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Activation of CPT-11 in Mice: Identification and Analysis of a Highly Effective Plasma Esterase[1]

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ABSTRACT

The camptothecin prodrug CPT-11 (irinotecan, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) is converted by esterases to yield the potent topoisomerase I poison SN-38 (7-ethyl-10-hydroxycamptothecin). Recently, a mouse strain (*Es1^e*) has been identified that demonstrates reduced plasma esterase activity, and we have monitored the ability of plasma from these mice to metabolize CPT-11. Total plasma esterase activity was reduced 3-fold in *Es1^e*mice in comparison to control mice, and this resulted in a 200-fold reduction in SN-38 production after incubation with CPT-11 *in vitro*. In addition, pharmacokinetic studies of CPT-11 and SN-38 in these animals demonstrated approximately 5-fold less conversion to SN-38. However, extracts derived from tissues from *Es1^e* animals revealed total esterase activities similar to those of control mice, and these extracts metabolized CPT-11 with equal efficiency. Northern analysis of RNA isolated from organs indicated that the liver was the primary source of *Es-1* gene expression and that very low levels of *Es-1* RNA were present in *Es1^e* mice. These results suggest that the reduced levels of Es-1 esterase present in *Es1^e* mice are due to down-regulation of gene transcription, and that this plasma esterase is responsible for the majority of CPT-11 metabolism in mice.

INTRODUCTION

The anticancer prodrug CPT-11 (irinotecan, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) is activated by esterases to yield SN-38 (7-ethyl-10-hydroxycamptothecin), a potent topoisomerase I poison (1–3). CPT-11 has demonstrated remarkable antitumor activity in immune-deprived animals bearing human tumor xenografts (4–9); consequently, this agent is undergoing Phase II/III trials in both adults and children. In addition, the Food and Drug Administration recently approved this drug for the treatment of refractory colon cancer in adults. The exact enzymes involved in CPT-11 activation in both humans and animals have not yet been identified, but it is clear that both CEs[4] and butyrylcholinesterases can metabolize this drug *in vitro* (2, 10, 11).

CPT-11 activation occurs very rapidly in rodents, resulting in very high initial plasma SN-38 concentrations (12, 13). In contrast, in patients given CPT-11 as a single high-dose infusion, the initial SN-38 plasma levels are much lower (14), indicating that there is considerable difference in the expression and/or structure of human and mouse esterases involved in CPT-11 metabolism. Hence, the analysis of antitumor responses observed with human tumors in immune-deprived mice may be complicated by the increased activation of CPT-11 by both mouse plasma and tissue esterases.

Recently, a mouse strain has been described that demonstrates reduced plasma esterase activity, and these mice maybe useful as a model for understanding CPT-11 metabolism. This strain of mice was originally generated in 1979 by alkylating agent-mediated mutagenesis and designated *Es1^e* (15). Zymogram patterns indicated a >95% reduction in esterase activity, and recent studies with glutathione-derived esters demonstrated dramatic stabilization of these compounds after administration to animals (16). We hypothesized that because rodent plasma is very efficient at CPT-11 metabolism, the Es-1 esterase may play a role in the activation of this drug.

Hence, we have analyzed the expression of the *Es-1* gene in both control and *Es1^e* mice and determined the ability of both plasma and tissue extracts to metabolize CPT-11. In addition, we have also performed pharmacokinetic studies with this drug to assess the role played by this plasma esterase in CPT-11 activation.

MATERIALS AND METHODS

Mice. Control B6D2F1 and *Es-1*-deficient DBA/2 × C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). These mice carry an allele of the esterase locus *Es-1* designated *Es1^e* (15) that results in reduced plasma esterase enzyme activity (16).

Mice were handled using an approved Institutional Animal Care and Use Committee protocol following the Guide to the Care and Use of Laboratory Animals.

CPT-11 and Inhibitors. CPT-11 was a generous gift from Dr. J. P. McGovren (Pharmacia and Upjohn, Kalamazoo, MI). It was dissolved in methanol and stored at −20°C until required. The total esterase inhibitor BNPP and the cholinesterase inhibitor tacrine were purchased from Sigma (St. Louis, MO).

Tissue Extract and RNA and DNA Preparation. Organs were removed from animals and minced immediately on ice. For preparation of whole tissue extracts, samples were sonicated on ice in appropriate volumes of 50 mM HEPES (pH 7.4) using an Ultrasonic Homogenizer 4710 (Cole Palmer, Chicago, IL) with a microtip probe. For RNA preparation, tissues were homogenized in 10 ml of RNAzol (Teltest, Friendswood, TX) using a Dounce homogenizer. RNA was isolated according to the manufacturer's recommendations, washed extensively with 75% ethanol, and stored at −80°C until required.

Northern Analysis. Northern analysis was performed using conventional protocols (17). Membranes were hybridized with a PCR-derived mouse *Es-1* or a G3PDH probe, and data were quantitated using ImageQuant software from PhosphorImager cassettes (Molecular Dynamics, Sunnyvale, CA).

RT-PCR Assays. cDNA was prepared from 1 μg of total RNA using random primers and avian myeloblastosis virus reverse transcriptase using a first-strand cDNA synthesis kit (Roche Molecular Biochemicals, Indianapolis, IN). Amplification of the mouse *Es-1* gene (GenBank accession number M57960) was accomplished using oligonucleotide primers designed at the ATG initiation and TAA termination codons and Taq polymerase. Conditions for the amplification were denaturation at 94°C for 45 s, annealing at 60°C for 1 min, and extension at 72°C for 1.5 min for a total of 35 cycles. Products were analyzed on 0.9% agarose gels and stained with ethidium bromide. Control reactions using primers that amplify mouse G3PDH cDNA (Clontech, Palo Alto, CA) were also performed under identical conditions.

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[4] The abbreviations used are: CE, carboxylesterase; BNPP, bis(4-nitrophenyl) phosphate; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; HPLC, high-performance liquid chromatography; RT-PCR, reverse transcription-PCR.

Table 1 *CE activity and CPT-11 conversion by control and Es1^e^ mouse plasma and tissue extracts*

CE activity was calculated from spectrophotometric assays using *o*-nitrophenyl acetate as a substrate and SN-38 conversion by HPLC.

Mouse	Tissue	CE activity (μmol/min/mg)	SN-38 (pmol/h/mg)	SN-38 (% of control)
Control	Plasma[a]	2983 ± 133	11207	
*Es1^e^*	Plasma[a]	792.5 ± 83.9	144.2	1
*Es1^e^*	Plasma[a] + BNPP[b]	327.6 ± 30.2	19.6	
*Es1^e^*	Plasma[a] + tacrine[b]	340.4 ± 17.3	77.5	
Control	Brain	40.4 ± 7.0	0.4	
*Es1^e^*	Brain	35.7 ± 4.4	0.1	25
Control	Heart	29.6 ± 3.7	2.8	
*Es1^e^*	Heart	41.0 ± 6.3	0.2	7
Control	Kidney	163.5 ± 11.7	7.5	
*Es1^e^*	Kidney	145.3 ± 29.7	3.1	41
Control	Liver	293.4 ± 50.0	45.5	
*Es1^e^*	Liver	303.1 ± 10.3	34.8	76
Control	Lung	61.2 ± 1.6	4.1	
*Es1^e^*	Lung	80.1 ± 5.6	0.8	20
Control	Small intestine	208.3 ± 13.9	10.0	
*Es1^e^*	Small intestine	554.3 ± 173.1	14.6	146
Control	Spleen	13.5 ± 3.6	1.3	
*Es1^e^*	Spleen	13.3 ± 1.0	0.1	8

[a] Values for plasma are expressed as μmol/min/ml for CE activity and pmol/h/ml for SN-38 conversion.
[b] Inhibitor concentrations were 1 mM.

Esterase Assays. Esterase activity was determined in whole tissue sonicates using a spectrophotometric assay with *o*-nitrophenol acetate as a substrate (10, 18). Protein concentrations were calculated using Bio-Rad protein assay reagent (Hercules, CA) with BSA as a standard. Enzyme activities were calculated as micromoles of *o*-nitrophenol produced/min/mg total protein.

***In Vitro* CPT-11 Activation Assays.** The conversion of CPT-11 to SN-38 by extracts *in vitro* was accomplished using 250 μg of total protein in a final volume of 200 μl of 50 mM HEPES (pH 7.4) containing 5 μM CPT-11. Reactions were incubated at 37°C for 18 h and terminated by the addition of an equal volume of cold acidified methanol. Reaction mixtures were stored at −80°C to precipitate proteins, and after centrifugation at 15,000 × *g* for 15 min, the amount of CPT-11 and SN-38 in the supernatant was determined by HPLC.

Quantitation of CPT-11 and SN-38 by HPLC. The concentrations of total CPT-11 and SN-38 in methanolic supernatants were determined by reverse-phase HPLC as described previously (19, 20). Briefly, samples were separated on a NovaPak C18 column using 25% acetonitrile/75 mM ammonium acetate (pH 4.0) as a mobile phase. CPT-11 and SN-38 were detected with a Jasco FP920 fluorescence detector with an excitation wavelength of 375 nm and an emission wavelength of 550 nm. The sensitivity of this system was 20 and 1.5 pg/μl for CPT-11 and SN-38, respectively.

Kinetic Analyses. K_m and V_{max} values for CPT-11 were determined as described previously (19). For plasma derived from control animals, reactions were performed using 10 μl of plasma in a total volume of 100 μl and incubated for 5 min at 37°C. For *Es-1*-deficient animals, reactions contained 50 μl of plasma in the same total volume, and incubations were extended to 1 h. In both cases, at least 10 different substrate concentrations were used, and reactions were performed and quantitated in duplicate. K_m and V_{max} values were determined from hyperbolic plots of data using the GraphPad Prism program. K_{cat}/K_m values were determined from linear regression of the initial data points of the hyperbolic plots.

Pharmacokinetic Analyses. Pharmacokinetic parameters for CPT-11 and SN-38 were determined as described previously (12). Briefly, a minimum of three Metafane-anesthetized mice were injected retro-orbitally with 10 mg/kg CPT-11 dissolved in 0.9% sodium chloride, and blood samples were collected after 0.083, 0.25, 1, 2, 4, and 6 h. After centrifugation at 15,000 × *g* for 2 min, an equal volume of cold methanol was added to the plasma, and the sample was stored at −80°C for 1 h. Particulate matter consisting of protein and lipid was removed by centrifugation at 50,000 × *g* for 20 min, and after acidification of the sample, CPT-11 and SN-38 were quantitated in duplicate by HPLC. A four-compartment model was used to determine the pharmacokinetic parameters including k_{13} (conversion of CPT-11 to SN-38), k_{30} (elimination rate constant), and V_c (volume of central compartment). $AUC_{0\to\infty}$ (area under the concentration-time curve from time 0 to infinity) and CL (CPT-11 drug clearance) were calculated using standard equations (12).

RESULTS

Esterase Expression in Control and *Es1^e^* Mice. To determine whether esterase activity was diminished in *Es1^e^* mice, we assessed CE activity values in plasma and various whole organ sonicates (Table 1). CE activity in control mouse plasma was approximately 3-fold higher than that observed with *Es1^e^* mouse plasma. The levels of CE protein were highest in liver, small intestine, and kidney and reduced in lung, brain, heart, and spleen; however, enzyme activity in *Es1^e^* mouse tissue extracts was comparable to that in extracts isolated from control mice. In addition, Western analysis of identical cell sonicates with an antiporcine esterase antibody confirmed the presence of readily detectable amounts of esterase protein in liver, kidney, and lung extracts isolated from both strains of animals (data not shown).

CPT-11 Conversion by Plasma and Tissue Extracts. To determine whether the reduced esterase activity in *Es-1*-deficient mouse plasma affected CPT-11 metabolism, we monitored conversion of the drug by HPLC. Plasma samples were incubated with 5 μM CPT-11 for up to 22 h, and SN-38 concentrations were determined by HPLC. Table 1 indicates that there is an approximately 100-fold reduction in the metabolism of CPT-11 by *Es-1*-deficient plasma. Incubation of *Es1^e^* plasma with the total esterase inhibitor BNPP reduced the amount of SN-38 produced to barely detectable levels. However, incubation of plasma with the cholinesterase inhibitor tacrine only reduced CPT-11 conversion activity by ~50% (Table 1), indicating that there is potentially another esterase present in plasma distinct from butyrylcholinesterase that can metabolize this drug.

To determine whether tissues derived from *Es1^e^* mice tissues could metabolize CPT-11 as efficiently as control organs, we incubated 250 μg of total protein from each sonicate with 5 μM CPT-11 for 18 h. SN-38 in the reaction mix was quantitated by HPLC, and the data are indicated in Table1. All tissues except small intestine produced less SN-38 than identical tissues isolated from control mice. Organs rich in esterases, such as the liver and kidney, produced relatively more SN-38 than other *Es1^e^* tissues; however, very little drug conversion was observed with other organs.

Kinetics of CPT-11 Bioactivation by Plasma. Because we observed dramatic differences in the levels of SN-38 produced from control and *Es-1*-deficient plasma, we determined the K_m and V_{max} values for the bioactivation of the drug. Table 2 indicates the value of these parameters for both control and *Es-1*-deficient plasma. Because the V_{max} value for the latter sample was very low and hence difficult to determine accurately, we calculated K_{cat}/K_m for both enzymes using the slope of the initial portion of the hyperbolic plots. As can be seen in Table 2, the catalytic efficiency of control plasma is ~650-fold greater than that of *Es-1*-deficient plasma. The K_m and V_{max}

Table 2 *K_m, V_{max}, and K_{cat}/K_m values for control and Es1^e^ mouse plasma with CPT-11*

SN-38 levels were determined by HPLC, and kinetic parameters were calculated from hyperbolic plots of *V versus* [S] for CPT-11 with each plasma sample.

Plasma	K_m (μM)	V_{max} (pmol SN-38/min/ml)	Goodness of fit (R^2)	K_{cat}/K_m[a] (s^{-1} l^{-1})
Control	2.35 ± 0.17	1761 ± 37.5	0.97	89.55 ± 7.85
*Es1^e^*	14.0 ± 1.40	31 ± 0.9	0.95	0.136 ± 0.011

[a] Determined from the initial slope of the *V versus* [S] hyperbolic plots. Units are s^{-1} l^{-1} because reactions were performed with unknown enzyme concentrations but known plasma volumes.

Table 3 *Pharmacokinetic model parameters for control and Es1*^{e} *mice*

A linear four-compartment pharmacokinetic model was fitted to CPT-11 and SN-38 plasma concentrations by maximum-likelihood estimation after administering a single retro-orbital dose of 10 mg/kg CPT-11 to control and *Es1*^{e} mice.

Pharmacokinetic parameter	Control	*Es1*^{e}
CPT-11		
$AUC_{0\to\infty}$ (ng/ml·h)	1090	1341
k_{13} (h^{-1})	4.17	0.89
CL (liters/h/m^2)	244.7	138.6
SN-38		
$AUC_{0\to\infty}$ (ng/ml·h)	118	60.2
k_{30} (h^{-1})	27.4	11.4

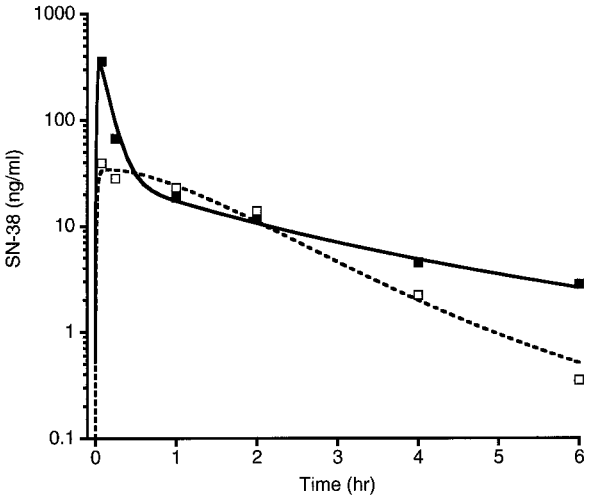

Fig. 1. SN-38 plasma concentrations *versus* time in control (*solid line* and ■) and *Es1*^{e} (*dashed line* and □) mice. *Symbols* represent measured plasma concentrations, and *lines* represent the best fit line from pharmacokinetic model estimations. A minimum of 18 mice received an injection of 10 mg/kg CPT-11, and plasma SN-38 concentrations were determined by HPLC.

values for conversion of CPT-11 by *Es1*^{e} plasma probably arise as combined data from other enzymes (*e.g.*, butyrylcholinesterase and potentially other esterases) present within the sample. However, these results demonstrate the dramatic difference in CPT-11 metabolism by the two plasma samples

Pharmacokinetics of CPT-11 and SN-38 after Retro-Orbital Injection. To confirm that reduced CPT-11 metabolism would occur in *Es1*^{e} mice, we monitored the formation of SN-38 after retro-orbital injection of 10 mg/kg CPT-11. As reported in Table 3, a 5-fold reduction in the conversion of CPT-11 to SN-38 (k_{13}) was observed. This translates to a 2-fold reduction in the SN-38 $AUC_{0\to\infty}$ in esterase-deficient mice as compared with control animals. As depicted in Fig. 1, the initial peak of SN-38 is absent in *Es1*^{e} mice, consistent with the hypothesis that plasma-mediated de-esterification of CPT-11 is attenuated in these animals.

Analysis of *Es-1* Gene Expression in Control and *Es1*^{e} Mice. Because the levels of esterases present in the control and *Es1*^{e} mouse tissues were similar, we examined the levels of *Es-1* transcripts in organs by both Northern analysis and RT-PCR. After hybridization, we observed high levels of *Es-1* mRNA 1.8 kilonucleotide in control mouse liver, approximately 5-fold higher than that in *Es1*^{e} mouse liver (Fig. 2; Table 4). Differences in other tissues were small, with only barely detectable *Es-1* expression in the kidney, heart, and lung. Comparison of mRNA levels with control mouse liver RNA (Table 4) indicated that very low levels of *Es-1* transcripts were present in other organs. However, because the nucleotide sequences of esterases are highly homologous, the potential exists that the probe used for the Northern analysis may hybridize with non-*Es-1*-encoded esterase mRNAs. To confirm the results we observed with the Northern analysis, we performed RT-PCR using liver RNA as a template and *Es-1*-specific primers.

Analysis of RT-PCR products derived from liver RNA of control and *Es1*^{e} mice demonstrated significantly reduced transcript levels in *Es1*^{e} tissues (Fig. 3*A*). Indeed, we were only able to detect expression of the *Es-1* gene in *Es1*^{e} mice after 35 cycles of amplification. In contrast, RNA from control liver yielded PCR products after as few as 20 cycles. These results clearly indicate the low level of *Es-1* transcript in *Es1*^{e} mouse liver and indicate that the probe used in the Northern analysis probably hybridizes with non-*Es-1*-encoded esterase transcripts. To confirm that approximately equal amounts of cDNA had been generated and subjected to amplification, PCR was performed using control primers that amplify mouse G3PDH cDNA. Similar levels of product were observed in both control and *Es1*^{e} liver RNA (Fig. 3*B*).

DISCUSSION

The metabolism of CPT-11 in rodents and humans is complex, yielding numerous metabolites. The metabolite SN-38 is responsible for the antitumor effect of this agent; therefore, most of the experimental studies with this drug have focused on its conversion to this active species. The levels of SN-38 produced when CPT-11 is administered to humans are much lower than those achieved in mice, indicating that the esterases involved in drug metabolism are either expressed at reduced levels or, more likely, that the enzymes are much less efficient at CPT-11 metabolism. We and others have used animal model systems to analyze the metabolism of CPT-11 and the response of human tumor xenografts to this drug (5, 9, 12, 20). However, in rodents, as much as 50% of the drug can be converted to SN-38 (12), making a direct comparison of antitumor and pharmacokinetics studies difficult to extrapolate to humans. For example, *in vitro* CPT-11

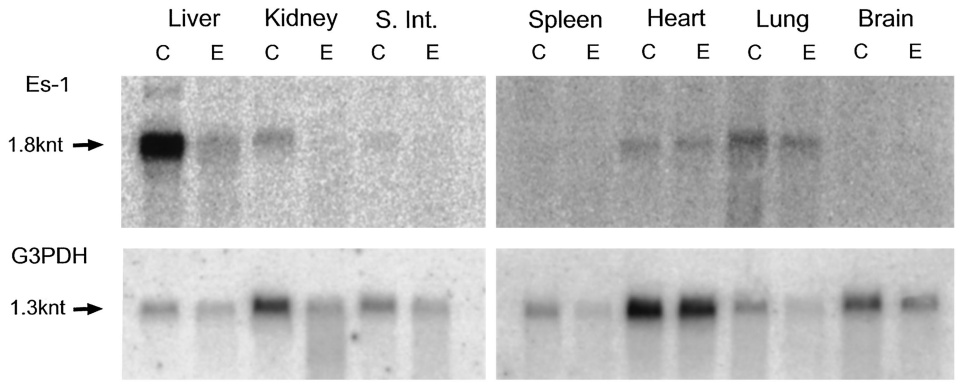

Fig. 2. Northern analysis of RNA isolated from control (*Lanes C*) and *Es1*^{e} (*Lanes E*) mouse tissues. Membranes were hybridized with either an *Es-1* probe or a G3PDH probe. The *Es-1* blots are deliberately overexposed to demonstrate the low levels of gene expression. Transcript sizes were estimated from 9.5 to 0.24 kilonucleotide RNA molecular weight markers (Life Technologies, Inc., Gaithersburg, MD).

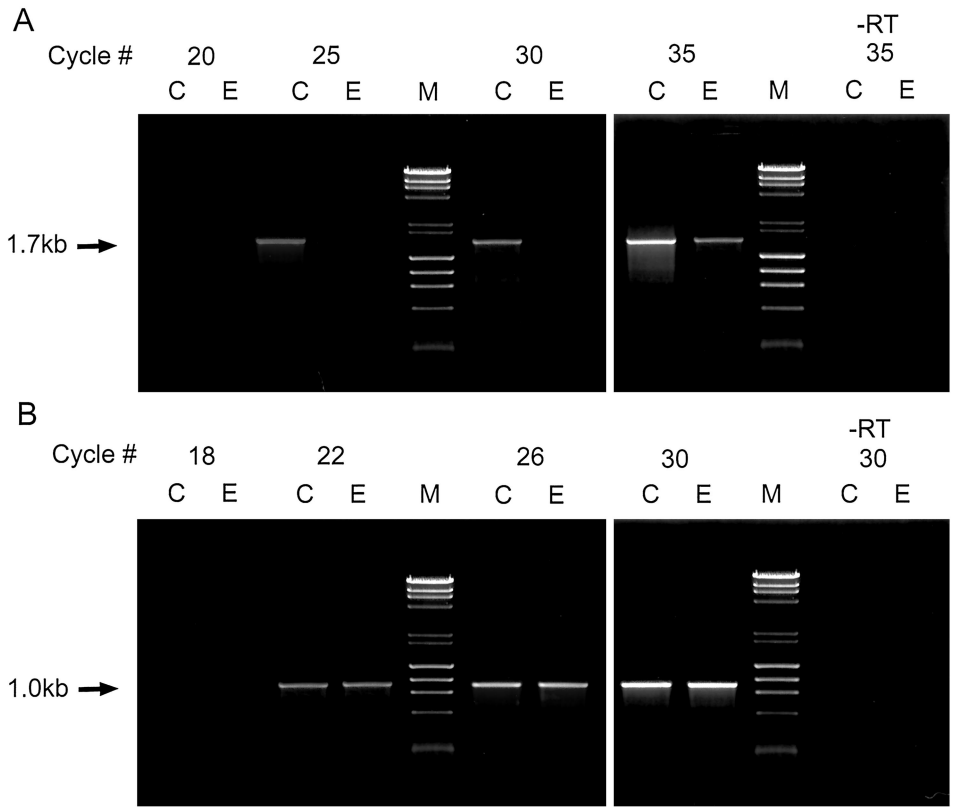

Fig. 3. RT-PCR analysis of *Es-1* gene expression in control (*Lanes C*) and *Es1^e^* (*Lanes E*) mouse liver. Expression of *Es-1* (*A*) and G3PDH (*B*) was monitored by amplification of cDNA for up to 35 cycles, and products were detected by agarose gel electrophoresis. The expected product sizes, 1.7 kb for *Es-1* and 1.0 kb for G3PDH, are indicated by the *arrows*. cDNA reactions lacking reverse transcriptase (−*RT*) were performed as controls. *Lanes M*, λ/*Hin*dIII and ϕX174/*Hae*III DNA molecular weight markers.

metabolism studies with human plasma samples demonstrate that the levels of SN-38 produced (300–700 pg/ml/h)[5] are comparable to those seen with *Es-1*-deficient plasma (2800 pg/ml/h). In contrast, control mouse plasma yields approximately 1.9×10^6 pg/ml/h of SN-38. Because *Es-1*-deficient animals demonstrate significantly reduced levels of CPT-11 activation in their plasma, they may represent a more accurate model of human drug metabolism.

Incubation of *Es1^e^* plasma with BNPP reduced SN-38 production to essentially zero, whereas tacrine only reduced the level by about 50%. Because tacrine is a potent inhibitor of butyrylcholinesterases but not of CEs, it indicates that there is another esterase present in *Es1^e^* plasma that can metabolize CPT-11. We have previously demonstrated the conversion of CPT-11 to SN-38 by butyrylcholinesterases (11); however, the role that this enzyme plays in drug activation in humans remains unclear. Recent reports by Furman *et al.* (21) have demonstrated that repeated low-dose administration of CPT-11 to pediatric patients can result in significant conversion to SN-38. This higher relative extent of drug conversion appears to be due to the low daily dose as compared with single bolus, high-dose infusions of CPT-11. We are currently evaluating the levels of SN-38 in control and *Es1^e^* mice plasma after administration of low doses of CPT-11 under repetitive schedules to determine whether this model system can simulate data observed in patients.

Liver, small intestine, and kidney isolated from both control and *Es1^e^* mice express significant amounts of esterase activity and demonstrate efficient conversion of CPT-11 to SN-38. High-level CE activity was observed in the small intestine of *Es1^e^* animals, and this may be a compensation mechanism for the reduced esterase activity in plasma. It is thought that esterases may be involved in the detoxification of xenobiotics (22), and, because the gut is a barrier to these agents, the small intestine may play a significant role in metabolizing these compounds. The same may be true for the lung, where inactivation of airborne toxins by esterases may provide a first-line defense for this tissue. We have observed significant amounts of CE activity in lung extracts from both control and *Es1^e^* mice. In addition, Northern analysis indicated the presence of esterase mRNAs isolated from lung samples derived from both strains of animal (Tables 1 and 4; Fig. 2).

The low levels of CE activity and SN-38 conversion by brain, heart, and spleen in control animals may be due to residual blood contaminating the tissue. Because the latter two organs are highly vascularized, small traces of plasma present within these samples would be detected by both the CE and CPT-11 conversion assays. Because the tissues were not perfused with saline before excision, blood would remain within the organ. However, because the levels of SN-38 produced by extracts derived from these tissues were low (Table 1),

Table 4 *Relative Es-1 RNA levels in control and Es1^e^ mouse tissues*

Es-1 specific RNA was quantitated by Northern analysis using ImageQuant software and corrected for loading differences using G3PDH.

Mouse	Tissue	RNA level[a]	Fold difference between organs	% of control liver
Control	Brain	2.4		0.4
*Es1^e^*	Brain	1.0	2.4	0.2
Control	Heart	3.6		0.6
*Es1^e^*	Heart	4.1	0.9	0.7
Control	Kidney	8.4		1.5
*Es1^e^*	Kidney	9.6	0.9	1.7
Control	Liver	561.9		100
*Es1^e^*	Liver	113.9	4.9	20.3
Control	Lung	57.7		19.3
*Es1^e^*	Lung	108.3	0.5	10.3
Control	Small intestine	2.9		0.5
*Es1^e^*	Small intestine	7.5	0.4	1.3
Control	Spleen	3.0		0.5
*Es1^e^*	Spleen	4.2	0.7	0.8

[a] The *Es1^e^*:G3PDH ratio for *Es1^e^* brain RNA was arbitrarily set as 1.

[5] S. D. Baker and P. M. Potter, manuscript in preparation.

the brain, heart, and spleen probably do not contribute significantly to the metabolism of CPT-11 *in vivo*.

The activation of CPT-11 by human plasma *in vitro* is poor, yielding very little SN-38.[5] This may be due to the fact that human plasma lacks CE activity (23). Because this observation cannot account for the level of SN-38 observed in patients treated with CPT-11 (14), it indicates that esterases located in tissues such as the liver and small intestine probably contribute to the majority of drug metabolism *in vivo*. However, it is clear that either the human homologue of the *Es-1* gene is expressed at very low levels in plasma or the amino acid sequence has diverged significantly from the mouse protein so that the enzyme is no longer efficient at CPT-11 catalysis. The significance of this loss of enzyme expression or sequence divergence in humans is unclear, but it may result from lack of evolutionary pressure on higher mammals to maintain enzymes required to metabolize complex organic molecules from vegetal sources.

Southern analysis of DNA isolated from both control and *Es1*^{e} mice has indicated no gross rearrangement in the *Es-1* gene structure (data not shown). However, *Es1*^{e} animals were generated by mutagenesis using the ethylating agent triethylenemelamine (15), and therefore we envisage that point mutation(s) would be responsible for loss of gene expression. RT-PCR products of the correct size were identified after amplification of *Es1*^{e} liver RNA, but only after an extended number of cycles, suggesting that a mutation, if present, may occur within the transcriptional control regions of the *Es-1* promoter. We are currently sequencing these PCR products to determine whether a point mutation exists within the catalytic domain of the *Es-1* esterase. If so, we may be able to identify amino acids crucial for CPT-11 recognition and activation in esterase-mediated catalysis.

In addition, we are currently assessing the formation of other metabolites of CPT-11 in *Es1*^{e} mice to further understand the disposition of this drug in these mice. Finally, we are comparing the effect of oral dosing of CPT-11 in control and *Es-1*-deficient animals to determine what role this esterase plays in peak plasma SN-38 concentrations after drug administration via this route.

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