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 μmol concentration neither inhibited nor stimulated CYP1A1/2-mediated 7-ethoxyresorufin O-deethylase, CYP2A6-mediated coumarin 7-hydroxylase, CYP2B6-mediated 7-benzyloxyresorufin O-debenzylation, CYP2C8/9-mediated tolbutamide methylhydroxylase, CYP2C19-mediated S-mephenytoin 4′-hydroxylation, CYP2D6-mediated bufuralol 1′-hydroxylation, CYP2E1-mediated chlorzoxazone 6-hydroxylation, CYP3A4-mediated nifedipine oxidation, or CYP3A4-mediated testosterone 6β-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) Multiplicity of 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 cephalosporines, but also the effect of cephalosporines 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-benzoxylresorufin, resorufin, tolbutamide, chlorpropamide, chlorzoxazone, and nifedipine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). S-Mephenytoin, 4′-hydroxy-mephenytoin, methylhydroxytolbutamide, 6-hydroxychlorzoxazone, and oxidized nifedipine were obtained from Ultrafine Chemicals (Manchester, U.K.). 2-Benzoxazoline and methytestosterone 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-benzyloxyresorufin O-debenzylation activity (CYP2B6), tolbutamide methylhydroxylase activity (CYP2C8/9), S-mephenytoin 4′-hydroxylation activity (CYP2C19), bufuralol 1′-hydroxylation activity (CYP2D6), chlorzoxazone 6-hydroxylation activity (CYP2E1), nifedipine oxidase activity (CYP3A4), and testosterone 6β-hydroxylation activity (CYP3A4) in the presence or absence of cefixime or cefdinir (2 μmol) were determined as described...
previously. The incubation mixture consisted of human microsomes, 2.5 mM NADP, 25 mM glucose-6-phosphate, 10 mM magnesium chloride, 0.2—1.0 mg/ml 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. The microsomal protein concentration in the mixture was 0.2 (for 7-ethoxyresorufin O-deethylation, coumarin 7-hydroxylation, and 7-benzyloxyresorufin O-debenzylolation), 0.5 (for tolbutamide methylhydroxylation, nifedipine oxidation, and testosterone 6β-hydroxylation), or 1 mg/ml (for S-mephénytoin 4’-hydroxylation, bufuralol 1’-hydroxylation, and chlorzoxazone 6-hydroxylation). Concentrations of 7-ethoxyresorufin, coumarin, 7-benzyloxyresorufin, tolbutamide, S-mephénytoin, bufuralol, chlorzoxazone, nifedipine, and testosterone were 0.5, 2, 1.5, 400, 100, 20, 100, 30, and 150 μM, respectively; which are around the expected Kᵢ values. Because cefdinir interfered with the assay of methylhydroxytolbutamide by the methods described previously, 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 μl of internal standard (20 μg/ml sodium phenobarbital) and 3 ml of ethylacetate/hexane (1 : 1) were added, and the mixtures were shaken for 10 min and centrifuged at 19000 g for 5 min. The organic phase (2.5 ml) obtained was evaporated under nitrogen, and the residue was dissolved in 200 μl of methanol/5 mM potassium dihydrogenphosphate (42 : 58), and 80 μl was applied to HPLC. HPLC analyses were performed using an Inertsil ODS-3 (5 μm, 150×4.6 mm I.D., GL Sciences, Tokyo, Japan) as a column and a TSK-guardgel ODS-80Ts (15×3.2 mm I.D., Tosoh Co., 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 potassium phosphate buffer (pH 7.4), and 0.1 mM EDTA in a final volume of 0.5 ml. After a 5-min incubation at 37 °C, the mixture was centrifuged at 105000 g for 60 min at 4 °C and the concentration of cefixime or cefdinir in the supernatant was measured by HPLC with an analytical column TSKgel ODS-80TS (150×4.6 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-deethylation, coumarin 7-hydroxylation, 7-benzyloxyresorufin O-debenzylation, tolbutamide methylhydroxylation, S-mephénytoin 4’-hydroxylation, bufuralol 1’-hydroxylation, chlorzoxazone 6-hydroxylation, nifedipine oxidation, and testosterone 6β-hydroxylation 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 Kᵢ 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:\(^\text{17,18}\)

\[
R = \frac{1}{1 + I / K_i}
\]

where \(I\) 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 \((I_{\text{in,max}})\) can be expressed as,

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

where \(I_{\text{max}}, k_a, D, Q_H,\) 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 \((I_{\text{max}})\) are 1.95 and 1.74 \(\mu\)g/ml, (4.3, 4.4 \(\mu\)M) respectively.\(^\text{19,20}\) Additionally, approximately 21.2—27.6% and 73.1%, respectively.\(^\text{21,22}\) Therefore, the predicted free fractions of cefixime and cefdinir in human serum is reported to be 70.2—72.0% except in the case of uncompetitive inhibition,\(^\text{17,18}\)

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