Effect of Cefixime and Cefdinir, Oral Cephalosporins, on Cytochrome P450 Activities in Human Hepatic Microsomes

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The effects of two kinds of oral cephalosporins, cefixime and cefdinir, on cytochrome P450 (CYP) activities in human hepatic microsomes were investigated. Both cefixime and cefdinir at 2 mM concentration neither inhibited nor stimulated CYP1A1/2-mediated 7-ethoxyresorufin *O*-deethylation, CYP2A6-mediated coumarin 7-hydroxylation, CYP2B6-mediated 7-benzoyloxyresorufin *O*-debenzylation, CYP2C8/9-mediated tolbutamide methylhydroxylation, CYP2C19-mediated *S*-mephenytoin 4'-hydroxylation, CYP2D6-mediated bufuralol 1'-hydroxylation, CYP2E1-mediated chlorzoxazone 6-hydroxylation, CYP3A4-mediated nifedipine oxidation, or CYP3A4-mediated testosterone 6 β -hydroxylation. The free fractions of cefixime and cefdinir in the incubation mixture, which were measured by ultracentrifugation, were 86.1–93.8% and 94.1–97.8%, respectively. These results suggest that both cefixime and cefdinir would not cause clinically significant interactions with other drugs, which are metabolized by CYPs, *via* the inhibition of metabolism.

Key words cefixime; cefdinir; human hepatic cytochrome P450; oral cephalosporin; drug interaction

Cefixime and cefdinir (Fig. 1), oral cephalosporins, are advanced-generation broad-spectrum antimicrobial agents.^{1–4)} Cefixime has broad and potent activity against various pathogens, especially gram-negative organisms, including beta-lactamase producing strains,^{2,3)} and cefdinir is highly active against many gram-positive and gram-negative bacteria.⁴⁾

Cytochrome P450s (CYP) comprise a superfamily of enzymes that catalyze the oxidation of a wide variety of xenobiotic chemicals, including drugs and carcinogens.^{5–7)} Multiple drug therapy is a common therapeutic practice, particularly in patients with several diseases or conditions, and many drug–drug interactions involving metabolic inhibition are being reported. Because antibiotics are co-administered in most cases, the possibility of interactions between them and other drugs exists. It is well known that macrolide antibacterials, especially troleandomycin and erythromycin, are inhibitors of CYP3A4-mediated metabolism.^{7,8)} Quinolone antibacterial agents, including enoxacin and pefloxacin, have an inhibitory effect on the metabolism of drugs mediated by CYP1A2.^{7,9–11)} In particular, ciprofloxacin inhibits CYP1A2-mediated metabolism, although more than 56% of

dosing is excreted in urine as unchanged drug, and ciprofloxacin metabolism by CYPs is of minor importance.^{12–14)} However, there are few reports describing not only the contribution of CYP to the metabolism of cephalosporins, but also the effect of cephalosporins on human hepatic CYP-mediated drug-metabolizing activity.

In the present study, we investigated the effects of cefixime and cefdinir on specific activities by CYP1A1/2, CYP2A6, CYP2B6, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human hepatic microsomes.

MATERIALS AND METHODS

Materials Pooled human hepatic microsomes from 10 donors were obtained from the Human and Animal Bridging Research Organization (HAB, Chiba, Japan).^{15,16)} Cefixime and cefdinir were synthesized and supplied by Fujisawa Pharmaceutical Co., Ltd. 7-Ethoxyresorufin, 7-benzoyloxyresorufin, resorufin, tolbutamide, chlorpropamide, chlorzoxazone, and nifedipine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). *S*-Mephenytoin, 4'-hydroxymephenytoin, methylhydroxytolbutamide, 6-hydroxychlorzoxazone, and oxidized nifedipine were obtained from Ultrafine Chemicals (Manchester, U.K.). 2-Benzoxazoline and methyltestosterone were purchased from Wako Pure Chemicals (Osaka, Japan), and 7-hydroxycoumarin was from Sumika Chemical Analysis Service, Ltd. (Osaka, Japan). All other reagents were of the highest purity commercially available.

Determination of Human CYP Activities 7-Ethoxyresorufin *O*-deethylase activity (CYP1A1/2), coumarin 7-hydroxylase activity (CYP2A6), 7-benzoyloxyresorufin *O*-debenzylation activity (CYP2B6), tolbutamide methylhydroxylase activity (CYP2C8/9), *S*-mephenytoin 4'-hydroxylase activity (CYP2C19), bufuralol 1'-hydroxylase activity (CYP2D6), chlorzoxazone 6-hydroxylase activity (CYP2E1), nifedipine oxidase activity (CYP3A4), and testosterone 6 β -hydroxylase activity (CYP3A4) in the presence or absence of cefixime or cefdinir (2 mM) were determined as described

Fig. 1. Chemical Structures of Cefixime and Cefdinir

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Table 1. Effect of Cefixime and Cefdinir on Metabolic Activities in Human Hepatic Microsomes

CYP	Reaction	Metabolic activity (pmol/min/mg)	Percent of control	
			Cefixime	Cefdinir
CYP1A1/2	7-Ethoxyresorufin <i>O</i> -deethylation	47.09	93.0	88.6
CYP2A6	Coumarin 7-hydroxylation	69.09	93.2	82.0
CYP2B6	7-Benzoyloxyresorufin <i>O</i> -debenzylation	4.86	100.8	112.1
CYP2C8/9	Tolbutamide methylhydroxylation	66.67	106.1	NA
	Tolbutamide methylhydroxylation ^{a)}	48.82	NA	101.6
CYP2C19	<i>S</i> -Mephenytoin 4'-hydroxylation	12.08	96.6	100.3
CYP2D6	Bufuralol 1'-hydroxylation	34.15	97.6	102.3
CYP2E1	Chlorzoxazone 6-hydroxylation	1384	89.3	92.3
CYP3A4	Nifedipine oxidation	1199	103.3	91.4
	Testosterone 6 β -hydroxylation	2180	102.6	92.3

NA: Not analyzed. *a*) Methylhydroxytolbutamide was analyzed by the method described in Materials and Methods. Results represent means of duplicate determinations.

previously.^{15,16)} The incubation mixture consisted of human microsomes, 2.5 mM NADP⁺, 25 mM glucose-6-phosphate, 10 mM magnesium chloride, 2 units of glucose-6-phosphate dehydrogenase, 100 mM potassium phosphate buffer (pH 7.4), 0.1 mM EDTA, and 0.01 ml of 200 mM sodium bicarbonate or 100 mM cefixime or cefdinir dissolved in 200 mM sodium bicarbonate in a final volume of 0.5 ml.^{15,16)} The microsomal protein concentration in the mixture was 0.2 (for 7-ethoxyresorufin *O*-deethylation, coumarin 7-hydroxylation, and 7-benzoyloxyresorufin *O*-debenzylation), 0.5 (for tolbutamide methylhydroxylation, nifedipine oxidation, and testosterone 6 β -hydroxylation), or 1 mg/ml (for *S*-mephenytoin 4'-hydroxylation, bufuralol 1'-hydroxylation, and chlorzoxazone 6-hydroxylation). Concentrations of 7-ethoxyresorufin, coumarin, 7-benzoyloxyresorufin, tolbutamide, *S*-mephenytoin, bufuralol, chlorzoxazone, nifedipine, and testosterone were 0.5, 2, 1.5, 400, 100, 20, 100, 30, and 150 μ M, respectively; which are around the expected K_m .^{15,16)} Because cefdinir interfered with the assay of methylhydroxytolbutamide by the methods described previously,^{15,16)} the effect of cefdinir on the methylhydroxytolbutamide formation was determined as follows. Briefly, after the reaction was terminated by the addition of 1 M HCl, 50 μ l of internal standard (20 μ g/ml sodium phenobarbital) and 3 ml of ethylacetate/hexane (1 : 1) were added, and the mixtures were shaken for 10 min and centrifuged at 1900 *g* for 5 min. The organic phase (2.5 ml) obtained was evaporated under nitrogen, and the residue was dissolved in 200 μ l of methanol/5 mM potassium dihydrogenphosphate (42 : 58), and 80 μ l was applied to HPLC. HPLC analyses were performed using an Inertsil ODS-3 (5 μ m, 150 $\times$ 4.6 mm I.D., GL Sciences, Tokyo, Japan) as a column and a TSK-guardgel ODS-80Ts (15 $\times$ 3.2 mm I.D., Tosoh Tokyo, Japan) as a guard-column, and the column temperature was maintained at 40 °C. The mobile phase was methanol/5 mM potassium dihydrogenphosphate (42 : 58) as eluent A and methanol/5 mM potassium dihydrogenphosphate (80 : 20) as eluent B. Gradient conditions were 0—12 min 0% B; 12—12.1 min 0—100% B (linear gradient); 12.1—20 min, 100% B; 20—20.1 min, 100—0% B (linear gradient); and 20—28 min, 0% B. The flow rate was held at 1 ml/min for 12 min and increased to 1.4 ml/min at 12.1 min. The UV detection wavelength was 240 nm.

Determination of Free Fraction in Incubation Mixture

The incubation mixture consisted of human microsomes (0.2—1.0 mg/ml), 2 mM cefixime or cefdinir, 100 mM potas-

Table 2. Free Fraction of Cefixime and Cefdinir in Reaction Mixture Containing Human Hepatic Microsomes

Protein concentration in reaction mixture (mg/ml)	Free fraction (%)	
	Cefixime	Cefdinir
0.2	90.2	94.3
0.5	86.1	97.8
1.0	93.8	94.1

Results represent means of duplicate determinations.

sium phosphate buffer (pH 7.4), and 0.1 mM EDTA in a final volume of 0.5 ml.¹⁵⁾ After a 5-min incubation at 37 °C, the mixture was centrifuged at 105000 *g* for 60 min at 4 °C,¹⁵⁾ and the concentration of cefixime or cefdinir in the supernatant was measured by HPLC with an analytical column TSKgel ODS-80T_M (150 $\times$ 4.6 mm I.D., Tosoh Co., Tokyo, Japan). The column temperature was set at 40 °C. The elution was conducted with 30% (for cefixime) or 20% (for cefdinir) methanol in 20 mM phosphate buffer (pH 2.5) at a flow rate of 1 ml/min, and detection was by UV absorbance at 295 nm.

RESULTS AND DISCUSSION

The inhibitory effects of cefixime and cefdinir on metabolic activities in human hepatic microsomes are summarized in Table 1. 7-Ethoxyresorufin *O*-deethylase, coumarin 7-hydroxylase, 7-benzoyloxyresorufin *O*-debenzylase, tolbutamide methylhydroxylase, *S*-mephenytoin 4'-hydroxylase, bufuralol 1'-hydroxylase, chlorzoxazone 6-hydroxylase, nifedipine oxidase, and testosterone 6 β -hydroxylase activities in the presence of cefixime or cefdinir at a concentration of 2 mM were 82.0—112.1% of the control, indicating that these cephalosporins had neither inhibitory nor stimulatory effects.

The free fractions of cefixime and cefdinir in the incubation mixture containing 0.2—1 mg/ml of microsomal protein were 86.1—93.8% and 94.1—97.8%, respectively, and the values were constant through the protein concentrations (Table 2). These results indicate that the protein binding of cefixime and cefdinir in the incubation mixture are of minor importance.

When the substrate concentration is much lower than the K_m value, the degree of inhibition (*R*) can be expressed by

the following equation, independent of the inhibition type, except in the case of uncompetitive inhibition,^{17,18)}

$$R = 1 / (1 + I_u / K_i)$$

where I_u and K_i are the unbound concentration of the inhibitor and the inhibition constant, respectively. Additionally, when the absorption rate is maximum, the maximum inflow concentration of the inhibitor into liver ($I_{in,max}$) can be expressed as,

$$I_{in,max} = I_{max} + (k_a \cdot D / Q_H) \cdot F_a$$

where I_{max} , k_a , D , Q_H , and F_a represent the maximum plasma concentration of the inhibitor in the circulation, absorption rate constant, dose, and hepatic blood flow, and the fraction absorbed from the gastrointestinal tract into the portal vein, respectively. After an oral dosing of 200 mg cefixime and cefdinir in healthy volunteers, the peak plasma concentrations (I_{max}) are 1.95 and 1.74 $\mu\text{g/ml}$, (4.3, 4.4 μM) respectively.^{19,20)} Additionally, approximately 21.2–27.6% and 25.9–33.3%, respectively, of dosing is excreted in urine unchanged, and no metabolites with antibacterial activity are detected in the serum or urine.^{19,20)} $I_{in,max}$ for cefixime and cefdinir are calculated using $k_a = 0.1 \text{ min}^{-1}$, $Q_H = 1610 \text{ ml/min}$, $F_a = 1$ to avoid false-negative predictions, to be 32 and 36 μM , respectively. In addition, protein binding of cefixime and cefdinir in human serum is reported to be 70.2–72.0% and 73.1%, respectively.^{21,22)} Therefore, the predicted free fractions of $I_{in,max}$ are much lower than the concentration investigated in this study (2 mM), suggesting that both cefixime and cefdinir might not affect the pharmacokinetics and metabolism of drugs metabolized by CYP1A1/2, CYP2A6, CYP2B6, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4.

In conclusion, the present study suggests that both cefixime and cefdinir would not cause clinically significant interactions with other drugs, which are metabolized by CYPs, via the inhibition of metabolism.

REFERENCES

- 1) Sakane K., Kawabata K., Inamoto Y., Yamanaka H., Takaya T., *Yakugaku Zasshi*, **113**, 605–626 (1993).
- 2) Markham A., Brogden R. N., *Drugs*, **49**, 1007–1022 (1995).
- 3) Matsumoto Y., *Chemotherapy*, **44** (Suppl. 1), 6–9 (1998).
- 4) Guay D. R. P., *Clin. Ther.*, **24**, 473–489 (2002).
- 5) Gonzalez F. J., *Pharmacol. Ther.*, **45**, 1–38 (1990).
- 6) Guengerich F. P., *FASEB J.*, **6**, 745–748 (1992).
- 7) Rendic S., *Drug Metab. Rev.*, **34**, 83–448 (2002).
- 8) Pea F., Furlanut M., *Clin. Pharmacokinet.*, **40**, 833–868 (2001).
- 9) Fuhr U., Anders E.-M., Mahr G., Sorgel F., Staib A. H., *Antimicrob. Agents Chemother.*, **36**, 942–948 (1992).
- 10) Fuhr U., Strobl G., Manaut F., Anders E., Sorgel F., Lopez-de-Brinas E., Chu D. T. W., Pernet A. G., Mahr G., Sanz F., Staib A. H., *Mol. Pharmacol.*, **43**, 191–199 (1992).
- 11) Kinzig-Schippers M., Fuhr U., Zaigler M., Dammeyer J., Rusing G., Labedzki A., Bulitta J., Sorgel F., *Clin. Pharmacol. Ther.*, **65**, 262–274 (1999).
- 12) McLellan R. A., Drobitch R. K., Monshouwer M., Renton K. W., *Drug Metab. Dispos.*, **24**, 1134–1138 (1996).
- 13) Vance-Bryan K., Guay D. R. P., Rotschafer J. C., *Clin. Pharmacokinet.*, **19**, 434–461 (1990).
- 14) Turnidge J., *Drugs*, **58**, 29–36 (1999).
- 15) Ikeda T., Nishimura K., Taniguchi T., Yoshimura T., Hata T., Kashiya E., Kudo S., Miyamoto G., Kobayashi H., Kobayashi S., Okazaki O., Hakusui H., Aoyama E., Yoshimura Y., Yamada Y., Yoshikawa M., Otsuka M., Niwa T., Kagayama A., Suzuki S., Satoh T., *Xenobio. Metab. Dispos.*, **16**, 115–126 (2001).
- 16) Umehara K., Shimokawa Y., Miyamoto G., *Biol. Pharm. Bull.*, **25**, 1112–1114 (2002).
- 17) Ito K., Iwatsubo T., Kanamitsu S., Ueda K., Suzuki H., Sugiyama Y., *Pharmacol. Rev.*, **50**, 387–411 (1998).
- 18) Ito K., Iwatsubo T., Kanamitsu S., Nakajima Y., Sugiyama Y., *Ann. Rev. Pharmacol. Toxicol.*, **38**, 461–499 (1998).
- 19) Nakashima M., Uematsu T., Takiguchi Y., Kanamaru M., *J. Clin. Pharmacol.*, **27**, 425–431 (1987).
- 20) Shimada K., Shishido A., Tsunoo M., *Chemotherapy*, **37** (Suppl. 2), 208–245 (1989).
- 21) Sakamoto H., Hirose T., Mine Y., Goto S., Nishida M., Kuwahara S., *Chemotherapy*, **33** (Suppl. 6), 157–168 (1985).
- 22) Sakamoto H., Hirose T., Nakamoto A., Hatano K., Mine Y., Kuwahara S., *Chemotherapy*, **37** (Suppl. 2), 165–178 (1989).