Irinotecan (CPT-11) is a prodrug that has proven efficacy against advanced colorectal and lung cancer (Johnson, 2001). CPT-11 is activated to the potent topoisomerase I poison SN-38 by ubiquitous carboxylesterases. CPT-11 can also be oxidized to a number of mostly inactive products (Rivory et al., 1996a; Dodds et al., 1997). SN-38 is glucuronon conjugated at the C-10 hydroxyl position to produce SN-38G. The reverse reaction is mediated by β-glucuronidases. Hence, production of SN-38 may occur through either pathway. In this study we conducted in vitro studies to examine these two reactions in neuroblastoma xenograft tumors (NB1691) and compared the rates of SN-38 production with those observed in the liver and plasma of the host SCID (severe-combined immunodeficient) mice. The rate of formation of SN-38 from CPT-11 by esterases slowed considerably during a 60-min incubation, consistent with the known deacylation-limited nature of this reaction. For xenograft tumor tissue, $K_m$ and $V_{max}$ values of 1.6 μM and 4.4 pmol/min/mg of protein, respectively, were observed. By comparison, these parameters were estimated to be 6.9 μM and 9.4 pmol/min/mg for mouse liver and 2.1 μM and 40.0 pmol/min/mg for mouse plasma, respectively. The formation of SN-38 from SN-38G was very pronounced in both liver and xenograft tumor tissue, in which it was nonsaturable (0.125–50 μM) and time-independent (0–60 min). The derived values of $V_{max}/K_m$ were 0.65 μl/min/mg for the tumor and 2.12 μl/min/mg for the liver preparations. Microdialysate experiments revealed the concentrations of SN-38G and CPT-11 in tumor to be comparable. At equal substrate concentrations, production of SN-38 from SN-38G in tumor extracts was comparable with that from CPT-11. Therefore, reactivation of SN-38 in the tumor by β-glucuronidases may represent an important route of tumor drug activation for CPT-11.

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It has been proposed that carboxylesterase activity within the tumor may be an important determinant of the activity of CPT-11 (Atsumi et al., 1995). Recent studies have demonstrated CPT-11 converting carboxylesterase activity in homogenized human colon and liver tumor samples (Ahmed et al., 1999; Guichard et al., 1999), human xenografts (Kojima et al., 1998), and human lung cancer cell lines (van Ark-Otte et al., 1998). Therefore, tumor tissue may contribute to the overall activation of CPT-11, which may explain the altered plasma pharmacokinetics of CPT-11 and SN-38 observed in xenograft-bearing animals (Stewart et al., 1997; Zamboni et al., 1998a). It is assumed that the major route of production of SN-38 is through esterolysis of CPT-11. However, regeneration of
Materials and Methods

Chemicals and Drugs

CPT-11 and APC were generously provided by Pharmacia & Upjohn Diagnostics (Kalama, MI), dissolved in 0.1 N HCl, and stored at −20°C until use. SN-38 and SN-38G were kindly provided by Aventis (Strasbourg, France). (2S)-Camptothecin (CPT) was purchased from Sigma-Aldrich (St. Louis, MO). SN-38 and CPT were dissolved in dimethyl sulfoxide and SN-38G in 0.1 N HCl, and these solutions were stored at −20°C until use. For i.v. administration, CPT-11 was dissolved in a solution of 0.26 ml of sorbitol (70%, w/w), 0.9 mg/ml lactic acid, and sterile water. The solution of 20 mg/ml was filtered, sterilized, and kept in a foil-wrapped sterile vial. Tetrabutylammonium phosphate (TBAP) was purchased from Waters (Milford, MA) as a ready-to-use solution (PIC A). All other chemicals and solvents used were analytical grade or better.

Animals and NB1691 Xenografts

Severely combined immunodeficient female mice (SCID) were obtained from Charles River Laboratories (Wilmington, MA). Human neuroblastoma NB1691 tumor pieces of 3 mm^3 were implanted in the subcutaneous space of both lateral flanks of female mice at 4 weeks of age to initiate tumor growth as described previously (Thompson et al., 1997). Each animal weighed 21 to 25 g at the time of the experiment. All procedures were approved by the Animal Care and Use Committee at St. Jude Children’s Research Hospital.

Preparation of Tissue Homogenate

Xenografts were grown until 2 cm in diameter. Animals were euthanized by methoxyflurane anesthesia followed by cervical dislocation. Tumor, plasma, and liver were harvested and frozen at −70°C until required. Liver extracts were prepared with a Dounce homogenizer after adding an equal volume of homogenization buffer (2 mM Tris-HCl, pH 7.3, 230 mM mannitol, and 20 mM sucrose). Thawed tumor samples were spun in an ultrafiltration apparatus from which the membrane had been removed (Pierce, Rockford, IL). This provided a simple method to collect tumor cells and extracellular fluid mostly free of the stromal component. The collected material was spun at 4000g to remove cell debris. Soluble protein content was then determined by the Lowry assay (Lowry et al., 1951).

SN-38 Production in Tissue Homogenates and Plasma

Reactions were performed in 50-μl final volumes of 0.1 M phosphate buffer (pH 7.4) in Eppendorf tubes. The lactone species of CPT-11 and SN-38G were added at final concentrations ranging from 0.13 to 20 and 0.13 to 50 μM, respectively. The reaction mix and drug were briefly incubated (10 min) at 37°C in a shaking water bath (~70 oscillations/min). The reactions were initiated with the addition of tissue extract (200 μg soluble protein) or blank murine plasma (5 μl, equivalent to 200 μg of soluble protein). The reaction was stopped by the addition of 100 μl of acetonitrile/methanol (50:50, v/v) containing 5 ng of CPT. Appropriate control reactions were performed at each concentration of substrate (without homogenate) in an identical fashion, and the concentration of SN-38 present was subtracted from that produced by the tissue.

Preliminary experiments on the formation of SN-38 were conducted to determine the effect of protein concentration (25, 50, 100, 150, 200, and 250 μg of soluble protein) with 5 μM CPT-11 and 25 μM SN-38G. The effect of incubation time (0, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 40, 50, and 60 min) was also evaluated for each source of tissue (tumor, liver, and plasma) with 5 μM CPT-11 and 25 μM SN-38G. Incubation periods of 10 and 20 min were selected for CPT-11 and SN-38G, respectively, from the initial linear portion of the curves. Intratumoral variability in the conversion to SN-38 was studied using four equivalent sections from one tumor. The velocity was expressed as the SN-38 produced divided by the incubation time and normalized per milligram of protein.

The reaction for carboxylesterases converting CPT-11 to SN-38 has been shown to follow the scheme (Rivory et al., 1996a):

\[
P_1 = \frac{EtS}{k_1} + \frac{k_2}{k_3} \frac{PtS}{E}\]

where \(E, S, E^*, P_1,\) and \(P_2\) are the free, Michaelis-Menten, and acylated enzyme species, respectively. \(S, P_1,\) and \(P_2\) refer to CPT-11, SN-38, and 4-piperidino-1-carboxylic acid piperidine, respectively. In the case of deacylation-limited kinetics, the concentration of the first product \(P_1\) at any time \(t\) is given by:

\[
P_1 = \frac{EtS}{k_1} + \frac{k_2}{k_3} \frac{PtS}{E} \left[1 + \left(\frac{\beta k_3}{\beta - \alpha k_3}\right) t + \left(\frac{(Q - r_2) \exp^{-r_2 t} - (Q - r_2) \exp^{-r_2}}{r_1 - r_2}\right)\right]
\]

where \(Et\) and \(S\) are the concentrations of total enzyme and substrate, respectively, and \(Q\) equals:

\[
\alpha^2 k_3 - \beta k_3 - \alpha \beta \alpha k_3 - \beta
\]

\(r_1\) and \(r_2\) are the roots of the quadratic equation:

\[
y = x^2 + ax + b
\]

\[
a = k_3S + k_2 + k_3 + k_1 - 1
\]

\[
\beta = k_3(k_1 + k_2 + 1) + k_3S(k_2 + k_3)
\]

These equations were fitted to the concentration versus time profiles by nonlinear regression (Excel; Microsoft, Redmond, WA) to determine \(k_1, k_2, k_3,\) and \(Et.\) The ratio of \((k_2 + k_3)k_3\) was calculated to determine the influence of deacetylation on the enzyme kinetics. A ratio \(> 1\) indicates deacylation-limited kinetics.

Kinetic parameters \((V_{max} \text{ and } K_m)\) were determined by fitting the velocity data to the Michaelis-Menten equation within Sigmaphot
In Vivo Plasma Pharmacokinetics and Microdialysis in NB1691 SCID Mice

The pharmacokinetics of CPT-11 and its metabolites were investigated in plasma of SCID mice bearing NB1691 xenograft tumor. Heparinized blood samples (~1 ml) were collected by cardiac puncture at 0.08, 0.125, 0.25, 1, 2, 4, and 6 h (three animals per time point) after the injection of CPT-11 (10 mg/kg) into a lateral tail vein. Samples were immediately centrifuged at 7700 g for 2 min, and plasma was separated and stored at −70°C until analysis.

The in vivo disposition of CPT-11 in tumor tissue was investigated in a separate but matching group of xenograft-bearing SCID mice. Each NB1691 tumor was 1 to 2 cm in diameter at the time of study. Microdialysis probes (two per animal; CMA-20/04; CMA/Microdialysis, Stockholm, Sweden) were implanted in each tumor as previously described by Zamboni et al. (1999b). CPT-11 (10 mg/kg) was injected i.v. as a bolus into a lateral tail vein. Successful microdialysis experiments were performed in one xenograft tumor from each of four animals. Perfusion solution (Ringer’s) was infused through the probe at 1 μl/min by way of a microinjection pump (CMA/100; CMA/Microdialysis). In vitro and in vivo recovery experiments showed that drug recovery was maximal at this flow rate (data not shown). Dialysate samples were collected in a microfraction collector (CMA-140; CMA/Microdialysis) every 25 min up to 8 h. An aliquot (20 μl) of each sample was then added to 80 μl of methanol to ensure maximum stability of sample at −70°C until analysis.

HPLC Analyses

Production of SN-38 by Tissue Preparations. The formation of total (lactone + carboxylate) SN-38 by the tissue homogenates was monitored by HPLC with fluorescence detection using CPT as an internal standard. This was achieved by a modification of the method of Rivory and Robert (1994) using a Waters Symmetry C8 reversed-phase column (3.8 × 150 mm, i.d. 5 μm) and matching guard column (Guard-Pak; Waters) at a flow rate of 1.0 ml/min. The HPLC system (Shimadzu, Sydney, Australia) consisted of an LC-10AT pump, DGU-12A in-line solvent degasser, SCL-10A system controller, SIL-10AXL autoinjector, and RF-10AXL fluorometric detector. Fluorescence detection was optimized for detection of SN-38 with excitation and emission wavelengths set at 380 and 530 nm, respectively. The mobile phase consisted of a 24:76 (v/v) mix of acetonitrile and 0.075 M ammonium acetate buffer (pH 6.0). Data were collected and analyzed using CLASS VP software (version 4.2, Shimadzu).

Standard samples of SN-38 were prepared by serial dilution of a stock solution into acetonitrile/0.05 M citric acid (50:50, v/v). Calibration standards were prepared for two standard curves with concentrations of 0.5 to 100 ng/ml and 50 to 1500 ng/ml, respectively. Standards were prepared in a manner similar to the incubation samples by being mixed with 100 μl of stop solution (acetonitrile/methanol, 50:50, v/v) containing 5 ng of CPT. These were centrifuged (8000g, 5 min), and the supernatant was acidified with 2 μl of 2 N HCl. An aliquot (50 μl) of the supernatant was injected onto the chromatograph.

Plasma Pharmacokinetics. Plasma concentrations of total CPT-11 and metabolites were determined using a slight variation of a validated method (Rivory and Robert, 1994). In this instance, the mobile phase consisted of 0.125 M ammonium acetate buffer (pH 7.4) and acetonitrile (19:81, v/v) with 15 mM TBAP and pumped at 1.5 ml/min.

Blank murine plasma was used to prepare calibration standards over a concentration range of 2.5 to 100 ng/ml for CPT-11, SN-38G, and SN-38, and 2.5 to 100 ng/ml for APC. An aliquot (50 μl) of plasma standards and samples were mixed with 100 μl ice-cold methanol/ACN (50:50, v/v) containing 5 ng of CPT, acidified, and centrifuged at 8000g for 5 min, and 20 μl was then injected onto the chromatograph.

Validation of this modified method was performed as previously described (Dodd et al., 2000). The assay was found to be linear, for each analyte, over the concentration range (r² ≥ 0.99, n = 56), with a lower limit of quantitation of 2.5 ng/ml for SN-38G (3.7 nM), APC (4 nM), and SN-38 (6.4 nM) and 10 ng/ml (17 nM) for CPT-11, respectively. Accuracy and total imprecision (n = 16) over the analytical range were 88.8 to 102.2% and 6.1 to 15.9%, respectively.

Pharmacokinetic Analysis

Pharmacokinetic analysis of both plasma and microdialysate was performed by noncompartmental methods. Specifically, the AUCO-T was determined by the trapezoidal method for each of the compounds of interest. For the plasma pharmacokinetics, the AUCs were extrapolated to infinity using the terminal rate constant estimated from a regression of the linear semilog concentration versus time profile of later time points (n = 3). The terminal half-life of elimination (t1/2T) was estimated as 0.693 divided by the terminal rate constant.

Physiological Modeling

The activation of SN-38 from CPT-11 in tumor, liver, and blood was estimated by assuming equilibrium distribution of CPT-11 in each of these organs based on a 25-g mouse with a 4-g tumor, 1-g liver, and 1.75 ml of blood. The contribution of tumor and liver to the clearance of CPT-11 in the mouse was calculated from the weight of tissue, the final volume of the homogenate, and the protein concentration of the homogenate. In the case of plasma, clearance was determined assuming a 0.4 hematocrit. The total clearance was then calculated as the sum of the clearances by each of the eliminating organs (Rowland and Tozer, 1989).

Statistics

The Michaelis-Menten and related parameters obtained from the tissue homogenate kinetic experiments were compared between their sources (i.e., liver, tumor, plasma) by analysis of variance with Bonferroni post hoc comparisons using SYSTAT (v7.0.1; SPSS Science). Statistical significance was considered to be reached with p < 0.05.

Results

SN-38 Production by Tissues. Formation of SN-38 from CPT-11 and SN-38G was linear with respect to the incubate protein concentration for all sources (tumor, liver and plasma; data not shown). Production of SN-38 from SN-38G was also linear with respect to time (data not shown). On the other hand, production of SN-38 from CPT-11 showed substantial slowing during a 60-min incubation, particularly for liver tissue (Fig. 1). This is consistent with deacetylation-limited kinetics, as has previously been reported for the rodent (SPSS Science, Chicago, IL). Values reported are means ± S.D. for at least three preparations.
and human carboxylesterase-mediated hydrolysis of CPT-11 (Tsuji et al., 1991; Rivory et al., 1996a). This was reflected by the ratio \(k_2 + k_3/k_2\) being \(>1\) for all tissue sources (Table 1). Substrate depletion was estimated and found to be \(<15\%\) at all times.

The concentration-dependent kinetics of carboxylesterase activation by tumor, liver, and plasma are shown in Fig. 2. Plasma was extremely active in comparison to liver and tumor homogenates. The relevant kinetic parameters are listed in Table 1. The \(V_{\text{max}}/K_m\) values derived for carboxylesterase-mediated conversion for each organ were in the order of: plasma \(>\) tumor \(>\) liver (Table 1).

The double reciprocal plots obtained for tumor, liver, and plasma were consistent with a single converting enzyme for all sources (Fig. 3). The kinetics of SN-38 formation varied somewhat among four replicate preparations from a single tumor with \(K_m\) and \(V_{\text{max}}\) values ranging from 1.8 to 3.2 \(\mu\)M and 2.03 to 6.08 pmol/min/mg of protein, respectively. The corresponding \(V_{\text{max}}/K_m\) values ranged from 1.1 to 2.7 \(\mu\)l/min/mg of protein. The calculated organ clearances of CPT-11, following the assumption in Materials and Methods, were 0.38, 0.08, and 0.81 \(\text{ml/min/mg}\) for tumor, liver, and plasma, respectively.

In contrast to the carboxylesterase-mediated hydrolysis of CPT-11, there was no evidence of any glucuronidase-mediated production of SN-38 in plasma. Also, the glucuronidase activity of liver and tumor was not saturable over the SN-38G concentration range of 0.13 to 50 \(\mu\)M (Fig. 4). As formation of product was not able to be saturated, the derived kinetic parameter used for comparison was \(V_{\text{max}}/K_m\) (microliters per minute per milligram of protein), which is equivalent to intrinsic clearance (Iwatsubo et al., 1997). This value was derived from the slope of the plot of the velocity of product formation as a function of substrate concentration (Table 1). The intratumoral variability observed for \(\beta\)-glucuronidase conversion of SN-38G to SN-38 resulted in \(V_{\text{max}}/K_m\) values ranging from 0.30 to 0.83 \(\mu\)l/min/mg of protein.

Preincubation of tumor and liver preparations with the specific \(\beta\)-glucuronidase inhibitor saccharolactone (100 \(\mu\)M) inhibited the production of SN-38 from SN-38G by \(>95\%\), confirming the role of \(\beta\)-glucuronidase in mediating this reaction.

**Pharmacokinetics in Plasma and Microdialysate.** Pharmacokinetic parameters for CPT-11, SN-38G, APC, and SN-38 in plasma and tumor extracellular microdialysate are summarized in Table 2. For comparison, although both lactone and carboxylate forms of these species were quantitated in the microdialysis studies, these concentrations are reported as total (lactone + carboxylate) concentrations.

![Fig. 1. SN-38 concentrations produced from the incubation of 5 \(\mu\)M CPT-11 with 200 \(\mu\)g of soluble protein in tumor (n = 4), liver (n = 3), and plasma (n = 3) homogenates.](image)

**TABLE 1**

Comparison of kinetic parameters for carboxylesterase and \(\beta\)-glucuronidase-mediated formation of SN-38 by human xenograft (NB1691) tumor, liver, and plasma

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Kinetic Parameter</th>
<th>Tumor (N = 4)</th>
<th>Liver (N = 3)</th>
<th>Plasma (N = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(V_{\text{max}}) (pmol/min/mg protein)</td>
<td>4.40 ± 1.08*</td>
<td>9.35 ± 2.00*</td>
<td>40.0 ± 2.51</td>
</tr>
<tr>
<td>CPT-11</td>
<td>(K_m) ((\mu)M)</td>
<td>1.63 ± 0.27†</td>
<td>6.92 ± 1.14</td>
<td>2.06 ± 0.15†</td>
</tr>
<tr>
<td></td>
<td>(k_2 + k_3/k_2)</td>
<td>12.3 ± 7.44</td>
<td>28.9 ± 13.18</td>
<td>10.3 ± 1.22</td>
</tr>
<tr>
<td>SN-38G</td>
<td>(V_{\text{max}}/K_m) ((\mu)l/min/mg protein)</td>
<td>2.70 ± 0.54*</td>
<td>1.43 ± 0.49*</td>
<td>19.5 ± 1.67</td>
</tr>
<tr>
<td></td>
<td>(V_{\text{max}}/V_m) ((\mu)l/min/mg protein)</td>
<td>0.65 ± 0.12</td>
<td>2.12 ± 0.65</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

* Significantly different from plasma (\(p < 0.0001\)).
† Significantly different from liver (\(p < 0.0001\)).
‡ Significantly different (\(p = 0.006\)).
Discussion

This is the first study to examine the potential contribution of the intratumoral activation of SN-38 by hydrolysis of its glucuronide metabolite. Although this pathway would still require the sequential production of SN-38 and SN-38G from CPT-11, the glucuronidase-mediated reactivation could provide a localized mechanism for production of SN-38 from systemic pools of SN-38G.

Two major differences were observed for SN-38 production by carboxylesterase and \( \beta \)-glucuronidase. First, CPT-11 hydrolysis was limited to some extent by the deacylation step of the catalytic cycle of carboxylesterase, whereas the production of SN-38 from SN-38G was linear with respect to time. This means that the contribution of SN-38 production from CPT-11 hydrolysis would be expected to decrease over time during prolonged exposure to CPT-11. The second difference was that the glucuronidase-mediated SN-38 production was not saturable over the concentration range investigated. This lack of saturability of glucuronidase has been observed with other glucuronide substrates (Lavrijsen et al., 1992; Tsai and Gorrod, 1999).

The kinetic properties of the tumor, liver, and plasma carboxylesterase for conversion of CPT-11 to SN-38 were different, although the tumor and plasma carboxylesterase activity shared similar \( K_m \) values. Carboxylesterase activity was deacylation-limited to some extent in all tissues, although the effect was not as marked as that previously reported for the human hepatic enzyme (Rivory et al., 1996a). The possibility that the tumor activity was partially due to the presence of the very active mouse plasma esterase

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Fig. 2. Rate of SN-38 production from CPT-11 in each preparation as estimated from a 10-min incubation. Tumor (\( n = 4 \)), liver (\( n = 3 \)), and plasma (\( n = 3 \)) homogenates were incubated with 0.13 to 20 \( \mu \)M CPT-11 lactone. The Michaelis-Menten parameters are listed in Table 1.

Fig. 3. Eadie-Hofstee plots for the hydrolysis of CPT-11 lactone in preparations from each organ. Data are presented as means of at least three preparations.
Critical analysis of CPT-11 and SN-38 disposition in tumor-bearing mice after intravenous administration of CPT-11 at 10 mg/kg. Data are expressed as means ± S.D.

**Table 2**: Plasma and tumor extracellular fluid pharmacokinetic parameters of CPT-11, SN-38G, SN-38, and APC in tumor-bearing mice after intravenous administration of CPT-11 at 10 mg/kg.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma (n = 3)</th>
<th>Tumor Extracellular Matrix (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT-11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCo-T (µg/ml · h)</td>
<td>2.08 ± 0.49</td>
<td>0.22 ± 0.19</td>
</tr>
<tr>
<td>Clearance (ml · min⁻¹)</td>
<td>2.06 ± 0.47</td>
<td>0.85</td>
</tr>
<tr>
<td>t1/2z (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SN-38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCo-T (µg/ml · h)</td>
<td>1.75 ± 0.50</td>
<td>0.43 ± 0.68</td>
</tr>
<tr>
<td>t1/2z (h)</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>SN-38G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCo-T (µg/ml · h)</td>
<td>0.69 ± 0.09</td>
<td>0.20 ± 0.12</td>
</tr>
<tr>
<td>t1/2z (h)</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>APC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCo-T (µg/ml · h)</td>
<td>0.15 ± 0.02</td>
<td>Not detected</td>
</tr>
<tr>
<td>t1/2z (h)</td>
<td>6.3</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. Production of SN-38 from SN-38G lactone (0.13–50 µM) in tumor (n = 4) and liver (n = 3) homogenates as estimated from a 20-min incubation. Data are expressed as means ± S.D.

Overall, our results suggest that plasma is the major source of carboxylesterase-mediated SN-38 formation in the mouse, with a rate exceeding that attributable to the liver by ~10-fold. The presence of the highly active plasma carboxylesterase in this species makes it difficult to extrapolate animal models of CPT-11 disposition to the clinical situation. Interestingly, our estimates also indicate that the tumors used in these experiments contribute significantly to carboxylesterase-mediated clearance. This may partly explain the observation that the kinetics of CPT-11 and SN-38 are modified by the presence of tumors (Zamboni et al., 1998a).

The estimated total clearance of CPT-11 based on the summation of the carboxylesterase-mediated in vitro clearances (tumor, liver, and plasma) was 1.3 ml · min⁻¹. This compares favorably with the 2 ml · min⁻¹ obtained with the pharmacokinetic analyses, the balance likely to be due to renal, biliary, and intestinal excretion. The observed in vivo clearance also compares favorably with other mouse plasma pharmacokinetic studies in which CPT-11 was administered intravenously at this dose (Kaneda et al., 1990; Stewart et al., 1997; Zamboni et al., 1998a).

The formation of SN-38 from CPT-11 and SN-38G yielded similar Vmax/Km values in the tumor preparations, indicating similar catalytic rates for these two pathways at very low substrate concentrations (which reflect clinically relevant concentrations). At the concentrations of CPT-11 and SN-38G observed in microdialysis, the local tumor formation of SN-38 from SN-38G would be comparable to that produced from CPT-11.

Although the production of SN-38 from SN-38G was highest for the liver, β-glucuronidase is normally located in the microsomal and lysosomal fractions, representing sites that are relatively inaccessible to polar conjugates in intact hepatocytes (Miyauchi et al., 1989). Homogenization of tissue results in the mechanical lysis of cells and release of these enzymes. In many tumors, on the other hand, there are significant extracellular levels of β-glucuronidase (Murder et al., 1997; Sperker et al., 2000), due to the liberation of the enzyme from the lysosomes of inflammatory cells and, to a much lower extent, disintegrating tumor cells (Bosslet et al., 1998). In non-necrotic tumors, the enzyme appears located only within intact granulocytes. Interestingly, in human tumor xenograft models, the tumoral β-glucuronidase activity could be identified as being of mouse rather than human origin (Bosslet et al., 1998).

In conclusion, significant activation of CPT-11 occurs in tumor, liver, and plasma of human xenograft-bearing mice. Although significant carboxylesterase-mediated activation of CPT-11 occurs within the tumor, reactivation from SN-38G by tumor glucuronidases represents a hitherto unrecognized but active pathway of intratumoral SN-38 production. Other groups are designing specific glucurononjugated prodrugs to take advantage of tumor glucuronidase-mediated activation, but our results indicate that this pathway may be equally applicable to drugs that are extensively conjugated as part of their metabolic profile. Experiments to examine the importance of glucuronidase-mediated activation of SN-38 in human tumor samples are currently under way.

**References**


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