Effect of Milk Thistle (*Silybum marianum*) on the Pharmacokinetics of Irinotecan

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Abstract

Purpose: Milk thistle (*Silybum marianum*) is one of the most commonly used herbal therapies, and its principal constituent silybin significantly inhibits cytochrome P450 isoform 3A4 (CYP3A4) and UDP glucuronosyltransferase isoform 1A1 (UGT1A1) *in vitro*. Here, we investigated whether milk thistle affects the pharmacokinetics of irinotecan, a substrate for CYP3A4 and UGT1A1, in humans.

Experimental Design: Six cancer patients were treated with irinotecan (dose, 125 mg/m^2) given as a 90-minute infusion once every week. Four days before the second dose, patients received 200 mg milk thistle, thrice a day, for 14 consecutive days. Pharmacokinetic studies of irinotecan and its metabolites 7-ethyl-10-hydroxycamptothecin (SN-38), 7-ethyl-10-[3,4,5-trihydroxy-pyran-2-carboxylic acid]-camptothecin (SN-38-glucuronide), and 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin were done during the first three irinotecan administrations.

Results: Short-term (4 days) or more prolonged intake of milk thistle (12 days) had no significant effect on irinotecan clearance (mean, 31.2 versus 25.4 versus 25.6 L/h; $P = 0.16$). The area under the curve ratio of SN-38 and irinotecan was slightly decreased by milk thistle (2.58% versus 2.23% versus 2.17%; $P = 0.047$), whereas the relative extent of glucuronidation of SN-38 was similar (10.8 versus 13.5 versus 13.1; $P = 0.64$). Likewise, the area under the curve ratio of 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin and irinotecan was unaffected by milk thistle (0.332 versus 0.285 versus 0.337; $P = 0.53$). The maximum plasma concentrations of silybin ranged between 0.0249 and 0.257 μmol/L.

Conclusions: Silybin concentrations after intake of milk thistle are too low to significantly affect the function of CYP3A4 and UGT1A1 *in vivo*, indicating that milk thistle is unlikely to alter the disposition of anticancer drugs metabolized by these enzymes.

In recent years, interest in complementary and alternative medicine has grown rapidly in the industrialized world. Some of the reasons for this increase relate to dissatisfaction with conventional allopathic therapies, a desire of patients to be involved more actively in their own health care, and because patients find these alternatives to be more congruent with their own philosophical orientations (1, 2). The Center for Disease Control and Prevention recently published data showing that 62% of the United States adult population has used complementary and alternative medicine within the preceding 12 months, and almost 19% have used a herbal remedy (3). Interestingly, complementary and alternative medicine use is more common among patients with cancer than among individuals in the general population (4). Indeed, surveys have shown that complementary and alternative medicine is used by cancer patients, with a prevalence of up to 83%, and for herbal preparations of up to 63% (5). Importantly, between 54% and 77% of cancer patients receiving conventional therapy use complementary and alternative medicine concurrently (6) and the majority of patients does not disclose this use to their treating physician (7). With the large number of cancer patients using herbal treatments in combination with conventional therapies, the risk for herb-drug interactions is a growing concern. The major cause of the concern is that many herbs contain phytochemicals that may interact with prescription drugs, altering their pharmacokinetic characteristics and leading to clinically significant interactions. Because of the high prevalence of herbal medicine use in cancer patients in the United States, we and others have propagated previously that physicians should include herb usage in their routine drug histories to have an opportunity to outline to individual patients which potential hazards should be taken into consideration (8, 9). However, with the exception of St. John's

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Received 6/14/05; revised 7/29/05; accepted 8/10/05.

Grant support: NIH grants P30 CA069773 and P50 AT00437 (S.D. Baker).

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doi:10.1158/1078-0432.CCR-05-1288

wort (10–12), *in vivo* assessments of the potential for the top-selling herbal supplements to interact with chemotherapeutic drugs are currently lacking.

Here, we determined the effect of short-term and long-term administration of milk thistle [*Silybum marianum* (L.) Gaertn. (Asteraceae)] on the pharmacokinetics of the anticancer drug irinotecan. Milk thistle is among the most popular herbs worldwide and it has been used for over 2,000 years as a tonic, demulcent, and antidepressant; it also assists in lactation (13). In the United States, it is currently listed at the 10th position of top-selling herbal supplements (14). A survey in cancer patients estimated that 7% of patients use milk thistle as an alternative remedy or in combination with cytotoxic chemotherapy (15), and it is the fourth most frequently used herb in this population. The principal constituent of milk thistle is silymarin, a mixture of flavonoids, including silybin, silydianin, and silycristin. Milk thistle components have been shown to inhibit a variety of cytochrome P450 (CYP) isozymes, including CYP isoform 3A4 (CYP3A4); UDP glucuronosyltransferases (UGT), including UGT isoform 1A1 (UGT1A1); and several efflux transporters, including ATP-binding cassette (ABC) transporter B1 (ABCB1, P-glycoprotein; refs. 16–22). Because these proteins are involved in the disposition pathway of irinotecan (Fig. 1), knowledge of a drug interaction between milk thistle and irinotecan is of clinical importance.

Materials and Methods

Patients. Eligibility for study entry included a diagnosis for which therapy with irinotecan is standard therapy (i.e., metastatic colorectal cancer) or for which the drug is a reasonable treatment option (e.g., mesothelioma and metastatic cervical, ovarian, head and neck, esophageal, and other gastrointestinal cancers). Eligible were patients ages 18 to 75 years, with a WHO performance status ≤2 and a life expectancy of at least 12 weeks. Patients were not allowed to receive chemotherapy, hormonal therapy, or radiation therapy within a period of 4 weeks before enrollment. No concurrent use of substances known or likely to interfere with the pharmacokinetics of irinotecan was allowed. All patients had adequate clinical functional reserve as defined by hemoglobin, ≥6.0 mmol/L; WBC, $\geq 3.0 \times 10^9$/L; neutrophils, $\geq 1.5 \times 10^9$/L; platelets, $\geq 100 \times 10^9$/L; creatinine clearance, ≥60 mL/min; bilirubin, within normal limits; and transaminases, less than or equal to twice the upper limit of normal (unless due to liver metastases, then less than or equal to thrice the upper limit of normal). The study was approved by the institutional ethics committee (Leiden, the Netherlands) and all patients gave written informed consent.

Study design. The study design was such that it allowed for an evaluation of short-term as well as long-term administration of milk thistle. All patients were treated with irinotecan (Sanofi-Aventis, Hoevelaken, the Netherlands), administered as a 90-minute i.v. infusion at a dose of 125 mg/m^2. For logistical reasons, the drug was given once every week for 4 consecutive weeks, followed by a 2-week rest period. This regimen differs from the recommended European regimen in which i.v. irinotecan is administered at a dose of 350 mg/m^2 on day 1 as a 90-minute infusion, given once every 3 weeks. However, a recent comparative phase III trial done in 291 patients with 5-fluorouracil refractory, metastatic colorectal cancer has shown that both irinotecan schedules of weekly and of once every 3 weeks result in similar efficacy and quality of life (23).

Four days before the second irinotecan administration, patients started taking standardized capsules from a single batch (GNC, Pittsburgh, PA; lot no. 4603KC3918), each containing 200 mg milk thistle seed extract (containing 80% silymarin), thrice per day with water (i.e., one capsule with each meal). The patients continued taking milk thistle while receiving the second irinotecan administration and stopped 2 days after the third administration of irinotecan. Irinotecan treatment continued as clinically indicated until progressive disease or unmanageable toxicity and a maximum of nine cycles of irinotecan were administered.

Irinotecan pharmacokinetics. Blood samples were collected for assessment of irinotecan pharmacokinetics in all patients upon the

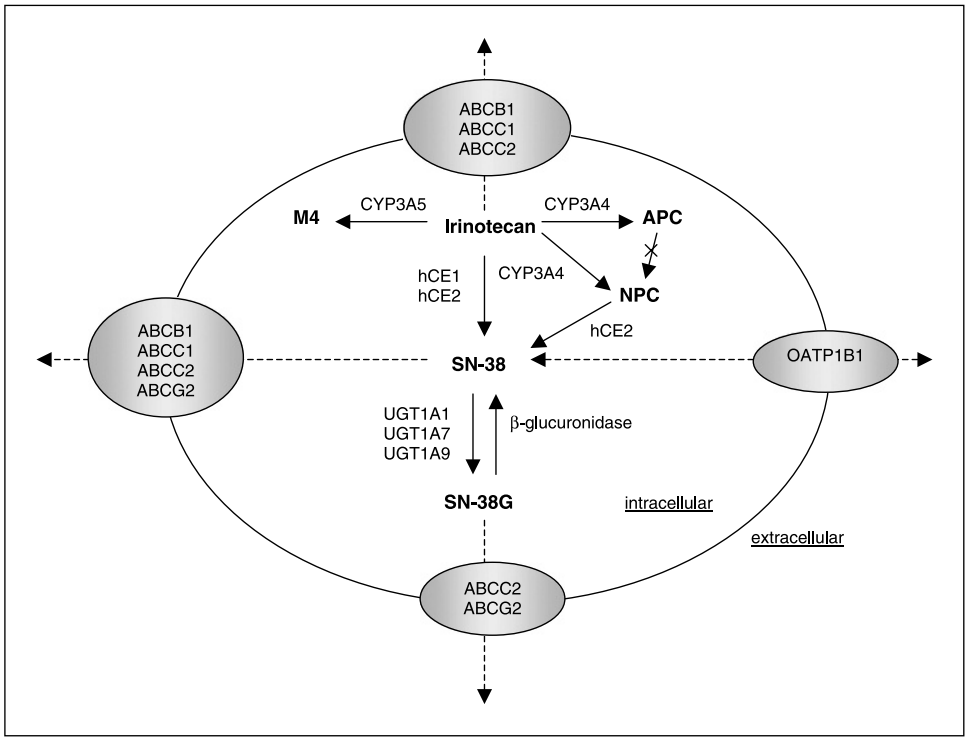

Fig. 1. Schematic diagram of irinotecan metabolism and transport. M4, unidentified metabolite; NPC, 7-ethyl-10-[4-(1-piperidino)-1-amino]-carbonyloxycamptothecin; hCE1, hCE2, human carboxylesterase isoforms 1 and 2; ABCC2, ABC transporter C2 (MRP2, cMOAT); ABCG2, ABC transporter G2 (ABCP, BCRP, MXR); and OATP1B1, organic anion transporting polypeptide 1B1 (OATP2, OATP-C, LST-1).

Table 1. Baseline patient characteristics

Characteristic	Value
Baseline screening	
Total studied	6
Age (y)	54 (40-69)
Sex (male/female)	2 (33)/4 (66)
Body-surface area (m^2)	1.8 (1.5-2.0)
Primary tumor site	
Colorectal	4 (66)
Cervix	1 (17)
Endometrial	1 (17)
Pretherapy chemistry	
Alanine aminotransferase (units/L)	34 (13-105)
Aspartate aminotransferase (units/L)	46 (15-98)
Total bilirubin (μmol/L)	6.3 (3.0-11)
Serum creatinine (μmol/L)	70 (38-100)
Creatinine clearance (mL/min)	102 (80-132)

NOTE: Continuous data are given as median with range in parenthesis and categorical data as number of patients with percentage of the total population in parenthesis.

first, second, and third weekly administrations of the drug. Samples were collected into heparin-containing tubes at the following time points: before the start of irinotecan administration; 30 minutes after start of infusion; 5 minutes before the end of infusion; and 10, 20, and 30 minutes; 1, 3, 5, 7, 24, and 48 hours after the end of infusion. The blood samples were centrifuged at 3,000 × *g* for 10 minutes and the resulting plasma was stored at −80°C until analysis. After analysis for irinotecan and metabolite concentrations, samples were then analyzed for presence of the milk thistle constituent silybin.

Analytic procedure for irinotecan and metabolites. Total concentrations (i.e., the total of lactone and carboxylate) of irinotecan and its metabolites 7-ethyl-10-hydroxycamptothecin (SN-38), 7-ethyl-10-[3,4,5-trihydroxy-pyran-2-carboxylic acid]-camptothecin (SN-38 glucuronide, SN-38G), and 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin (APC) in plasma were determined by reversed-phase high-performance liquid chromatography with fluorescence detection using a modification of a procedure described previously (24). The method was validated according to the Food and Drug Administration guidance document for bioanalytic methods (http://www.fda.gov/cvm/guidance/published.htm) on 4 consecutive days using freshly prepared calibrators and quality control samples. Each of the runs included a calibration curve processed in duplicate and quality control samples at four different concentrations in processed in quintuplicate. The lower limit of quantitation was 1 ng/mL for irinotecan and SN-38, and 2 ng/mL for APC. SN-38G was measured indirectly by quantitation of the peak area at the retention time of SN-38G using the SN-38 calibration curve as previously described (24). A limited amount of SN-38G was available to confirm the retention time of this metabolite (Aventis Pharma, Vitro-sur-Seine Cedex, France). Over 4 days, within- and between-day precision were determined to be <10% for irinotecan and the metabolites.

Analytic procedure for milk thistle constituents. The phytochemical content of the milk thistle capsules from the lot administered to the patients as well as an additional lot from GNC (1357CE4563) was determined according to a method described by the Institute for Nutraceutical Advancement (http://www.nsfina.org/methods/milkthistle.html). Silybin (A and B mixture) was obtained from Sigma-Aldrich (St. Louis, MO), and silychristin and silydianin were obtained from Chromadex, Inc. (Santa Ana, CA). Calibrators and quality control samples were made by dissolving the analytes in 50% methanol in water. Calibration curves were constructed over the concentration range of 2 to 100 μg/mL, and duplicate quality control samples were made at low, medium, and high concentrations. A calibration curve and duplicate quality control samples were analyzed on 7 separate days.

For measurement of analytes in the milk thistle capsules, five capsules were analyzed on 3 consecutive days. Capsule weight and content in milligrams were recorded, 10 mg of the powder content was extracted with methanol, then sonicated for 30 minutes, shaken for 30 minutes, and then centrifuged for 10 minutes. The top organic layer (100 μL) was mixed 1:3 with deoinized water, mixed for 30 seconds, and transferred to a high-performance liquid chromatography vial. Analytes were extracted from the capsule content with methanol and analytes were quantitated in calibrators, quality control samples, and capsule content using an assay based on high-performance liquid chromatography with UV detection at 288 nm as previously described (25). On the chromatogram, two distinct peaks were observed for silybin A and silybin B; the area of both peaks were combined at each calibrator and quality control sample concentration. The milk thistle constituent iso-silibinin (A and B) was measured indirectly off of the calibration curve for silibin. Over 7 days, the within- and between-day accuracy and precision were <5% for all constituents. The percentage of the individual constituents in each capsule was calculated as $C \times FV \times D \times 100 / W$, where C is the constituent concentration (mg/mL), FV is the final volume (mL), D is the dilution factor, and W is the sample (powder) weight (mg).

Concentrations of silybin were quantitated in plasma using a newly developed and validated method based on high-performance liquid chromatography with tandem mass spectrometric detection. Silybin and the internal standard, temazepam, were extracted from calibrators, quality control samples, and unknown plasma samples using methyl *t*-butyl ether. Separation was achieved on a Waters X-Terra MS C_{18} column (50 × 2.1 mm internal diameter) packed with a 3.5 μm particle-size octadecyl stationary phase (Waters, Milford, MA), and protected by a stainless-steel guard column packed with 3 μm RP18 material (20 × 2.1 mm internal diameter; Waters). The mobile phase used for the chromatographic separation was composed of a mixture of acetonitrile-water (70:30, v/v) containing 0.1% formic acid and was delivered isocratically at a flow rate of 0.15 mL/min. Silybin eluted at 3.0 ± 0.3 minutes; under the method conditions, only one peak was observed representing the silybin diastereoisomers A and B. The internal standard eluted at 3.9 ± 0.3 minutes and the total run time was 30 minutes. Identification of drug was through a positive-ion mode and multiple reaction monitoring mode at *m/z*+ 483→162.8 for silybin and *m/z*+ 301.2→255 for the internal standard. The method was validated on 3 consecutive days using freshly prepared calibrators in duplicate and quality control samples at four different concentrations in

Table 2. Content of phytochemical marker compounds in milk thistle

Compound	Lot no. 4603KC3918	Lot no. 1357CE4563
Silybins A and B	8.08 ± 0.71	8.67 ± 0.52
Silychristin	3.53 ± 0.44	3.85 ± 0.32
Silydianin	0.82 ± 0.26	0.69 ± 0.06
Isosilybins A and B	1.79 ± 0.15	1.72 ± 0.10
Total (%)	14.2 ± 1.27	14.9 ± 0.99
Total (mg/capsule)	98.0 ± 10.1	102 ± 7.71

NOTE: Content is expressed as percentage per capsule and given as mean ± SD.

quintuplicate. The calibration curve covered the concentration range of 1 to 100 ng/mL, and the within- and between-day precision and accuracy were always <12%.

Pharmacokinetic analysis. Individual plasma concentrations of irinotecan and metabolites were analyzed using noncompartmental methods as implemented in the computer software program WinNonlin version 4.0 (Pharsight, Inc., Mountain View, CA). Calculated pharmacokinetic variables included peak concentration, area under the plasma concentration-time curve extrapolated to infinity (AUC), clearance (defined as the ratio of dose administered in milligrams and AUC), the rate constant of the terminal disposition phase (k), and the half-life of the terminal disposition phase (defined as ln 2 / k). Metabolic ratios were calculated from plasma AUC values and included the relative extent of conversion of irinotecan to its active metabolite SN-38 (i.e., $AUC_{SN\text{-}38}/AUC_{irinotecan}$), the relative extent of glucuronidation of SN-38 (i.e., $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$), and the relative extent of metabolism of irinotecan to APC (i.e., $AUC_{APC}/AUC_{irinotecan}$). Because inhibition or induction in one of the biotransformation pathways by coadministration of milk thistle will have its effect on both the concentrations of the metabolite and the compound being metabolized, application of metabolic ratios is very sensitive to detect effects of milk thistle on irinotecan pharmacokinetics.

Silybin concentrations were summarized as the minimum and maximum over the 48-hour sampling period after irinotecan dosing on days 8 and 15.

Statistical considerations. Based on the SD of the changes expected in irinotecan disposition (s_d), a power ($1 - \beta$) of 0.8 (80%), a clinically relevant change in the AUC of SN-38 of 30% (δ; standardized difference, $2\delta / s_d$), and a two-sided significance level (α) of 0.05 (5%), a patient sample size of six was required in a paired, two-sided analysis. All pharmacokinetic data are presented as mean values ± SD unless stated otherwise. Differences in pharmacokinetic variables as a function of milk thistle coadministration were evaluated with a repeated-measures ANOVA model using NCSS 2001 (J. Hintze, Number Cruncher Statistical Systems, Kayesville, UT). Two-tailed P values of <0.05 were considered to be statistically significant.

Results

General observations. A total of six patients were included in the study. Baseline characteristics of these patients are shown in Table 1. All patients completed the study within the scheduled time. The median absolute dose of irinotecan in each of the study periods was 225 mg (range, 190-250 mg). Severe and unexpected side effects upon concurrent use of milk thistle to the therapy with irinotecan were not observed (data not shown).

Phytochemical content of milk thistle extract. Before use, it was confirmed that the four principle constituents of silymarin—namely silybin A and B, silychristin, silydianin, and isosilybins A and B—were present in the milk thistle extracts (Table 2). The product content for these constituents was relatively consistent between different capsules (precision and accuracy, <10%) and between the two lots tested, and was also very similar to that reported previously for other commercial milk thistle preparations (26).

Effect of milk thistle on irinotecan pharmacokinetics. Short-term (i.e., 4 days) or more prolonged intake of milk thistle (i.e., 12 days) had no pronounced effect on the pharmacokinetics of irinotecan and its metabolites when evaluating mean values (Table 3) or when evaluating individual values (Fig. 2). In the absence of milk thistle, the mean clearance of irinotecan was 31.2 L/h, whereas on day 8 it was 25.4 L/h, and on day 15 it was 25.6 L/h ($P = 0.16$). Milk thistle also did not influence the

Table 3. Summary of irinotecan pharmacokinetic parameter estimates

Parameter	Day 1	Day 8	Day 15	P
Irinotecan				
C_{max} (μg/mL)	1.82 ± 0.852	1.96 ± 0.593	1.88 ± 0.647	0.70
AUC (μg·h/mL)	9.15 ± 3.93	10.6 ± 3.84	10.4 ± 3.73	0.46
CL (L/h)	31.2 ± 19.6	25.4 ± 14.6	25.6 ± 13.6	0.16
V_{ss} (L)	264 ± 217	188 ± 129	198 ± 102	0.18
$T_{1/2,z}$ (h)	11.4 ± 1.32	9.58 ± 2.50	11.1 ±1.29	0.25
SN-38				
C_{max} (ng/mL)	37.1 ±31.5	31.0 ± 16.6	29.6 ± 17.4	0.45
AUC (ng·h/mL)	239 ± 154	233 ± 151	226 ± 136	0.071
REC (%)	2.58 ± 1.26	2.23 ± 1.18	2.17 ± 1.08	0.047
SN-38G				
C_{max} (μg/mL)	0.234 ± 0.204	0.222 ± 0.091	0.240 ± 0.104	0.93
AUC (μg·h/mL)	2.47 ± 1.76	2.44 ± 1.09	2.18 ± 0.854	0.75
REG	10.8 ± 3.21	13.5 ± 7.03	13.1 ±9.08	0.64
APC				
C_{max} (μg/mL)	0.294 ± 0.233	0.302 ± 0.169	0.381 ±0.241	0.20
AUC (μg·h/mL)	3.01 ±2.07	3.11 ±2.10	3.46 ± 2.18	0.67
REM	0.332 ± 0.215	0.285 ± 0.140	0.337 ± 0.177	0.53

NOTE: Pharmacokinetic data are given as mean ± SD and were obtained on day 1 (irinotecan alone), day 8 (4 days after start of milk thistle), and day 15 (12 days after start of milk thistle). P values were obtained from a repeated-measures ANOVA model.

Abbreviations: AUC, area under the concentration-time curve; C_{max}, peak plasma concentration; CL, clearance; V_{ss}, volume of distribution at steady state; $T_{1/2z}$, half-life of the terminal disposition phase; REC, AUC ratio of SN-38 to irinotecan (relative extent of conversion); REG, AUC ratio of SN-38G to SN-38 (relative extent of glucuronidation); REM, AUC ratio of APC to irinotecan (relative extent of metabolism).

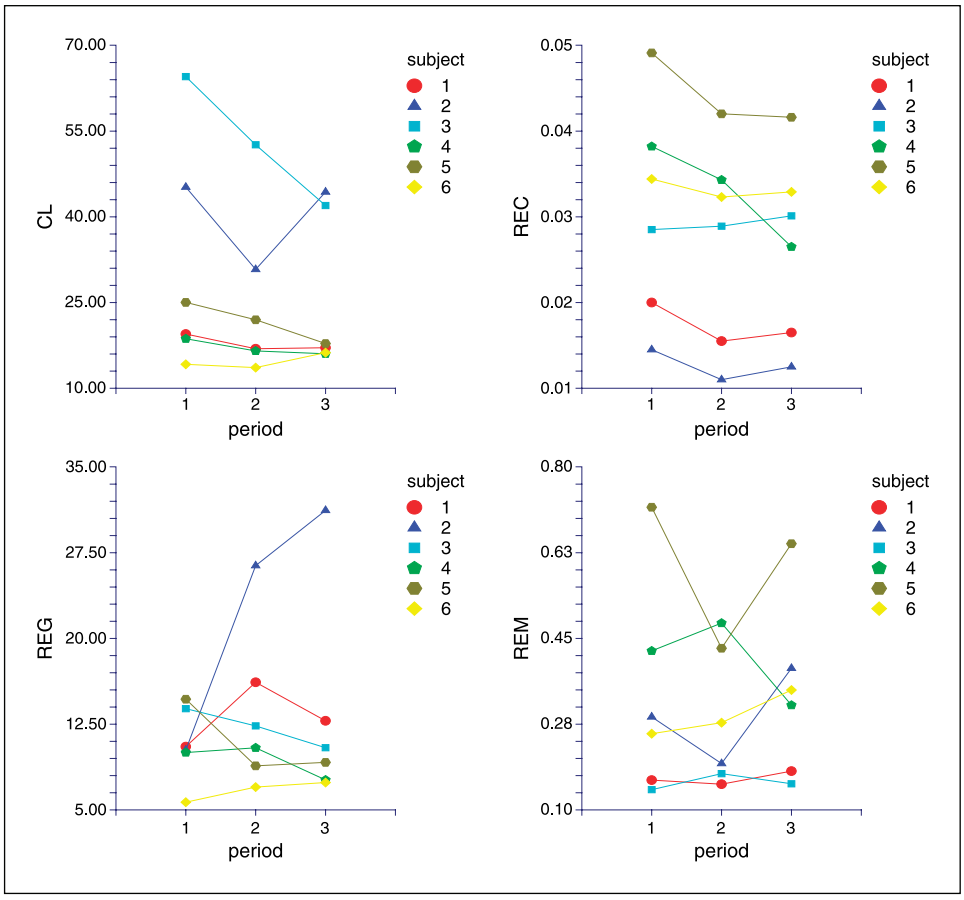

Fig. 2. Comparison of individual irinotecan pharmacokinetic variables obtained before milk thistle administration (*period 1*), and 4 days (*period 2*) and 12 days (*period 3*) after start of milk thistle intake. CL, irinotecan clearance; REC, relative extent of conversion of irinotecan to its active metabolite SN-38 (i.e., $AUC_{SN\text{-}38}/AUC_{irinotecan}$); REG, relative extent of glucuronidation of SN-38 (i.e., $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$); and REM, relative extent of metabolism of irinotecan to APC (i.e., $AUC_{APC}/AUC_{irinotecan}$).

CYP3A4-mediated metabolic pathway in which APC is formed; the AUC ratio of APC to irinotecan was not significantly different on days 1, 8, and 15 (relative extent of metabolism values, 0.332 versus 0.285 versus 0.337, respectively; $P = 0.53$). The UGT1A-mediated metabolism of SN-38, expressed in the AUC ratio of SN-38G to SN-38, was also similar on the three study periods [10.8 (day 1) versus 13.5 (day 8) versus 13.1 (day 15); $P = 0.64$]. The only effect observed after the administration of milk thistle was a slightly changed AUC ratio of SN-38 to irinotecan, which progressively decreased from 2.58% (day 1) to 2.23% (day 8) and 2.17% (day 15); $P = 0.047$.

Exposure to silybin after milk thistle administration. Because silybin concentrations could be detected in the plasma of all patients (Table 4), adherence to the intake of milk thistle was shown in all study participants. The maximum concentration of silybin in plasma at steady state were highly variable ($\sim$10-fold) and ranged between 0.0249 and 0.257 μmol/L, which is consistent with previously reported estimates following oral administration of milk thistle (27, 28). Silybin steady-state concentrations on days 8 and 15 were not statistically significantly associated with AUC ratio of SN-38 to irinotecan ($R^2 < 0.092$, $P > 0.56$).

Discussion

This study shows that the concurrent use of the herbal supplement milk thistle does not significantly influence the pharmacokinetics of irinotecan in patients with cancer. Irinotecan was selected as a model drug in this investigation because, as the enzymes and transporters involved in the metabolism and excretion of irinotecan (Fig. 1) are important for a large variety of other widely used anticancer drugs, interactions with such agents have the potential to occur frequently.

A recently published *in vitro* study showed that milk thistle inactivates the CYP3A4 and CYP2C9 isoforms (16–19) and also inhibits β-glucuronidase (29) as well as the UGT1A1, UGT1A6, UGT1A9, UGT2B7, and UGT2B15 isoforms (19, 20). Similarly, it has been reported that milk thistle inhibits ABCB1 (P-glycoprotein; ref. 21) and ABCC1 (MRP1; ref. 22) in some *in vitro* model systems. However, *in vivo* evidence for enzyme- or transporter-mediated interactions caused by milk thistle constituents is less compelling. The administration of milk thistle for 12 days (30), 21 days (31), or 28 days (32) did not result in clinically significant changes in the pharmacokinetics of the CYP3A4 and ABCB1 substrate drug indinavir in three independent studies done in healthy volunteers. Prior studies have also indicated that prolonged administration of milk thistle had no effect on the pharmacokinetics of the nonspecific CYP probes aminopyrine and phenylbutazone (33), or on the CYP3A4 substrate midazolam (26). Because irinotecan is highly susceptible to CYP3A4 and ABCB1 inhibition (34) and induction (10) in humans, the results of the current study confirm that milk thistle does not seem to modulate the activity and/or expression of these proteins. This is consistent with the notion that the milk thistle constituent silybin is not a ligand activator of the pregnane X receptor (PXR, SXR, or NR1I2; ref. 35), a steroid- and

Table 4. Observed steady-state concentrations of silybin in plasma (ng/mL) following milk thistle administration

Patient no.	Day 8		Day 15	
	$C_{ss,\ min}$	$C_{ss,\ max}$	$C_{ss,\ min}$	$C_{ss,\ max}$
1	1.19	101	2.34	27.6
2	2.12	23.6	3.13	23.9
3	2.50	24.2	2.73	14.2
4	16.1	124	9.11	89.6
5	1.11	12.3	1.82	31.8
6	4.12	48.2	3.75	36.2
Mean	4.53	55.5	3.81	37.2
SD	5.79	46.2	2.68	26.7

Abbreviations: $C_{ss,\ min}$, minimum plasma concentration; $C_{ss,\ max}$, maximum steady-state concentration.

xenobiotic-regulated transcription factor that is a key regulator of constitutive expression and induction of CYP3A4, UGT1A, and ABCB1. Our results also provide additional, previously unavailable, support for an apparent lack of effect of milk thistle on the function of human carboxylesterase isoform 2–mediated hydrolysis and UGT1A-mediated phase II conjugation pathways in humans.

The underlying reasons for the inconsistency between the various *in vitro* and *in vivo* observations related to the effect of milk thistle on enzyme and transporter function are currently unknown. A previous study indicated that currently available milk thistle dosage forms show rapid disintegration (26), suggesting that poor dissolution characteristics can be excluded as a contributing mechanism. It is possible that the lack of effects *in vivo* is related to poor absorption in conjunction with large interindividual variability of the various milk thistle constituents. Indeed, the oral absorption of silybin has previously been reported to be low due to the poor solubility (27, 28). In our patients, the highest concentration of silybin measured at steady state in plasma was ~0.26 μmol/L. Prior *in vitro* studies showed that silybin concentrations of >1.4 μmol/L are required for inhibition of UTG1A1, whereas only concentrations of >32 μmol/L are associated with inactivation of CYP3A4 function (19). Therefore, the plasma concentrations of silybin at the manufacturer-recommended doses of milk thistle as applied in our study may be too low to affect the various disposition pathways of irinotecan.

The present study was not randomized for treatment cycles, which might be regarded as a limitation of the study design. In addition, a separate control group receiving a placebo could have provided additional information of potential changes in the elimination pathways after repeat exposure to irinotecan alone. However, because irinotecan was given as a single 90-minute infusion with an interval of 1 week and patients were only exposed to a total of three irinotecan administrations, autoinduction or autoinhibition of enzymes and transporters are not likely to occur (36).

None of the patients in this study receiving the combination of irinotecan and milk thistle showed an aberrant irinotecan metabolic disposition profile compared with other patients receiving irinotecan alone. Therefore, it is not expected that genetic heterogeneity in drug metabolism or excretion is confounding the results of the present study. However, because UGT1A1 activity is at least in part determined by genetic factors and is also known to affect the relative extent of SN-38 glucuronidation (37), a differential effect of the milk thistle-irinotecan interaction depending on UGT1A1 genotype status cannot be entirely excluded.

In conclusion, the present findings show that milk thistle does not substantially affect the disposition of irinotecan. The milk thistle constituent silymarin has the potential to affect the function of many enzymes and transporters but the systemic concentrations after intake of the recommended doses of milk thistle are presumably too low to significantly exert this effect. Collectively, this study indicates that milk thistle poses little risk of interfering with the pharmacokinetic profile of chemotherapeutic agents that are substrates for CYP3A4 and UGT1A1.

Acknowledgments

We thank Carol Hartke, Alex Mnatsakayan, and Yelena Zabelina (Baltimore, MD) for expert analytic assistance and Jan Ouwerkerk and the oncology nursing staff (Leiden, the Netherlands) for collecting samples and for patient care.

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