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Advances in Brief

Characterization of CPT-11 Hydrolysis by Human Liver Carboxylesterase Isoforms hCE-1 and hCE-2[1]

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Abstract

7-Ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin (irinotecan; CPT-11) is a prodrug activated by carboxylesterase enzymes. We characterized the hydrolysis of CPT-11 by two recently identified human carboxylesterase (hCE) enzymes, hCE-1 and hCE-2. K_m and V_{max} for hCE-1 and hCE-2 are 43 μM and 0.53 nmol/min/mg protein and 3.4 μM and 2.5 nmol/min/mg protein, respectively. hCE-2 has a 12.5-fold higher affinity for CPT-11 and a 5-fold higher maximal rate of CPT-11 hydrolysis when compared with hCE-1. In cytotoxicity assays, incubation of 1 μM CPT-11 with hCE-2 (3.6 μg/ml) resulted in a 60% reduction in survival of SQ20b cells. No significant reduction in cell survival was observed after incubation of CPT-11 with hCE-1. These data indicate that hCE-2 is a high-affinity, high-velocity enzyme with respect to CPT-11. hCE-2 likely plays a substantial role in CPT-11 activation in human liver at relevant pharmacological concentrations.

Introduction

CE[3] enzymes are members of a group of serine esterases found in numerous animal species and a variety of mammalian tissues (1, 2). These enzymes hydrolyze many different endogenous and xenobiotic compounds and play a role in the metabolism of numerous drugs. The anticancer agent CPT-11 is a prodrug that is activated by CE enzymes. Hydrolysis of the bulky piperidino side chain by CE produces SN-38, a potent inhibitor of topoisomerase I (3, 4).

The conversion of CPT-11 to SN-38 has been characterized in several mammalian species (5), but the specific enzyme(s) responsible for activation of CPT-11 in humans has not been clearly defined. Recently, Kojima *et al.* (6, 7) and Kroetz *et al.* (8) reported the expression of a previously cloned human liver CE enzyme in human tumor cell lines. Overexpression of this enzyme resulted in increased activation of CPT-11 to SN-38 and enhanced cytotoxicity. However, conversion of CPT-11 to SN-38 by this enzyme was determined at 100 μM, a concentration significantly higher than pharmacologically relevant plasma concentrations observed after CPT-11 administration to patients. Additionally, cytotoxicity was observed in these cell lines at CPT-11 concentrations higher than typically observed clinically. IC_{50}s ranged from approximately 2 to 20 μM after a 72-h continuous exposure. Several other recent reports have examined the conversion of CPT-11 by rabbit liver CE and compared its activity with that of the above human CE enzyme (9–11). Although the rabbit and human enzymes were quite similar (81% sequence identity), the rabbit enzyme was found to be 100-1000-fold more efficient at converting CPT-11 to SN-38 *in vitro* and 12–55-fold more efficient in sensitizing transfected cells to CPT-11. Both the human and rabbit CE enzymes are being developed in enzyme/prodrug combinations with CPT-11 (6, 7, 9).

Recently, Dean *et al.* (12) and Takai *et al.* (13) have reported the purification and partial characterization of two distinct human CE enzymes. These human liver CE enzymes, designated hCE-1 and hCE-2, are both members of the M_r 60,000 serine esterase superfamily, but they differ substantially. Sequence homology between the two enzymes is only 48% (Fig. 1). hCE-1 is a M_r 180,000 trimer with an isoelectric point of 5.8, whereas hCE-2 is a monomer with an isoelectric point of 4.9 (14, 15). Substantial differences in substrate specificity also exist between the two isoforms. For example, hCE-1 hydrolyzes the methyl ester of cocaine, and hCE-2 hydrolyzes the benzoyl ester (14, 15). Additionally, hCE-2 hydrolyzes aspirin (acetyl salicylic acid) and procaine, whereas hCE-1 does not (13). Sequence comparisons of hCE-1 and hCE-2 with the human CE enzyme cloned previously by Kroetz *et al.* (8) and used in the above studies by Kojima *et al.* (6, 7) and Danks *et al.* (9, 10) identify it as hCE-1.

To date the kinetics of CPT-11 hydrolysis to SN-38 by the two human liver CE isoforms hCE-1 and hCE-2 and the relative roles of the two enzymes in the activation of CPT-11 have not been reported. In this study, we have determined the kinetics of CPT-11 hydrolysis by the two human liver CE isoforms using purified enzymes from human liver and have examined the effect of these two enzymes on CPT-11 cytotoxicity.

Materials and Methods

Purification of Human Liver CE Enzymes. Procedures for purification of human liver CE enzymes were modified from a method described previously (15). All buffers were purged with helium and contained 1 mM benzamidine, 1 mM EDTA, and 1 mM DTT. Frozen human liver (60 g) obtained at autopsy was homogenized in 60 ml of 50 mM HEPES at pH 6.8. The homogenate supernatant, prepared by centrifugation at 125,000 × *g* for 35 min, was filtered through two layers of Miracloth (Calbiochem Corp., La Jolla, CA). The filtrate was applied to a DEAE-cellulose anion exchange column (150 g) equilibrated with 50 mM HEPES (pH 6.8). Bound protein was eluted with 250 mM potassium phosphate (pH 6.8), and fractions were assayed for 4-methylumbelliferyl acetate hydrolase activity using a spectrophotometric assay. The buffer of the active fractions was exchanged for 75 mM Tris-HCl (pH 7.6), using a Minitan ultrafiltration system (M_r 30,000 membrane; Millipore Corp., Bedford, MA), and the sample was loaded onto a Q Sepharose Fast Flow column (5.5 × 8 cm). CE activity was eluted with a 900-ml linear gradient of 0–250 mM NaCl in 75 mM Tris-HCl. Two peaks of activity were recovered: the first peak corresponding to hCE-1 and the second peak corresponding to hCE-2.

Fractions containing hCE-1 and hCE-2 were separately pooled and 1 mM of $MgCl_2$, 1 mM $CaCl_2$, and 1 mM $MnSO_4$ were added to the enzyme. Each isoenzyme sample was purified by the following procedure. The sample was loaded onto a ConA Sepharose 4B column (2.5 × 6 cm; Pharmacia, Piscataway, NJ), washed with 75 mM Tris-HCl plus 0.15 M NaCl, and bound protein was eluted with 0.5 M methyl-α-mannopyranoside in 10 mM potassium phos-

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[3] The abbreviations used are: CE, carboxylesterase; hCE, human CE; CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin (irinotecan); SN-38, 7-ethyl-10-hydroxy-camptothecin; HPLC, high-performance liquid chromatography.

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hce1    1 GHP.SP.VVD .VH.K...KF .SLE.FAQP. AIF.....G. ........T. .Q.A.P..F. KNA.SY.P.. 70
hce2    1 -QDSASPIRT THTGQVLGSL VHVKGANAGV QTFLGIPFAK PPLGPLRFAP PEPPESWSGV RDGTTHPAMC 69

hce1   71 T..PKAGQLL ..LFTNRKEN IPLK-L.... ...N.....D LTKKNR.... ......G.MV .A..T...LA 139
hce2   70 LQD--LTAVE SEFLSQFNMT FPSDSMSEDC LYLSIYTPAH SHEGSNLPVM VWIHGGALVF GMASLYDGSM 137

hce1  140 ........V. T......IW. ......EHSR ....H..... ......D... S.....GS.. ........E. 209
hce2  138 LAAHENVVHV IIQYRLGVLG FFSTGDKHAT GNWGYLDQVA ALRWVQQNIA HFGGNPDRVT IFGESAGGTS 207

hce1  210 .....L..LA KN...R..S. .....TSV.V KKGDVKPLAE QI.ITAG.KT TT.AVM.H.. .Q.TE..L.E 279
hce2  208 VSVLVVSPIS QGLFHGAIME SGVALLPGLI -ASSADVIST VVANLSACDQ VDSEALVGCL RGKSKEEILA 276

hce1  280 TTLKM.FLSL DLQGDPRESQ PLLGT.I..M L.LKT.E..Q .ERN.HT..Y M..I.KQ... ....MQL.SY 349
hce2  277 INKPFK---- ---------- -MIPGVVDGV FLPRHPQELL ASADFQPVPS IVGVNNNEFG WLIP-KVMRI 330

hce1  350 PLSEGQL.QK TAMSL.W.SY P.VCIAKELI PEAT.K.L.G TD.TVKKKDL .LDLI..V.. GV.SVIV.RN 420
hce2  331 YDTQKEMDRE ASQAALQKML TLLMLPPTFG DLLREEYIGD NGDPQTLQAQ FQEMMADSMF VIPALQVAHF 400

hce1  421 HRDAG..T.M ....YR..FS SDMK.KTVIG ......FS.. GAP.--LKEG AS...IR..K MV..F..... 489
hce2  399 QC-SRAPVYF YEFQHQPSWL KNIRPPHMKA DHGDELPFVF RSFFGGNYIK FTEEEEQLSR KMMKYWANFA 469

hce1  490 .......... ....EYN.K. G...IGANTQ A.QK..DKEV A..TNLFAK. AV.-KP.QTE .I..        552
hce2  470 RNGNPNGEGL PHWPLFDQEE QYLQLNLQPA VGRALKAHRL QFWKKALPQK IQELEEPEER HTEL        533
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Fig. 1. Alignment of amino sequences of hCE-1 and hCE-2. Amino acids corresponding to hCE-1 and hCE-2 are numbered, beginning with the first residue of mature protein. Those amino acids common to both enzymes are indicated by *dots*. Active site residues (Ser^{202}, Glu^{319}, His^{431}) are in *bold*. Gaps inserted to maximize alignment are indicated by *hyphens*. A 15-residue gap is inserted after residue 283. Potential glycosylation sites (Asn-X-Ser/Thr) are in *bold* and *singly underlined*. Two cysteine pairs are *doubly underlined*, and the COOH-terminal tetrapeptide is in *bold*.

phate (pH 7.0). The sample was concentrated to 10 ml, loaded onto a hydroxylapatite column (2.5 × 25 cm; Bio-Rad Laboratories, Hercules, CA), and washed with 10 mM potassium phosphate (pH 7.0). hCE-1 was eluted with a linear gradient of 30–250 mM potassium phosphate (pH 7.0). hCE-2 was eluted from a separate column with 30 mM potassium phosphate (pH 7.0). All purified enzyme samples were concentrated, and buffer was exchanged into 50 mM sodium phosphate (pH 7.0), sterile filtered, and stored at 4°C. Purified hCE-1 and hCE-2 exhibited single bands (>90% purity) on SDS-PAGE (M_r 70,000 subunit size) and nondenaturing PAGE after staining for protein. The specific activity of hCE-1 preparations ranged from 6 to 10 units/mg (14) and that of hCE-2 ranged from 40 to 140 units/mg (15). The wide range of specific activity for hCE-2 occurred because it is much more labile during purification.

Esterase Activity. Enzyme activity was determined by measuring hydrolysis of 500 μM 4-methylumbelliferone acetate by purified hCE enzymes, as described previously (15).

CPT-11 Hydrolysis. Human CE enzymes, hCE-1 or hCE-2 (0.1 unit), were incubated with increasing concentrations of CPT-11 at 37°C in 50 mM sodium phosphate buffer (pH 7.4). At selected incubation times, the reaction was stopped by mixing 0.5 ml of reaction solution with 2.0 ml of ice-cold methanol and placing the solution on ice. Two hundred μl of internal standard (camptothecin 1 μg/ml stock in 0.1 N HCl) was added. Samples were evaporated to dryness under nitrogen and reconstituted in 400 μl of HPLC mobile phase. SN-38 was quantitated by HPLC.

K_m and V_{max} values were calculated from nonlinear regression analysis of kinetic data to the Michaelis-Menten equation using the GraFit program (GraFit Version 4.0, 1998; R. J. Leatherbarrow, Erithacus Software Ltd., Staines, United Kingdom).

Quantitation of SN-38 Production. CPT-11 and SN-38 concentrations were determined by HPLC as modified from Gupta *et al.* (16). Briefly, CPT-11 and SN-38 were separated using a Partisphere 10 μM C_{18} column (4.5 × 250 mm; Whatman, Inc., Clifton, NJ) with a mobile phase consisting of 27% acetonitrile:73% 0.1 M potassium dihydrogen phosphate containing 3 mM sodium heptane sulfonate (pH 4.0). Detection was monitored using a Hitachi F1050 fluorescence detector (Hitachi Instruments, Naperville, IL) with λ_{ex} = 375 nm and λ_{em} = 566 nm. Standard curves of CPT-11 and SN-38 were linear (r = 0.99) within the range of 15–2500 ng/ml and 2.0–250 ng/ml, respectively.

Colony-forming Cytotoxicity Assay. CPT-11 at varying concentrations (0.25–1 μM) was incubated for 1 h at 37°C in the presence of 3.6 μg/ml hCE-1 or hCE-2 or no enzyme in serum-free cell culture medium (3:1 DMEM:Hank's F-12K, 100 μg/ml penicillin-streptomycin, and 0.4 μg/ml hydrocortisone). The medium was then filtered through a 0.22 μm disc filter into a sterile 15-ml conical tube. 0.5 ml was removed for quantitation of SN-38. SQ20b cells plated 16–18 h earlier at a density of 5×10^5 cells/25 cm^2 were exposed to the filtrate (5 ml) for 4 h at 37°C. After the 4-h incubation, another 0.5-ml aliquot was removed for SN-38 quantitation. The cells were washed with fresh medium containing 20% serum, trypsinized, and replated in culture dishes at a density of 100-1000 cells/100 mm^2 dish. Cell colonies (>50 cells) were counted 10–12 days later after staining with crystal violet. Control samples (no CPT-11 or enzyme) were treated identically.

Results

Enzyme Kinetics of Human CE Isoforms. To compare the kinetics of CPT-11 hydrolysis by hCE-1 and hCE-2, 0.1 unit of purified enzyme was incubated with concentrations of CPT-11 ranging from 0.1 μM to 50 μM at 37°C for up to 40 min. SN-38 production was determined at three separate time points for each CPT-11 concentration to ensure linear kinetics. The amount of SN-38 produced was <10% of total initial CPT-11 concentrations.

The K_m for hCE-1 and hCE-2, respectively, are 43 and 3.4 μM (Table 1). V_{max} values of 78 and 18 pmol/min/unit activity were obtained for the respective isoforms from the original fit of the data. These values were then normalized to pmol/mg/min based upon rates determined previously of 4-methylumbelliferone acetate hydrolysis by hCE-1 and hCE-2 enzymes of 6.8 units/mg protein (14) and 140 units/mg protein (15), respectively. V_{max} values for hCE-1 and hCE-2 were calculated to be 530 and 2500 pmol/mg/min, respectively. Assuming a subunit molecular weight of M_r 59,000 and one active site per subunit, the turnover number of the enzymes would be 0.031 and 0.160 min^{-1}, respectively. Hence, hCE-2 has a 12.5-fold higher affinity for CPT-11 and a 5-fold higher maximal rate of CPT-11 hydrolysis compared with hCE-1. The differences between the two

Table 1 *Kinetic constants for hydrolysis of CPT-11 by hCE isoforms hCE-1 and hCE-2*

Isoform	K_m μM[a]	V_{max} pmol/mg/min	V_{max} min^{-1}	Catalytic efficiency $min^{-1}\mu M^{-1}$
hCE-1	42.7 ± 6.8	530 ± 40	0.031 ± 0.0024	0.73×10^{-3}
hCE-2	3.4 ± 1.4	2500 ± 400	0.160 ± 0.024	47×10^{-3}

[a] Data represent pooled values from three separate experiments.

isoforms are best exhibited by comparing catalytic efficiency (V_{max}/K_m). The catalytic efficiency of hCE-2 (47×10^{-3} $min^{-1}\mu M^{-1}$) is 60-fold higher than that of hCE-1 (0.74×10^{-3} $min^{-1}\mu M^{-1}$).

Timed Comparison of CPT-11 Hydrolysis. The conversion of CPT-11 to SN-38 by hCE-1 and hCE-2 was compared at pharmacologically relevant CPT-11 concentrations of 0.5 and 1.0 μM using 0.1 unit of purified enzyme at 37°C. The amount of SN-38 produced was measured at various time points (Fig. 2). At 1 μM CPT-11, the rate of hydrolysis by hCE-1 and hCE-2 measured after 10 min was 11 and 700 pmol/mg protein/min, respectively. Thus, at pharmacologically relevant doses, the rate of CPT-11 conversion by hCE-2 is significantly greater than that by hCE-1.

Cytotoxicity of CPT-11 after Incubation with hCE Isoforms. CPT-11 at therapeutic or subtherapeutic concentrations of 0.25–1 μM was preincubated with either hCE-1 or hCE-2 or no enzyme in serum-free cell culture medium for 1 h at 37°C. Equivalent amounts of hCE-1 and hCE-2 (3.6 μg/ml) were added. SQ20b squamous cells were exposed to the products of the incubation for 4 h. After the exposure, SQ20b cells were replated at varying concentrations to determine cloning efficiency (Fig. 3*A*). Survival of cells exposed to CPT-11 in the absence of CE enzyme was similar to that of cells in the control group (no CPT-11). Incubation with hCE-1, likewise, produced no significant cytotoxicity up to 1 μM of CPT-11. Incubation of CPT-11 in the presence of hCE-2, however, resulted in significantly greater cytotoxicity at all three CPT-11 concentrations examined. At 1 μM CPT-11, only 38% of cells survived after incubation with hCE-2, whereas 88% of cells survived after incubation with an equivalent amount of hCE-1. Ninety-five % of control cells (no hCE exposure) survived after incubation with 1 μM CPT-11. At the lower concentrations of CPT-11, 0.25 and 0.5 μM, 62 and 44% of cells survived, respectively, after incubation with hCE-2.

Concentrations of SN-38 in the medium were measured after the initial 1-h preincubation (Fig. 3*B*) and after the final 4-h incubation on the SQ20b cells (Fig. 3*C*). SN-38 ranged from 5 to 15 nM after 1 h of incubation and 12 to 30 nM after 4 h of incubation in the presence of hCE-2. Concentrations of SN-38 were <1 nM after exposure to hCE-1 at both time points. The additional production of SN-38 during the 4-h incubation on the SQ20b cells is attributed to residual CE enzyme in the filtered incubation medium. No measurable conversion of CPT-11 to SN-38 was evident in the absence of added hCE enzyme during preincubation or during exposure to the SQ20b cells (data not shown).

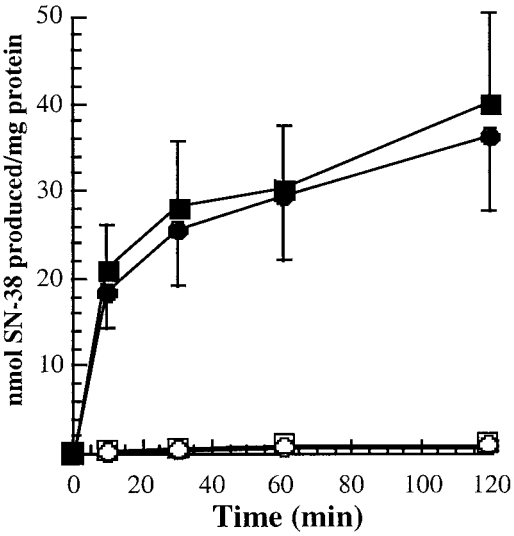

Fig. 2. Timed comparison of CPT-11 conversion to SN-38 by hCE-1 and hCE-2. One μM (*squares*) and 0.5 μM (*circles*) CPT-11 were incubated with 0.1 unit of hCE-1 (*open symbols*) and hCE-2 (*filled symbols*) in 50 mM potassium phosphate buffer (pH 7.4). Aliquots were removed at designated time points, and SN-38 was determined by HPLC. Values represent the average of data from three separate experiments; *bars*, SD.

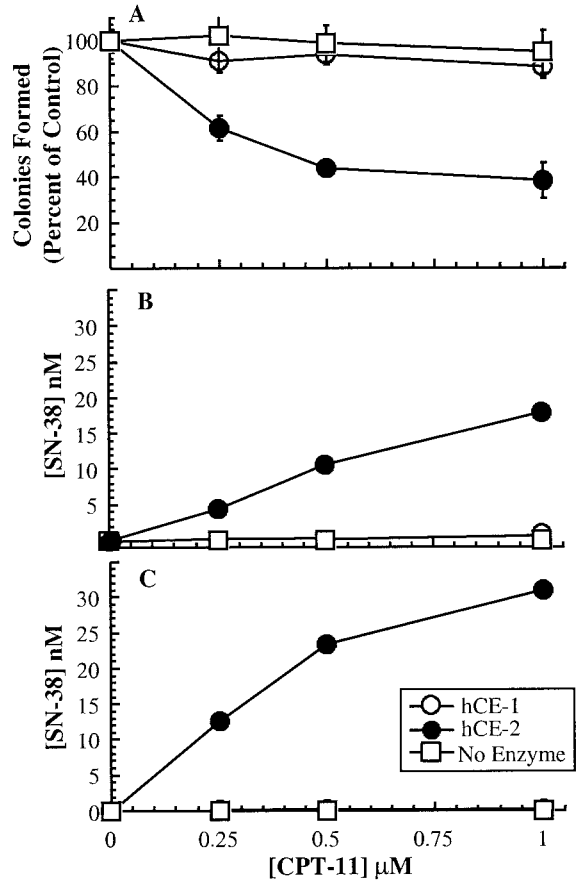

Fig. 3. CPT-11 cytotoxicity after incubation with hCE-1 and hCE-2. CPT-11 at the indicated concentrations was preincubated with 3.6 μg/ml of hCE-1 or hCE-2 or no enzyme in serum-free medium for 60 min. SQ20b cells were then exposed to filtered medium for 4 h at 37°C. Colony-forming efficiency of the exposed cells was then determined. *A*, colony-forming efficiency of SQ20b cells after exposure to CPT-11. *Bars*, SD. *B*, SN-38 concentrations in cell culture medium after 1-h preincubation. *C*, SN-38 concentrations in cell culture medium after 4 h incubation in SQ20b cells.

Discussion

In this study, we report the kinetics of CPT-11 conversion to SN-38 by two distinct hCE enzymes, hCE-1 and hCE-2. We have demonstrated that hCE-2 is a higher affinity and higher velocity enzyme, when compared with hCE-1. The K_ms are 3.4 and 43 μM for hCE-2 and hCE-1, respectively. The catalytic efficiency of hCE-2 is 60-fold higher than that of hCE-1. At pharmacologically relevant concentrations, hCE-2 converts CPT-11 to SN-38 at a 30–60-fold higher rate than hCE-1. Finally, incubation of low, pharmacologically relevant concentrations of CPT-11 with hCE-2 results in increased cytotoxicity when compared with that seen with hCE-1.

The hydrolysis of CPT-11 to SN-38 by CEs has been studied extensively using a variety of mammalian enzymes. In humans, the majority of studies have focused on a single CE enzyme. Both Satoh *et al.* (5) and Rivory *et al.* (17) purified and characterized a CE enzyme from human liver. They reported single K_ms of 169 and 52.9 μM, respectively. In contrast, Slatter *et al.* (18) characterized the kinetics of CPT-11 hydrolysis using human liver microsomes and identified the presence of two CE isoforms. Modeling of the kinetic data for CPT-11 hydrolysis by liver microsomes fit a two-enzyme model, with a high-affinity isoform (K_m = 1.4–3.9 μM) and a low-affinity isoform (K_m = 129–164 μM). These investigators proposed

that the higher affinity enzyme was most likely responsible for CPT-11 activation. Consistent with the data reported by Slatter *et al.* (18), we now report both a low-affinity (hCE-1) and a high-affinity (hCE-2) isoform of human liver CE. The K_m for purified hCE-2 of 3.4 μM is almost identical to that reported by Slatter *et al.* (18) for the high-affinity enzyme. The K_m for hCE-1 of 43 μM is ~3-fold lower than that reported by Slatter *et al.* (18) for the lower affinity isoform. This small difference may reflect the use of purified enzyme *versus* liver microsomes.

Our studies with purified enzymes also show that hCE-2 has a 5-fold higher V_{max} than hCE-1. Thus, not only does hCE-2 activate CPT-11 at lower concentrations, it does so at a faster rate. This is illustrated by direct comparison of the hydrolysis of therapeutic concentrations of CPT-11 by the two human enzymes (Fig. 2). Considering the lower K_m of 3 μM for hCE-2 (which is more consistent with plasma levels measured in patients receiving CPT-11) and the higher hydrolytic rate, hCE-2 (and not hCE-1) is most likely responsible for activation of CPT-11 to SN-38 in the human liver. However, final confirmation of the roles of hCE-1 and hCE-2 in CPT-11 activation will depend upon relative expression of the two isoforms in the liver, and perhaps, other tissues, particularly tumor tissues.

In our cytotoxicity experiments, SQ20b cells, which are not sensitive to 1 μM CPT-11, show increased sensitivity to CPT-11 after incubation with hCE-2. A similar increase in sensitivity to CPT-11 was not observed after incubation with an equivalent amount of hCE-1. When 20-fold higher concentrations of hCE-1 protein were added, the cytotoxic effect was similar to, but still less than, that observed with hCE-2 (data not shown).

Enzyme prodrug combinations with CPT-11 are currently being developed using both hCE-1 and a purified rabbit CE enzyme in several laboratories (6, 7, 9). Transfection of tumor cell lines with either the rabbit CE enzyme or hCE-1 increases sensitivity to CPT-11. In the case of hCE-1, increased sensitivity to CPT-11 was observed in transfected human lung cancer cell lines. The reported IC_{50} for A549 cells transfected with hCE-1 is ~2 μM (6). However, the cells were exposed to CPT-11 continuously for 72 h, a duration of exposure that far exceeds that observed in a clinical setting. The half-life of CPT-11 in humans is 8–10 h (19). Additionally, *in vivo* cytotoxicity was not reported with systemic dosing of CPT-11 but only after direct injection of tumors with 7 μg/ml (~12 μM) CPT-11 (6). Ideally, an effective enzyme/prodrug combination would be toxic to transfected tumor cells at lower than normal plasma concentrations, minimizing toxicities and increasing the therapeutic index.

The rabbit CE enzyme is much more efficient at converting CPT-11 to SN-38 than hCE-1 and may be a better candidate than hCE-1 for development in an enzyme prodrug combination. Rh30 cells transfected with the rabbit CE were 100-1000-fold more active in converting CPT-11 to SN-38 than those transfected with hCE-1. Transfection of rabbit CE produced an 8-fold increase in sensitivity to CPT-11, shifting the IC_{50} from 4.3 to 0.57 μM. Xenograft experiments also show enhanced cytotoxicity in cell lines transfected with the rabbit CE enzyme when compared with controls or those transfected with hCE-1. Nevertheless, transfection of a nonhuman protein in the clinical setting may lead to an immunological response and subsequent enzyme inactivation. Transfection of a higher affinity, higher efficiency human enzyme such as hCE-2 may overcome these limitations.

Finally, the catalytic properties of hCE-1 and hCE-2 are clearly different for a variety of substrates. hCE-2 is 20-fold more efficient than hCE-1 in hydrolyzing 4-methylumbelliferyl acetate, a compound considered to be a relatively nonspecific esterase substrate (15). Aspirin, (acetylsalicylic acid), procaine, and oxybutynin are also specific substrates for hCE-2 (13). Meperidine is a specific substrate for hCE-1 (20). Identification of additional substrate specificities and elucidation of binding site structure will provide important information about the roles of these two isoforms in the metabolism of other drugs and may ultimately lead to the specific design of ester prodrugs.

In conclusion, we have shown that the hCE enzyme hCE-2 clearly plays a role in the conversion of CPT-11 to SN-38 in the human liver. On the basis of enzyme kinetic profiles, hCE-2 exhibits the highest catalytic efficiency for CPT-11 activation. Additional experiments are being conducted to further define the roles of hCE-2 and hCE-1 in the activation of CPT-11 and other chemotherapeutic ester prodrugs. The expression of hCE-1 and hCE-2 in normal human tissues and tumors is also being investigated.

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