HYDROLYSIS OF IRINOTECAN AND ITS OXIDATIVE METABOLITES, 7-ETHYL-10-[4-N-(5-
AMINOPENTANOIC ACID)-1-PIPERIDINO] CARBONYLOXYCAMPTOTHECIN AND 7-
ETHYL-10-[4-(1-PIPERIDINO)-1-AMINO]-CARBONYLOXYCAMPTOTHECIN, BY HUMAN
CARBOXYLESTERASES CES1A1, CES2, AND A NEWLY EXPRESSED CARBOXYLESTERASE ISoenzyme, CES3

Sonal P. Sanghani, Sara K. Quinney, Tyler B. Fredenburg, Wilhelmina I. Davis, Daryl J. Murry, and William F. Bosron

Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana (S.P.S., T.B.F., W.I.D., W.F.B.); Purdue University, School of Pharmacy and Pharmacal Sciences, Department of Pharmacy Practice, Indianapolis, Indiana (S.K.Q.); and University of Iowa, College of Pharmacy, Iowa City, Iowa (D.J.M.)

Received November 24, 2003; accepted February 4, 2004

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

ABSTRACT:

Carboxylesterases metabolize ester, thioester, carbamate, and amide compounds to more soluble acid, alcohol, and amine products. They belong to a multigene family with about 50% sequence identity between classes. CES1A1 and CES2 are the most studied human isoenzymes from class 1 and 2, respectively. In this study, we report the cloning and expression of a new human isoenzyme, CES3, that belongs to class 3. The purified recombinant CES3 protein has carboxylesterase activity. Carboxylesterases metabolize the carbamate produg 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxyacamptothecin (CPT-11; irinotecan) to its active metabolite 7-ethyl-10-hydroxyacamptothecin (SN-38), a potent topoisomerase I inhibitor. CYP3A4 oxidizes CPT-11 to two major oxidative metabolites, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxyacamptothecin (APC) and 7-ethyl-10-[4-(1-piperidino)-1-aminocarbonyloxycamptothecin (NPC). In this study, we investigate whether these oxidative metabolites, NPC and APC, can be metabolized to SN-38 by purified human carboxylesterases, CES1A1, CES2, and CES3. We find that CPT-11, APC, and NPC can all be metabolized by carboxylesterases to SN-38. CES2 has the highest catalytic activity of 0.012 min⁻¹ μM⁻¹ among the three carboxylesterases studied for hydrolysis of CPT-11. NPC was an equally good substrate of CES2 in comparison to CPT-11, with a catalytic efficiency of 0.005 min⁻¹ μM⁻¹. APC was a very poor substrate for all three isoenzymes, exhibiting a catalytic activity of 0.015 × 10⁻³ min⁻¹ μM⁻¹ for CES2. Catalytic efficiency of CES3 for CPT-11 hydrolysis was 20- to 2000-fold less than that of CES1A1 and CES2. The relative activity of the three isoenzymes was CES2 > CES1A1 >> CES3, for all three substrates.

CPT-11 is a water-soluble carbamate produg of camptothecin and is activated in vivo to SN-38, a potent topoisomerase I inhibitor (Kunimoto et al., 1987). CPT-11 either alone or in combination with other chemotherapeutic agents has shown promising clinical activity against several solid tumors (Rothenberg, 2001). Currently, CPT-11 in combination with 5-fluorouracil and leucovorin is approved by the FDA for the treatment of metastatic carcinoma of colon or rectum [Camptosar (irinotecan hydrochloride) package insert, Pharmacia & Upjohn Co., 2002 May].

CPT-11 undergoes extensive hepatic metabolism as shown in Fig. 1. Two major human liver carboxylesterases (E.C.3.1.1.1), CES1A1 and CES2, can hydrolyze CPT-11 to generate the active form of the drug, SN-38. CES2 is 64 times more efficient in metabolizing CPT-11 than CES1A1 (Humerickhouse et al., 2000). Human plasma esterases can hydrolyze CPT-11 in vitro (Kehrer et al., 2000; Morton et al., 2000). However, the peak plasma CPT-11 concentration is about 50 times greater than that of SN-38 after intravenous administration of drug (Slatter et al., 2000); thus, hydrolysis in plasma seems ineffi-
CPT-11 is oxidized by cytochrome P450 3A4 (CYP3A4) isozyme to produce 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (APC) and 7-ethyl-10-[4-(1-piperidino)-1-amino]carbonyloxycamptothecin (NPC). NPC, APC, and CPT-11 are metabolized by carboxylesterases (CES) to produce active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38). SN-38 is inactivated by UDP-glucuronosyltransferase isoform 1A1 to SN-38 glucuronide.

Several uridine diphosphate glucuronosyltransferase (UGT) isoforms inactivated by glucuronidation to form SN-38 glucuronide (SN-38G). Several uridine diphosphate glucuronosyltransferase (UGT) isoforms were studied, and UGT1A1 was found to be at least 10 times more active than other isoforms (Hanioka et al., 2001). In vitro studies suggested that NPC, but not APC, is metabolized by liver microsomes and/or CES1A1 to produce SN-38 (Rivory et al., 1996; Dodds et al., 1998).

Upon intravenous administration of [14C]CPT-11, 30% of the dose was recovered in the urine and 62% of the dose was excreted through feces (Slatter et al., 2000). The major excretion product was unchanged CPT-11, accounting for 55% of the administered dose, followed by APC (10.5%), SN-38 (8.7%), SN-38G (3.3%), and NPC (1.5%) (Slatter et al., 2000). Interindividual variation in CPT-11 pharmacokinetics is reported in several studies (Sparreboom et al., 1998; Kehrer et al., 2000; Slatter et al., 2000). Analysis of 24 human primary and metastatic colon tumor samples indicated a 56-fold variation in CPT-11 hydrolysis activity (Sanghani et al., 2003). This variation in activity correlated significantly with CES2 but not CES1A1 gene expression (Sanghani et al., 2003). The major dose-limiting toxicities of CPT-11 therapy are diarrhea and leukopenia. Mick et al. (1996) showed that the predicted biliary index of SN-38 correlated with the intestinal toxicity associated with CPT-11 therapy. The biliary index is the product of total CPT-11 area under the curve and the relative area ratio of SN-38 to SN-38G. The expression of CPT-11 carboxylesterases (Ahmed et al., 1999) and UGT1A1 (Iyer et al., 2001) in gastrointestinal tissue will determine the local conversion of CPT-11 to SN-38 and SN-38 glucuronide. Therefore, the intestinal levels of CPT-11 carboxylesterases and UGT1A1 may be determinants of the diarrhea side effect of CPT-11 therapy. Significant response has been observed in patients with low plasma SN-38 concentrations, and therefore, it has been suggested that tumor CPT-11 hydrolase activity may be important for efficacy of CPT-11 (Ratain, 2000). Hence, the expression of CPT-11 carboxylesterases may be a determinant of both the therapeutic efficacy and toxicity of CPT-11 therapy for colorectal cancer.

Carboxylesterases belong to a multigene family. These isoenzymes are responsible for metabolism of a variety of ester, carbamate, thioester, and amide drug compounds (Satoh and Hosokawa, 1998). Although substrate specificity of these isoenzymes is overlapping, they do show substrate preference (Bosron and Hurley, 2002). Human CES1A1 prefers substrates with a smaller alcohol moiety and larger acyl substituent such as meperidine or methylphenidate, whereas CES2 prefers a large alcohol and small acyl moiety such as CPT-11 or heroin (Satoh et al., 2002). Multi-tissue Northern blot analysis in human (Satoh et al., 2002) and rat (Sanghani et al., 2002) shows that carboxylesterase isoenzymes are most abundantly expressed in the liver, but some isoenzymes are expressed in a tissue-specific manner.

The overall goal of this study was to determine the relative contribution of individual carboxylesterases in SN-38 formation. We are reporting the cloning and expression of a new human carboxylesterase isozyme, CES3. Purified CES3 has carboxylesterase activity and is expressed in human liver and colon tissues by Northern blot analysis. The role of human liver carboxylesterases CES1A1, CES2, and CES3 in hydrolysis of CPT-11, APC, and NPC was investigated by steady-state kinetics.

Materials and Methods

Cloning of CES3. The CES3 gene (GI: 7019977) was cloned from cDNA generated by reverse transcription of human liver RNA (OriGene Technologies, Inc., Rockville, MD) using oligo-dT primer. A 50-μl reverse transcription reaction containing 2.5 μg of total human liver RNA was performed using a GeneAmp RNA PCR kit (Applied Biosystems, Foster City, CA). The reaction contained 2.5 μM oligo-dT primer, 5 mM magnesium, 1 mM concentration of each deoxynucleotide triphosphate, 50 U of RNase inhibitor, and 2.5 U/μl final concentration of murine leukemia virus reverse transcriptase. The reverse transcription conditions were 10 min at room temperature, 60 min at 42°C, 10 min at 68°C, and 5 min at 95°C. The cDNA was purified with a QIAquick PCR purification kit (Qiagen, Valencia, CA). The CES3 gene was amplified from human liver cDNA using the following forward 5′-GAAACCAGTTGTAAGGGAAATGGAGA-3′ and reverse 5′-CTGGGACTCTGCCACCATCTG3′.
GAA-3 primers. The 1.8-kb PCR product was gel purified with a QiAquick gel extraction kit (QIAGEN) and cloned into pCR-Blunt TOPO II vector (Invitrogen, Carlsbad, CA). Clones 11, 13, and 18 were sequenced in both directions.

Expression and Purification of CES3. The full-length 1.8-kb CES3 gene was obtained by complete digestion of clone 18 with BamHI and partial digestion with PsI, and was inserted into the pAcMP2 transfer vector (BD Biosciences PharmMingen, San Diego, CA). The pAcMP2-CES3 transfer vector, 2.5 μg, was cotransfected with 0.5 μg of linearized Baculogold DNA into 2.5 × 10^8 Sf9 cells using the Baculogold kit (BD Biosciences PharmMingen). The recombinant viruses were collected on day 5 and purified by plaque assay. One virus plaque was amplified, titrated by plaque assay, and used to infect log phase Sf9 cells in serum-free medium (Cambrex Corp., East Rutherford, NJ) at a multiplicity of infection of 1.

CES3 was purified from Sf9 cell extracts by a two-step purification protocol involving concanavalin A affinity chromatography followed by preparative nondenaturing gel electrophoresis. Briefly, Sf9 cells were harvested at 65 to 69 h postinfection, and immediately frozen in liquid nitrogen and stored at −70°C until further use. The frozen cell pellet from a 1-liter culture was resuspended in 40 ml of 20 mM Tris buffer, pH 7.4, containing 0.1% Triton X-100, 1 mM benzamidine, and 1 μM leupeptin. The cells were lysed by sonication and the lysate was centrifuged at 40,000 g for 45 min. One milliliter concentration each of Ca^2+, Mg^2+, and Mn^2+ ions was added to the clear supernatant and loaded onto a 20-ml concanavalin A column (Sigma-Aldrich, St. Louis, MO) column equilibrated in 20 mM Tris buffer, pH 7.4, with 0.2 M NaCl (buffer A). The affinity resin was washed with buffer A and the enzyme was eluted with a linear gradient of 150 ml of buffer A and 150 ml of buffer A containing 0.1 M methyl-α-D-mannopyranoside. The activity was monitored by enzyme assay using α-naphthyl acetate substrate, the active fractions were pooled and concentrated, and buffer was exchanged into 20 mM Tris buffer, pH 7.4. Concentrated protein from concanavalin A was separated by preparative nondenaturing gel electrophoresis on an 8-cm column of 6% polyacrylamide running gel and 1 cm of 4% polyacrylamide stacking gel prepared in a 0.25 mM Tris acetate running gel and 1 cm of 4% polyacrylamide stacking gel prepared in a 0.25 mM Tris acetate

4-Methylumbelliferyl Acetate Hydrolase Assay. Total carboxylesterase activity was monitored by a spectrophotometric method described by Brzezinski et al. (1997). Briefly, the enzyme was incubated at 37°C with 0.5 mM 4-methylumbelliferyl acetate in 90 mM KH_2PO_4, 40 mM KCl, pH 7.4. The formation of product, 4-methylumbelliferone, was monitored spectrophotometrically at 350 nm, ε_350 = 12.2 mM cm^−1. Protein was quantitated by the Bradford dye-binding method with bovine serum albumin as standard (Bio-Rad). The specific activity is expressed as μmol min^−1 mg^−1.

CESA1A1 purified from human liver exhibited a specific activity of about 7 U/mg (Brzezinski et al., 1994) and CES2 exhibited a specific activity of about 140 U/mg (Humerickhouse et al., 2000) with 4-naphthyl acetate solution in acetone. Formation of the azo dye was monitored at 490 nm, ε_490 = 18.33 mM cm^−1.

Steady-State Kinetics with CPT-11, NPC, and APC. The ability of carboxylesterases to hydrolyze CPT-11 and its metabolites was investigated by detecting the product, SN-38, using an Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA). Purified carboxylesterases were incubated with CPT-11 (0–200 μM), NPC (0–200 μM), or APC (0–100 μM) in 20 mM Hepes buffer, pH 7.0, with 10% ethylene glycol at 37°C from 2 to 24 h in 250 μl of reaction volume. The reaction was stopped by addition of 250 μl of acetonitrile. Ten microliters of 0.14 mM camptothecin (internal standard) was added, and the mixture was centrifuged at 1400g for 8 min. We found that the APC stock was contaminated with camptothecin; therefore, a control with no enzyme was studied for each concentration of APC analyzed. For CPT-11 and NPC, 100 μl of 2.5% perchorlic acid was added to 400 μl of supernatant to convert the drugs to the lactone form and extracted with 2 × 5 ml of CHCl_3. The CHCl_3 phase from each extraction was pooled and dried under nitrogen. For APC, a solid phase extraction method was used after quenching the reaction with 670 μl of 0.1 N HCl. The mixture was loaded on an Oasis HLB column (Waters, Milford, MA) that had been prepared by washing twice with methanol and equilibrated in water. The columns were washed twice with water and 10% methanol before eluting SN-38 with 1.6 ml of methanol. The samples from all the assays were dried under nitrogen and reconstituted in 100 μl of 0.005% perchloric acid containing 23% acetonitrile. SN-38 standards were prepared using the same procedure as described for the samples, and 45 μl was injected onto a 5-μm C18, 150 × 4.6 mm Luna column (Phenomenex, Torrance, CA). The mobile phase was 28.5% acetonitrile in 0.1 M KH_2PO_4, pH 4.0, with 3 mM heptane sulfonic acid. The compounds were eluted at a flow rate of 1 ml/min and monitored by fluorescence (excitation = 375 nm, emission = 560 nm). The data were fit to the Michaelis-Menten equation (GraFit 4.0; Erithacus Software Ltd., Surrey, UK), and the K_M and k_cat values for the three isoenzymes with the three substrates were determined.

Results

Cloning, Expression, and Purification of CES3. The CES3 gene was recently identified (GI: 7019977) during the New Energy and Industrial Technology Development Organization human cDNA sequencing project. We amplified the CES3 gene from human liver cDNA to generate a 1.8-kb PCR product including the start and stop codons. Sequencing in both directions confirmed that it was identical to the reported sequence (GI: 7019977). The Baculovirus expression system was used to overexpress the CES3 protein in Sf9 insect cells. CES3 was purified to homogeneity by a two-step procedure involving concanavalin A affinity chromatography and preparative nondenaturing PAGE (Table 1). Most of the activity in the cell lysate was from

HYDROLYSIS OF CPT-11 AND ITS METABOLITES BY CARBOXYLESTERASES

507
insect cell esterases. Concanavalin A column selectively binds the CES3 protein, and the insect cell esterases were separated in the flow-through. The specific activity of purified CES3 was 5.8 U/mg with α-naphthyl acetate as substrate. We used α-naphthyl acetate as substrate because 4-methylumbelliferyl acetate was found to be a very poor substrate for CES3. The protein yield from 1 liter of insect cell culture was ∼0.7 mg (Table 1). The carboxylesterase was purified 44-fold from the transfected Sf9 cell extract, and it exhibited a major band (>90% purity) of 60 kDa on SDS-PAGE (Fig. 2). Endoproteinase Glu-C or trypsin cleavage of this 60-kDa protein after SDS-PAGE resulted in enzyme with similar specific activity with CPT-11, NPC, and APC as substrates.

**Discussion**

Carboxylesterases belong to a multigene family (Satoh and Hosokawa, 1998), and the two main human carboxylesterase isoenzymes, CES1A1 (GI: 16905523) and CES2 (GI: 37622884), have been well characterized in human liver (Brzezinski et al., 1994; Pindel et al., 1997; Satoh et al., 2002). The CES3 gene was identified in colon tissue during the New Energy and Industrial Technology Development Organization human cDNA sequencing project (GI: 7019977). However, the expression of CES3 has not been reported. Hence, this is the first report of the cloning of the entire coding region of CES3 gene from human liver cDNA, expression of the CES3 carboxylesterase in insect cells, and characterization of CES3 catalytic activity with CPT-11, NPC, and APC as substrates.

CES1A1 and CES2 are mapped to human chromosome 16, q13–q22.1 and q21, respectively. The National Center for Biotechnology Information’s Spidey mRNA-to-genomic alignment program (http://www.ncbi.nlm.nih.gov/IEB/Research/Ostell/Spidey/index.html) was used to identify the location of CES3 gene (GI: 7019977). The mRNA mapped to the Homo sapiens chromosome 16 (GI: NT_010478) locus with no mismatches in the coding region. Thirteen exons were identified spanning about 12 kb of genomic sequence near the CES2 gene. The exon size and location of cDNA sequences are shown in Fig. 5.

Sequence identity analysis of carboxylesterase isoenzymes showed that CES3 has about 40% identity with both CES1A1 and CES2 isoenzymes. Phylogenetic analysis of CES3 indicates that it belongs to a new class of human carboxylesterase isoenzyme, and it has 63% identity with mouse esterase 31 (GI: 38511890), which was classified as Class 3 by Satoh and Hosokawa (1995). Hence, we call this isoenzyme CES3.
exposure.

The autoradiograph was developed after 8 days of exposure.

The predicted amino acid sequence of the CES3 gene is shown in Fig. 3. The active site residues Ser229, Glu347, and His460 and the four conserved cysteine residues were identified by alignment with rat and human carboxylesterase isoenzymes (Omiga 1.1; Accelrys, San Diego, CA). Analysis of the CES3 protein with ScanProsite (http://us.expasy.org/tools/scanprosite/) identified a conserved carboxylesterase motif, PS00122 (amino acids 216–231), and one potential glycosylation site at Asn105, shown in bold and underlined in Fig. 3. Mammalian carboxylesterases have a C-terminal tetra peptide QEDL does, however, function as an endoplasmic reticulum retention signal. This peptide contains an asparagine residue at position 105, shown in bold and underlined. The peptides that were identified by MALDI-TOF mass spectrometry of Glu-C and tryptic peptides of the recombinant protein are underlined.

During purification, recombinant CES3 protein binds to concanavalin A, verifying that it is glycosylated. Consistent with other carboxylesterases, a 26-amino acid N-terminal signal peptide was identified by Signal P 1.1 software (http://www.cbs.dtu.dk/services/SignalP/). In support of this, the most N-terminal peptide was identified during MALDI-TOF mass spectrometry of the tryptic peptides but not the leader sequence (Fig. 3). Mammalian carboxylesterases have a C-terminal microsomal retention sequence that is usually a variation of the ERp (Mazzarella et al., 1994). It is not clear whether QDEL functions more commonly as a microsomal retention signal. Northern-blot analysis (Fig. 4) showed that the tissue distribution of CES3 is very similar to that of CES2 (Satoh et al., 2002), with liver > colon > small intestine. Based on the exposure time and determination of relative expression in colon tissue (Sanghi et al., 2003), we conclude that the abundance of CES3 is much less than that of CES2 in colon. In the same study, we did not find any correlation between CPT-11 hydrolysis activity and CES3 message in colon tumor samples.

In vitro cytotoxicity studies show that the IC_{50} for CPT-11 is about 10^3 higher (less efficient) than for the active form of the drug, SN-38 (Sparreboom et al., 1998). The oxidative metabolism of CPT-11 by the action of CYP3A4 (Fig. 1) generates two major metabolites, APC and NPC (Santos et al., 2000). The IC_{50} values of NPC and APC are also about 10^3 higher than SN-38 (Rivory et al., 1996; Dodds et al., 1998). Enzymes involved in the glucuronidation of SN-38 (Fig. 1) have been extensively studied with respect to inactivation of SN-38 (Hanioka et al., 2001; Tukey et al., 2003). Hence, overall SN-38 concentration is precisely determined by a balance between generation of SN-38 from CPT-11, APC, and NPC and inactivation by glucuronidation.

The identity and roles of specific carboxylesterases in the in vivo activation of NPC or APC to form SN-38 have not been established. We find that CPT-11, NPC, and APC are converted to SN-38 by all three human liver carboxylesterases but with different catalytic efficiencies, CES2 > CES1A1 > CES3 (Table 3). In vitro studies demonstrated that CPT-11 is metabolized by carboxylesterases and that CES2 is 64 times more efficient than CES1A1 (Humerickhouse et al., 2000). CES3 can hydrolyze CPT-11, but the relative rate is much lower than those of CES2 and CES1A1 (Table 3). The relative abundance of carboxylesterases...
The substrates, CPT-11 (0–200 μM), NPC (0–200 μM), or APC (0–1000 μM) were incubated in 20 mM Hapes buffer, pH 7.0, with 10% ethylene glycol at 37°C for 2–24 h in 250 μl of reaction volume. The product, SN-38, was extracted with 2 × 5 ml CHCl₃, or with Oasis HLB columns and quantitated on an Agilent 1100 HPLC equipped with a fluorescence detector, with excitation set at 375 nm and emission monitored at 560 nm. The concentration of SN-38 was determined from the standard curve generated under identical conditions with each experiment. The substrate consumption was always less than 6%.

One hundred percent of the CDNA sequence (GI: 7019977) matched with the genomic sequence. The cDNA was distributed over 13 exons (shown as boxes) with 100% identity and with no gaps and no mismatches. The mRNA coordinates based on GI: 7019977 and genomic coordinates based on Contig; GI: NT_010478 for each exon are as follows: exon 1, 1–100, 632,917–633,016; exon 2, 101–305, 634,806–635,010; exon 3, 306–444, 635,118–635,256; exon 4, 445–578, 635,429–635,562; exon 5, 579–732, 635,984–636,137; exon 6, 733–837, 636,250–636,354; exon 7, 838–939, 637,871–637,972; exon 8, 940–1080, 638,352–638,492; exon 9, 1081–1161, 641,306–641,386; exon 10, 1162–1309, 642,799–642,946; exon 11, 1310–1459, 643,983–644,132; exon 12, 1460–1538, 644,295–644,373; exon 13, 1539–2093, 644,481–645,035. Blasting the human genome with the GI: 7019977 also identified this gene on human chromosome 16 and adjacent to CES2 gene.

**TABLE 3**

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>Substrate</th>
<th>( K_M )</th>
<th>( k_{cat} )</th>
<th>Catalytic Efficiency</th>
<th>Normalization of Catalytic Efficiency to CES2 for Each Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CES1A1</td>
<td>CPT-11</td>
<td>39 ± 3</td>
<td>4.9 ± 0.1</td>
<td>0.13</td>
<td>1</td>
</tr>
<tr>
<td>CES2</td>
<td>CPT-11</td>
<td>1.1 ± 0.1</td>
<td>13 ± 0.2</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>CES3</td>
<td>CPT-11</td>
<td>137 ± 11</td>
<td>0.9 ± 0.4</td>
<td>0.0063</td>
<td>0.05</td>
</tr>
<tr>
<td>CES1A1</td>
<td>NPC</td>
<td>80 ± 6</td>
<td>1.8 ± 0.06</td>
<td>0.023</td>
<td>0.5</td>
</tr>
<tr>
<td>CES2</td>
<td>NPC</td>
<td>3.2 ± 0.2</td>
<td>16 ± 0.2</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>CES3</td>
<td>NPC</td>
<td>460 ± 50</td>
<td>0.26 ± 0.01</td>
<td>0.00056</td>
<td>0.01</td>
</tr>
<tr>
<td>CES2</td>
<td>APC</td>
<td>270 ± 50</td>
<td>0.2</td>
<td>0.015</td>
<td></td>
</tr>
</tbody>
</table>

*Independent estimations of \( K_M \) and \( k_{cat} \) values were made for each enzyme and substrate. The values for one such experiment with standard error for the fit are reported. The estimations were made by nonlinear regression analysis of the data to the Michaelis-Menten equation (Grafit 4.0; Erithacus Software Ltd.).

**Fig. 5.** The exon-intron map of CES3 gene on human chromosome 16 (Contig; GI: NT_010478) as determined by Spidey software.

This study conclusively shows that CPT-11 and its metabolites, APC and NPC, are metabolized by carboxylesterases to SN-38. We also find that the new isoenzyme, CES3, is expressed in liver and colon tissue but has very poor activity for CPT-11, APC, and NPC and therefore will not play a significant role in CPT-11 metabolism. Based on the steady-state kinetics results and the tissue distribution and abundance of carboxylesterases determined by Northern analysis (Sanghani et al., 2003), we predict that CES2 isoenzyme is the most important isoenzyme in CPT-11 metabolism.

**Acknowledgments.** We thank Dr. Patrick McGovren, Pharmacia Corp., for providing CPT-11, APC, and NPC, and SN-38 for these studies.

**References**


glucuronosyltransferase isoforms involved in the glucuronidation of 7-ethyl-10-
Humerickhouse R, Lohrbach K, Li L, Bosron WF, and Dolan ME (2000) Characterization of
CPT-11 hydrolysis by human liver carboxylesterase isoforms hCE-1 and hCE-2. Cancer Res
60:1189–1192.
Iyer L, King CD, Whittington PF, Green MD, Roy SK, Tephly TR, Coffman BL, and Ratain MJ
diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metab-
Johnston KJ and Ashford AE (1980) A simultaneous-coupling azo dye method for the quanti-
Factors involved in prolongation of the terminal disposition phase of SN-38: clinical and
Kunimoto T, Nitta K, Tanaka T, Uehara N, Baba H, Takeuchi M, Yokokura T, Sawada S,
Miyasaka T, and Mutai M (1987) Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-
piperidino]carbonyloxy-camptothecin, a novel water-soluble derivative of camptothecin,
Mazarella RA, Marcus N, Haugejorden SM, Balcarek JM, Baldassare JJ, Roy B, Li LJ, Lee AS,
and Green M (1994) ERp61 is GRP58, a stress-inducible luminal endoplasmic reticulum
protein, but is devoid of phosphatidylinositol-specific phospholipase C activity. Arch Bio-
chem Biophys 308:454–460.
pharmacokinetics: pharmacodynamics: prediction of biliary index and intestinal toxicity. J Clin
Morton CL, Wierdl M, Oliver L, Ma MK, Danks MK, Stewart CF, Eisenman JL, and Potter PM
Pindel EV, Kedishvili NY, Abraham TR, Brzezinski MR, Zhang J, Dean RA, and Bosron WF
(1997) Purification and cloning of a human liver carboxylesterase (hCE-2) that catalyzes the