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ORIGINAL ARTICLE

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Human plasma carboxylesterase and butyrylcholinesterase enzyme activity: correlations with SN-38 pharmacokinetics during a prolonged infusion of irinotecan

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Abstract *Purpose*: To characterize the relationships between human plasma irinotecan carboxylesterase-converting enzyme activity, caboxylesterase-mediated hydrolysis of *p*-nitrophenyl acetate (pNPA), and the butyrylcholinesterase-mediated hydrolysis of butyrylthiocholine in human plasma and to test the ability of these in vitro tests to predict the variability in SN-38 pharmacokinetics in adult patients during a prolonged infusion of irinotecan. *Methods*: Individual plasma-converting enzyme activity was measured in 20 adult cancer patients participating in a pharmacokinetic and phase I clinical trial of a prolonged 96-h intravenous infusion of irinotecan. The pNPA and butyrylthiocholine hydrolysis in patient plasma was also assayed. *Results*: The irinotecan carboxylesterase-converting enzyme in human plasma had a V_{max} of 89.9 ± 22.7 pmol/h per ml plasma and a Km of 207 ± 56 μ*M* (mean ± SD, $n = 3$). The mean value of the specific activity of this enzyme in 20 adult cancer patients was 10.08 ± 2.96 pmol/h per ml plasma ranging from 5.43 to 15.39 pmol/h per ml. The area-under-the-concentration-versus time curve (AUC) ratio of SN-38 to irinotecan ($AUC_{SN\text{-}38}/AUC_{CPT\text{-}11}$) was used to assess the relative SN-38 exposure to the active metabolite in individual patients. Pharmacokinetic variations in the relative exposure to SN-38 did not correlate with the measured carboxylesterase-converting enzyme activity nor with plasma butyrylcholinesterase activity in our patient population. However, it did correlate with the measured pNPA hydrolysis activity in patient plasma ($r^2 = 0.350$, $P = 0.0124$, $n = 18$). *Conclusions*: Determination of patient plasma pNPA hydrolysis activity may have utility in predicting SN-38 pharmacokinetics during prolonged infusions of irinotecan.

Key words Irinotecan · SN-38 · Carboxylesterase · Butyrylcholinesterase

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Introduction

Irinotecan is a topoisomerase I poison with clinical utility in advanced colon cancer. Irinotecan is an inactive prodrug that must first be enzymatically activated by a carboxylesterase-converting enzyme. Cleavage of the bulky dipiperidino sidechain at the carbon-position generates the active metabolite, SN-38, which is the biologically active species responsible for stabilizing topoisomerase I protein-bound to DNA in a cleavable complex [1]. In humans, the enzymatic activation of irinotecan is relatively inefficient, with only a small percentage (3–4%) of the total administered drug being converted into the active metabolite, following a standard 90-min infusion [2].

Human liver microsomal carboxylesterase (E.C. 3.1.1.1) is presumed to be the principal enzyme responsible for the clinical activation of irinotecan. This enzyme has been cloned [3] and its irinotecan-metabolizing activity has been extensively studied in purified protein preparations [4] and in human liver microsomes [5]. The efficiency of irinotecan activation by human liver carboxylesterase is quite low compared with carboxylester-

ases from other species [6, 7] and its kinetics are complex demonstrating a rate-limiting deacylation step and autoinhibitory activity in the presence of irinotecan [4]. Furthermore, carboxylesterase-mediated metabolism studies can be further complicated by the ubiquitous nature of these enzymes, which can exist in multiple different subtypes, each with broad substrate specificity [8]. For example, recent studies by Slatter et al. suggest that high- and low-affinity carboxylesterase isoforms are present in human liver with both demonstrating irinotecan-converting enzyme activity [9].

Human liver carboxylesterase activity can also vary substantially in different individuals. For example, the variation in carboxylesterase activity in human liver microsomes prepared from 11 different donors was shown to range from 5-fold to 45-fold, depending upon the specific substrate analyzed [10]. Because the pharmacokinetic exposure to the active metabolite, SN-38, is also highly variable in individual patients, we and others [11] have hypothesized that differences in irinotecan pharmacokinetics may be due in part to variations in human carboxylesterase-converting enzyme activity. Performing liver biopsies to measure human carboxylesterase activity in cancer patients is not practical for widespread clinical use, but it may be possible to use surrogate measurements to assess individual variation in carboxylesterase-converting enzyme activity in patients. For example, clearance of 5-fluorouracil is principally mediated by the enzyme, dihydropyrimidine dehydrogenase (DPD), which is the key rate-limiting step for endogenous pyrimidine catabolism [12]. Measurement of DPD enzyme activity in peripheral blood mononuclear cells as a marker for systemic enzyme activity, can predict clinically significant differences in 5-FU pharmacokinetics [13]. Therefore, we sought to determine if similar surrogate assays for individual carboxylesterase activity in patients could also predict the pharmacokinetics of SN-38 in patients receiving irinotecan.

Serum irinotecan carboxylesterase-converting enzyme activity is quite high in some species, such as rats, but its activity in human serum is very much less [6]. However, as we discovered during our phase I and pharmacologic trial of irinotecan administered as a prolonged intravenous infusion [14], irinotecan carboxylesterase-converting enzyme activity can be detected in human plasma. This finding led us to further characterize the kinetics of this plasma enzyme activity and to examine its interpatient variability in a population of adult solid tumor patients participating in our phase I clinical trial. We also measured the plasma hydrolysis of *p*-nitrophenyl acetate (pNPA), a general substrate for carboxylesterase activity, in our patients. During the conduct of these studies, Morton et al. found that another plasma enzyme, butyrylcholinesterase (E.C. 3.1.1.8), also has measurable irinotecan-converting enzyme activity [15]. These authors characterized the kinetics of purified butyrylcholinesterase and concluded this may be the principal enzyme responsible for the conversion of irinotecan to SN-38 in cancer patients [15]. Therefore, we also measured the variation in plasma butyrylcholinesterase activity in our study population. Finally, we examined the predictive value of these measurements as markers for explaining the interindividual variability in SN-38 pharmacokinetics in 20 patients receiving irinotecan in our phase I clinical trial.

Materials and methods

Reagents

Irinotecan (CPT-11) and SN-38 were provided by Pharmacia-Upjohn. HPLC grade methanol, acetonitrile and water were obtained from Fisher (Fair Lawn, N.J.) and all other chemicals and supplies were obtained from Sigma Chemicals (St. Louis, Mo.), unless otherwise stated.

Measurement of carboxylesterase-converting enzyme activity in human plasma

Irinotecan carboxylesterase-converting enzyme activity was determined by measuring the formation of SN-38 during incubation of irinotecan in human plasma. To 800 µl 20 m*M* Tris-HCl buffer, pH 7.5, was added 10 µl 2.5 m*M* irinotecan and the mixture was incubated at 37 °C for 30 min to allow equilibrium to be reached between the lactone and carboxylate drug forms. Then 200 µl human plasma was added and the samples were incubated for 24 h at 37 °C. At the end of the incubation, 100-µl aliquots of the mixture were deproteinated by adding 500 µl acidified methanol (1% HCl), and then 25 µl of the sample supernatant was analyzed via HPLC. Analytic separation was achieved using a 250 × 4.6 mm Lichrosorb C-18 reversed-phase HPLC column (Alltech, Deerfield, Ill.) with a Waters 600 E pump and a Waters 470 fluorescence detector (Waters Corporation, Milford, Mass.) set at an excitation wavelength of 375 nm and emission wavelength of 535 nm. Five SN-38 calibration standards were prepared at concentrations of 10, 50, 100, 500, and 1000 n*M*. Individual plasma irinotecan carboxylesterase-converting enzyme activity for each patient was determined from the amount of SN-38 produced during the incubation period and is expressed as picomoles per hour per milliliter plasma.

Carboxylesterase-converting enzyme kinetics in human plasma

Single-donor human plasma (200 µl) was added to 800 µl 20 m*M* Tris-HCl buffer, pH 7.5, and different concentrations of irinotecan were added to give final concentrations of 10, 50, 100, 200, 400, and 800 µ*M*. The tubes were vortexed, incubated for 24 h at 37 °C, and then 25 µl of the reaction mixture was removed and the amount of SN-38 generated was determined as described above. The Michaelis-Menten kinetics of SN-38 formation was determined by nonlinear regression of the untransformed, nonlinearized data using SigmaPlot for Windows, version 5.0 (SPSS, Chicago, Ill.).

Hydrolysis of pNPA in human plasma

Human plasma carboxylesterase activity was assessed using a modification of the method developed by Krisch to measure the hydrolysis of pNPA [16]. Briefly, 18.1 mg of pNPA was dissolved in 0.8 ml of acetonitrile and the volume raised to 50 ml with distilled water. Then, 0.8 ml of the above solution was added to 0.2 ml 100 m*M* Tris-HCl buffer, pH 7.5, for a final substrate pNPA concentration of 1.6 m*M*. The reaction was started by the addition 10 µl patient plasma. Release of *p*-nitrophenol was measured every 30 s using visible spectroscopy at a wavelength of 405 nm at 25 °C for up to 30 min. A calibration standard consisting of *p*-nitrophe-

nol dissolved in the same reaction mixture was used to convert the absorbance units to a molar concentration of *p*-nitrophenol.

Measurement of butyrylcholinesterase activity in human plasma

Human plasma butyrylcholinesterase activity was measured using a visible spectroscopic method based upon the conversion of butyrylthiocholine to thiocholine, which reacts with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) to generate 5-thio-2-nitrobenzoate, a yellow substance [17, 18]. Briefly, 40 µl was added to 10 µl buffer containing 200 µ*M* DTNB, and 1 ml of this preparation was placed in a cuvette and butyrylthiocholine at a final concentration of 200 µ*M* was added. The visible absorbance at a wavelength of 415 nm at 25 °C was measured every 30 s for up to 30 min. A calibration standard consisting of 2 m*M* L-cysteine dissolved in buffer containing DTNB and plasma was used to convert absorbance units to an equivalent molar concentration of thiocholine.

Patient pharmacokinetics and correlation studies

In association with our phase I clinical trial of 96-h weekly infusions of irinotecan, the plasma pharmacokinetics of total irinotecan and SN-38 were measured using a reversed-phase HPLC-based assay as described previously [14]. Patients were included in the current study only if they had undergone validated pharmacokinetic monitoring and if sufficient stored plasma was available to assay for irinotecan carboxylesterase-converting enzyme activity. These criteria were met for 20 patients consecutively enrolled in our phase I study. Ten of these patients were included in our previous report on infusional irinotecan [14], but ten patients in the current study group were entirely new and not previously described. One patient who was included in our previous report received treatment at 12.5 mg/m^2 per day, but had insufficient pretreatment plasma for our current assay, and thus could not be included in the present analysis. Noncompartmental pharmacokinetic parameters were determined using WinNonLin, Version 2.1 (Pharsight Corporation, Cary, S.C.). The relative exposure of each patient to the active metabolite was determined by calculating the SN-38 formation-clearance ratio, which was defined as the AUC ratio of SN-38 to irinotecan ($AUC_{SN\text{-}38}/AUC_{CPT\text{-}11}$) [19]. Linear regression was used to analyze the relationship between the formation-clearance ratio and the measured plasma irinotecan carboxylesterase-converting enzyme, pNPA hydrolysis, and butyrylthiocholine hydrolysis.

Results

Characterization of human plasma irinotecan carboxylesterase-converting enzyme activity

Fresh human donor plasma incubated with concentrations of irinotecan ranging from 12.5 to 200 µ*M* under the conditions described in Materials and methods produced increasing amounts of SN-38 over time (Fig. 1). The overall enzyme activity was low, with only minimal amounts of SN-38 generated compared to control samples over the initial few hours. However, after 24 h and 26 h of incubation, a consistent and easily measured amount of SN-38 was generated under the assay conditions. Intraassay variability was assessed by measuring irinotecan hydrolysis over 24 to 48 h in human donor plasma in triplicate on three successive occasions. Intraday coefficients of variation ranged from 1.7% to 9.3%, demonstrating a high robustness of the analytic method.

The stability of human plasma irinotecan carboxylesterase-converting enzyme activity was compared in single-donor fresh and frozen human plasma samples stored for up to 6 months. Repeated measurements of the carboxylesterase-converting enzyme activity from the same plasma sample varied about the mean value by less than 16% over a 6-month time period with no temporally related trends ($n = 4$). Thus, storage of human plasma at –20 °C for prolonged periods of time did not appreciably alter the measured plasma converting enzyme activity.

Human plasma irinotecan carboxylesterase-converting enzyme kinetics

The kinetics of human plasma carboxylesterase-converting enzyme were examined in fresh samples of

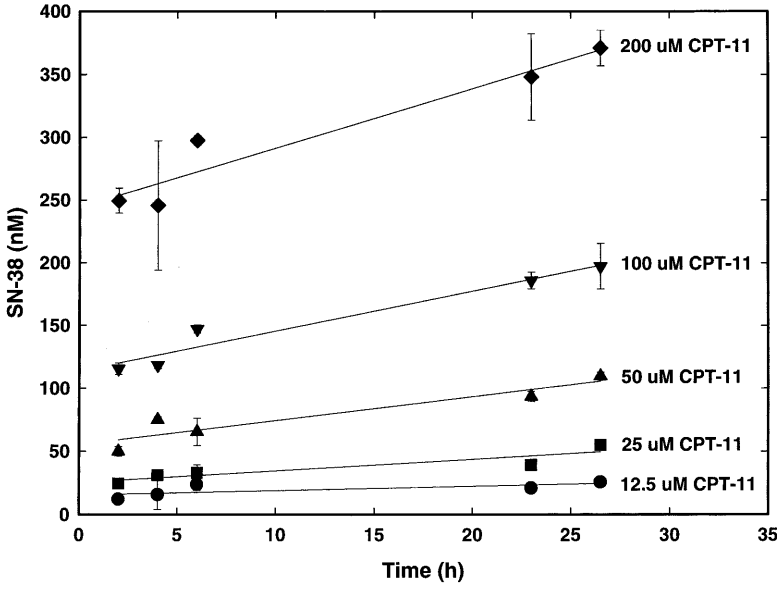

Fig. 1 Formation of SN-38 over time. Various concentrations of irinotecan (CPT-11) were incubated with 10 µl fresh human donor plasma in 20 m*M* Tris-HCl, pH 7.5 buffer at 37 °C for 0, 3.5, 6, 24, and 26 h and the amount of SN-38 generated plotted as a function of time as described in Materials and methods. Samples were corrected for nonenzymatic hydrolysis measured in control samples

plasma from a single donor performed on three separate occasions. Complex kinetic behavior has been described for irinotecan hydrolysis by human liver carboxylesterase enzymes. During the first few minutes, the reaction velocity is governed by the rate of the hydrolysis reaction. However, after this initial phase, the rate-limiting step of enzyme deacylation dictates the reaction velocity [4]. Because of the long 24-h incubation time used here, variations in the enzyme velocity during the initial incubation period did not interfere with the overall assessment of enzyme activity. Using our assay, the kinetics of conversion of irinotecan to SN-38 were fitted by the Michaelis-Menten equation with a V_{max} of 89.9 ± 22.7 pmol/h per ml plasma and a Km of 207 ± 56 μM (mean $\pm$ SD, $n = 3$; Fig. 2).

Assessment of human carboxylesterase-converting enzyme activity in patient plasma

Human plasma samples obtained immediately prior to treatment with irinotecan were obtained from 20 patients enrolled on our phase I study of a 96-h irinotecan infusion administered weekly for 2 of 3 weeks. Overall, 12 men and 8 women were tested with a median age of 57 years and a range from 28 to 79 years. Further demographics for this population are shown in Table 1. Patients were treated at either 10 or 12.5 mg/m^2 per day, and plasma samples were stored at –20 °C for up to 6 months prior to analysis for carboxylesterase-converting enzyme activity as described in Materials and methods.

Human plasma irinotecan carboxylesterase-converting enzyme varied by about threefold in the 20 patients analyzed in our study (Table 2). The mean value was 10.08 ± 2.96 pmol/h per ml plasma and ranged from 5.43 to 15.39 pmol/h per ml plasma. No correlation could be detected between measured carboxylesterase-converting enzyme activity in plasma and age, gender, diagnosis, prior treatment, liver transaminase levels (AST, ALT, alkaline phosphatase, total bilirubin), total protein, or albumin concentrations (data not shown). Five patients underwent repeated measurement of irinotecan carboxylesterase-converting enzyme activity in plasma obtained prior to the start of the irinotecan infusion on weeks 1 and 2. No difference in the mean plasma irinotecan carboxylesterase-converting enzyme activity was seen when week 1 was compared with week 2: 10.7 ± 3.2 versus 9.9 ± 4.7 pmol/h per ml plasma, respectively; $P = 0.72$, paired t-test).

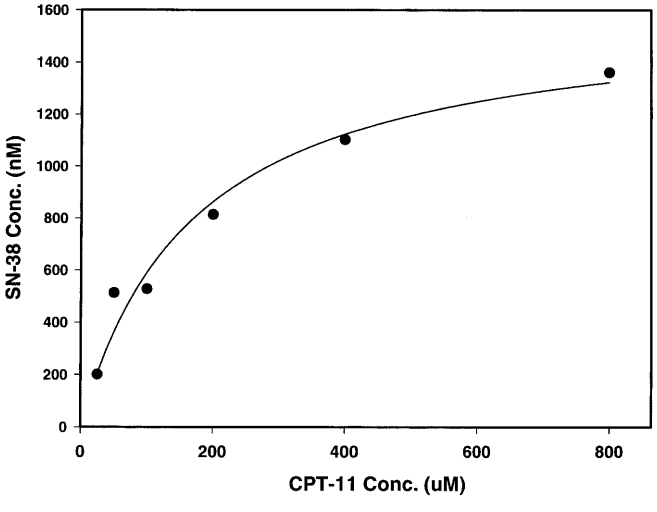

Fig. 2 Carboxylesterase-converting enzyme activity kinetics in human plasma. Irinotecan (CPT-11) at concentrations of 25, 50, 200, 400 and 800 μM was incubated with fresh human donor plasma for 24 h and the amount of SN-38 generated was determined as described in Materials and methods. The data presented are from a single representative of three separate experiments

pNPA hydrolysis in human plasma

Because of the weak activity of human plasma irinotecan carboxylesterase-converting enzyme, the assay for

Table 1 Patient demographics ($n = 20$)

Age (years)	
Median	60
Range	28 to 79
Gender	
Male	12
Female	8
Performance status (ECOG)	
0	3
1	16
2	1
Histologic tumor type	
Colon	8
Rectal	1
Non-small-cell lung	4
Pancreatic	1
Other	6
Prior therapy	
None	1
Chemotherapy only	11
Chemotherapy and radiation therapy	8
Serum creatinine (mg/dl)	
Median	0.8
Range	0.50–1.2
AST (U/l)	
Median	24
Range	15–76
ALT (U/l)	
Median	27.5
Range	10–64
Total bilirubin (mg/dl)	
Median	0.5
Range	0.3–1.0
Alkaline phosphatase (U/l)	
Median	95
Range	60–932
Serum albumin (g/dl)	
Median	4.0
Range	3.1–4.8
Total protein (g/dl)	
Median	7.0
Range	5.5–8.3

this activity required a prolonged 24-h incubation time. However, previous investigators have reported a correlation between human irinotecan-converting enzyme activity in liver and the carboxylesterase-mediated hydrolysis of pNPA [5]. This rapid, easy to measure assay for general carboxylesterase activity releases the yellow *p*-nitrophenol product within minutes and can be monitored using visible absorbance spectroscopy. Therefore, we examined overall plasma carboxylesterase activity using the pNPA assay in 18 of the 20 patients studied above (Table 2). However, in our patient population, plasma irinotecan carboxylesterase-converting enzyme activity did not correlate with carboxylesterase-mediated pNPA hydrolysis ($r^2 = 0.001$, $P = 0.898$).

Butyrylcholinesterase activity in patient plasma

Recent reports suggesting that human plasma butyrylcholinesterase activity in plasma may be responsible for the conversion of irinotecan to SN-38 in patients [15] prompted us to analyze this enzymatic activity in our patient population. Hydrolysis of butyrylthiocholine to thiocholine was measured in the plasma of 18 patients (Table 2). However, as before, the activity of plasma butyrylcholinesterase did not correlate with the conversion of irinotecan to SN-38 ($r^2 = 0.034$, $P = 0.463$). Furthermore, butyrylcholinesterase was also not related to the measured activity of pNPA hydrolysis ($r^2 = 0.0001$, $P = 0.965$).

Pharmacokinetics of prolonged infusions of irinotecan

Pharmacokinetic data were collected from a subset of 20 patients enrolled in our phase I study of a prolonged 96-h infusion given weekly for 2 of 3 weeks. Of these 20 patients, 15 were treated with 10 mg/m^2 per day irinotecan and 5 received 12.5 mg/m^2 per day. Noncompartmental pharmacokinetic parameters for these patients are shown in Table 3. Interpatient variability in SN-38 pharmacokinetics during the prolonged infusion schedule was high, as evidenced by coefficients of variation ranging from 38% to 60% (Table 3), which is consistent with the variability seen during shorter infusions of irinotecan [11].

The formation-clearance ratio $AUC_{SN\text{-}38}/AUC_{CPT\text{-}11}$ was calculated in order to determine the relative exposure of individual patients to the active metabolite SN-38 as a function of the total systemic exposure to irinotecan. The $AUC_{SN\text{-}38}/AUC_{CPT\text{-}11}$ ratio during the first week of therapy in 20 patients ranged from 0.067 to 0.269 with a mean value of 0.134 ± 0.057. Linear regression analysis showed no relationship between this assessment of relative exposure to SN-38 and either the relative plasma activity of irinotecan-converting enzyme activity (Fig. 3A) or the plasma butyrylcholinesterase activity (Table 4). However, a significant relationships was noted between pNPA hydrolysis and relative exposure to SN-38 ($r^2 = 0.350$, $P = 0.0124$, $n = 18$; Fig. 3B).

Discussion

We successfully characterized the kinetics of irinotecan activation in human plasma. Although its activity was low, it was reproducibly measured in plasma after a prolonged incubation and it varied by about threefold in different patients. This variation did not correlate with patient covariates, including age, gender, liver function, total protein or serum albumin levels. In previous studies, human irinotecan carboxylesterase activity has been characterized in liver, tumors and in various organs [4, 5, 7, 20, 21]. Rivory et al. have reported that human

Table 2 Activity of irinotecan carboxylesterase-converting enzyme, pNPA hydrolysis, and butyrylthiocholine hydrolysis in human plasma (*ND* no data available)

Patient no.	Irinotecan hydrolysis (pmol/h/ml plasma)	pNPA hydrolysis (nmol/s/ml plasma)	Butyrylthiocholine hydrolysis (μmol/s/ml plasma)
1	9.65	3.50	0.48
2	15.39	7.20	0.30
3	8.47	7.50	0.59
4	6.83	10.23	0.60
5	9.29	9.37	0.37
6	5.43	5.07	0.47
7	6.82	8.02	0.33
8	7.48	11.07	0.52
9	7.77	10.26	1.08
10	8.49	2.05	0.32
11	12.55	3.22	0.71
12	13.37	5.79	1.12
13	13.55	5.04	1.03
14	6.65	3.78	0.40
15	14.49	7.12	0.43
16	10.93	5.89	0.97
17	8.80	5.04	0.86
18	11.32	ND	ND
19	10.62	ND	ND
20	13.66	ND	ND

Table 3 Irinotecan and SN-38 pharmacokinetics (*CPT-11* irinotecan, *AUC* area-under-concentration versus time curve, *CL* clearance, *Css* steady-state plasma concentration)

Dose level	*n*	CPT-11			SN-38	
		AUC (μg·h/l)	Css (μg/l)	CL (l/h)	AUC (μg·h/l)	Css (μg/l)
10	15	1631 ± 1000	24.14 ± 15.82	39.01 ± 21.03	184.3 ± 80.4	2.50 ± 0.95
12.5	5	2087 ± 485	29.22 ± 7.06	34.41 ± 9.96	304.5 ± 115.9	3.67 ± 1.47
Totals	20	–	–	37.86 ± 18.73	–	–

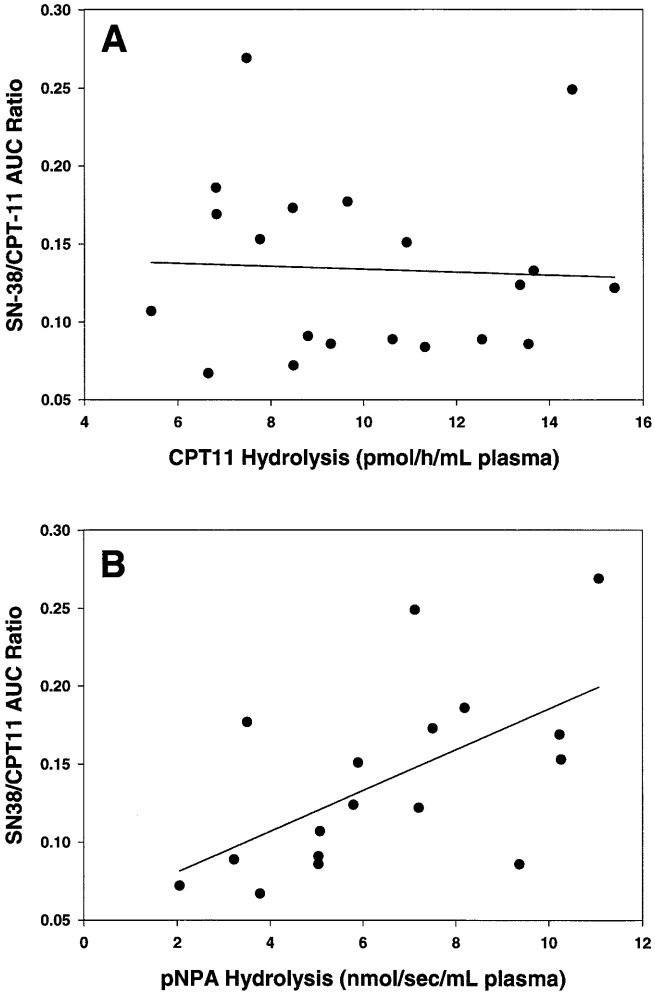

Fig. 3A, B Plot of SN-38 formation-clearance ratio (AUC_{SN-38}/AUC_{CPT-11}) measured in adult cancer patients receiving a prolonged 96-h infusion of irinotecan versus the measured human plasma activity of irinotecan carboxylesterase-converting enzyme ($n = 20$) (**A**) and plasma pNPA hydrolysis ($n = 18$) (**B**)

Table 4 Correlation between SN-38 formation clearance ratio (AUC_{SN-38}/AUC_{CPT-11}) and irinotecan carboxylesterase-converting enzyme activity, pNPA hydrolysis activity, and butyrylthiocholine hydrolysis activity in human plasma

Plasma activity	AUC_{SN-38}/AUC_{CPT-11}	
	r^2	*P*-value
Irinotecan hydrolysis	0.002	0.839
pNPA hydrolysis	0.350	0.012
Butyrylthiocholine hydrolysis	0.012	0.676

liver has relatively high irinotecan carboxylesterase-converting enzyme activity with complex kinetics [4]. An initially rapid irinotecan hydrolysis phase occurs during first 10 min, followed by a much slower and longer deacylation-limited kinetic phase which predominates at later time-points [4]. Whether a similar process occurs in plasma enzymes is not known, but because our assay used a long 24-h incubation time, only the late kinetic phase was profiled in our patients. Furthermore, the rates of enzymatic hydrolysis of irinotecan lactone and open-ring carboxylate are known to differ [5]. However, our assay only examined total (lactone + carboxylate) irinotecan concentrations. Nonetheless, our experiments demonstrated a relatively low, but consistent and reproducible, rate of enzymatic activation of irinotecan in individual patient's plasma with saturable kinetics at high micromolar concentrations of substrate.

Plasma hydrolysis of irinotecan did not correlate with the hydrolysis of pNPA, a general carboxylesterase substrate, or with the hydrolysis of butyrylthiocholine, a specific substrate for butyrylcholinesterase. At present, we do not know the exact enzymes(s) responsible in plasma for irinotecan activation. However, our results make it very unlikely that human plasma irinotecan carboxylesterase-converting enzyme activity is mediated by human plasma butyrylcholinesterase as previously hypothesized by Morton et al. [15].

A major goal of our investigation was to determine whether individual irinotecan-converting enzyme activity is useful in predicting the variations in patient exposure to the active metabolite, SN-38. The SN-38 formation-clearance ratio (AUC_{SN-38}/AUC_{CPT-11}), which is highly variable in individual patients [11], was used in this study as an indicator of the relative exposure of our patients to the biologically active metabolite. For our novel schedule of prolonged irinotecan administration, the SN-38 formation-clearance ratio of 0.134 ± 0.057 was much higher than that seen during short 90-min infusion of irinotecan (0.03–0.04) [2]. Thus, the prolonged low-dose infusion of irinotecan resulted in more efficient conversion of irinotecan to SN-38. However, the measured plasma irinotecan carboxylesterase-converting enzyme activity in individual patients did not correlate with the SN-38 formation-clearance ratio. Furthermore, preliminary physiologic-based compartmental pharmacokinetic modeling analysis demonstrated that the enzyme activities measured in our patients' plasma was too low to account for the observed SN-38 plasma concentrations (data not shown). These

observations suggest that the plasma is not the major locus for irinotecan activation in humans. In addition, although plasma irinotecan-converting enzyme activity varies by up to threefold in adult solid tumor patients, this plasma activity is not a good surrogate marker for predicting the overall extent of drug activation.

Instead, a significant correlation between the hydrolysis of pNPA in patient plasma and the relative exposure to SN-38 (Fig. 3B) was found in our study. This observation was mildly surprising, in that the actual drug substrate, irinotecan, was less predictive than a surrogate carboxylesterase substrate, pNPA. One possible explanation is that our plasma assay may reflect the activity of several different enzymes that are all weak metabolizers of irinotecan. These could potentially include butyrylcholinesterase, human carboxylesterase and other as-yet-unidentified enzymes. Although pNPA acetate is also a substrate for different plasma esterases [22], the overall plasma hydrolysis activity assessed by the pNPA assay may more closely parallel the activity of tissue carboxylesterases, such as human liver carboxylesterase, which may represent the major site for in vivo drug activation. The modest strength of the correlation between pNPA hydrolysis and the relative exposure to SN-38 (Fig. 3B) makes it unlikely that individualized predictions of irinotecan pharmacokinetics can be based only upon the raw data obtained from this assay. However, the identification of more specific esterase inhibitors or the use of substrates with greater specificity for irinotecan carboxylesterase-converting enzyme activity might improve the predictive power of these types of in vitro tests. Furthermore, the clinical impact of the pNPA hydrolysis assay on predicting the pharmacokinetics of irinotecan and its metabolites may be better assessed by incorporating this parameter as a covariate in a population pharmacokinetic mixed-effect modeling approach as implemented in a software program such as NONMEM [23]. Currently, we are examining population models for predicting the pharmacokinetics of irinotecan and SN-38 based upon the data obtained in our phase I trial [14].

Another important factor in determining the interindividual variability in exposure to SN-38 is variation in metabolic clearance instead of differences in metabolite formation. Hepatic glucuronidation [24, 25] and biliary excretion [26] are major pathways for SN-38 clearance, and phenotypic variations in liver function and/or pharmacogenetic differences in drug conjugation pathways may also contribute to variations in exposure to SN-38 in cancer patients. Recently, the specific 1A1 isoform of uridine diphosphate glucuronosyltransferase (UGT) in human liver has been identified as the major isoform involved in SN-38 glucuronidation [27]. Pharmacogenetic variations in the expression of this specific UGT isoform have been identified, and further tests of the clinical relevance of these observations in predicting SN-38 exposure are in progress [28]. In our patients, studies are ongoing to measure the plasma concentrations of SN-38 glucuronide to better understand the potential clinical importance of variations in hepatic glucuronidation in individual patients.

In summary, we characterized the activity of irinotecan carboxylesterase-converting enzyme in human plasma in 20 adult solid tumor patients. Despite its variability in individual patients, this measurement was not predictive of the pharmacokinetics of SN-38. Instead, a modest correlation between the hydrolysis of the carboxylesterase substrate, pNPA, and the relative exposure to SN-38 was observed in our adult solid tumor patients receiving prolonged 96-h intravenous infusions of irinotecan. The clinical utility of these measurements must still be determined. Future studies will also focus on whether these relationships are valid for other more commonly utilized schedules of irinotecan administration, such as 90-min intravenous infusions given weekly or every 3 weeks.

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