MOUSE LIVER AND KIDNEY CARBOXYLESTERASE (M-LK) RAPIDLY HYDROLYZES ANTITUMOR PRODRUG IRINOTECAN AND THE N-TERMINAL THREE QUARTER SEQUENCE DETERMINES SUBSTRATE SELECTIVITY

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ABSTRACT:

Antitumor prodrug irinotecan is used for a variety of malignancies such as colorectal cancer. It is hydrolyzed to the metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), which exerts its antineoplastic effect. Several human and rodent carboxylesterases are shown to hydrolyze irinotecan, but the overall activity varies from enzyme to enzyme. This report describes a novel mouse liver and kidney carboxylesterase (M-LK) that is highly active toward this prodrug. Northern analyses demonstrated that M-LK was abundantly expressed in the liver and kidney and slightly in the intestine and lung. Lysates from M-LK transfected cells exhibited a markedly higher activity on irinotecan hydrolysis than lysates from the cells transfected with mouse triacylglycerol hydrolase (TGH) (6.9 versus 1.3 pmol/mg/min). Based on the immunostaining intensity with purified rat hydrolase A, M-LK had a specific activity of 173 pmol/mg/min, which ranked it as one of the most efficient esterases known to hydrolyze irinotecan. A chimeric carboxylesterase and its wild-type enzyme (e.g., M-LKm and M-LK), sharing three quarters of the entire sequence from the N-terminus, exhibited the same substrate preference toward irinotecan and two other substrates, suggesting that the N-terminal sequence determines substrate selectivity. M-LK transfected cells manifested more severe cytotoxicity than TGH transfected cells upon being exposed to irinotecan. Topoisomerase I inhibitors such as irinotecan represent a promising class of anticancer drugs. Identification of M-LK as an efficient carboxylesterase to activate irinotecan provides additional sequence information to locate residues involved in irinotecan hydrolysis and thus facilitates the design of new analogs.

Carboxylesterases represent a large class of hydrolytic enzymes that play important roles in the metabolism of endogenous lipids, pharmacological determination of therapeutic agents and detoxication of organophosphorus insecticides (Satoh et al., 2002). In addition to hydrolyzing numerous carboxylic acid esters, amides, and thioesters, some carboxylesterases have been shown to catalyze transesterification reaction, which accounts for the conversion of cocaine (a methyl ester) to a longer lasting metabolite, ethylcocaaine (the corresponding ethyl ester) (Randall, 1992; Xu et al., 1994). Carboxylesterase activity is widely distributed in mammalian tissues, with the highest levels present in liver microsomes. High abundance of carboxylesterases in the liver is linked to certain cellular structural roles, particularly in directing protein trafficking. For example, egasyn, a liver microsomal carboxylesterase, binds to β-glucuronidase (normally targeted to the lysosome), resulting in the sequestration of this enzyme in the endoplasmic reticulum (Zhen et al., 1995; Islam et al., 1999). The microsomal β-glucuronidase has been shown to hydrolyze glucuronidated hormones (e.g., steroids), an effective mechanism that recycles physiologically important molecules (Zhen et al., 1995; Zhu et al., 1996; Islam et al., 1999).

Hydrolysis by carboxylesterases is increasingly used as a basis for drug design, particularly on pro-drugs containing functional groups such as carboxylic acid ester (Buchwald and Bodor, 2000, 2002; Senter et al., 2001). Introduction of ester linkages generally improves bioavailability of therapeutic agents or targets them to specific tissues or cell types based on hydrolytic activation (Buchwald and Bodor, 2000, 2002). Irinotecan (CPT-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin), for example, is an ester derivative of antineoplastic camptothecin linking to a bipteridino carbonyl moiety (Masuda et al., 1996; Humerickhouse et al., 2000). Such a modification not only improves significantly the polarity of the molecule but also decreases toxicity associated with the parent compound. However, irinotecan itself has little antitumor activity, but its hydrolytic metabolite (SN-38) is a potent inhibitor of topoisomerase I. Therefore, hydrolytic biotransformation plays a determinant role in the overall antitumor activity of irinotecan. Experimentally forced expression of human and rabbit carboxylesterases increases the sensitivity of xenografted tumors or tumor cell lines to irinotecan (Danks et al., 1999; Humerickhouse et al., 2000; Wu et al., 2002). More importantly, the increased sensitivity is proportionally correlated with the catalytic velocity of a carboxylesterase toward irinotecan. Carboxylesterases HCE-2 and rCE, from human and rabbit, respectively,

1 Abbreviations used are: Irinotecan, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (CPT-11); HCE, human carboxylesterase; rCE, rabbit carboxylesterase; M-LK, mouse liver and kidney carboxylesterase; TGH, triacylglycerol hydrolase; HPLC, high performance liquid chromatography; LDH, lactate dehydrogenase; HS, anti-hydrolase S antibody; 4PP, 4-piperidino-piperidine; SN-38, 7-ethyl-10-hydroxycamptothecin.
are found to effectively catalyze irinotecan hydrolysis (Senter et al., 2001).

In this study, we report the tissue distribution, immunochemical cross-reactivity, and enzymatic characterization of a novel mouse liver and kidney carboxylesterase (M-LK). This carboxylesterase has a restricted tissue distribution and is highly active toward irinotecan. The N-terminal three quarter sequence appears to be responsible for such a high efficient hydrolysis. Along with HCE-2 and rCE, M-LK is the third member of carboxylesterases that are known to effectively activate irinotecan.

Materials and Methods

Chemicals and Supplies. TRI REAGENT RNA extraction solution, para-nitrophenylacetate and para-nitrophenylbutyrate were from Sigma-Aldrich (St. Louis, MO); kit for primer extension labeling, nitro blue tetrazolium, and 5-bromo-4-chloro-3-indolyl-phosphate were from Promega (Madison, WI). The goat anti-rabbit-IgG conjugated with alkaline phosphatase was from Promega (Madison, WI). The cDNAs encoding M-LK and triacylglycerol hydrolase (TGH) were isolated by screening a mouse liver cDNA library as described previously (Hu and Yan, 1999). The library was constructed with the SPORT mammalian expression vector (Invitrogen). The cDNAs shared a HindIII site at the location encoding amino acids 434 and 435. This endonuclease site enabled the sequence encoding the C-terminal residues of both enzymes to be switched with each other (108 and 112 residues for M-LK and TGH, respectively). As a result, M-LK and its chimeric enzyme M-LKhn shared the N-terminal 434 residues but differed on the C-terminal 108 residues. The same was true with TGH and its chimeric enzyme TGHn. The resultant chimeric constructs were subjected to sequencing analyses.

Plasmid Constructs. The cDNAs encoding M-LK and triacylglycerol hydrolase (TGH) were isolated by screening a mouse liver cDNA library as described previously (Hu and Yan, 1999). The library was constructed with the SPORT mammalian expression vector (Invitrogen). The cDNAs shared a HindIII site at the location encoding amino acids 434 and 435. This endonuclease site enabled the sequence encoding the C-terminal residues of both enzymes to be switched with each other (108 and 112 residues for M-LK and TGH, respectively). As a result, M-LK and its chimeric enzyme M-LKhn shared the N-terminal 434 residues but differed on the C-terminal 108 residues. The same was true with TGH and its chimeric enzyme TGHn. The resultant chimeric constructs were subjected to sequencing analyses.

Transfection. Human embryonic kidney cells (293T) were plated at a density of 60% in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. After reaching 80% confluence, cells were transfected by LipofectAMINE and Plus Reagent. A plasmid construct or the empty vector (4 μg) was added to a monolayer of 293T cells. After a 3-h incubation, the media were replaced with fresh media containing various concentrations of irinotecan. After an additional 3-h incubation, the media were collected and centrifuged at 4°C for 5 min to remove any debris. To determine the total cellular activity of lactate dehydrogenase (LDH), the same amount of medium (500 μl) was added to each well, and the cells were then subjected to freezing-thawing to completely lyse the cells. Likewise, the supernatants were prepared by centrifugation and used to assess the intracellular LDH. The activity of LDH was determined with a CytoTox 96 kit (Promega) as described by the manufacturer. The ratios of the released LDH over the intracellular LDH were calculated.

Other Assays. Protein concentration was determined with Micro BCA Reagents (Ference Chemical) as described by the manufacturer. Three antibodies against distinct carboxylesterases were described elsewhere (Morgan et al., 1994; Yan et al., 1995b; Xie et al., 2002). Northern and Western analyses were performed as described previously (Zhu et al., 2000). Data are presented as mean ± S.D. of at least three separate experiments, except where results of blots are shown in which case a representative experiment is depicted in the figures. Comparisons between two values were made with Student’s t test at p < 0.05.

Results

Molecular Cloning of M-LK. In an effort to isolate cDNAs encoding distinct mouse carboxylesterases, cDNA trapping experiments were performed to screen a mouse liver library with two oligonucleotides (TGTGACCATCTTTGAGAGTC, TTTGGC-GAGTCTGCGGTTGCC). These oligonucleotides targeted the region encoding the conserved motif GXXG among carboxylesterases (Satoh et al., 2002), therefore, cDNAs encoding several distinct carboxylesterases were isolated. One of the cDNAs encoded a novel carboxylesterase and was identical to the cDNA recently deposited to the GenBank (accession number BC 013479). Based on subsequent analyses on its tissue distribution, the encoded carboxylesterase by this cDNA was designated M-LK. Like other carboxylesterases, M-LK has several structural features (Satoh et al., 2002). It has an N-terminal signal peptide (18 amino acids) commonly seen in secretory proteins, five cysteine residues with four of them presumed to form intramolecular disulfide bonds, and two putative N-glycosylation sites. A catalytic triad (Ser203, His448, and Glu335) is located based on sequence acting as a retention signal in the endoplasmic reticulum (Yan et al., 1995b). Sequence alignment analyses show that M-LK has a ~70% sequence identity with other mouse carboxylesterases except Es-M, a carboxylesterase that is abundantly expressed in the male liver and that has a markedly lower sequence identity (~45%) with any other mouse carboxylesterases (Ovnic et al., 1991a,b; Aida et al., 1993; Ellinghaus et al., 1998; Dolinsky et al., 2001). Based on the classification proposed by Satoh and Hosokawa (1998), M-LK is a member of the CES1B subfamily.

Tissue Distribution. We next examined the tissue distribution of M-LK by Northern analysis. As a comparison, the tissue distribution of TGH (another mouse carboxylesterase) was simultaneously determined (Dolinsky et al., 2001). TGH was chosen because Western analysis demonstrated that M-LK and TGH were immunologically distinct (below). As shown in Fig. 1, M-LK was abundantly expressed in the liver and kidney but only slightly in the intestine and lung. In contrast, TGH had a broader tissue distribution. High levels of TGH mRNA were detected not only in the liver and kidney but also in the lung. In addition, TGH was moderately expressed in the heart and slightly in the intestine and testis. Neither M-LK nor TGH mRNA was detected in the brain or spleen. It was interesting to notice that a
Tissue RNA was pooled from 5 mice, and an aliquot (20 μg) was subjected to 2.2 M formaldehyde-agarose gel electrophoresis and transferred to a Nytran nylon membrane (Midwest Scientific, Valley Park, MO) with a vacuum-blotting system. The mRNA encoding M-LK or TGH was detected with 32P-labeled cDNA probe. As a control for loading, the RNA gel was visualized with ethidium bromide before being transferred to the membrane.

previous study reported the absence of TGH in the lung (Dolinsky et al., 2001). We used two different mouse strains (CD-1 and B6C3F1) and high levels of TGH mRNA in the lung were detected in both cases. The tissue distributions and the relative abundance in each organ established by Northern analyses were confirmed by Western analyses (data not shown).

Immunocross-Reactivity. M-LK shares an 85% sequence identity with rat hydrolase B whereas TGH shares 98% with human carboxylesterase HCE-3 (Yan et al., 1994; Mori et al., 1999; Dolinsky et al., 2001). Therefore, it was hypothesized that the antibody against purified hydrolase B cross-reacted with M-LK whereas the antibody against a peptide from human HCE-3 cross-reacted with TGH. To test this possibility, transient transfection experiments were performed with cDNAs encoding M-LK, TGH, and two chimeric enzymes, M-LK n and TGH n. The chimeric constructs were prepared by fusing the N-terminal sequence of M-LK to the C-terminal sequence of TGH or vice versa. Figure 2A shows the diagrammatic presentation of M-LK, TGH, M-LK n, and TGH n.

Immunocross-reactivity of M-LK, TGH, M-LK n, and TGH n was determined with the following three antibodies: anti-HCE-3, anti-hydrolase B, and anti-hydrolase S. As expected, the anti-HCE-3 antibody recognized TGH and M-LK n (Fig. 2B) because they shared the C-terminal sequence in which a peptide was derived for preparing this antibody (Fig. 2A). In contrast, the anti-hydrolase B antibody (against purified hydrolase B) reacted strongly with M-LK but only weakly with TGH (Fig. 2B). In addition, the anti-hydrolase B antibody cross-reacted strongly with M-LK n and TGH n as they shared with M-LK the N-terminal and C-terminal sequence, respectively (Fig. 2A). These results provide direct evidence that M-LK and TGH are immunologically distinct based on their reactivity toward anti-HCE-3 and hydrolase B. In contrast, rat hydrolase S is ~70% identical to M-LK, TGH, and the chimeric enzymes (Yan et al., 1995b; Dolinsky et al., 2001); therefore, the anti-hydrolase S antibody (HS) reacted comparably with all four carboxylesterases (Fig. 2B). The immunocross-reactivity shown by the recombinant carboxylesterases established that M-LK n and TGH n were indeed chimeric carboxylesterases. No cross-reactive protein was detected by any antibodies in the lysates from the vector-transfected cells (Fig. 2B).

Hydrolysis of Irinotecan, para-Nitrophenylbutyrate and para-Nitrophenylacetate. We next examined whether the recombinant carboxylesterases were enzymatically active. Three substrates were used including irinotecan, para-nitrophenylbutyrate, and para-nitrophenylacetate. Irinotecan is a water-soluble anticancer prodrug whereas para-nitrophenylbutyrate and para-nitrophenylacetate are standard substrates that are widely used for assaying hydrolytic activity. In relation to human carboxylesterases, transfection and subsequent enzymatic assays were performed with human HCE-1. As shown in Fig. 3A, transfection with the empty vector caused little hydrolytic activity toward all substrates. In contrast, transfection with plasmid constructs encoding carboxylesterases caused a marked increase on hydrolysis. The overall hydrolytic activity, however, varied markedly from enzyme to enzyme and substrate to substrate as well. M-LK and TGH comparably hydrolyzed para-nitrophenylbutyrate whereas M-LK was markedly more active than irinotecan (5-fold) and para-nitrophenylacetate (2-fold). Although the chimeric enzymes exhibited a similar substrate preference as the respective wild-type carboxylesterases (e.g., M-LK n and M-LK), the overall hydrolytic activity of the chimeras was lower than that of the wild-type carboxylesterases (Fig. 3A). M-LK was generally more active than human HCE-1 toward all substrates, particularly on irinotecan (6.9 versus 0.1 pmol/mg/min).

To determine whether such a difference on the overall hydrolytic activity was due to the variation on the expression levels, the same amount (2.5 μg) of the lysates for each carboxylesterase was analyzed for the relative abundance with the anti-hydrolase S antibody. Various amounts of purified rat hydrolase A were used to serve as a basis for normalization. The anti-hydrolase S antibody was chosen because hydrolase S has a similar sequence identity (~70%) with all carboxylesterases to be analyzed, and we have demonstrated that this antibody cross-reacted comparably with an array of mammalian carboxylesterases (Xie et al., 2002). As shown in Fig. 3B, all lysates yielded a

![Figure 1](image1.png)

**Fig. 1.** Tissue distribution of M-LK and TGH. Total RNA was isolated from brain, heart, intestine (small), kidney, liver, lung, spleen, and testis of 9-week-old male CD-1 mice.

![Figure 2](image2.png)

**Fig. 2.** Diagrammatic presentation and immunocross reactivity of M-LK, TGH, M-LK n, and TGH n.
Fig. 3. Hydrolytic activity of lysates from carboxylesterase-transfected cells and immuno-quantitative determination of the abundance of each recombinant enzyme in the lysates.

A. Hydrolytic rate Lysates (330 μg for CPT-11 and 20 μg for other substrates) from cells transfected with the empty vector or a cDNA construct encoding a carboxylesterase were assayed for their activity to hydrolyze irinotecan, para-nitrophenylacetate (p-NpAc) and para-nitrophenylbutyrate (p-NpBu). The hydrolytic activity toward irinotecan was determined by HPLC whereas the activity toward other two substrates was spectrophotometrically determined as described under Materials and Method. All assays were performed in triplicate with three transfection experiments. Hydrolytic rates were expressed as the mean ± S.D. (pmol or μmol/ng of protein/min). B. Immuno-quantitative determination of recombinant M-LK, TGH, M-LKn, and TGHn Lysates (2.5 μg) from the transfected cells or purified rat hydrolase A (25–200 ng) were subjected to SDS-polyacrylamide gel electrophoresis and transferred electrophoretically to a Trans-Blot nitrocellulose membrane. The immunoblot was detected with the anti-hydrolase S antibody as described above.

Similar intensity on the immunoblot, suggesting that the concentrations of the recombinant carboxylesterases in their lysates were similar. Based on densitometrical scanning of the immunostaining intensity against various amounts of purified rat hydrolase A, it was estimated that ~4% of the protein in the lysates was recombinant carboxylesterases.

Cytotoxicity Induced by Irinotecan. We next examined whether expression of M-LK would sensitize cells to irinotecan-induced toxicity. Cells were transfected with the vector, M-LK, or TGH construct. The transfected cells were exposed to various concentrations of irinotecan for 30 h, and the released and intracellular LDH was determined. The ratio of the released LDH over the intracellular LDH was calculated and the irinotecan-induced toxicity was expressed as a percentage of an increase on the ratios in the presence over the absence of irinotecan. As shown in Fig. 4, transfection with either M-LK or TGH caused a marked increase on LDH release by as much as 80%. In any concentrations, transfection with the M-LK construct resulted in a significantly higher LDH release than that with TGH, particularly when lower concentrations (e.g., 0.37 μM) were used (~3 fold difference). To determine whether such differential sensitivities were due to the variations on the expression levels, the supernatants from the lysed cell pellets were analyzed for the abundance of the recombinant M-LK and TGH. As depicted in Fig. 4, transfection with M-LK or TGH resulted in a similar immunostaining intensity although the transfected cells, exposed to the highest concentration of irinotecan, had slightly lower levels of the recombinant carboxylesterases (likely due to higher toxicity). Apparently the hydrolytic metabolite has direct cytotoxic effect although topoisomerase inhibitors are generally considered as anti-proliferative agents (Wu et al., 2002). As expected, no immuno-reactive protein was detected in the vector-transfected cells.

Discussion

This report describes the tissue distribution, immunochemical cross-reactivity, and substrate preference of a novel mouse carboxylesterase M-LK. Rapid hydrolysis of irinotecan by M-LK ranks this carboxylesterase as one of the most efficient esterases known to hydrolyze this prodrug. Several mammalian carboxylesterases are found to catalyze irinotecan hydrolysis, but the overall activity differs markedly from enzyme to enzyme (Humerickhouse et al., 2000; Senter et al., 2001; Wadkins et al., 2001). Human HCE-2, for example, is 26-fold more active than human HCE-1 (92 versus 3.5 pmol/mg/min). A rCE is even more active than HCE-2 and is 40 or 130 times as active as HCE-1 depending on the laboratories in which the enzymatic assays were performed (Senter et al., 2001; Wadkins et al., 2001). Based on the immunostaining intensity against various amounts of purified rat hydrolase A (Fig. 3), we estimate that M-LK and HCE-1 have a specific activity of 173 and 2.5 pmol/mg/min, respectively. Such estimation ranks M-LK as one of the most efficient esterases known to hydrolyze irinotecan, although purified M-LK is required to precisely determine its specific activity. Similarly, TGH is estimated to have a specific activity of 33 pmol/mg/min, which is 13
FIG. 5. Partial sequence alignment of M-LK, TGH, rCE, HCE-1, and HCE-2.

Amino acids are shown in single letters and the sequence corresponding residues 121 to 453 of rCE is aligned. Amino acids proposed to form the side door in rCE are boxed whereas the shaded residues are assumed to participate in facilitating the exit of the leaving group 4PP. These residues are conserved or substituted by similar amino acids among M-LK, rCE, and HCE-2 but not HCE-1 and TGH. Gaps are introduced for maximal alignment.

TABLE 1

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Acyl Pocket*</th>
<th>Hydrophobic Subsite</th>
<th>Peripheral Anionic</th>
<th>Reference</th>
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<tr>
<td>M-LK</td>
<td>LSLOGDDKNSQDFVTS</td>
<td>KFLWDLDAKHT</td>
<td>This study</td>
<td></td>
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<tr>
<td>TGH</td>
<td>LDLDGKSEYPFPLFT</td>
<td>NYWLDV</td>
<td>Dolinsky et al., 2001</td>
<td></td>
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<td>NYWLDV</td>
<td>Mori et al., 1999</td>
<td></td>
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<tr>
<td>HCE-1</td>
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<tr>
<td>HCE-2</td>
<td>LDLDGKSEYPFPLFT</td>
<td>NYWLDV</td>
<td>Robbi and Beaufay, 1994</td>
<td></td>
</tr>
</tbody>
</table>

HA, hydrolase A; HE, hydrolase E.

*The acyl pocket is equivalent to the region harboring phenylalanines 288 and 290 (the acyl pocket) in acetylcholinesterase (Harel et al., 1992).
times as active as HCE-1. Mouse TGH shares a 98% sequence identity with human HCE-3 (Mori et al., 1999; Dolinsky et al., 2001), and presumably both have a similar activity toward irinotecan. Therefore, HCE-3 likely contributes significantly to the hydrolytic activation of irinotecan.

The substrate preference shared by the chimeric and its respective wild-type carboxylesterase (e.g., M-LKn and M-LK) suggests that the N-terminal sequence contains residues involved in enzyme-substrate interactions as observed with its counterpart in acetyl- and butyrylcholinesterases (Oakeshott et al., 1999). Carboxylesterases and cholinesterases both belong to a superfamily of α/β fold proteins; they share a moderate sequence identity (~30%); and they use two-step serine hydrolase mechanism and are highly sensitive to serine enzyme inhibitors (Sussman et al., 1991; Ordentlich et al., 1993; Oakeshott et al., 1999; Satoh et al., 2002). X-ray crystallographic studies with acetylcholinesterase, and recently with rCE, reveal that the catalytic triad is located at the base of a deep catalytic gorge (Sussman et al., 1991). Amino acid residues lining the inner surface or the rim of the gorge form several functional subsites. Site-directed mutagenesis studies demonstrate that the nonconserved residues in the subsites (e.g., the acyl pocket) are largely responsible for the difference between acetyl- and butyrylcholinesterases on the substrate preference and inhibitor reactivity (Ordentlich et al., 1993; Oakeshott et al., 1999; Satoh et al., 2002). Therefore, it is conceivable that carboxylesterases, like cholinesterases, contain functional subsites that play determinant roles in substrate selectivity. Several lines of evidence support this notion. X-ray crystallographic study demonstrates that rCE and ace-tylcholinesterase are similar on the secondary structural elements within the catalytic domain although they show significant deviation on other regions (e.g., the αβ domain) (Sussman et al., 1991; Bencharit et al., 2002). In this report, the chimeric enzymes share with the respective wild-type carboxylesterases the N-terminal sequence (a region harboring all the subsites) and exhibit the same selectivity toward irinotecan, para-nitrophenylacetate, and para-nitrophenylbutyrate (Fig. 3A). Conversely, M-LK and TGH differ on 14 of 25 residues composing the subsites (the hydrophobic subsite, the acyl pocket, and the peripheral anionic subsite) and exhibit different substrate preference toward these subsites (Fig. 3A, Table 1). In addition, we have previously made similar observations with 12 human and rodent carboxylesterases (Xie et al., 2002). For example, hydrolase A and HCE-3, sharing all of the 25 residues, preferably hydrolize para-nitrophenylbutyrate over para-nitrophenylacetate. In contrast, hydrolase A differs from rat hydrolase E by 6 of the 25 residues (particularly in the acyl pocket), and they exhibit opposite preference toward these two substrates (Xie et al., 2002; Table 1).

Hydrolysis of irinotecan is likely further limited by its rather bulky leaving group 4-piperidino-piperidine (4PP), which contrasts small/linear aliphatic chains of standard substrates such as β-nitrophenylacetate. Recent X-ray crystallographic studies with rCE reveal that this carboxylesterase has a gate, so called “side door,” for the leaving group 4PP to exit (Bencharit et al., 2002). Such a side door resembles “the back door” proposed for acetylcholinesterase but is located ~180° away from the back door (Gilson et al., 1994; Bencharit et al., 2002). The side door, composed that consists of different residues. Studies with chimeric enzymes of carboxylesterases such as HCE-1 have a threonine at all three positions (Fig. 5). Interestingly, two of the three residues are directly related to the residues forming the side door proposed for rCE (Bencharit et al., 2002). The residue 253 is located between two of the side-door-forming residues (Leu252 and Ser254). The proline 411, although 12 residues apart from Leu424 (one of the side-door-forming residues), is located on the same α-helix (α-12). Proline is usually found in the bends of folded proteins and is known to restrict range of allowed conformation. Therefore, the proline 411 likely ensures such a side door to be open and thus facilitates the exit of the leaving group 4PP. Finally, the residue 298 is located on α-helix 8, which is part of the catalytic domain as shown by the X-ray crystallographic structure (Bencharit et al., 2002).

In summary, we report the tissue distribution, immunocomunochemical cross-reactivity, and enzymatic characterization of a novel mouse carboxylesterase M-LK. This carboxylesterase is highly expressed in the liver and kidney and is a third member of carboxylesterases known to efficiently hydrolyze produg irinotecan. Topoisomerase I inhibitors such as irinotecan represent a promising class of anticancer drugs. Identification of M-LK as an efficient carboxylesterase to activate irinotecan provides additional sequence information to locate residues involved in irinotecan hydrolysis and thus facilitates the design of new analogs. Furthermore, M-LK is likely used in viral-directed enzyme-prodrug combing therapy.

References


Dolinsky VW, Siepne S, Lehner R, and Vance DE (1999) The cloning and expression of a carboxylic ester hydrolase A differs from rat hydrolase E by 6 of the 25 residues (e.g., the acyl pocket) and the peripheral anionic subsite) and exhibit different substrate preference toward these subsites (Fig. 3A, Table 1). In addition, we have previously made similar observations with 12 human and rodent carboxylesterases (Xie et al., 2002). For example, hydrolase A and HCE-3, sharing all of the 25 residues, preferably hydrolize para-nitrophenylbutyrate over para-nitrophenylacetate. In contrast, hydrolase A differs from rat hydrolase E by 6 of the 25 residues (particularly in the acyl pocket), and they exhibit opposite preference toward these two substrates (Xie et al., 2002; Table 1).

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In summary, we report the tissue distribution, immunocomunochemical cross-reactivity, and enzymatic characterization of a novel mouse carboxylesterase M-LK. This carboxylesterase is highly expressed in the liver and kidney and is a third member of carboxylesterases known to efficiently hydrolyze produg irinotecan. Topoisomerase I inhibitors such as irinotecan represent a promising class of anticancer drugs. Identification of M-LK as an efficient carboxylesterase to activate irinotecan provides additional sequence information to locate residues involved in irinotecan hydrolysis and thus facilitates the design of new analogs. Furthermore, M-LK is likely used in viral-directed enzyme-prodrug combing therapy.


