Irinotecan (CPT-11) Metabolites in Human Bile and Urine

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ABSTRACT

A female patient was treated with irinotecan (CPT-11) for liver metastatic colon carcinoma. She had a percutaneous biliary catheter because of extrahepatic biliary obstruction. The patient was treated with CPT-11 for three courses at doses of 350 mg/m^2 for the first course and 300 mg/m^2 for the remaining courses, given as a 30-min i.v. infusion. Metabolism studies in bile and urine were performed by coupling high-performance liquid chromatography to electrospray mass spectrometry. Conventional spectra [liquid chromatography/mass spectrometry (LC/MS)] allowed on-line molecular mass determination of CPT-11 and its main metabolites, whereas structural information was obtained by tandem mass spectrometry (LC/MS/MS).

At least 16 metabolites were detected in bile, while 8 of them were also detected in urine. Three compounds were identified as the parent drug, the active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38), and SN-38 glucuronide. The major metabolic pathway consists in oxidations of the terminal piperidine ring of the CPT-11 side chain, which eventually results in the formation of a primary amine. Other metabolites result from oxidation of the camptothecin nucleus. Finally, decarboxylation of the carboxylate form of CPT-11 was also observed. Several metabolites result from combinations of these pathways.

The structures of the identified metabolites indicate for the first time a major role of monooxygenases in the elimination of a camptothecin derivative in humans. This finding will allow better understanding of interindividual variability in pharmacokinetics and intestinal toxicity of CPT-11.

INTRODUCTION

CPT-11[2] (compound 1) is a semisynthetic derivative of 20(*S*)-camptothecin, an alkaloid originally extracted from the Chinese tree *Camptotheca acuminata*. Camptothecin and its derivatives act by inhibition of topoisomerase I, an enzyme involved in DNA duplication. CPT-11 has shown significant activity against various types of tumors in clinical studies and is thus far the only camptothecin drug to be marketed (1, 2).

CPT-11 differs from natural camptothecin by a 10-[1,4′-bipiperidine]-1′-carbonyloxy side chain and a 7-ethyl group. The synthetic side chain of CPT-11 renders the drug water soluble, which makes i.v. administration possible and also improves tissue distribution. However, the antitumour activity of CPT-11 results mainly from its hydrolysis by carboxylesterases to SN-38 (compound 2), a compound that is at least a 200 times more potent inhibitor than CPT-11 of topoisomerase I *in vitro* (3–5). In the rat, up to 40% of an i.v. administered dose of CPT-11 can be converted to SN-38 at low doses (6). SN-38 can be excreted in the bile and urine or conjugated by UDP-glucuronosyltransferases (7).

Studies in cancer patients have shown that CPT-11 can also be converted to SN-38 and SN-38 glucuronide in humans (8–10). However, the relative exposure to SN-38 is lower in humans than in rodent species. This is possibly caused by lower carboxylesterase levels in human plasma (5). Moreover, we showed previously that the sum of CPT-11, SN-38, and SN-38 glucuronide excreted in bile, feces, and urine accounted for only 25–50% of the administered dose in patients (8). Therefore, due to the lack of information on the metabolism of camptothecin derivatives in humans, we investigated the metabolic pathways of CPT-11 in a patient *in vivo*.

MATERIALS AND METHODS

Chemicals. CPT-11, SN-38, RPR 112524, and RPR 112526 were supplied by Rhône-Poulenc Rorer.

Patient. A 53-year-old woman was admitted with an extrahepatic biliary obstruction due to liver metastatic colon carcinoma (treating physician, Dr. J. P. Armand, Institut Gustave Roussy, Villejuif, France). The plasma bilirubin level was 14 μmol/liter. A percutaneous biliary catheter was placed to enable a rapid decrease and normalization of total bilirubin. Prior to chemotherapy, plasma levels of proteins and transaminases were normal. Plasma γ-glutamyl-transferase levels were four times the normal limit. The biological parameters remained stable between two infusions of CPT-11.

The patient received 350 mg/m^2 for the first course and 300 mg/m^2 for the second and third courses, administered at 3-week intervals. CPT-11 was given as a 30-min i.v. infusion.

Bile and urine were collected over a 48-h period, pooled, and stored at −20°C before analysis.

HPLC. Bile and urine samples were analyzed according to a HPLC technique modified from Barilero *et al.* (11). Briefly, samples were processed using a solid-phase (C_{18}) extraction step. The extracts were chromatographed on a C_{18} reversed-phase column with a mobile phase consisting of acetonitrile: deionized water (40:60), adjusted to pH 4 with 2 N HCl, using spectrofluorimetric detection (excitation 380 nm, emission 500 nm). Because of the acidification, only the lactone forms (closed rings) of CPT-11 and its metabolites were assayed.

To detect glucuronoconjugates, 100 μl of bile and urine

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[2] The abbreviations used are: CPT-11, irinotecan, (+)-7-ethyl-10-hydroxycamptothecin-10-[1,4′-bipiperidine]-1′-carboxylate; SN-38, 7-ethyl-10-hydroxycamptothecin; HPLC, high-performance liquid chromatography; amu, atomic mass units; LC, liquid chromatography; MS, mass spectrometry.

were treated overnight at 37°C with 1000 Sigma units of β-glucuronidase (*Escherichia coli*, type IX; Sigma Chemical, St. Louis, MO). The samples were then reanalyzed in the same conditions, and the chromatographic profiles were compared with the original samples.

Metabolite Identification. The chromatographic conditions for LC/MS and LC/MS/MS experiments were identical to those described above. Electrospray spectra were recorded in the positive ion mode on a Perkin Elmer Sciex API III triple stage quadrupole mass spectrometer. The voltage at the tip of the capillary used in the ionization process at atmospheric pressure was 5000 V. Nitrogen was added as nebulizer gas along the tip of the capillary with a flow rate of 0.6 liters/min. Data were acquired in the profile mode and the instrument was calibrated with polypropylene glycol.

LC/MS and LC/MS/MS experiments were carried out using a split at the outlet of the column (1:24) to obtain a flow rate of 40 μl/min into the mass spectrometer. The mass range for LC/MS experiments was 200–800 amu, with a step size of 0.1 amu. Scan time was 2 s. For LC/MS/MS, the first quadrupole selected only protonated molecular ions of the different metabolites identified by LC/MS, whereas the third quadrupole filtered ions after fragmentation in the argon-filled collision cell.

Mass spectra were reconstituted by averaging the scans (approximately 10.30 s) of each compound and subtraction of the background measured at both sides of the chromatographic peak.

RESULTS

Biliary and Urinary Profiles. Bile and urine samples from each course were analyzed using HPLC. Representative chromatograms with spectrofluorimetric detection of biliary and urine extracts are shown in Fig. 1. LC/MS analysis actually pointed out that some peaks consisted of a mixture of several metabolites (*e.g.*, compounds 9–12, 13–14). Urine samples from all courses presented an identical profile, with eight main components. Bile samples from each course also showed similar profiles, but with 16 components. A common numbering has been used for both bile and urine profiles, with CPT-11 and SN-38 corresponding to compounds 1 and 2, respectively. Glucuronidase treatment resulted in the conversion of SN-38 glucuronide into its aglycone, whereas none of the other peaks was affected (data not shown).

Mass Spectrometry Analyses. The molecular mass of the different metabolites was determined by LC/MS. Structural information was obtained by comparison of their fragmentation pattern on LC/MS/MS with the daughter spectrum of CPT-11 which is shown in Fig. 2.

Daughter spectra leading to a loss of 85 amu, corresponding to the terminal piperidine, in combination with the presence of *m/z* 84, 167 (bipiperidine), and 195 [(1,4′-bipiperidine)-1′-carboxyl] fragments, indicate that structural modification of metabolites occurred on the 7-ethyl-camptothecin nucleus. Conversely, from the persistence of the fragment *m/z* 502, corresponding to the 7-ethyl-camptothecin nucleus plus the proximal piperidine, it can be concluded that the terminal piperidine was modified. This observation is generally confirmed by a shift of the mass of the *m/z* 167 and 195 fragments equal to the difference in mass between CPT-11 and the corresponding metabolite. Finally, a lack of any loss of 44 amu in daughter spectra indicates that the lactone ring has been modified.

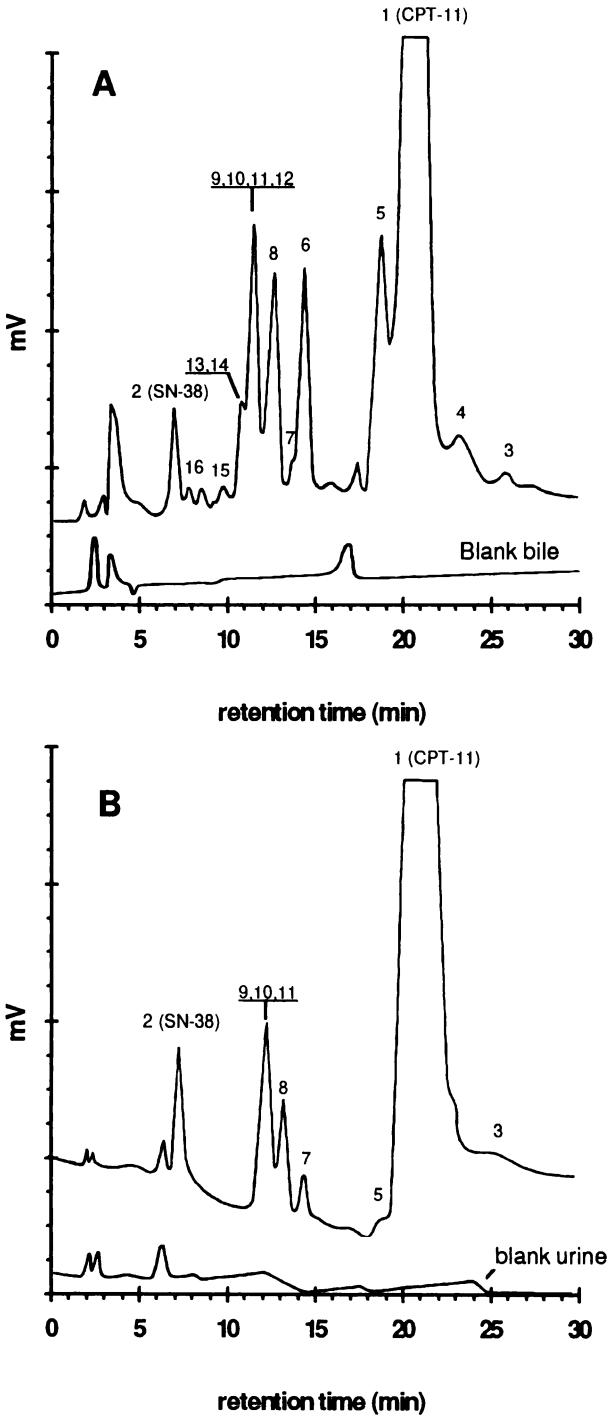

Fig. 1 HPLC chromatograms using spectrofluorimetric detection of a bile (A) and an urine sample (B) from a cancer patient before and after receiving CPT-11 treatment.

Metabolite Identification. The main fragment ions of the different metabolites, in agreement with the metabolite num-

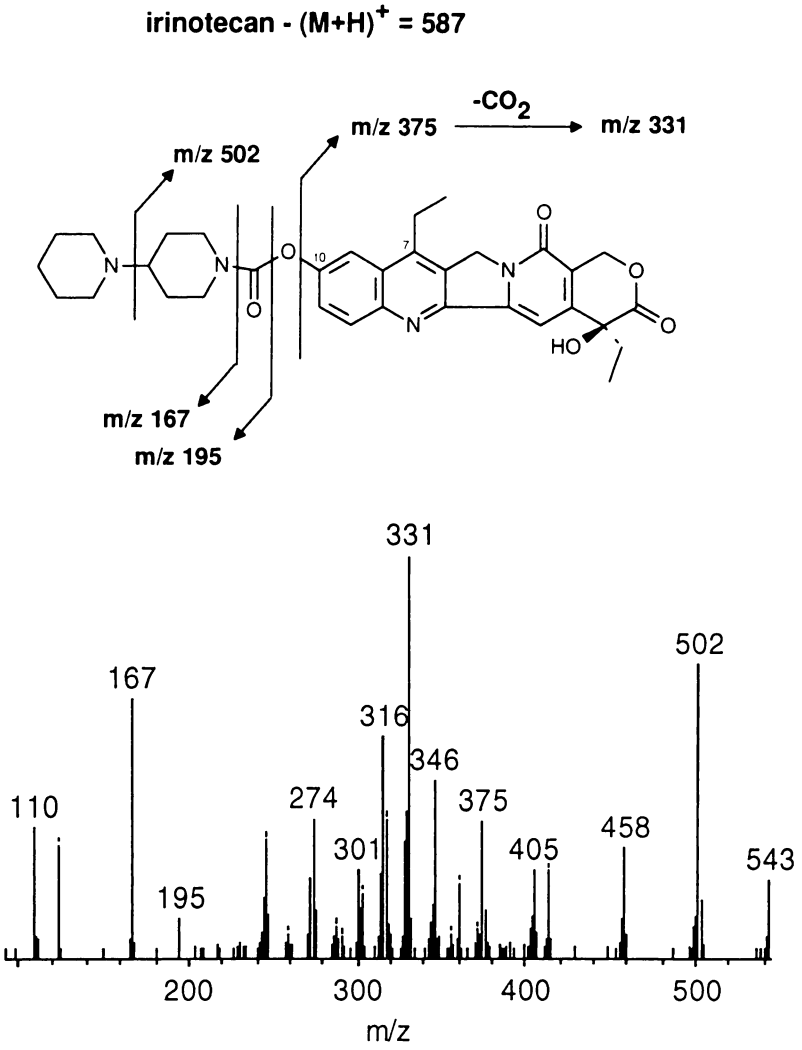

Fig. 2 MS/MS daughter spectrum of CPT-11 and a description of its fragmentation pattern.

bering used in Fig. 1, are summarized in Table 1 along with the most likely structure assignment. Some MS/MS daughter spectra of metabolites which allowed structural identification are shown in Fig. 3.

Five metabolites correspond to a single oxidation of CPT-11 (molecular mass of 602 or M + 16). The two major compounds result from oxidation of the terminal piperidine (metabolites 8 and 9, 7-ethyl-10-hydroxycamptothecin-10-[4-(*x*-hydroxy-1-piperidino)-1-piperidino]-1′-carboxylate), whereas metabolite 10 with a molecular mass of 618 results from a double oxidation of this ring. Finally, a double *N*-dealkylation in the terminal piperidine, resulting from oxidations of both α carbons, leads to metabolite 11, the primary amine 7-ethyl-10-hydroxy-camptothecin-10-(4-amino-1-piperidino)carboxylate with a molecular mass of 518.

The fragmentation pattern of two minor monooxidized metabolites (compounds 7 and 13) indicates that these are modified on the camptothecin moiety. The position of this additional oxygen could be precisely determined for metabolite 7 by comparison to the MS/MS spectrum of the reference compound RPR 112524 and corresponds to 5-hydroxy-CPT-11 (Fig. 3). The other metabolite can either result from oxidation of one of the two ethyl groups, of the aromatic rings of CPT-11, or correspond to a *N*-oxide.

Two other major metabolites only found in biliary extracts are of particular interest, since they correspond to a loss of 28 and 26 mass units from CPT-11 (compounds 5 and 6, respectively). Their MS/MS spectra indicate that structural modification has occurred on the camptothecin skeleton and, more precisely, on the lactone ring, as shown by the absence of a loss of 44 amu (CO_2) in the fragmentation spectrum. The MS/MS spectrum of metabolite 5 was effectively identical to that of the hemiketal compound RPR 112526 shown in Fig. 3. These metabolites thus result from decarboxylation of the open ring form of CPT-11. Moreover, a metabolic pathway with modifications of the bipiperidine side chain similar to that of CPT-11 was also observed for compound 5 (metabolites 4, 12, and 14–16).

The results of these analyses lead to a proposed metabolic scheme of CPT-11 in humans, which is presented in Fig. 4.

Table 1 Identification of CPT-11 metabolites in bile and urine

Bile and urine extracts were analyzed by LC/MS and LC/MS/MS. Structure assignments are based on the fragmentation pattern of the parent drug.

Compound	Molecular mass	MH^+ daughter spectrum	Comments	Peak intensity	
				Bile	Urine
1	586	*m/z* 543 ($-CO_2$) *m/z* 502 (−85 amu) *m/z* 84, 167, 195	CPT-11	High	High
2	392	–	SN-38	High	High
3	602	*m/z* 559 ($-CO_2$) *m/z* 502 [−(85 + 16) amu] *m/z* 183 (167 + 16 amu)	Oxidation of terminal piperidine	Low	Low
4	574	*m/z* 557 (−18 amu) *m/z* 474 [−(85 + 16) amu] *m/z* 183 (167 + 16 amu)	Oxidation of terminal piperidine of metabolite 5	Low	nd[a]
5	558	*m/z* 541 (−18 amu) *m/z* 474 (−85 amu) *m/z* 84, 167, 195	Decarboxylated: −28 amu on camptothecin nucleus; modification of the lactone ring	High	Low
6	560	*m/z* 543 (−18 amu) *m/z* 476 (−85 amu) *m/z* 84, 167, 195	Decarboxylated: −26 amu on camptothecin; modification of the lactone ring	High	nd
7	602	*m/z* 559 ($-CO_2$) *m/z* 518 (−85 amu) *m/z* 84, 167, 195	Oxidation of the camptothecin nucleus	Low	Low
8	602	*m/z* 559 ($-CO_2$) *m/z* 502 [−(85 + 16) amu] *m/z* 183 (167 + 16 amu)	Oxidation of terminal piperidine	High	High
9					
10	618	*m/z* 575 ($-CO_2$) *m/z* 502 [−(85 + 32) amu] *m/z* 393 [$(SN\text{-}38 + H)^+$] *m/z* 349 (393-CO_2) *m/z* 227 (195 + 32 amu)	Double oxidation of terminal piperidine	High	High
11	518	*m/z* 393 [$(SN\text{-}38 + H)^+$] *m/z* 349 (393-CO_2) *m/z* 99, 127	Loss of terminal piperidine	High	High
12	574	*m/z* 557 (−18 amu) *m/z* [−(85 + 16) amu] *m/z* 183 (167 + 16 amu)	Oxidation of terminal piperidine of metabolite 5	High	nd
13	602	*m/z* 559 ($-CO_2$) *m/z* 518 (−85 amu) *m/z* 84, 167, 195	Oxidation of the camptothecin nucleus	Low	Low
14	574	*m/z* 557 (−18 amu) *m/z* 474 [−(85 + 16) amu] *m/z* 183 (167 + 16 amu)	Oxidation of terminal piperidine of metabolite 5	Low	nd
15	490	No data obtained	Could correspond to metabolite 11 with the lactone ring of CPT-11 modified as metabolite 5	Low	nd
16	574	*m/z* 557 (−18 amu) *m/z* 490 (−85 amu) *m/z* 84, 167, 195	Oxidation on camptothecin nucleus of metabolite 5	Low	nd

[a] nd, not detected.

DISCUSSION

Drug disposition studies play an important role in the understanding of clinical pharmacology. Metabolite identification allows one to detect possibly active compounds and indicates the main enzymatic pathways involved in drug activation, detoxification, and elimination. This in turn may lead to a better understanding and thus control of interpatient variability in pharmacokinetics and drug response. This is particularly important for anticancer drugs, since therapeutic doses are often close to the maximum tolerated dose.

In the case of CPT-11, knowledge of metabolism and biliary excretion is even more important because of the clinical effects of the drug: CPT-11 has been shown to be active in colorectal cancer, while diarrhea is the dose-limiting toxicity in patients. Bile analysis may thus give an indication of luminal exposure of intestine tissue. Indeed, Gupta *et al.* (10) recently found a positive correlation between a biliary index based on SN-38 and SN-38 glucuronide plasma concentrations and the severity of diarrhea, indicating the importance of glucuronidation in detoxification. However, since only 5% of a therapeutic dose of CPT-11 is excreted in the form of SN-38 and its glucuronide and less than 50% as parent drug, other metabolic pathways are of major interest (8).

The results of this study show that biotransformation of the

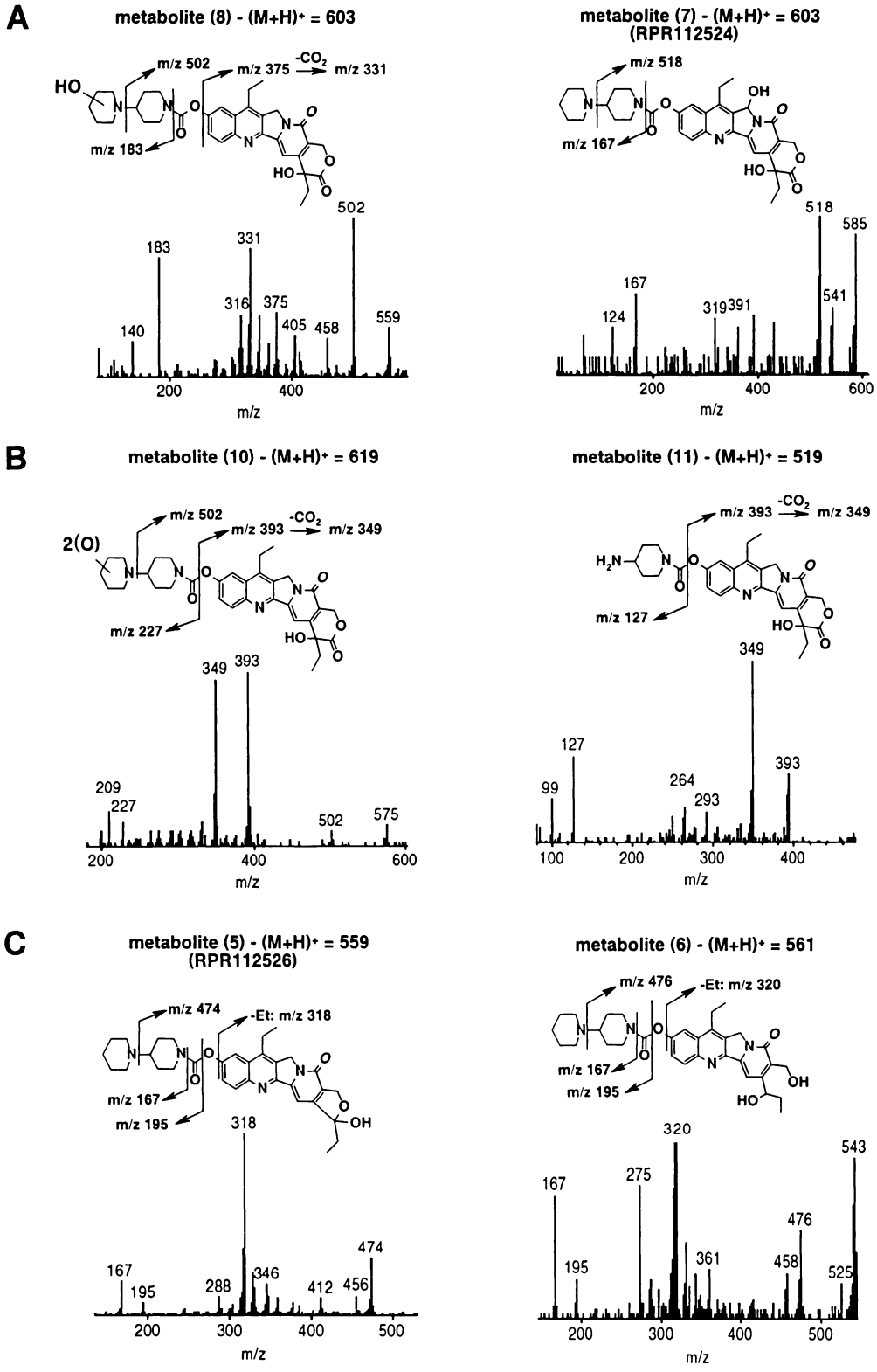

Fig. 3 Typical daughter spectra of CPT-11 metabolites obtained by LC/MS/MS. *A*, single oxidations on the terminal piperidine (*left*, 7-ethyl-10-hydroxycamptothecin-10-[4-(*x*-hydroxy-1-piperidino)-1-piperidino]-1′-carboxylate, compound 8) and on the camptothecin nucleus [*right*, RPR 112524, 5-hydroxy-CPT-11 or (+)-(*S*)-4,11-diethyl-4,9,12-trihydroxy-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-b]quinoline-3,14(4*H*,12H)-dione 9-[1,4′-bipiperidine]-1′-carboxylate, compound 7]. *B*, characterization of a double oxidation on the terminal piperidine (*left*, compound 10) and double *N*-dealkylation leading to the 4-amine-monopiperidine derivative [*right*, 7-ethyl-10-hydroxycamptothecin-10-[4-amino-1-piperidino]carboxylate, compound 11]. *C*, decarboxylation of the acid form of the lactone ring of CPT-11 can lead to the hemiketal analogue [*left*, RPR 112526, (+)-(*S*)-3,10-diethyl-3,8-dihydroxy-1*H*,3*H*-furo[3′,4′:6,7]indolizino[1,2-b]quinoline-13-one 8-[1,4′-bipiperidine]-1′-carboxylate, compound 5] and probably to the corresponding open ring form [*right*, (+)-(*S*)-12-ethyl-2-hydroxy-8-hydroxymethyl-7-(1-hydroxypropyl)-indolizino[1,2-b]quinoline-9-one 2-[1,4′-bipiperidine]-1′-carboxylate, compound 6].

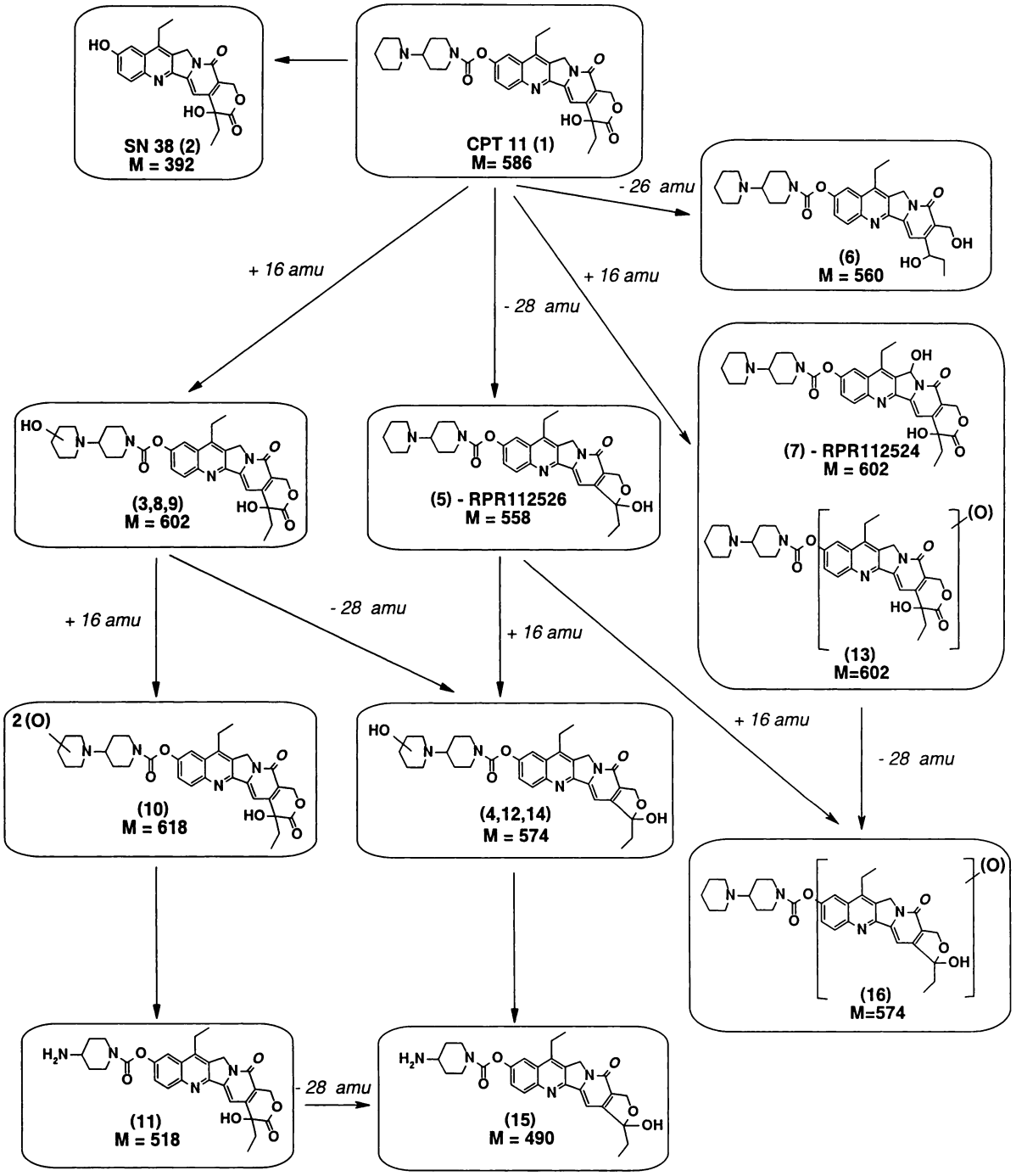

Fig. 4 Proposed metabolic pathways of CPT-11 in a human patient. Chemical names of major metabolites are given in the legend of Fig. 3, except for compound 15: (+)-(*S*)-3,10-diethyl-3,8-dihydroxy-1*H*,3*H*-furo[3′,4′:6,7]indolizino[1,2-b]quinoline-13-one 8-[4-amino-1-piperidino-carboxylate.

parent drug occurs at least on three sites in addition to ester hydrolysis: (*a*) decarboxylation of the lactone ring; (*b*) oxidation of the camptothecin nucleus or the 7-ethyl group; and (*c*) multiple oxidations of the bipiperidine side chain.

Decarboxylation of CPT-11 leads to the hemiketal metabolite 5 and to metabolite 6, most likely a ring-opened diol analogue. Oxidation of the camptothecin nucleus is evidenced by the fact that at least two minor metabolites, 7 and 13, had a molecular mass 16 amu more than the parent drug, whereas the fragment corresponding to the side chain was unchanged. The fragmentation spectrum of compound 7 was indeed identical to that of 5-hydroxy-CPT-11. However, MS/MS analysis did not

allow us to determine the precise structure of all metabolites, and the quantities recovered from the bile samples were not sufficient for nuclear magnetic resonance or infrared spectroscopic analysis.

Oxidation of the bipiperidine side chain appears to be the major metabolic pathway for CPT-11. Several metabolites resulting form oxidation of the terminal ring were detected. Oxidation of both α carbons leads via *N*-dealkylation to ring opening and the formation of the primary amine derivative, metabolite 11. Such a reaction is not uncommon for piperidine rings (12). Interestingly, all of these metabolites resulting from modifications of the side chain are still potential substrates for the carboxylesterases and could thus still be converted into the active metabolite SN-38. Combinations of the described reactions led to several minor metabolites.

The results of this study show that oxidative metabolism plays an important role in the elimination of CPT-11. It is therefore concluded that, besides the carboxylesterases and UDP-glucuronosyl transferases, a third class of enzymes is involved in CPT-11 metabolism, probably from the cytochrome P450 enzyme family. Some of these metabolites are likely to be systemically available, since they are excreted in urine. All of them will lead to intestinal exposure following biliary excretion. The exact cause of toxicities resulting from CPT-11 administration such as early and delayed diarrhea have not been elucidated, but a cholinergic effect of parent CPT-11 not related to cytotoxic activity has been suggested (13, 14). For these reasons, a pharmacological or toxic effect of Phase I metabolites of CPT-11 excreted in bile cannot be excluded.

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