regarded as linear processes, the rate constants can be related to the statistical moments as follws

$$X_1^\infty = k_{1e}FD/(k_1+k_{1e}) \quad (3)$$

$$MRT_1 = 1/k_a + 1/(k_1+k_{1e}) \quad (4)$$

$$X_2^\infty = k_1k_{2e}X_1^\infty/k_{1e}(k_2+k_{2e}) \quad (5)$$

$$MRT_2 = 1/(k_2+k_{2e}) + MRT_1 \quad (6)$$

$$X_3^\infty = k_2X_2^\infty/k_{2e} \quad (7)$$

$$MRT_3 = 1/k_{3e} + MRT_2 \quad (8)$$

where D is dose, F is the fraction of the dose absorbed, the subscripts a, e, 1, 2 and 3 specify absorption, urinary excretion, unchanged penicillin, penicilloic acid and penamaldic acid, respectively, and X's and k's are the excreted amounts and rate constants specified by the subscripts, respectively. In using Eqs. 1 and 2, the moments were calculated by rectangular

Table VI. Pharmacokinetic Parameters for AB–PC, IPAB–PC, TA–PC, AM–PC and AC–PC (500 mg) administered to J.H.

	AB–PC	IPAB–PC	TA–PC	AM–PC	AC–PC
X_T^∞ (mg)	166.6	324.7	414.5	420.7	460.1
X_1^∞ (mg)	132.0	260.9	337.5	297.4	399.4
X_2^∞ (mg)	23.9	44.2	54.8	80.3	60.7
X_3^∞ (mg)	10.7	19.6	22.6	43.0	—
F	0.333	0.649	0.830	0.842	0.920
f_1	0.793	0.804	0.814	0.707	0.868
f_2	0.144	0.136	0.132	0.191	0.132
f_3	0.064	0.060	0.054	0.102	—
T_0 (h)	0.21	0.21	0.25	0.22	0.22
MRT_1 (h)	2.70	2.87	2.73	2.49	1.29
MRT_2 (h)	4.15	3.98	3.85	3.66	2.46
MRT_3 (h)	5.82	5.64	5.48	5.54	—
MRT_2-MRT_1 (h)	1.45	1.11	1.12	1.17	1.17
MRT_3-MRT_2 (h)	1.67	1.66	1.63	1.88	—
k_a (h^{-1})	1.22	1.28	1.50	1.56	2.71
k_{1e} (h^{-1})	0.42	0.43	0.45	0.38	0.94
k_{2e} (h^{-1})	0.56	0.62	0.63	0.68	1.05
k_{3e} (h^{-1})	0.60	0.60	0.61	0.53	—
k_1 (h^{-1})	0.11	0.10	0.10	0.16	0.14
k_2 (h^{-1})	0.25	0.28	0.26	0.36	—

f: fraction of total excretion amount.
T_0: lag time.

integration with extrapolation of the time course curve to infinite time according to a monoexponential equation, which was determined by the least-squares method using the last three to eight points on the urinary excretion rate–time curve. The calculation of the moments according to Eqs. 1 and 2 and the estimation of pharmacokinetic parameters from the moments

Table VII. Pharmacokinetic Parameters for AB–PC, IPAB–PC, TA–PC, AM–PC and AC–PC (500 mg) administered to Y.K.

	AB–PC	IPAB–PC	TA–PC	AM–PC	AC–PC
X_T^∞ (mg)	168.7	304.7	377.0	331.3	421.6
X_1^∞ (mg)	137.1	244.0	309.0	243.9	355.5
X_2^∞ (mg)	21.1	42.2	46.6	59.1	66.1
X_3^∞ (mg)	10.5	18.5	21.4	28.3	—
F	0.337	0.609	0.754	0.663	0.842
f_1	0.813	0.801	0.820	0.736	0.843
f_2	0.125	0.139	0.123	0.179	0.157
f_3	0.062	0.061	0.057	0.085	—
T_0 (h)	0.18	0.22	0.18	0.22	0.00
MRT_1 (h)	2.40	2.78	2.17	2.18	1.01
MRT_2 (h)	3.62	3.98	3.59	3.20	2.02
MRT_3 (h)	4.98	5.14	4.78	4.29	—
MRT_2-MRT_1 (h)	1.22	1.20	1.42	1.02	1.01
MRT_3-MRT_2 (h)	1.36	1.16	1.19	1.09	—
k_a (h^{-1})	1.54	1.64	2.12	2.28	4.67
k_{1e} (h^{-1})	0.52	0.41	0.54	0.48	1.06
k_{2e} (h^{-1})	0.44	0.58	0.48	0.67	0.99
k_{3e} (h^{-1})	0.95	0.87	0.84	0.92	—
k_1 (h^{-1})	0.12	0.10	0.11	0.17	0.20
k_2 (h^{-1})	0.22	0.25	0.22	0.32	—

f: fraction of total excretion amount.
T_0: lag time.

Table VIII. Pharmacokinetic Parameters for AB–PC, IPAB–PC, TA–PC, AM–PC and AC–PC (500 mg) administered to Y.M.

	AB–PC	IPAB–PC	TA–PC	AM–PC	AC–PC
X_T^∞ (mg)	203.6	329.5	461.0	468.5	468.6
X_1^∞ (mg)	176.9	271.9	386.7	340.9	394.1
X_2^∞ (mg)	19.1	43.6	50.6	96.4	74.5
X_3^∞ (mg)	7.6	14.0	23.7	31.2	—
F	0.398	0.659	0.922	0.937	0.937
f_1	0.869	0.825	0.839	0.728	0.841
f_2	0.094	0.132	0.110	0.206	0.159
f_3	0.037	0.042	0.051	0.067	—
T_0 (h)	0.24	0.23	0.22	0.17	0.17
MRT_1 (h)	3.73	3.53	3.29	3.68	1.97
MRT_2 (h)	4.80	4.42	3.92	4.47	2.95
MRT_3 (h)	6.21	5.76	5.11	5.78	—
MRT_2-MRT_1 (h)	1.07	0.89	0.63	0.78	0.98
MRT_3-MRT_2 (h)	1.41	1.34	1.19	1.31	—
k_a (h^{-1})	0.87	0.96	1.20	0.92	1.11
k_{1e} (h^{-1})	0.37	0.36	0.37	0.30	0.93
k_{2e} (h^{-1})	1.25	0.87	1.07	0.96	1.02
k_{3e} (h^{-1})	0.52	0.75	0.84	0.76	—
k_1 (h^{-1})	0.06	0.08	0.07	0.11	0.18
k_2 (h^{-1})	0.49	0.27	0.50	0.31	—

f: fraction of total excretion amount.
T_0: lag time.

according to Eqs. 3—8 were carried out on a personal computer (PET 2001, Commodore) with programming in BASIC. The results thus obtained are given in Tables VI—VIII.

Discussion

Although many papers have described some pharmacokinetic features of amino-penicillins in man, none have referred to the rate profiles of the metabolites, whose amounts are as largeb as 20—30% of the total excretion amounts. Further, the methods so far used for the metabolite assays have been limited to the determination of AM–PA by HPLC,[14] or to the indirect determination of AB–PA by fluorometry.[4] None could detect, much less assay, the urinary excretion of penamaldic acid. The present results allow us to discuss the overall pharmacokinetic features of amino-penicillins in man.

AB-PC and Prodrugs

Tables VI—VIII show that the average excretion ratios at infinite time, *i.e.* the ratios of the excreted amounts of penicillin (X_1^∞), penicilloic acid (X_2^∞) and penamaldic acid (X_3^∞) per dose (D=500 mg) as an average of three subjects, are: (1) AB–PC: 29.4%, 4.3%, 2.0% (total 35.6%); (2) IPAB–PC: 51.8%, 8.7%, 3.9% (total 63.9%); and (3) TA–PC: 68.8%, 10.2%, 4.5% (total 83.5%), respectively. The penicilloic acid and penamaldic acid metabolites of the prodrugs are designated as AB–PA and AB–PM, respectively. These results clearly show that the total excretions of IPAB–PC and TA–PC are 1.8 times and 2.4 times larger than that of AB–PC, respectively, reflecting the better absorption of these prodrugs. However, the metabolite fractions, f_1, f_2 and f_3 show that there are no significant differences among AB–PC, IPAB–PC and TA–PC. This means that from a quantitative viewpoint, these prodrugs are absorbed better than but metabolized in the same way as AB–PC.

From a kinetic viewpoint, the analysis of variance for rate constants and mean residence times indicated that k_a of TA–PC was appreciably larger than those of AB–PC and IPAB–PC ($p<0.05$), whereas there were no significant differences in the rate constants for urinary excretion (k_{1e}, k_{2e}, k_{3e}) and metabolism (k_1, k_2), and in mean residence time (MRT_1, MRT_2, MRT_3) among AB–PC, IPAB–PC and TA–PC. The values of MRT_2-MRT_1 and MRT_3-MRT_2, which represent mean times for the biotransformations from penicillin to penicilloic acid and from penicilloic acid to penamaldic acid, respectively, also did not show significant differences. Thus, it follows that TA–PC and IPAB–PC are absorbed better than AB–PC, and that TA–PC is absorbed faster than IPAB–PC and AB–PC, while their rate processes of metabolism and urinary excretion are equivalent. This is in accordance with the well-known facts that TA–PC and IPAB–PC are rapidly changed to AB–PC soon after absorption through the GI tract.

AB-PC, AM-PC and AC-PC

The excretion ratios of unchanged penicillin, penicilloic acid and penamaldic acid per dose (500 mg) differed greatly among AB–PC, AM–PC and AC–PC. The values calculated from Tables VI—VIII are: (1) AB–PC: unchanged from 29.4%, AB–PA 4.3% and AB–PM 2.0% (total 35.6%); (2) AM–PC: unchanged from 58.9%, AM–PA 15.6% and AM–PM 6.9% (total 81.4%); and (3) AC–PC: unchanged form 76.6% and AC–PA 13.4% (total 90.0%). It is readily apparent that AM–PC and AC–PC are better absorbed and excreted on average, 2.3 and 2.5 times better than AB–PC. The results for metabolite fractions indicate that AM–PC shows somewhat different behavior from AB–PC and AC–PC; the analysis of variance for f_1, f_2 and f_3 values indicated that the extents of biotransformation of AM–PC to penicilloic acid and penamaldic acid are significantly larger than those of AB–PC and AC–PC ($p<0.05$). This was also confirmed from the viewpoint of rate constants. The values for the mean residence time of unchanged penicillin and penicilloic acid (*i.e.* MRT_1 and MRT_2) of AC–PC are significantly smaller than those of AB–PC and AM–PC, while the excretion rate constant of

unchanged AC–PC (k_{1e}) is larger than those of AB–PC and AM–PC. The differences between them were found to be significant at the 1% level. Comparison of mean time (*i.e.* $MRT_2 - MRT_1$) and rate constant (k_1) for the metabolism of the parent penicillin to penicilloic acid indicated that the hydrolysis of AB–PC occurred significantly more slowly than those of AM–PC and AC–PC ($0.01 < p < 0.05$). No significant differences were observed in other cases. Thus, it follows that AC–PC undergo rapid absorption and rapid excretion as an intact form, resulting in a short residence time compared with those of AB–PC and AM–PC while AM–PC undergoes fairly good absorption but is metabolized somewhat more readily than AB–PC and AC–PC. AB–PC shows a comparatively low extent of absorption and undergoes slow metabolism to penicilloic acid. The small bioavailability of AB–PC can thus be improved by the use of prodrugs.

The present investigations on the urinary excretions of amino-penicillins and prodrugs in man revealed that these penicillins are metabolized to penicilloic acids, which are further transformed into penamaldic acids. The excretion of penamaldic acid is about 2—7% of the dose which corresponds to about 40% of the amount of penicilloic acid. Detailed analysis of the excretion rates made possible a comparative discussion on the overall pharmacokinetic profiles.

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