DETERMINATION OF DRUG INTERACTIONS OCCURRING WITH THE METABOLIC PATHWAYS OF IRINOTECAN

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(Received October 25, 2001; accepted February 26, 2002)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

Irinotecan or CPT-11 [7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecine] is a derivative of camptothecine used in the treatment of advanced colorectal cancer. It requires activation to SN-38 (7-ethyl-10-hydroxycamptothecine) by carboxylesterase. Irinotecan and SN-38 are detoxified through two major metabolic pathways: the first one leads to oxidative degradation compounds, APC [7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecine] and NPC [7-ethyl-10-[4-amino-1-piperidino]carbonyloxycamptothecine], and involves cytochrome P450 3A4 (3A4 isoform); the second one leads to SN-38 glucuronide (SN-38G) and involves UDP-glucuronosyltransferase (UGT). Using human hepatic microsomes, we studied the interactions of 15 drugs of common use in colorectal cancer patients on these metabolic pathways. Only nifedipine had a significant effect on SN-38 formation, decreasing carboxylesterase activity by 50% at 100 μM and 35% at 10 μM. Three drugs had a significant effect on SN-38G formation: clonazepam increased UGT activity by 50% at 100 μM and 30% at 10 μM, and nifedipine and vinorelbine inhibited the activity by 65 and 55% at 100 μM, respectively, with no effect at 10 μM. Five drugs exerted a significant inhibition on SN-38 formation at 100 μM: clonazepam (70%), methylprednisolone (50%), nifedipine (80%), omeprazole (85%), and vinorelbine (100%). Only omeprazole and vinorelbine still exerted a significant inhibition at 10 μM (30 and 90%, respectively), whereas only vinorelbine had a significant effect at 2 and 0.5 μM (70 and 40%, respectively). In conclusion, potential clinical interactions with the metabolism of irinotecan are likely to be important for vinorelbine, which strongly inhibits irinotecan catabolism by CYP3A4 at clinically relevant concentrations, but not for the other drugs, which exert an effect at concentrations not achievable in patients.

Irinotecan or CPT-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecine is a water-soluble derivative of camptothecine (Sawada et al., 1991) that is currently used in the treatment of advanced colorectal cancer. Irinotecan is a prodrug that needs to be converted to SN-38 (7-ethyl-10-hydroxycamptothecine; Kawato et al., 1991) through the action of carboxylesterases (Rivory et al., 1996b; Haaz et al., 1997b). SN-38 is a very potent inhibitor of topoisomerase I to stabilize the cleavable complexes DNA-topoisomerase I. Irinotecan and SN-38 are detoxified through two major metabolic pathways. The first one independently leads to oxidative degradation compounds, APC [7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecine; Rivory et al., 1996b] and NPC [7-ethyl-10-[4-amino-1-piperidino]carbonyloxycamptothecine; Dodds et al., 1998]; in both cases, the reaction involves cytochrome P450 3A4 (3A4 isoform) (Haaz et al., 1998a,b). The second one leads to a glucuronide of SN-38 (SN-38G; Rivory and Robert, 1995) and involves UDP-glucuronosyltransferase (UGT) (isoenzyme 1A1 and other 1A isoforms) (Haaz et al., 1997a; Iyer et al., 1998).

The availability of SN-38 to its targets is, therefore, determined by a variety of enzyme activities, both for its formation and its detoxification. Since these enzymes are subjected to a wide individual variability, due to both genetic and environmental factors, there should be a similar variability in SN-38 availability, which could explain in turn at least part of the variability in response to irinotecan (about 20% responders in untreated as well as in 5-fluouracil-pretreated patients as stated by Rougier et al., 1997). In addition, the enzyme activities responsible for irinotecan metabolism could be affected by interactions with various drugs that are commonly combined with irinotecan in therapeutics. Using the model of human liver microsomes, we studied interactions of 15 drugs with the three major pathways involving irinotecan: formation of SN-38 by carboxylesterase, formation of NPC by CYP3A4, and formation of SN-38G by UGT.

Materials and Methods

Chemicals and Reagents. Pure irinotecan, SN-38, and NPC were supplied by Aventis (Vitré-sur-Seine, France). 20(S)-Camptothecine was obtained from Sigma-Aldrich Chimie (Saint-Quentin-Fallavier, France). The drugs used for interaction studies were obtained from various sources: carbamazepine, clonazepam, dexamethasone, flurafur, methylprednisolone, nifedipine, omeprazole, phenobarbital, phenytoine, ranitidine, valproic acid, and warfarin were obtained as pure chemicals from Sigma-Aldrich Chimie, as was bilirubin; capectabine, gemcitabine, and vinorelbine were obtained as clinical formula-

This work was supported by a grant from Aventis Oncology.

1 Abbreviations used are: SN-38, 7-ethyl-10-hydroxycamptothecine; SN-38G, SN-38 glucuronide; APC, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecine; NPC, 7-ethyl-10-[4-amino-1-piperidino]carbonyloxycamptothecine; UGT, UDP-glucuronosyltransferase.

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tions from Produits Roche (Neuilly-sur-Seine, France), Lilly-France (Saint-Cloud, France), and Pierre-Fabre Oncologie (Boulogne-sur-Seine, France), respectively. They were dissolved either in water or in methanol when they were not soluble in water. Solvents and reagents were of the highest grade commercially available.

**Human Liver Microsomes.** Microsomes were prepared according to a standard fractionation procedure (Berthou et al., 1989) from human liver samples obtained after approval by the relevant institutional ethical committee. We used a pool of five individual preparations obtained from Dr C. Riché (Faculty of Medicine, Brest, France) containing 17.4 mg of proteins per milliliter.

**Incubations.** Drug interactions were studied at fixed concentrations of irinotecan or SN-38 (25 or 5 μM, respectively) and of each potentially interfering drug (100 μM). When an effect was detected under these conditions, three other concentrations of the interfering drug were tested (0.5, 2, and 10 μM). Two different conditions were used: simultaneous incubation of the drug and irinotecan or SN-38 with the microsomes before the start of the reaction and preincubation of the drug with the microsomes for 20 min before the start of the reaction. Two independent experiments were performed in each condition. Control experiments were always carried out in the presence of an equivalent amount of the drug solvent (methanol or water).

For the study of the activation of irinotecan to SN-38 by carboxylesterases, microsomes were preincubated for 10 min on ice with 0.5% Triton X-100 (v/v). Aliquots of 0.32 mg of proteins were added to 0.1 M Tris buffer, pH 7.2, containing the drug tested or its solvent (final volume: 320 μl) and incubated in a shaking water bath. Irinotecan (25 μM) was then added to start the reaction. Incubation was performed for 1 h, and 37.5-μl samples were taken every 15 min for analysis.

To study the glucuronidation of SN-38 by UGT, microsomes were incubated for 30 min on ice with 0.5 mg/ml of proteins Brij 35 (Sigma-Aldrich Chimie). Aliquots of 0.32 mg of microsomal proteins were added to 0.1 M Tris buffer, pH 7.4, 37°C, containing the drug tested or its solvent (final volume: 320 μl) and incubated in a water bath. SN-38 (5 μM) and saccharolactone (4 mM), an inhibitor of β-glucuronidases, were then added. The reaction was started by the addition of uridine diphosphate β-glucuronic acid (4 mM). Incubation was performed for 1 h, and 37.5-μl samples were taken every 15 min for analysis. The effect of bilirubin was also tested on UGT activity in the same conditions, with concentrations ranging from 1 to 100 μM.

For the study of the conversion of irinotecan to NPC by CYP3A4, incubations were performed in 0.1 M phosphate buffer, pH 7.2, containing NADPH (1 mM), the drug tested or its solvent, and irinotecan (25 μM) (final volume: 320 μl). The reaction was started by adding the microsomes (0.32 mg of proteins). Incubation was performed for 20 min, and 37.5-μl samples were taken every 5 min for analysis.

**High-Performance Liquid Chromatography Analysis.** The samples of 37.5 μl were placed in 0.5-ml tubes containing 75 μl of ice-cold methanol/acetoneitrile (1:1, v/v) and 50 ng of camptothecin (internal standard). The mixture was vortex-mixed and centrifuged at 8000 g for 10 min. The supernatants were injected onto the high-performance liquid chromatography system (ThermoQuest, Saint-Quentin-en-Yvelines, France). Samples were processed with an automated sampler (Spectra Series AS300, ThermoQuest, and the compounds were separated with a Radial-Pak NovaPak C-18 reversed-phase column (Waters Associates, Saint-Quentin-en-Yvelines, France).

Elution was performed at a constant flow rate (1.5 ml/min). For SN-38 and SN-38G, the mobile phase was isocratic and contained 78% (v/v) of 0.075 M ammonium acetate buffer, pH 5.3, 22% acetonitrile, and tetrabutylammonium phosphate (Waters) at a final concentration of 5 mM (Rivory and Robert, 1994). For NPC, elution was performed with a programmed linear gradient over 18 min, containing 85 to 70% (v/v) of 0.075 M ammonium acetate, pH 6.0, and 15 to 30% acetonitrile, together with tetrabutylammonium phosphate, at a final concentration of 5 mM.

Detection was achieved with a spectrofluorometer (Spectra Series F1000) with excitation and emission wavelengths, respectively, set at 356 and 516 nm (for SN-38, 380 and 532 nm). Concentrations were calculated by reference to standard calibration curves.

**Results and Discussion**

**Drug Interactions at the Level of SN-38 Formation by Carboxylesterases.** SN-38 formation was linear between 15 and 60 min, following an initial burst characteristic for this enzyme (Rivory et al., 1996a; Haaz et al., 1997b). Carboxylesterase had a mean activity in the pool of 1.15 ± 0.05 pmol/min/mg of proteins (mean ± S.E.M.). Only one drug had a significant effect on SN-38 formation, nifedipine, which at 100 μM altered by about 50% the carboxylesterase activity, both with and without a 20-min preincubation of the microsomes with the drug. No effect was detected at 0.5 and 2 μM nifedipine, whereas a 35% reduction was evidenced at 10 μM.

In a prior study undertaken to characterize this activity (Haaz et al., 1997b), no major drug interactions were observed with a panel of 11 other drugs including anticancer drugs, antiemetics, antiadiaretics, antibiotics, analgesics, and nonsteroidal anti-inflammatory drugs. Only loperamide and ciprofloxacin exerted a significant inhibition of ca. 50% at 100 μM. In another study, Slater et al. (1997) identified no relevant interactions with another group of 13 different drugs. Few data are known about drug interactions with this enzyme activity, except for the known inhibitors of carboxylesterases, bis-nitrophenylphosphate and phytostigmine (Tsujii et al., 1991; Satoh et al., 1994). Nifedipine is the only drug in our panel to contain a carboxylester bond between two moieties of the molecule. This may explain the inhibitory effect exerted on SN-38 formation. Nevertheless, nifedipine probably cannot play a role in the clinical setting since, at clinically relevant concentrations (2 μM), this effect was not significant. Capecitabine contains an amide bond, which is cleaved by carboxylesterase for drug (Miwa et al., 1998). Since there was no interference of capecitabine on SN-38 formation, the enzyme responsible for capecitabine activation is probably distinct from that responsible for irinotecan activation, which would allow the combination between these drugs in the clinical setting.

**Drug Interactions at the Level of SN-38G Formation by UGT.** SN-38G formation was linear between 0 and 60 min. UGT had a mean activity in the pool of 5.0 ± 0.6 pmol/min/mg of proteins (mean ± S.E.M.). This is about 10 times lower than expected from experiments published earlier (Haaz et al., 1997a). Such a difference can only be explained by the existence of large individual variations or by a different purification yield of the microsomal preparation. Only three drugs had a significant effect on SN-38G formation at 100 μM: clonazepam, which increased the activity about 50%, and nifedipine and vinorelbine, which inhibited the activity by 65 and 55%, respectively. There was no additional effect of the 20-min preincubation of the microsomes with the drug. No effects were detected at 10 μM or below, except for clonazepam, for which a 30% stimulation of the activity was evidenced at 10 μM. Bilirubin, a known substrate of UGT1A1, inhibited the glucuronidation of SN-38, with 50% inhibition at 100 μM.

Loperamide, morphine, and paracetamol were the only drugs exerting a significant effect in our previous study (Haaz et al., 1997a). It could be expected that drug competition at the level of the glucuronidation reaction would explain the inhibition of SN-38G formation. However, many drugs on the total of 26 are glucuronidated in vivo, although the isoenzyme responsible has not always been identified. In addition, bilirubin, a known substrate of UGT1A1, has a limited effect on the formation of SN-38G, with an IC50 of about 100 μM. This may be due to the fact that UGT isoforms other than 1A1 can be involved in the glucuronidation of SN-38 (Cotti et al., 1999). It is worth noting that clonazepam exerts a stimulation of the glucuronidation of SN-38, which is already significant at 10 μM. No such direct stimulation of
this enzyme activity has been mentioned in the literature to our knowledge.

**Drug Interactions at the Level of NPC Formation by Cytochrome P450.** We chose NPC rather than APC formation as an indication of CYP3A4 activity because this metabolite was more easily detected and accurately quantified than APC. NPC formation was linear between 5 and 20 min with a constant y-axis intercept of about 50 nM. NPC was formed at a mean rate of 6.34 ± 0.50 pmol/min/mg of proteins (mean ± S.E.M.), which is lower than expected from the results previously published (Haaz et al., 1998a).

Again, this difference can be explained by the existence of individual variations in CYP3A activity. Indeed, we had observed a 40-fold difference in the rates of formation of NPC when studying individual microsomes preparations; the presence of a very active preparation in only one of the pools would thus substantially increase the global results. Five drugs had a significant effect on SN-38 formation at this concentration: clonazepam, methylprednisolone, nifedipine, omeprazole, and vinorelbine, which inhibited NPC formation by 70, 50, 80, 85, and 100%, respectively, both with and without a 20-min preincubation of the microsomes with the drug. Only omeprazole and vinorelbine still exerted a significant effect at 10 μM, whereas only vinorelbine also had a significant effect at 2 and 0.5 μM. Figure 1 presents the drug interactions as a function of their concentration. Using Dixon plots, it was possible to evaluate by nonlinear regression the Ki value of vinorelbine on NPC formation, which was 1.31 ± 0.01 μM. The Ki values were calculated by nonlinear regression and were as follows: vinorelbine, 0.85 μM; omeprazole, 22 μM; nifedipine, 50 μM; clonazepam, 60 μM; and methylprednisolone, 100 μM.

**References**


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