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High Performance Liquid Chromatographic Analysis and Pharmacokinetic Investigation of Oxacillin and Its Metabolites in Man

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Pharmacokinetic evaluation of oxacillin in man was carried out by detailed investigation of the urinary excretion profiles of unchanged oxacillin and metabolites. A new metabolite, penicilloic acid of the 5-hydroxymethyl derivative of oxacillin, was discovered in human urine excreted after oral administration of oxacillin. This metabolite was formed by cleavage of the β-lactam ring of the 5-hydroxymethyl derivative (a known active metabolite of oxacillin), not by hydroxylation of the 5-methyl group on the isoxazolyl moiety of penicilloic acid of oxacillin. The time courses of excretion of the new metabolite as well as unchanged oxacillin and known metabolites (penicilloic acid and 5-hydroxymethyl derivative) were measured by HPLC analysis of urine excreted after oral administration of oxacillin tablets to human subjects. The values for cumulative excretion amount at infinite time (X^{∞}) and mean residence time (MRT) for each species were estimated by moment analysis of excretion rate *vs.* time curves. The rate constants for absorption, metabolism, and urinary excretion were calculated by non-linear least-squares fittings of the time course data using a one compartment model. The results indicate that the excretion ratio (X^{∞}/D, $D=500$ mg) and MRT value as averages of five subjects are 27.4% and 1.76 h for unchanged oxacillin, 16.1% and 3.79 h for penicilloic acid, 22.2% and 2.04 h for 5-hydroxymethyl derivative, and 22.4% and 3.89 h for penicilloic acid of the 5-hydroxymethyl derivative. The conversion ratio at each elimination step and the MRT value intrinsic to each metabolite were evaluated from the results of moments.

Keywords——oxacillin; penicilloic acid; active metabolite; metabolism; excretion; pharmacokinetics; moment analysis; mean residence time; rate constant; high performance liquid chromatography

The investigation of drug metabolism can provide important information in establishing rational dosage regimens in clinical chemotherapy. Oxacillin is one of the isoxazolylpenicillins effective in treating infections caused by penicillin G-resistant *staphylococci*.[1] This drug is known to be biotransformed to a considerable extent in man to an active metabolite,[2] whose antibiotic activity is of almost the same order of magnitude as that of the parent penicillin.[3] Van Harken *et al.* indicated that dicloxacillin is metabolized in man to 6-[3-(2,6-dichlorophenyl)-5-hydroxymethyl-4-isoxazolecarboxamido]-penicillanic acid *i.e.* the 5-hydroxymethyl derivative of the parent penicillin.[4] However, it was not clear whether the active metabolite of oxacillin has a similar structure, although the retention behavior on a reversed phase thin-layer chromatography suggested an analogous structure.[5] Thijssen subsequently found that the active metabolites of isoxazolylpenicillins in the rat are 5-hydroxymethyl analogs common to oxacillin, cloxacillin, dicloxacillin, and flucloxacillin.[6] In the previous paper we reported that a similar metabolism of oxacillin occurs in man, and we achieved the isolation and gas chromatography–mass spectrum (GC–MS) identification of the active metabolite as the 5-hydroxymethyl derivative of oxacillin.[7] Recently Thijssen described high performance liquid chromatographic (HPLC) analysis of isoxazolylpenicillins and their metabolites in serum and urine,[8] but did not detect the presence of a new metabolite which is revealed in the present paper to be penicilloic acid of the 5-hydroxymethyl derivative of oxacillin. Since the excreted amount of this new metabolite accounts for more than 20% of the dose, which is comparable to those of the parent penicillin and known active metabolite, it should be taken into account in the pharmacokinetic evaluation of oxacillin. The pharma-

cokinetic features of oxacillin in man, however, are not yet known in detail, although pharmacokinetic parameters for isoxazolylpenicillins were estimated by using a one compartment model[9] and a two compartment model[10] without regard to their metabolism, the plasma half-life of unchanged cloxacillin was measured by using ^{35}S-labeled compound,[11] and the differences in blood levels[12] and in urinary recoveries[13] among isoxazolylpenicillins were comparatively discussed. Recently Thijseen evaluated pharmacokinetic parameters for cloxacillin and flucloxacillin taking their penicilloic acids and active metabolites into account, but unfortunately the results were obtained from only one subject and did not include oxacillin.[8]

The present paper describes the discovery of a new metabolite of oxacillin in man and simultaneous HPLC determination of the new metabolite along with unchanged oxacillin and known metabolites (penicilloic acid and 5-hydroxymethyl derivative), and presents a pharmacokinetic reevaluation through moment analysis and compartment model analysis of their urinary excretion rate *vs.* time curves.

Experimental

Reagents and Materials——Oxacillin sodium used as a standard material and oxacillin tablets (Staphcillin V, 250 mg as potency) given to subjects were supplied by Banyu Seiyaku Co. (Tokyo). Acetic acid and sodium acetate were commercial products of reagent grade. Methanol and water were purified by distillation and degassed prior to preparation of the mobile phase for chromatography.

High Performance Liquid Chromatography——A high performance liquid chromatograph (ALC/GPC 204, Waters Assoc.) equipped with a UV-detector (254 nm, Model 440, Waters Assoc.) was used in a reversed phase mode with a stationary phase of LiChrosorb RP-18 (E. Merck Co.) packed in a stainless steel tube (4.6 mm i.d. × 25 cm) and a mobile phase of 0.03 M acetate buffer (pH 5.6)/methanol = 2/1 (v/v), whose flow rate was maintained at 1.5 ml/min. A short precolumn (4.6 mm i.d. × 5 cm) packed with LiChrosorb RP-2 was used to guard the main column. All operations were carried out under ambient conditions.

Drug Administration and Sample Preparation——Oxacillin tablets were given orally with 200 ml water to five healthy male subjects (each receiving 250 mg × 2), 22 to 31 years of age, weighing 55 to 68 kg. The subjects had remained drug-free for at least one week previously, and had fasted for 12 h before administration. Urine samples were collected just before and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, and 10.0 h after administration. After measurement of volume, the urine was passed through a 0.45 μm pore size membrane filter (Fuji Photo Film Co.), and a 5.0 μl portion of the filtrate was subjected to HPLC analysis. The remaining portion was reserved for isolation of active metabolite.

Isolation of the 5-Hydroxymethyl Derivative of Oxacillin——About 250 ml of urine collected at 1 to 2.5 h after administration was chromatographed in portions on a reversed phase column (2.5 cm i.d. × 31 cm) packed with LiChroprep RP-8 (40 to 63 μm particle diameter, E. Merck Co.) using 0.1 M acetate buffer (pH 5.1)/methanol = 6/5 (v/v) as a developing solvent. The eluate was analyzed by HPLC and the fraction containing active metabolite (peak 3 in Fig. 1) was collected. After removal of the solvent by careful evaporation at 35°C under reduced pressure, the residue was dissolved in a small portion of water and rechromatographed on the same column with 0.2 M acetate buffer (pH 5.2)/methanol = 5/3 (v/v). The metabolite fraction was collected and concentrated in the same manner as above, and developed again on the same column with water/methanol = 1/1 (v/v) in order to remove buffer salt. The fraction following the rapidly eluted buffer salt was collected. Removal of the solvent followed by lyophilization finally gave a small amount (not weighed) of a fleecy white solid. The HPLC analysis indicated a single peak with a retention time equivalent to that of peak 3 in Fig. 1. This metabolite had been identified by GC–MS as the 5-hydroxymethyl derivative of oxacillin.[7]

Calibration Graphs——The urinary concentrations of unchanged oxacillin and metabolites were determined by referring to the regression lines (peak height *vs.* concentration) obtained by using 5.0 μl portions of the following standard solutions. The standard solutions of oxacillin and its 5-hydroxymethyl derivative were prepared by dissolving accurately weighed amounts of standard oxacillin and the isolated metabolite in control urine to make several different concentrations ranging from 83 to 1620 μg/ml and from 74 to 920 μg/ml, respectively. The standard solutions of penicilloic acid of oxacillin and penicilloic acid of the 5-hydroxymethyl derivative were prepared by adding 1.0 ml of 1 N NaOH to 1.0 ml of each of the above mentioned standard solutions, and keeping the mixture at 37°C for 7 min followed by neutralization with 2.0 ml of 0.5 N HCl. This procedure resulted in 100% hydrolysis of the β-lactam ring to yield corresponding penicilloic acids without further degradation. These standard solutions covered concentrations of penicilloic acid of oxacillin and penicilloic acid of the 5-hydroxymethyl derivative ranging from 21 to 405 μg/ml and from 18 to 230 μg/ml as oxacillin equivalent, respectively. All the calibration graphs thus obtained passed through

the origin with correlation coefficients above 0.999.

Calculation of Statistical Moments and Rate Constants——The cumulative excretion amount at infinite time (X^∞) and the mean residence time (MRT) have been defined in terms of the zero and first normal moments,[14] respectively, as

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

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

where dX/dt represents a function expressing the urinary excretion rate *vs.* time curve. The mathematical manipulation to obtain the moments from experimental data has been described in the previous paper.[14] The rate constants for absorption, metabolism, and urinary excretion were estimated using a linear one compartment model (Fig. 2) by means of non-linear least-squares fitting of the excretion time course data. The computations were carried out on a personal computer (PET 2001, Commodore Co.) with programming in BASIC. The program (MULTI) allows the simultaneous calculation of parameters involved in five different time course equations. The details of MULTI are described elsewhere.[15]

Results and Discussion

HPLC Analysis

The HPLC separation of urine excreted at 2.5 h after administration of oxacillin tablet to a subject (Y.M., see Table I) is shown in Fig. 1, where the dotted curves indicate the background due to control urine. The peaks 2, 3, and 4 were assigned, respectively, to penicilloic acid of oxacillin, the 5-hydroxymethyl derivative of oxacillin, and unchanged oxacillin by comparing their retention times with those of standard materials. The assignment of the new metabolite (peak 1) was made on the basis of enzymatic hydrolysis of the 5-hydroxymethyl derivative of oxacillin; when the isolated active metabolite was incubated with penicillinase at 37°C for 30 min, the chromatogram indicated the emergence of a single peak having a retention time coincident with that of peak 1. The same treatment of the urine of Fig. 1 resulted in the disappearance of peaks 3 and 4 with concomittant marked increase in the intensities of peaks 1 and 2. These results indicate that peak 1 in Fig. 1 arose from cleavage of the β-lactam ring of the 5-hydroxymethyl derivative of oxacillin. The same chromatographic results were obtained when the hydrolysis reaction was carried out in aqueous alkaline solution instead of enzyme solution. Thus, the new metabolite was assigned as penicilloic acid of the 5-hydroxymethyl derivative of oxacillin. Cleavage of the β-lactam ring to yield penicilloic acid, in general, results in inactivation of β-lactam antibiotics and enhancement of hydrophilicity. The latter effect is responsible for the short retention time of peak 1 on the hydrophobic stationary phase. This is possibly why the presence of this metabolite in human urine was not noticed in the previous work[8] (*i.e.*, owing to insufficient separation from endogenous urinary components).

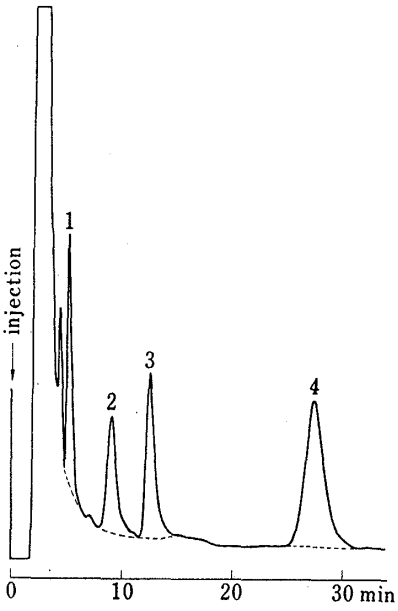

Fig. 1. HPLC Separation of Human Urine excreted after Oral Administration of Oxacillin Tablet

Peak assignments: 1, penicilloic acid of the 5-hydroxymethyl derivative of oxacillin; 2, penicilloic acid of oxacillin; 3, the 5-hydroxymethyl derivative of oxacillin; 4, oxacillin. HPLC conditions: see text.

Metabolic Pathways

The metabolic pathway leading to the new metabolite was investigated by HPLC analysis

of the rat urine excreted after intraperitoneal administrations of oxacillin, the 5-hydroxymethyl derivative, and penicilloic acid of oxacillin. The results indicated that penicilloic acid of the 5-hydroxymethyl derivative was excreted only when oxacillin and the 5-hydroxymethyl derivative were administered to the rats, whereas penicilloic acid of oxacillin was not transformed into the corresponding 5-hydroxymethyl derivative. It follows, therefore, that the new metabolite is produced by hydrolysis of the β-lactam ring of the 5-hydroxymethyl derivative of oxacillin, not by hydroxylation of the 5-methyl group on the isoxazolyl moiety of penicilloic acid of oxacillin. Judging from a similarity in metabolism of isoxazolylpenicillins between man[7] and rat,[6] we conclude that the metabolic pathways of oxacillin in man are depicted in Fig. 2.

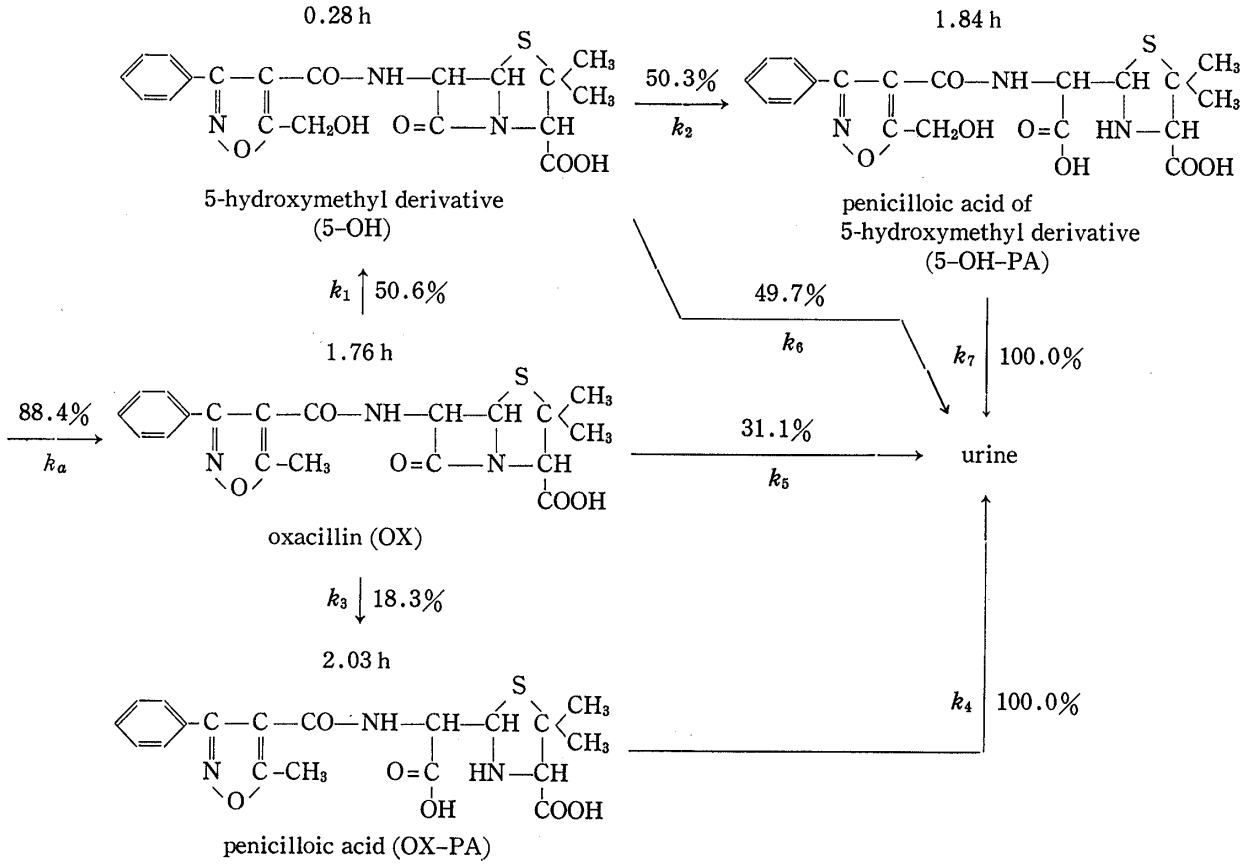

Fig. 2. Metabolic Pathways of Oxacillin

Figures above structural formulas are intrinsic MRT values, and those on arrows are elimination ratios with respect to the immediate parent compound.

Pharmacokinetic Evaluation

Table I lists the results for urinary excretion amounts of unchanged oxacillin and three metabolites in each of five subjects and their mean values, where the values for metabolites are given as equivalent to parent penicillin. The time course curves of excretion rates and cumulative excretion amounts for each species (average of five subjects) are shown in Figs. 3 and 4.

The statistical moments were calculated from the excretion rate *vs.* time curves. The results are given in Table II, where F is the fraction of the dose excreted in urine (X^{∞}/D), and MRT is the mean residence time of the excretion rate *vs.* time curves for each species. The results indicate that an average of 27.4% of the dose was excreted in urine as unchanged form, 16.1% as peniciloic acid, 22.2% as the 5-hydroxymethyl derivative, and 22.4% as penicilloic acid of the 5-hydroxymethyl derivative. Since the total accounts for 88.4% of

TABLE I. Urinary Excretion Amounts (mg) of Unchanged Oxacillin and Metabolites after an Oral Dose of Oxacillin (500 mg) to Human Subjects

Time (h)	Y.M.	M.M.	H.M.	Y.T.	T.H.	Mean	S.D.
Unchanged oxacillin							
0—0.5	3.54	11.98	7.46	12.33	14.46	9.95	3.93
0.5—1.0	42.31	44.83	44.52	82.51	36.54	50.14	16.46
1.0—1.5	28.34	30.61	21.10	42.23	24.24	29.30	7.25
1.5—2.0	15.07	18.70	11.69	21.52	13.31	16.06	3.59
2.0—2.5	6.91	7.82	9.02	9.26	9.54	8.51	0.99
2.5—3.0	4.47	5.06	6.84	4.50	3.31	4.84	1.15
3.0—3.5	3.45	4.04	5.49	3.44	2.86	3.86	0.90
3.5—4.0	2.43	2.81	4.18	2.65	1.51	2.72	0.86
4.0—5.0	4.12	4.87	6.74	2.75	1.95	4.09	1.67
5.0—6.0	3.06	2.74	5.33	1.13	1.28	2.71	1.52
6.0—8.0	3.98	1.04	6.21	1.11	1.63	2.79	2.02
8.0—10.0	n.d.	n.d.	1.88	n.d.	0.60	0.50	0.73
Total	117.68	134.50	130.46	183.43	111.23	135.46	25.42
5-Hydroxymethyl derivative of oxacillin							
0—0.5	0.46	2.81	1.75	1.47	6.07	2.51	1.93
0.5—1.0	17.60	27.19	23.71	26.08	47.98	28.51	10.28
1.0—1.5	31.65	27.41	26.66	23.94	32.14	28.36	3.11
1.5—2.0	22.42	19.12	15.25	12.73	18.43	17.59	3.33
2.0—2.5	9.12	9.81	9.69	6.93	11.80	9.47	1.56
2.5—3.0	5.27	6.59	5.89	3.44	7.98	5.83	1.50
3.0—3.5	3.69	4.78	3.99	2.27	5.21	3.99	1.02
3.5—4.0	2.50	3.19	3.32	1.67	3.30	2.80	0.64
4.0—5.0	4.87	4.36	5.01	2.81	3.37	4.08	0.86
5.0—6.0	3.22	3.17	3.89	1.03	1.86	2.63	1.04
6.0—8.0	3.64	1.97	5.59	1.06	2.07	2.87	1.59
8.0—10.0	1.38	n.d.	2.44	0.43	0.64	0.98	0.86
Total	105.82	110.40	107.19	83.86	140.85	109.62	18.22
Penicilloic acid of oxacillin							
0—0.5	n.d.	n.d.	0.62	0.64	0.67	0.39	0.32
0.5—1.0	4.62	6.65	7.74	12.80	3.96	7.15	3.13
1.0—1.5	8.12	9.71	9.16	17.59	6.49	10.21	3.85
1.5—2.0	9.21	12.19	9.93	17.54	5.84	10.94	3.88
2.0—2.5	6.22	10.83	7.35	15.36	4.11	8.77	3.95
2.5—3.0	4.02	8.96	5.13	12.41	3.17	6.74	3.46
3.0—3.5	3.40	7.94	4.49	10.30	2.67	5.76	2.90
3.5—4.0	2.40	7.04	3.60	9.40	2.52	4.99	2.77
4.0—5.0	4.36	9.39	6.15	13.92	3.37	7.44	3.83
5.0—6.0	3.02	7.30	4.32	9.55	2.38	5.31	2.71
6.0—8.0	5.25	5.80	5.19	9.86	2.68	5.76	2.32
8.0—10.0	4.14	2.70	2.57	5.16	0.97	3.11	1.44
Total	54.76	88.51	66.25	134.53	38.83	76.58	33.19
Penicilloic acid of 5-hydroxymethyl derivative of oxacillin							
0—0.5	n.d.	n.d.	n.d.	n.d.	n.d.	—	—
0.5—1.0	3.75	6.85	8.23	6.66	5.86	6.27	1.47
1.0—1.5	12.57	15.49	14.24	13.52	8.93	12.95	2.22
1.5—2.0	19.13	21.49	19.72	14.78	8.73	16.77	4.59
2.0—2.5	13.56	19.49	13.10	13.34	8.69	13.64	3.44
2.5—3.0	8.58	15.12	10.01	10.48	7.96	10.43	2.52
3.0—3.5	6.61	13.65	7.56	9.08	6.93	8.77	2.59
3.5—4.0	4.60	10.65	6.75	8.13	6.42	7.31	2.01
4.0—5.0	8.32	13.20	9.45	10.25	8.38	9.92	1.79
5.0—6.0	5.12	9.92	7.44	7.88	5.33	7.14	1.77
6.0—8.0	9.07	8.62	5.90	10.41	6.00	8.00	1.77
8.0—10.0	6.64	3.32	3.20	7.28	2.43	4.57	1.98
Total	97.95	137.80	105.60	111.81	75.66	105.76	20.15

Amounts of metabolites are given as equivalent to unchanged oxacillin. n.d. means not detected.

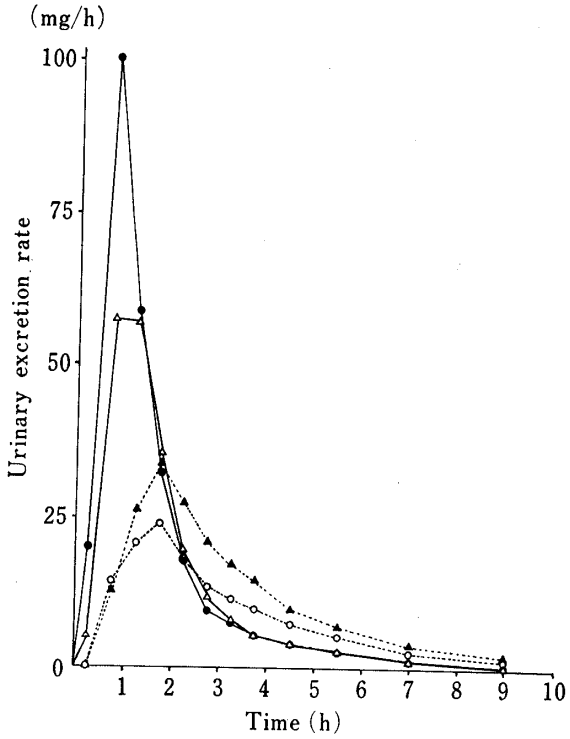

Fig. 3. Time Course Curves of Urinary Excretion Rates of Oxacillin and Metabolites (average of five subjects)

●: unchanged oxacillin, △: the 5-hydroxymethyl derivative of oxacillin, ○: penicilloic acid of oxacillin, ▲: penicilloic acid of the 5-hydroxymethyl derivative of oxacillin.

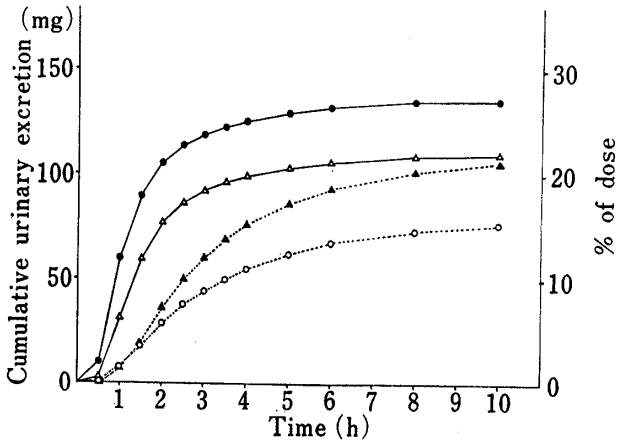

Fig. 4. Time Course Curves of Cumulative Excretion Amounts of Oxacillin and Metabolites (average of five subjects)

Symbols: see Fig. 3.

TABLE II. Statistical Moments and Rate Constants of Oxacillin and Metabolites

	Y.M.	M.M.	H.M.	Y.T.	T.H.	Mean	S.D.
F_{OX} (%)	24.6	27.0	26.5	36.8	22.3	27.4	4.96
$F_{5\text{-OH}}$	21.4	22.3	22.1	16.8	28.3	22.2	3.66
$F_{OX\text{-PA}}$	13.0	18.1	13.8	27.9	7.9	16.1	6.71
$F_{5\text{-OH-PA}}$	22.2	28.0	21.6	24.8	15.6	22.4	4.10
F_{total}	82.3	95.5	83.9	106.2	74.1	88.4	11.2
MRT_{OX} (h)	2.19	1.57	2.24	1.30	1.51	1.76	0.38
$MRT_{5\text{-OH}}$	2.26	1.96	2.61	1.70	1.69	2.04	0.35
$MRT_{OX\text{-PA}}$	5.38	3.44	3.49	3.53	3.12	3.79	0.81
$MRT_{5\text{-OH-PA}}$	4.78	3.30	3.22	4.56	3.57	3.89	0.65
k_a (h^{-1})	1.45 (1.12)	1.13 (1.81)	0.87 (1.52)	1.21 (2.25)	0.87 (2.83)	1.11 (1.91)	0.22 (0.59)
k_1	0.54 (0.70)	0.92 (0.58)	0.78 (0.45)	0.86 (0.46)	1.65 (0.50)	0.95 (0.54)	0.37 (0.09)
k_2	5.11	8.01	7.57	7.44	6.14	6.85	1.07
k_3	0.26 (0.33)	0.38 (0.24)	0.27 (0.15)	0.60 (0.33)	0.31 (0.10)	0.36 (0.23)	0.13 (0.09)
k_4	0.17	0.33	0.52	0.44	0.81	0.45	0.21
k_5	0.32 (0.41)	0.50 (0.32)	0.46 (0.26)	0.79 (0.43)	0.87 (0.27)	0.59 (0.34)	0.21 (0.07)
k_6	5.27	6.07	7.17	5.04	11.56	7.02	2.39
k_7	0.65	0.62	0.77	0.39	0.70	0.63	0.13

$F=X_\infty/D$. MRT, see Eq. 2. Subscripts: OX, oxacillin; 5-OH, 5-hydroxymethyl derivative of oxacillin; OX-PA, penicilloic acid of oxacillin; 5-OH-PA, penicilloic acid of 5-hydroxymethyl derivative of oxacillin. k_a, k_1 to k_7, see Fig. 2; the rate constants in parentheses are those due to a "flip flop."

the dose, it is suggested that the oxacillin tablet is absorbed through the GI tract to at least this extent. This result is significantly higher than those reported previously.[13,17–19] However, only a half of the dose remains efficient, since the two penicilloic acids are inactive metabolites.

The elimination ratio at each successive step involved in Fig. 2 was calculated from the F values mentioned above. The results are also shown in Fig. 2. It was found that 50.6% of absorbed oxacillin is transformed to the 5-hydroxymethyl derivative, 18.3% to penicilloic acid, and 31.1% is excreted in the urine as the unchanged form. A 50.3% portion of the 5-hydroxymethyl derivative further undergoes hydrolysis of the β-lactam ring to yield penicilloic acid of the 5-hydroxymethyl derivative and the rest (49.7%) is excreted in the urine. The two penicilloic acids thus formed are all excreted in the urine, because they are the final products.

The MRT value for a metabolite given in Table II represents the mean time from administration of oxacillin to excretion of the metabolite, that is, the mean overall time required for absorption, distribution, metabolism, and excretion. Therefore, in general, the time period intrinsic to a transformed product is given by the difference of MRT value from the immediate parent compound, since MRT can be additive in linear systems.[14] For instance, the intrinsic MRT value for penicilloic acid of oxacillin is estimated as $MRT_{\text{ox-PA}}-MRT_{\text{ox}}=$ 2.03 h, and similarly those for the 5-hydroxymethyl derivative and penicilloic acid of the 5-hydroxymethyl derivative are given by $MRT_{\text{5-OH}}-MRT_{\text{ox}}=0.28$ h and $MRT_{\text{5-OH-PA}}-MRT_{\text{5-OH}}=1.84$ h, respectively. These results (also given in Fig. 2) indicate that the 5-hydroxymethyl derivative of oxacillin remains in the human body for a shorter period of time than penicilloic acid, that is, this active metabolite is as easily eliminatable intermediate. The rate profile for absorption, metabolism, and urinary excretion can be viewed in terms of their rate constants. The results are shown in Table II, where k_a is the absorption rate constant, k_1 to k_3 are metabolic rate constants, k_4 to k_7 are excretion rate constants as specified in Fig. 2, and the figures in parentheses are another set of rate constants due to a "flip flop." It was found that hydroxylation of the 5-methyl group on the isoxazole ring proceeds faster than hydrolysis of the β-lactam ring of intact oxacillin, and that cleavage of the β-lactam ring is accelerated by the hydroxylation. Among the four species found in urine, the 5-hydroxymethyl derivative is excreted most rapidly. This result is consistent with the intrinsic MRT value mentioned above. Such rapid elimination of this metabolite may be related to its antibiotic activity.

Pharmacokinetic investigation of a drug is useful to clarify the rate profile of its bioavailable activity. The antimicrobial activities of isoxazolylpenicillins depend considerably on their active metabolites, *i.e.* 5-hydroxymethyl derivatives. For instance, the activity against *S. lutea* of the 5-hydroxymethyl derivative of oxacillin excreted in urine after an oral administration of oxacillin has been reported to account for 20.7% of the total activity, and the susceptibility of *S. lutea* to the metabolite, though dependent on the bacterial strain, is comparable to that to the parent penicillin.[3] However, it is difficult, in general, to analytically express activity as a function of urinary excretion rate. As the simplest case, provided that the total activity of oxacillin may be expressed as the sum of activities of unchanged oxacillin and 5-hydroxymethyl derivative, and that the activity may be regarded as proportional to dX/dt for each active species within a certain limited area, it follows from Eqs. 1 and 2 that

$$MRT_{\text{a}}=\frac{pX_{\text{ox}}^{\infty}MRT_{\text{ox}}+qX_{\text{5-OH}}^{\infty}MRT_{\text{5-OH}}}{pX_{\text{ox}}^{\infty}+qX_{\text{5-OH}}^{\infty}},$$

where MRT_{a} means MRT of total activity *vs.* time curve, p and q denote the proportionality factors relating urinary excretion rate to activity, which are assumed to be time-independent, and $pX_{\text{ox}}^{\infty}+qX_{\text{5-OH}}^{\infty}$ represents total bioavailable activity at infinite time. The tentative substitution of $p=q$ to the above equation gives $MRT_{\text{a}}=1.89$ h.

It is known that absorption of isoxazolylpenicillins is enhanced by progressive substitution of halogen on the phenyl ring.[16] However, the present results for oxacillin show significantly higher absorption than those for other isoxazolyl-penicillins.[9,13,16,20] This arises because the urinary excretion of the new metabolite is taken into pharmacokinetic consideration. It seems likely, however, that other isoxazolylpenicillins may be transformed to corresponding new metabolites to a lesser degree than oxacillin, because the latter undergoes a higher extent of hydroxylation, yielding the precursor of the new metabolite. As a result, it is considered that orally dosed isoxazolylpenicillins are more effectively absorbed, and the variation of the degree of absorption is smaller, than previously reported.

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References and Notes

1) W.M.M. Kirby, L.S. Rosenfeld, and J. Brodie, *J. Am. Med. Assoc.*, **181**, 739 (1962).
2) G.N. Rolinson and F.R. Batchelor, *Antimicrob. Ag. Chemother.*, **1963**, 654.
3) H.H.W. Thijssen and H. Mattie, *Antimicrob. Ag. Chemother.*, **10**, 441 (1976).
4) D.R. Van Harken, C.W. Dixon, and J.M. Essery, *Pharmacologist*, **12**, 220 (1970).
5) H.H.W. Thijssen, *Arzneim. Forsch.*, **28**, 1065 (1978).
6) H.H.W. Thijssen, *J. Antibiot.*, **32**, 1033 (1979).
7) Y. Murai, T. Nakagawa, and T. Uno, *Chem. Pharm. Bull.*, **28**, 362 (1980).
8) H.H.W. Thijssen, *J. Chromatogr.*, **183**, 339 (1980).
9) E.H. Nauta and H. Mattie, *Br. J. Clin. Pharmac.*, **2**, 111 (1975).
10) L.W. Dittert, W.O. Griffen, J.C. LaPiana, F.J. Shainfeld, and J.T. Doluisio, *Antimicrob. Ag. Chemother.*, **1970**, 42; J.T. Doluisio, J.C. LaPiana, G.R. Wilkinson, and L.W. Dittert, *Antimicrob. Ag. Chemother.*, **1970**, 49.
11) K. Hellström, A. Rosén, and A. Swahn, *Eur. J. Clin. Pharmacol.*, **7**, 125 (1974).
12) J.E. Rosenblatt, A.C. Kind, J.L. Brodie, and W.M.M. Kirby, *Arch. Intern. Med.*, **121**, 345 (1968).
13) M. Cole, M.D. Kenig, and V.A. Hewitt, *Antimicrob. Ag. Chemother.*, **3**, 463 (1973).
14) K. Yamaoka, T. Nakagawa, and T. Uno, *J. Pharmacokin. Biopharm.*, **6**, 547 (1978).
15) K. Yamaoka, Y. Tanigawara, T. Nakagawa, and T. Uno, *J. Pharm. Dyn.*, **4**, 879 (1981).
16) R. Sutherland, E.A.P. Croydon, and G.N. Rolinson, *Br. Med. J.*, **4**, 455 (1970).
17) P. Naumann, *Antimicrob. Ag. Chemother.*, **1966**, 937.
18) C.M. Kunin, *Antimicrob. Ag. Chemother.*, **1966**, 1025.
19) H. Graber, T. Perényi, M. Arr, and E. Ludwig, *Int. J. Clin. Pharmacol.*, **14**, 284 (1976).
20) G.P. Bodey, C. Vallejos, and D. Stewart, *Clin. Pharmacol. Ther.*, **13**, 512 (1972).