INTERACTION OF IRINOTECAN (CPT-11) AND ITS ACTIVE METABOLITE 7-ETHYL-10-HYDROXYCAMPTOTHECIN (SN-38) WITH HUMAN CYTOCHROME P450 ENZYMES

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ABSTRACT:

The inhibition and mechanism-based inactivation potencies of irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin; CPT-11) and its active metabolite (7-ethyl-10-hydroxycamptothecin; SN-38) for human cytochrome P450 (P450) enzymes were investigated to evaluate the potential for drug interactions involving CPT-11 using microsomes from insect cells expressing specific human P450 isoforms. The mechanism and potential for interaction were examined by Lineweaver-Burk analysis, and NADPH-, time- and concentration-dependent effects were observed. CPT-11 and SN-38 competitively inhibited CYP3A4 (testosterone 6β-hydroxylation) activity with \( K_i \) values of 129 and 121 \( \mu \)M, respectively. CYP2A6 (cumarin 7-hydroxylation) and CYP2C9 (diclofenac 4'-hydroxylation) activities exhibited a mixed type of inhibition comprising competitive and noncompetitive components in response to SN-38, the \( K_i \) values being 181 and 156 \( \mu \)M, respectively. On the other hand, CYP1A2 (phenacetin O-deethylation), CYP2B6 (7-ethoxycoumarin O-deethylation), CYP2C8 (paclitaxel 6β-hydroxylation), CYP2C19 (S-mephenytoin 4'-hydroxylation), CYP2D6 (bufuralol 1'-hydroxylation), and CYP2E1 (chlorzoxazone 6-hydroxylation) were hardly affected by either compound. Furthermore, CPT-11 and SN-38 were suggested to be mechanism-based inactivators of CYP3A4. The \( k_{\text{inact}} \) and \( K_i \) values of CPT-11 and SN-38 were 0.06 min\(^{-1}\) and 24 \( \mu \)M and 0.10 min\(^{-1}\) and 26 \( \mu \)M, respectively. However, no inactivation of CYP2A6 and CYP2C9 by SN-38 was observed. These results mean that CPT-11 and SN-38 interact with human P450 isoforms, such as CYP2A6, CYP2C9, and CYP3A4, in vitro and imply that the significant drug interactions involving CPT-11 may be caused by a mechanism-based inactivation of CYP3A4 by SN-38 as an active metabolite of CPT-11 rather than competitive inhibition.

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin; CPT-11) (Fig. 1) is a semisynthetic water-soluble derivative of camptothecin, with a heterocyclic ring structure isolated from the Chinese tree Camptotheca acuminata (Sawada et al., 1991). CPT-11 has been shown to have strong antitumor activity through inhibition of topoisomerase I (Creemers et al., 1994; Pommier et al., 1994). This drug is currently registered for use in Japan, Europe, and the United States in patients with metastatic colorectal cancer refractory to several first-line therapies, including 5-fluorouracil, and shows clinical activity against several other types of solid tumors (Slichenmyer et al., 1993; Wiseman and Markham, 1996).

After intravenous administration, CPT-11 is metabolized by carboxylesterases in the liver and small intestine to an active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38) (Fig. 1), which is 100- to 1000-fold more potent than CPT-11 as a topoisomerase I inhibitor in various in vitro system (Creemers et al., 1994; Pommier et al., 1994; Mathijsen et al., 2001). SN-38 is further conjugated by UDP-glucuronosyltransferases primarily in liver to an inactive β-glucuronide.

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Abbreviations used are: CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin; CYP, cytochrome P450; SN-38, 7-ethyl-10-hydroxycamptothecin; APC, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxy-camptothecin; NPC, 7-ethyl-10-[4-(1-piperidino)-1-amino]carbonyloxy-camptothecin; HPLC, high-performance liquid chromatography; \( b_o \), cytochrome \( b_6 \); L-754,394, N-(2(R)-hydroxy-1(S)-indanyl)-5-(2(S)-1,1-dimethylallylamino)carbonyl)-4-(furo(2,3-b)pyridin-5-yl)(methyl)piperazin-1-yl)-4(S)-hydroxy-2(R)-phenyl(methyl)pentanamide.

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Fig. 1. Chemical structures of CPT-11 and SN-38.
derivative and excreted into urine and bile (Rivory, 2000; Mathijssen et al., 2001). Hydrolysis of SN-38 glucuronide by the intestinal microflora can occur and allows possible recycling of SN-38 in humans (Rivory, 2000; Mathijssen et al., 2001). However, CPT-11 unexpectedly causes severe toxicity of leukopenia or diarrhea, and it has been suggested that these side effects are due to an accumulation of SN-38 in various tissues (Rothenberg, 1997; Mathijssen et al., 2001).

Another pathway of CPT-11 metabolism consists of a cytochrome P450 (P450)-mediated oxidation of the bipiperidine side chain attached to the core structure (Haaz et al., 1998a,b; Rivory, 2000). The main metabolites resulting from this pathway have been identified as the aminopentane carboxylic acid and primary amine derivatives of CPT-11, namely APC and NPC, respectively (Rivory, 2000; Santos et al., 2000; Slatter et al., 2000). Although these metabolites have been regarded to have little cytotoxicity like CPT-11, NPC but not APC can be converted into SN-38 by hepatic carboxylesterases and, as such, may contribute to the overall production of the pharmacologically active species (Rivory, 2000). P450 consists of a superfamily of heme-containing monoxygenases and is responsible for the metabolism of many drugs, environmental chemicals, and endogenous substances (Nelson et al., 1996). Although the number of individual isoforms that have been identified and characterized is increasing, the drug metabolism in humans is handled mainly by CYP1, CYP2, and CYP3 (Spatzenegger and Jaeger, 1995; Nelson et al., 1996). In the case of CPT-11, CYP3A4 has been suggested to be the predominant isoform in oxidative metabolism (Haaz et al., 1998a,b; Santos et al., 2000). By contrast, the products and isoforms involved in the P450-mediated metabolism of SN-38, the active metabolite of CPT-11, in humans have not been identified, although the UDP-glucuronosyltransferase isoforms involved in glucuronidation have (Iyer et al., 1998; Rivory, 2000; Mathijssen et al., 2001).

Clinically relevant drug interactions are often the result of the effects of P450 enzymes during metabolism and can cause severe complications (Muck, 1994). However, there is little information available about the clinical interactions of CPT-11 with other drugs, and there are no reports directly comparing the efficiencies of CPT-11 and SN-38 to interfere with the biological functions of P450 isoforms and there are no reports directly comparing the efficiencies of CPT-11 with other drugs, effects of P450 enzymes during metabolism and can cause severe complications.

Materials. CPT-11 (lot 115122) and SN-38 (lot 970326) were kindly supplied by Yakult Honsha Co. (Tokyo, Japan). The purity (≥99%) of each compound was confirmed by analytical HPLC. Phenacetin, 4-acetamidophenol, 3-acetamidophenol, N-phenylanthranilic acid, chlorzoxazone, and 4-nitrophenol were purchased from Aldrich Chemical Co. (Milwaukee, WI). Coumarin, 7-hydroxycoumarin, 7-ethoxycoumarin, corticosterone, glutathione, and deferoxamine were purchased from Sigma Chemical Co. (St. Louis, MO). Paclitaxel, difenofenac, and testosterone were obtained from Wako Pure Chemical Industries (Osaka, Japan). 6a-Hydroxycyclopipecolate and 4′-hydroxydifenofenac were obtained from Gentest (Woburn, MA). S(-)-Mephenytin, (+)-3′-4′-hydroxymephenytin, (+)-butafural, (-)-1′-hydroxybutafural, 6′-hydroxycortisone, and 6β-hydroxysterosterone were purchased from Ultrafine Chemicals and Research (Manchester, UK). 7,13-Diacetyl baccatin III was obtained from Alexis Biochemicals (San Diego, CA), phenobarbital from Tokyo Kasei Kogyo Co. (Tokyo, Japan), and catalase from Calbiochem-Novabiochem Co. (San Diego, CA). NADP, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast Co. (Tokyo, Japan). All other chemicals and organic solvents were of the highest quality commercially available. Microsomes from baculovirus-infected insect cells expressing CYP1A2, CYP2A6 + b6, CYP2B6 + b6, CYP2C8 + b6, CYP2C9*1 + b6, CYP2C19 + b6, CYP2D6*1, CYP2E1 + b6, and CYP3A4 + b6 were obtained from GENTEST. All enzymes were coexposed with NADPH-cytochrome P450 oxidoreductase. The P450 contents were as described in the data sheets provided by the manufacturer.

Enzyme Assays. P450-dependent activities were determined by HPLC, as described previously with some modifications (Chauret et al., 1997; Busby et al., 1999; Nakajima et al., 1999). The standard incubation mixture contained 50 mM potassium phosphate buffer, pH 7.4, substrate, human P450-expressing microsomes, inhibitor/inactivator, and NADP+ generating system (1.0 mM NADP+, 10 mM glucose 6-phosphate, 2.0 units/ml glucose-6-phosphate dehydrogenase, and 5.0 mM MgCl2) in a final volume of 500 μl. All substrates were dissolved in methanol. CPT-11 and SN-38 as inhibitors/inactivators were dissolved in dimethyl sulfoxide, with the solvent being used as the control. The final concentration of organic solvent (methanol and dimethyl sulfoxide) in the incubation mixture was 0.8% (v/v). The substrate concentrations for the determination of residual P450 activities were set up to be close to the KM value for each enzyme activity obtained in the preliminary kinetic analysis.

Substrate and microsomal P450 concentrations for the assays were as follows: CYP1A2, 20 μM phenacetin and 20 pmol of P450/ml; CYP2A6, 0.80 μM coumarin and 5.0 pmol of P450/ml; CYP2B6, 100 μM 7-ethoxycoumarin and 20 pmol of P450/ml; CYP2C8, 5.0 μM paclitaxel and 20 pmol of P450/ml; CYP2C9, 2.0 μM difenofenac and 10 pmol/ml P450; CYP2C19, 20 μM S-mephenytin and 20 pmol/ml CYP2D6, 1.5 μM bufuralol and 5.0 pmol of P450/ml; CYP2E1, 600 μM chlorzoxazone and 50 pmol of P450/ml; CYP3A4, 50 μM testosterone and 10 pmol of P450/ml. The substrate concentrations for the determination of Km were 0.2 to 3.2 μM for CYP2A6, 0.50 to 8.0 μM for CYP2C9, and 10 to 160 μM for CYP3A4.

The reaction was initiated by the addition of the NADPH-generating system after a 1-min preincubation at 37°C. After incubation for 20 min (CYP2C8 and CYP2C19), 15 min (CYP1A2, CYP2C9, and CYP2E1), or 10 min (CYP2A6, CYP2D6, and CYP3A4) at 37°C, the reaction was terminated by the addition of 2.5 ml of ethyl acetate (CYP1A2, CYP2C8, CYP2C9, CYP2E1, and CYP3A4), 20 μl of 2.0 M phosphoric acid and 2.5 ml of t-butyl methyl ether (CYP2C9), and 20 μl of 2.0 M phosphoric acid (CYP2A6, CYP2B6, and CYP2D6). Samples for the determination of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2E1, and CYP3A4 activities were spiked with internal standards (5.0 nmol of 3-acetamidophenol for CYP1A2, 2.0 mmol of 7,13-diacetyl baccatin III for CYP2C8, 2.0 mmol of N-phenanthrylanilic acid for CYP2C9, 2.0 mmol of phenobarbital for CYP2C19, 5.0 nmol of 4-nitrophenol for CYP2E1, and 5.0 nmol of corticosterone for CYP3A4) and vigorously vortexed for 2 min. After centrifugation at 2000g for 10 min, the organic phase was evaporated to dryness under a gentle stream of nitrogen at 35°C. The residues were dissolved in 200 μl of methanol/water (50:50, v/v) and analyzed by HPLC. Samples for determination of CYP2A6, CYP2B6, and CYP2D6 activities were centrifuged at 6000g for 20 min. The supernatant was filtered with a polytetrafluoroethylene membrane filter with a 0.2 μm pore size (Millipore, Bedford, MA) and analyzed by HPLC.

HPLC Analysis. HPLC analysis was performed using a Shimadzu SCL-10A system controller (Kyoto, Japan) consisting of three LC-10AD pumps, an SIL-10A auto injector with sample cooler, an SPD-10AV UV-VIS detector, a CTO-10A column oven, a DGU-14A degasser, and a C-R7A chromatopac integrator. The samples were cooled at 4°C, and 20-μl aliquots were injected into an Inertsil ODS-80A column (150 × 4.6-mm i.d.; GL Sciences, Tokyo, Japan). The column was kept at 40°C. The calibration curves were established using authentic metabolites. The analytical conditions under which the products did not overlap with interfering peaks originating from inhibitors/inactivators and their metabolites were examined in a preliminary study.

The product (4-acetamidophenol) for CYP1A2 was eluted isocratically with 20 mM phosphate buffer, pH 5.4/methanol/acetonitrile (90:7.3, v/v/v) for 15 min, followed by a 15-min linear gradient to 20 mM phosphate buffer, pH 5.4/methanol/acetonitrile (62:14.24, v/v/v), and held for 20 min at a flow rate of 1.1 ml/min. UV detection was performed at 245 nm. The product (7-hydroxycoumarin) for CYP2A6 and CYP2B6 was eluted isocratically with 20 mM sodium perchlorate, pH 2.5/methanol (68:32, v/v) for 10 min, followed by a 10-min linear gradient to 20 mM sodium perchlorate, pH 2.5/methanol/acetonitrile (53:32:15, v/v/v), and held for 20 min at a flow rate of 1.1 ml/min.
Fluorometric detection was performed at a 330-nm excitation and 297-nm emission. The product (6'-hydroxybufuralol) for CYP2D6 was eluted isocratically with 20 mM potassium dihydrogenphosphate/methanol/acetonitrile (63:34:3, v/v/v) for 30 min, followed by a 10-min linear gradient to 20 mM potassium dihydrogenphosphate/methanol/acetonitrile (50:26:24, v/v/v), and held for 20 min at a flow rate of 1.0 ml/min. Fluorometric detection was performed at a 380-nm excitation and 454-nm emission. The protective effects of trapping agents, such as glutathione (a}
nucleophilic-trapping agent), deferoxamine (a free-iron scavenger), and catalase (a scavenger of reactive oxygen species), on the inactivation of CYP3A4 by CPT-11 and SN-38 were also evaluated (Table 3). All of the exogenously added trapping agents failed to prevent CYP3A4 from being inactivated by CPT-11 and SN-38.

**Discussion**

Although CPT-11 is a useful anticancer agent for numerous malignancies, such as colon and lung cancers, it can cause severe leukopenia or diarrhea due to an accumulation of SN-38, the active metabolite of CPT-11, in various tissues (Rothenberg, 1997). CPT-11 is clinically administered either alone or in combination with other drugs, including anticancer agents. However, coadministration often causes drug interactions leading to harmful side effects (Muck, 1994). Thus, the prediction or evaluation of the potential for drug interactions is an important aspect of individualized drug therapy. One of the most important mechanisms of drug interactions is the decrease of hepatic metabolism catalyzed by P450 enzymes (Guengerich, 1997). In the present study, the selectivity of inhibition or mechanism-based inac-
activation of human P450 enzymes by CPT-11 and SN-38 was investigated using microsomes from insect cells expressing P450s. To this end, we used nine P450-dependent activities as markers of P450 isoforms that mainly catalyze xenobiotics. The P450-dependent activities were phenacetin O-deethylation for CYP1A2, coumarin 7-hydroxylation for CYP2A6, 7-ethoxycoumarin O-deethylation for CYP2B6, paclitaxel 6α-hydroxylation for CYP2C8, diclofenac 4′-hydroxylation for CYP2C9, S-mephenytoin 4′-hydroxylation for CYP2C19, bufuralol 1′-hydroxylation for CYP2D6, chlorozoxazine 6-hydroxylation for CYP2E1, and testosterone 6β-hydroxylation for CYP3A4 (Wrighton and Stevens, 1992; Wrighton et al., 1993; Busby et al., 1999).

It has been reported that the most predominant P450 isoform involved in the oxidative metabolism of CPT-11 is CYP3A4 (Haaz et al., 1998a,b; Santos et al., 2000). The present results showed that CPT-11 effectively inhibits only CYP3A4 activity among the P450 isoforms tested, through competitive inhibition. SN-38, however, inhibited CYP2A6, CYP2C9, and CYP3A4 activities with a mixed or competitive type of inhibition. These observations may suggest that not only CYP3A4 but also CYP2A6 and CYP2C9 contribute to SN-38 metabolism. However, the inhibitory effects of CPT-11 and SN-38 on these P450 activities were not so strong; the K_i values (121–181 μM) were higher than the K_m values (18–111 μM) for the formation of APC and NPC in P450-mediated metabolism of CPT-11 using microsomes from human livers and mammalian cells expressing CYP3A4 in previous reports (Haaz et al., 1998a,b; Santos et al., 2000). On the other hand, Haaz et al. (1998a,b) have found that the oxidative metabolism of CPT-11 in human liver microsomes is remarkably inhibited by drugs such as loperamide and ondansetron, which are mainly metabolized by CYP3A4. The reason for the discrepancy between our results and previous reports may be a difference in the marker enzyme reactions, source of the enzyme, and/or CYP3A4 properties. Like CYP3A4, CYP2A6 and CYP2C9 also catalyze many drugs used clinically, such as tegafur, warfarin, and phenytoin (Rendic and Di Carlo, 1997). To predict drug interactions involving CPT-11, therefore, it is necessary to identify the rate and metabolites of the oxidative metabolism of SN-38 in humans and the P450 isoforms that are involved.

Drug metabolism catalyzed by P450 enzymes can be inhibited by other mechanisms in addition to competitive inhibition. One example is the inactivation of P450 by the metabolite of a drug that covalently binds to the enzyme to form a complex with P450, leading to irreversible inhibition (Silverman, 1988). In this case, as the drug has to be metabolically activated by an enzyme and covalently binds to the enzyme to form a complex, leading to irreversible (Silverman, 1988). In this case, as the drug has to be metabolically activated by an enzyme and covalently binds to the enzyme to form a complex, leading to irreversible inhibition. This may imply that the decrease in CYP2A6 and CYP2C9 activities is not due to the formation of a metabolic intermediate complex. Accordingly, it is possible that CPT-11 and SN-38 are moderate mechanism-based inactivators of human CYP3A4.

The mechanism-based inactivation requires more attention than competitive inhibition because the inhibitory effects remain after the elimination of the inhibitor from blood and tissue. It has been reported that the terminal elimination half-life of CPT-11 in plasma after intravenous administration is 14 to 15 h, and the value of SN-38 increases 1.7- to 2.1-fold relative to CPT-11 (Sparreboom et al., 1998; Kehrer et al., 2000; Slatter et al., 2000). Furthermore, the excretion rate of SN-38 into urine and bile has been reported to be clearly slower than that of unchanged CPT-11, although the total urinary and fecal excretion as free/conjugated forms is 6.5 to 12% of the dose (Sparreboom et al., 1998; Slatter et al., 2000). These findings may mean that SN-38 is extensively distributed and accumulated in the tissues and that the metabolite rather than parent drug causes drug interactions. However, although the K_i values of CPT-11 and SN-38 for CYP3A4 activities obtained in this study differed from the plasma concentration of CPT-11 and its metabolites (0.17–7.5 μM) (Sparreboom et al., 1998; Kehrer et al., 2000), we could not identify the relationship between in vitro and in vivo data.

In conclusion, we studied the selectivity of the inhibition or inactivation of human P450 isoforms by CPT-11 and its active metabolite SN-38. CPT-11 and SN-38 blocked CYP3A4 activity and CYP2A6, CYP2C9, and CYP3A4 activities, respectively, through competitive or mixed-type inhibition. The K_i values were >100 μM, suggesting that the drug interactions involving CPT-11 caused by competitive inhibition are clinically insignificant. However, a moderate mechanism-based inactivation of CYP3A4 by CPT-11 and SN-38 was observed (SN-38 > CPT-11). Therefore, our findings imply that significant drug interactions involving CPT-11 results from a mechanism-based inactivation of CYP3A4 by SN-38 as an active metabolite of CPT-11 rather than competitive inhibition. The pharmacokinetic interactions of CPT-11 based on the induction of CYP3A4 by anticonvulsants and micellae encapsulation of anticancer agents in formulation vehicles have been reported (Friedman et al., 1999; van Zuylen et al., 2001). Further studies are underway in our laboratory to clarify the relationship between the metabolic profile of CPT-11 and the inhibition or inactivation selectivity of P450 isoforms and to predict in vivo interactions of CPT-11 from in vitro data for the suitable use of CPT-11 in combination with other drugs.

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References


