International Journal of Pharmaceutics, 15 (1983) 309–320
Elsevier

High-performance liquid chromatographic determination and moment analysis of urinary excretion of flucloxacillin and its metabolites in man

Y. Murai, T. Nakagawa *, K. Yamaoka and T. Uno **

Faculty of Pharmaceutical Sciences, Kyoto University, Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto, 606 (Japan)

(Received July 20th, 1982)
(Accepted December 27th, 1982)

Summary

Pharmacokinetic evaluation of flucloxacillin in man has been achieved through detailed investigation of urinary excretions of unchanged flucloxacillin and 3 metabolites (penicilloic acid, 5-hydroxymethyl derivative, and penicilloic acid of the 5-hydroxymethyl derivative). The last metabolite was newly found from human urine. The time courses for excretion of unchanged flucloxacillin and the metabolites were measured by HPLC analysis of urine excreted after oral administration of flucloxacillin capsules to 5 human subjects. The values for cumulative excretion amount at infinite time (X_u^∞) and mean residence time (MRT) for each species were estimated by moment analysis of excretion rate vs time curves. The results indicate that the average values of excretion ratio (X_u^∞/D, $D = 500$ mg) and MRT are, respectively, 64.8% and 2.30 h for unchanged flucloxacillin, 3.8% and 4.51 h for penicilloic acid, 10.5% and 2.72 h for 5-hydroxymethyl derivative, and 1.0% and 4.04 h for penicilloic acid of the 5-hydroxymethyl derivative. The metabolic pathways of flucloxacillin are depicted, and the transfer ratio at each elimination step and the MRT value intrinsic to each metabolite were evaluated from the results of moments.

Introduction

The isoxazolyl group of semisynthetic penicillins has been used in the oral therapy of infections caused by Gram-positive cocci, including penicillinase-produc-

* To whom correspondence should be addressed.
** Present address: Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 4–16 Edagawa-cho, Nishinomiya 663, Japan.

ing Staphylococci (Kirby et al., 1965; Sutherland et al., 1970). Flucloxacillin, a later entrant in the isoxazolyl group of penicillins, is well absorbed in man after administration by both oral and intramuscular routes, and shows a high degree of binding to serum proteins (Sutherland et al., 1970; Bodey et al., 1972). Evaluating flucloxacillin in comparison with its predecessors, Sutherland et al. (1970) reported that the relationship of the total serum levels provided by a given oral dose of oxacillin, cloxacillin, dicloxacillin, and flucloxacillin was approximately 1 : 2 : 4 : 4. However, when the protein binding was taken into consideration, the levels of the unbound penicillins in the serum were approximately 1 : 2 : 2 : 4. Nauta and Mattie (1975) presumed that other factors besides absorption could play an important role in causing higher concentrations after oral administration of flucloxacillin, and concluded that this arose from its slower renal and extra-renal elimination.

On the other hand, the isoxazolyl penicillins are known to be biotransformed to a considerable extent in man to active metabolites (Rolinson et al., 1963) whose antibiotic activities are almost the same order of magnitude as those of parent penicillins (Thijssen et al., 1976). In the previous paper (Murai et al., 1980) we reported that the active metabolite of oxacillin in man is 6-(3-phenyl-5-hydroxymethyl-4-isoxazolecarboxamide)-penicillanic acid, i.e. the 5-hydroxymethyl derivative of parent penicillin. We also discovered a new metabolite, penicilloic acid of the 5-hydroxymethyl derivative of oxacillin, in human urine excreted after oral administration of oxacillin, and achieved accurate pharmacokinetic investigation of oxacillin in man (Murai et al., 1981).

The aim of the present paper is to investigate the metabolism of flucloxacillin in man by HPLC and GC-MS analysis of urine, and to achieve pharmacokinetic evaluation by the moment analysis of excretion rate–time curve.

Experimental

Reagents and materials

Flucloxacillin sodium used as a standard material and flucloxacillin capsules (Floxapen, 250 mg as potency) given to subjects were supplied by Banyu Seiyaku (Tokyo). Tetrabutylammonium bromide (TBAB) and other chemicals used for HPLC were commercial products of reagent grade. Phenyl methyl silicone (OV-17, Nishio Kogyo, Tokyo, Japan) and hexamethyl disilazane (HMDS, Wako Pure Chemicals, Osaka, Japan) were used for GC-MS analysis. Methanol and water were purified by distillation and degassed prior to preparation of mobile phase.

Drug administration and sample preparation

Five healthy male adults of 22–27 years old, weighing 55–59 kg, participated in this experiment. Each subject, who had been drug-free at least one week and fasted overnight, received flucloxacillin capsules (250 mg × 2) orally with 200 ml water. 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 and 8.0 h after administration. After measurement of volume, the urine was passed through a 0.45 μm pore size membrane filter (Fuji Photo Film), and a 5.0 μl

portion of the filtrate was subjected to HPLC analysis. The remaining portion was reserved for isolation of active metabolite.

High-performance liquid chromatography

A high-performance liquid chromatograph (ALC/GPC 204, Waters Ass.) equipped with a UV-detector (254 nm, Model 440, Waters Ass.) was used in a reversed phase mode with a stationary phase of LiChrosorb RP-18 (E. Merck) packed in a stainless steel tube (4.6 mm i.d. × 25 cm) and a mobile phase of aqueous 5 mM TBAB + 1/120 M Na_2HPO_4 + 1/120 M KH_2PO_4 solution mixed with acetonitrile at a volume ratio of 3/1 (pH 7.48), whose flow rate was maintained at 3.0 ml/min (2800 psi). A short column (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.

The urinary concentrations of unchanged flucloxacillin 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 flucloxacillin and its 5-hydroxymethyl derivative were prepared by dissolving accurately weighed amounts of standard flucloxacillin and the isolated metabolite (see below) in control urine to make several different concentrations ranging from 41 to 3750 μg/ml and from 15 to 670 μg/ml as flucloxacillin equivalent, respectively.

The standard solutions of penicilloic acid of flucloxacillin and penicilloic acid of 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 flucloxacillin and penicilloic acid of the 5-hydroxymethyl derivative ranging from 10 to 136 μg/ml and from 4 to 80 μg/ml as flucloxacillin equivalent, respectively. All the calibration graphs thus obtained passed through the origin with correlation coefficients above 0.999, and S.D. values less than 0.5 ($n = 3$ for each concentration), and the day-to-day reproducibility was well conserved by washing the column with methanol and water after use and by repacking the guard column sometimes.

Isolation of the 5-hydroxymethyl derivative of flucloxacillin

About 1000 ml of urine collected at 1–3.5 h after administration was chromatographed in portions on a reversed-phase column (2.5 cm i.d. × 31 cm) packed with LiChroprep RP-8 (40–63 μm particle diameter, E. Merck) using 0.2 M acetate buffer (pH 5.2)/methanol (4 : 3 v/v) as a developing solvent. The eluate was analyzed by HPLC and the fraction containing active metabolite (peak 3 in Fig. 2) 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 0.03 M acetate buffer (pH 5.6)/acetonitrile (15 : 4 v/v). The metabolite fraction was collected and concentrated in the same manner as above,

and developed again on the same column with aqueous 5 mM TBAB + 1/120 M Na_2HPO_4 + 1/120 M KH_2PO_4 solution/acetonitrile (10:3 v/v). In order to remove buffer salt, the metabolite fraction was collected, concentrated, and developed again on the same column with water/methanol (4:1 v/v). The fraction (between 255 ml and 285 ml of eluate) following the rapidly eluted buffer salts (eluted between 35 ml and 50 ml) was collected. Removal of the solvent followed by lyophilization gave a small amount (not weighed) of a fleecy white solid, whereas the fraction between 50 ml and 255 ml gave no solid substances. The HPLC analysis of the white solid indicated a single peak with a retention time equivalent to that of peak 3 in Fig. 2. This metabolite was identified by GC-MS (see below) as the 5-hydroxymethyl derivative of flucloxacillin.

Sample preparation for GC-MS analysis

The 5-hydroxymethyl derivative thus isolated was acidified to pH 2.5 with use of ammonium sulfate/sulfuric acid solution, and extracted with dichloroethane. After evaporation of the solvent, the dried extract was trimethylsilylated in hexamethyldisilazane (HMDS)/pyridine solution and subjected to GC-MS analysis.

Gas chromatography-mass spectrometry (GC-MS)

A mass spectrometer (JEOL JMS-01SG-2) combined with a gas chromatograph (JGC-20k) was used under the following operating conditions: gas chromatographic stationary phase — 1.5% OV-17 coated on Chromosorb W (100–120 mesh) packed in a 100 cm × 2 mm i.d. glass tubing; carrier gas — helium (30 ml/min); column temperature — 275°C; injector temperature — 295°C; ionization voltage — 50 eV; and acceleration voltage — 8 kV.

Estimation of statistical moments

Urinary excretion amount at infinite time (X_u^∞) and mean residence time (MRT) are given by the zero and first normal moments, respectively, as:

$$X_u^\infty = \int_0^\infty (dXu/dt)dt \tag{1}$$

$$MRT = \int_0^\infty t(dXu/dt)dt/X_u^\infty \tag{2}$$

where dX_u/dt is a function expressing urinary excretion rate vs time curve. In using the above equations, the moments were calculated by trapezoidal 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 3–6 data points on the time course curve. The details of the mathematical operations to obtain the moments from the experimental data were described in our previous paper (Yamaoka et al., 1978). The computations were carried out on a personal computer (PET2001, Commodore) with programming in BASIC.

Results and Discussion

GC-MS analysis of active metabolite

Fig. 1 depicts the mass spectrum for the peak of TMS-derivative of the isolated

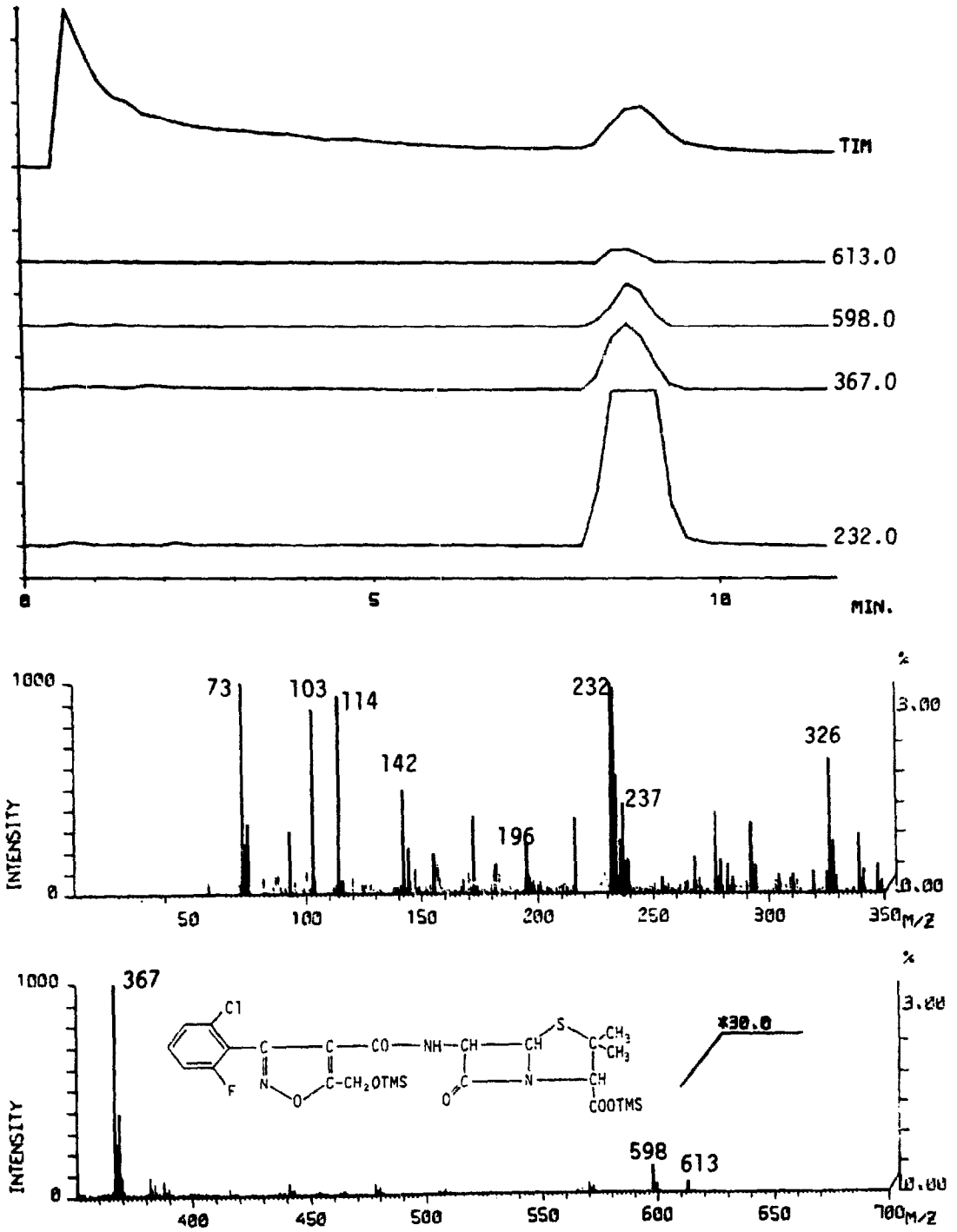
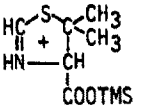
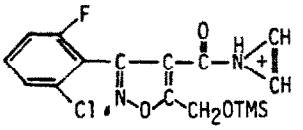
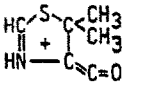
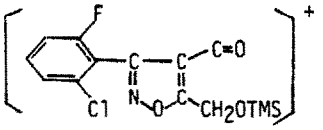
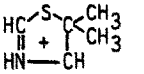
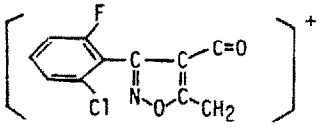
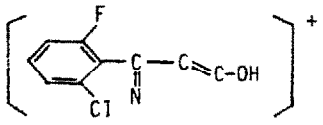


Fig. 1. Mass spectrum and mass chromatogram of active metabolite of flucloxacillin.

TABLE 1

ASSIGNMENTS OF MASS SPECTRAL PEAKS

m/z	assignment	m/z	assignment
613	M^+	232	
598	$(M-CH_3)^+$		
367		142	
326		114	
		103	CH_2^+-O-TMS
237		73 (base peak)	TMS^+
196			

metabolite with the elapsed time of 8.8 min on TIM-chromatogram, and the mass chromatograms monitored at m/z 613, 598, 367 and 232. The assignments of characteristic peaks and fragmentations are summarized in Table 1. The peak at m/z 232 is characteristic of the protonated ion of TMS-derived thiazolidine resulted from cleavage of the β-lactam ring, which corresponds to protonated ion at m/z 174 found with methyl-ester of thiazolidine (Mitscher et al., 1975; Thijssen, 1979). The peaks at m/z 367 and 326 are characteristic of TMS-derived 5-hydroxymethyl isoxazole moiety, and the peak at m/z 237 is assignable to $(326\text{-OTMS})^+$. The occurrence of the latter peak obviously indicates that the metabolite is the hydroxymethyl derivative.

From these observations, the isolated metabolite is identified to 5-hydroxymethyl isoxazolyl derivative of flucloxacillin, which is the same with the active metabolite found from rat urine by Thijssen (1979).

HPLC analysis

Fig. 2 depicts the chromatogram of urine collected at 4 h after administration of flucloxacillin capsule to a subject (T.H. see Table 2), where the dotted curves indicate the background due to control urine. The peaks 2, 3 and 4 were assigned, respectively, to penicilloic acid of flucloxacillin (FX-PA), the 5-hydroxymethyl derivative of flucloxacillin (5-OH), and unchanged flucloxacillin (FX) by comparing

their capacity factors ($k'_2 = 17.2$, $k'_3 = 27.0$, $k'_4 = 50.5$) with those of standard materials. The assignment of the penicilloic acid of the 5-hydroxymethyl derivative of flucloxacillin (5-OH-PA; peak 1, $k'_1 = 15.4$) was made on the basis of enzymatic

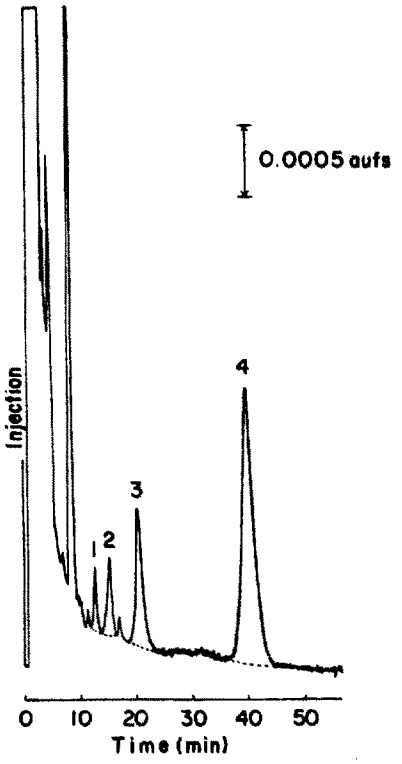


Fig. 2. HPLC separation of human urine excreted after oral administration of flucloxacillin capsule. Peak assignments: 1, penicilloic acid of the 5-hydroxymethyl derivative of flucloxacillin; 2, penicilloic acid of flucloxacillin; 3, the 5-hydroxymethyl derivative of flucloxacillin; and 4, flucloxacillin. HPLC conditions: see text.

hydrolysis and alkaline hydrolysis of the 5-OH as follows. When the isolated 5-hydroxymethyl derivative was incubated with penicillinase at 37°C for 30 min, the chromatogram indicated the emergence of a single peak having a retention time (12.9 min) coincident with that of peak 1. The same treatment of the urine of Fig. 2 resulted in the disappearance of peaks 3 and 4 with concomitant marked increase in the intensities of peaks 1 and 2. These results indicate that peak 1 arose from β-lactam ring opening of the 5-hydroxymethyl derivative of flucloxacillin. The same chromatographic results were obtained when the hydrolysis reaction was carried out in aqueous alkaline solution instead of enzyme solution, and when using several different mobile phase conditions. The new metabolite was thus assigned to penicilloic acid of the 5-hydroxymethyl derivative of flucloxacillin.

Metabolic pathways

Prior to pharmacokinetic analysis of the time course data, the metabolic pathway leading to the new metabolite was investigated by HPLC analysis of the rat urine

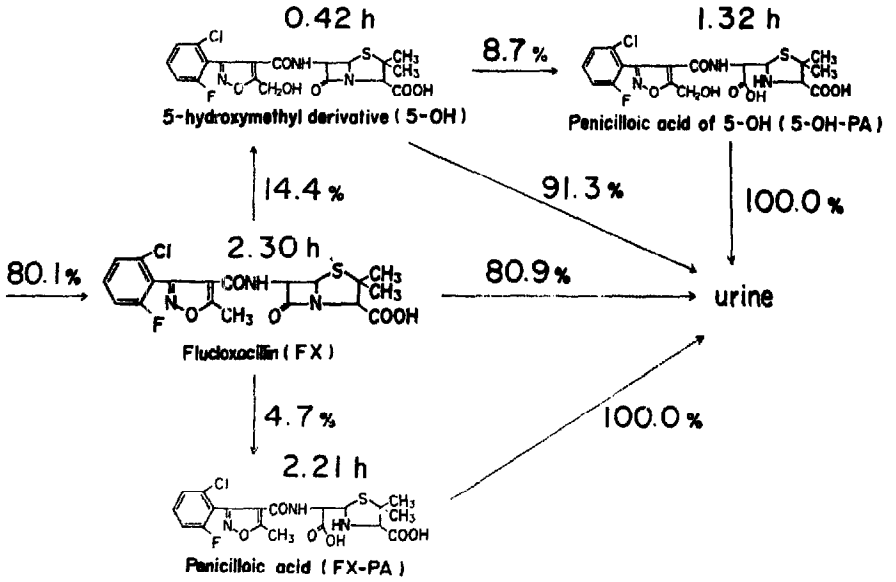


Fig. 3. Metabolic pathways of flucloxacillin. Figures above structural formulae are intrinsic MRT values, and those on arrows are elimination ratios with respect to the immediate parent compound.

excreted after intraperitoneal administrations of flucloxacillin, the 5-hydroxymethyl derivative, and penicilloic acid of flucloxacillin. The results revealed that the excretion of penicilloic acid of the 5-hydroxymethyl derivative was observed when flucloxacillin and 5-hydroxymethyl derivative were administered, whereas penicilloic acid of flucloxacillin was not transformed to corresponding 5-hydroxymethyl derivative. It follows, therefore, that the new metabolite was produced by β-lactam ring opening of the 5-hydroxymethyl derivative of flucloxacillin, not by hydroxylation of 5-methyl group on isoxazolyl moiety of penicilloic acid of flucloxacillin. Judging from a similarity in metabolism of isoxazolyl penicillins between man and rat, we may figure the metabolic pathways of flucloxacillin in man as given in Fig. 3.

Time course of urinary excretion

Figs. 4 and 5 illustrate, respectively, the time course curves of excretion rates and cumulative excretion amounts for each species (average of 5 subjects). It is found that the urinary excretion rate reaches maximum at 1–1.5 h after dosing for unchanged flucloxacillin, at 1.5–2 h for the 5-hydroxymethyl derivative and penicilloic acid of flucloxacillin, and 2–2.5 h for penicilloic acid of the 5-hydroxymethyl derivative of flucloxacillin, and that their excretion is almost completed within 8 h after dosing.

Pharmacokinetic evaluation

The statistical moments were calculated from the excretion rate vs time curves. The results are shown in Table 2, where F is the fraction of the dose excreted in urine (X_u^∞/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 64.8% of the dose was

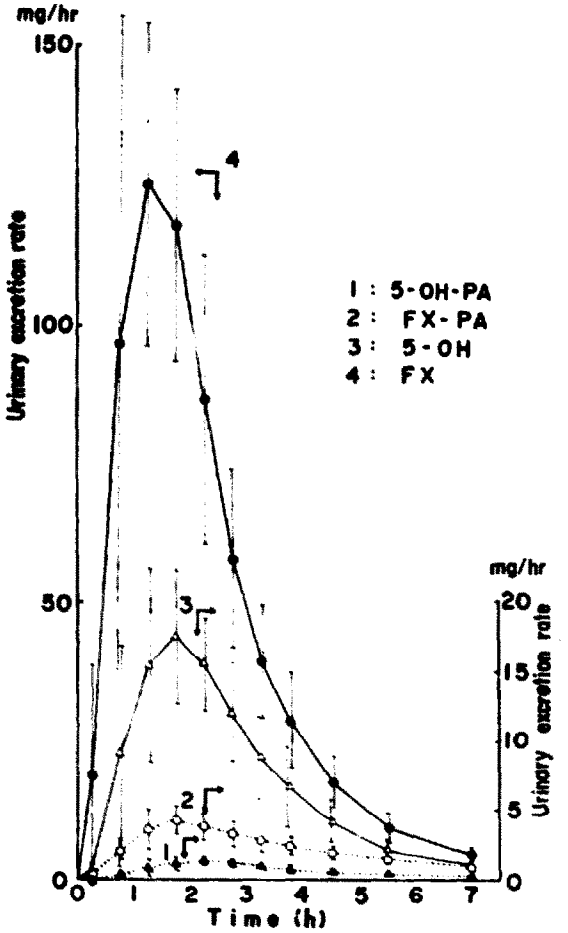


Fig. 4. Time course curves of urinary excretion rates of flucloxacillin and metabolites (average of 5 subjects). ●, unchanged flucloxacillin; △, the 5-hydroxymethyl derivative of flucloxacillin; ○, penicilloic acid of flucloxacillin; ▲, penicilloic acid of the 5-hydroxymethyl derivative of flucloxacillin.

excreted in urine as the unchanged form, 10.5% as the 5-hydroxymethyl derivative, 3.8% as penicilloic acid, and 1.0% as penicilloic acid of the 5-hydroxymethyl derivative. The transfer ratio at each successive step involved in Fig. 3 was calculated from the F values mentioned above. The results are also given in Fig. 3, which shows that 14.4% of absorbed flucloxacillin is transformed to the 5-hydroxymethyl derivative, 4.7% to penicilloic acid, and 80.9% is excreted in the urine as the unchanged form. A 8.7% 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 (91.3%) is excreted in the urine. The two penicilloic acids thus formed are all excreted in the urine, because they can be regarded as the final products.

As found from the definition, the MRT value for a metabolite given in Table 2 represents the mean time from administration of flucloxacillin to urinary excretion of the metabolite, that is, the mean overall time required for absorption, distribution, metabolism and excretion. Therefore, in general, the intrinsic MRT value for a

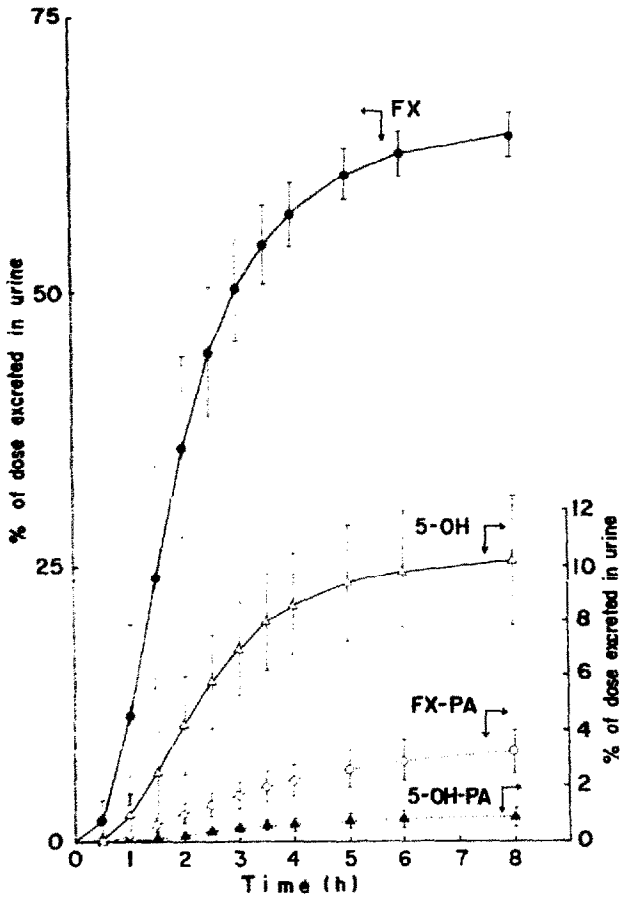


Fig. 5. Time course curves of cumulative excretion amounts of flucloxacillin and metabolites (average of 5 subjects). Symbols: see Fig. 4.

metabolite can be represented by the difference in MRT value between the metabolite and its immediate precursor, since MRT can be additive in linear systems (Yamaoka et al., 1978). For instance, the intrinsic MRT (i-MRT) value for penicilloic acid of flucloxacillin is estimated as $MRT_{FX\text{-}PA} - MRT_{FX} = 2.21$ h, and similarly those for the 5-hydroxymethyl derivative and penicilloic acid of the 5-hydroxymethyl derivative are given as $MRT_{5\text{-}OH} - MRT_{FX} = 0.42$ h and $MRT_{5\text{-}OH\text{-}PA} - MRT_{5\text{-}OH} = 1.32$ h, respectively. These results (also given in Fig. 3) indicate that the 5-hydroxymethyl derivative of flucloxacillin remains in the human body for a shorter period of time than penicilloic acid, that is, this active metabolite is an easily eliminatable intermediate. It is also found that hydroxylation of the 5-methyl group on the isoxazole ring proceeds faster than hydrolysis of the β-lactam ring of intact flucloxacillin, and that cleavage of the β-lactam ring is accelerated by the hydroxylation. Among the 4 species found in urine, the 5-hydroxymethyl derivative is excreted most rapidly.

The antimicrobial activities of isoxazolyl penicillins depend considerably on their active metabolites, i.e. 5-hydroxymethyl derivatives. For instance, the activity against

TABLE 2

FRACTION EXCRETED AND MEAN RESIDENCE TIME OF FLUCLOXACILLIN AND METABOLITES

	Y.M.	M.S.	H.M.	Y.T.	T.H.	Mean	S.D.
F_{FX} [a] (%)	62.7	63.8	64.0	68.8	64.6	64.8	2.10
$F_{5\text{-}OH}$	11.4	8.8	10.8	7.2	14.4	10.5	2.44
$F_{FX\text{-}PA}$	2.1	4.2	4.7	4.2	3.9	3.8	0.90
$F_{5\text{-}OH\text{-}PA}$	1.0	0.8	1.2	0.4	1.7	1.0	0.43
F_{total}	77.2	77.6	80.7	80.6	84.6	80.1	2.67
MRT_{FX} [b] (h)	2.85	1.82	2.19	2.57	2.06	2.30	0.37
$MRT_{5\text{-}OH}$	3.34	2.26	2.48	2.83	2.68	2.72	0.37
$MRT_{FX\text{-}PA}$	4.67	5.01	4.02	4.12	4.72	4.51	0.38
$MRT_{5\text{-}OH\text{-}PA}$	4.13	3.90	3.76	3.87	4.52	4.04	0.27

[a] $F = \lambda_u^\infty/D$.

[b] MRT; see Eqn. 2.

Subscripts: FX, flucloxacillin; 5-OH, 5-hydroxymethyl derivative of flucloxacillin; FX-PA, penicilloic acid of flucloxacillin; 5-OH-PA, penicilloic acid of 5-hydroxymethyl derivative of flucloxacillin.

S. lutea of the 5-hydroxymethyl derivative of flucloxacillin excreted in urine after an oral administration of flucloxacillin has been reported to account for 10.2% 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 (Thijssen et al., 1976). 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 flucloxacillin may be expressed as the sum of activities of unchanged flucloxacillin and 5-hydroxymethyl derivative, and that the activity may be regarded as proportional to dX_u/dt for each active species within a certain limited area, it follows from Eqns. 1 and 2 that

$$MRT_a = \frac{pX_{FX}^\infty MRT_{FX} + qX_{5\text{-}OH}^\infty MRT_{5\text{-}OH}}{pX_{FX}^\infty + qX_{5\text{-}OH}^\infty}, \quad (3)$$

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 $pX_{FX}^\infty + qX_{5\text{-}OH}^\infty$ represents total bioavailable activity at infinite time. The tentative substitution of $p = q$ to Eqn. 3 gives $MRT_a = 2.36$ h. The corresponding value for oxacillin was 1.89 h (Murai et al., 1981).

It is known that absorption of isoxazolyl penicillins is enhanced by progressive substitution of halogen on the phenyl ring (Sutherland et al., 1970). However, we considered in a previous paper (Murai et al., 1981) that when the urinary excretion of the new metabolite (i.e. 5-OH-PA) is taken into pharmacokinetic consideration, the orally dosed isoxazolyl penicillins are more effectively absorbed, and the variation of the degree of absorption is smaller, than previously reported (Sutherland

et al., 1970; Bodey et al., 1972; Cole et al., 1973; Nauta et al., 1975). The present results indicate that the absorption of flucloxacillin (80.1%) is comparable to that of oxacillin (88.4%), while the active forms of flucloxacillin are retained much longer in the body with higher total amounts. This corresponds to the results of slower elimination of flucloxacillin reported by Nauta et al. (1975) and Thijssen (1980). The detailed comparative discussion on the pharmacokinetic behavior of isoxazolyl penicillins including cloxacillin and dicloxacillin will follow before long.

Acknowledgement

This work was supported in part by a Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture, Japan, and in part by a grant from Takeda Pharmaceutical Ind. Co. Ltd. The authors are grateful to Miss N. Ugumori for her technical assistance, and also to Dr. N. Akimoto for measuring GC-MS spectra.

References

Bodey, G.P., Vallejos, C. and Stewart, D., Flucloxacillin: a new semisynthetic isoxazolyl penicillin. Clin. Pharmacol. Ther., 13 (1972) 512–515.

Cole, M., Kenig, M.D. and Hewitt, V.A., Metabolism of penicillins to penicilloic acids and 6-aminopenicillanic acid in man and its significance in assessing penicillin absorption. Antimicrob. Ag. Chemother., 3 (1973) 463–468.

Kirby, W.M.M., Gravenkemper, C.F., Bennett, J.V. and Brodie, J.L., Dicloxacillin: in vitro and pharmacologic comparisons with oxacillin and cloxacillin. Arch. Intern. Med., 116 (1965) 340–345.

Mitscher, L.A., Showalter H.D.H. and Shirahata, K., Chemical-ionization mass spectrometry of β-lactam antibiotics. J. Antibiot. 28 (1975) 668–675.

Murai, Y., Nakagawa, T. and Uno, T., GC-MS identification of active metabolite of oxacillin in man. Chem. Pharm. Bull., 28 (1980) 362–364.

Murai, Y., Nakagawa, T., Yamaoka, K. and Uno, T., High performance liquid chromatographic analysis and pharmacokinetic investigation of oxacillin and its metabolites in man. Chem. Pharm. Bull., 29 (1981) 3290–3297.

Nauta, E.H. and Mattie, H., Pharmacokinetics of flucloxacillin and cloxacillin in healthy subjects and patients on chronic intermittent haemodialysis. Br. J. Clin. Pharmacol., 2 (1975) 111–121.

Rolinson, G.N. and Batchelor, F.R., Penicillin metabolites. Antimicrob. Ag. Chemother., 1962 (1963) 654–660.

Sutherland, R., Croydon, E.A.P. and Rolinson, G.N., Flucloxacillin, a new isoxazolyl penicillin, compared with oxacillin, cloxacillin, and dicloxacillin. Br. Med. J., 4 (1970) 455–460.

Thijssen, H.H.W. and Mattie, H., Active metabolites of isoxazolyl-penicillins in humans. Antimicrob. Ag. Chemother., 10 (1976) 441–446.

Thijssen, H.H.W., Identification of the active metabolites of the isoxazolylpenicillins by means of mass-spectrometry. J. Antibiot., 32 (1979) 1033–1037.

Thijssen, H.H.W., Analysis of isoxazolyl penicillins and their metabolites in body fluid by high-performance liquid chromatography. J. Chromatogr., 183 (1980) 339–345.

Yamaoka, K., Nakagawa, T. and Uno, T., Statistical moments in pharmacokinetics. J. Pharmacokin. Biopharm., 6 (1978) 547–558.