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Research Paper

St. John's Wort Modulates the Toxicities and Pharmacokinetics of CPT-11 (Irinotecan) in Rats

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Purpose. CPT-11 is a DNA topoisomerase I inhibitor for the therapy of colorectal cancer, whereas St. John's Wort (*Hypericum perforatum*, SJW) is a widely used herbal anti-depressant. This study aimed to investigate the effects of co-administered SJW on the toxicities and pharmacokinetics of CPT-11 and the underlying mechanisms.
Methods. The body weight loss, gastrointestinal and hematological toxicities induced by CPT-11, and the pharmacokinetic parameters of CPT-11 were evaluated in rats pretreated with SJW or vehicle.
Results. Rats treated with CPT-11 alone experienced rapid decrease in body weight, whereas co-administration of SJW with CPT-11 resulted in lesser body weight loss. The gastrointestinal and hematological toxicities following CPT-11 injection were both alleviated in the presence of SJW. The rat pharmacokinetics of both CPT-11 and its metabolite SN-38 were significantly altered in presence of SJW.
Conclusions. In conclusion, co-administered SJW significantly ameliorated the toxicities induced by CPT-11. The protective effect of SJW may be partially due to pharmacokinetic interaction between CPT-11 and SJW.

KEY WORDS: CPT-11; pharmacokinetics; St. John's wort; toxicity.

INTRODUCTION

Irinotecan (CPT-11, Camptosar, 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin) is a semisynthetic and water soluble derivative of camptothecin (CPT). As a potent DNA topoisomerase (Topo) I inhibitor (1,2), CPT-11 induces tumor cell death due to the stabilization of Topo I complex and the generation of permanent DNA strand breaks (3). This agent has shown a wide spectrum of antitumor activity (4–6). It has been worldwide approved for the treatment of colorectal cancers as a first-line therapy in combination with 5-fluorouracil. CPT-11 also presented considerable clinical responses for many other malignancies, including lung, gastric, pancreatic, cervical, and ovarian cancer, leukemia and lymphoma (7–11). The metabolic pathways of CPT-11 in rats and humans are complicated but similar, despite the presence of some species differences in the contribution of individual pathways and enzymes involved. As shown in Fig. 1, in both rats and humans CPT-11 as a prodrug is rapidly hydrolyzed by carboxylesterases to its active metabolite, 7-ethyl-10-hydroxy-camptothecin (SN-38) (12–17). The resultant metabolite, SN-38, is approximately 100–1,000-fold more cytotoxic than the parent molecule (18,19). It is subsequently conjugated to form SN-38 glucuronide (SN-38G) by uridine diphosphate glucuronosyltransferases (UGT1A1/1A9) (19–22). Notably, SN-38G can be converted to SN-38 by intestinal β-glucuronidase and reabsorbed into the plasma. In both rats and humans, a second metabolism pathway of CPT-11 is cytochrome P450 (CYP) 3A-mediated bipiperidine side chain oxidation, resulting in 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino)] carbonyloxy-camptothecin and 7-ethyl-10-[4-amino-1-piperidino carbonyloxy-camptothecin (20,23–27). However, there are some species differences in the contribution of individual pathways, enzymes and transporters involved. Thus, caution should be taken when extrapolating the results from rats to humans.

The major dose-limiting toxicities of CPT-11 are myelosuppression and gastrointestinal toxicity, in particular unpredictable severe diarrhea (28,29). The latter may have

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ABBREVIATIONS: CPT-11, rinotecan; DMSO, dimethyl sulfoxide; LOQ, limit of quantification; SJW, St. John's wort; SN-38G, SN-38 glucuronide; Topo, topoisomerase.

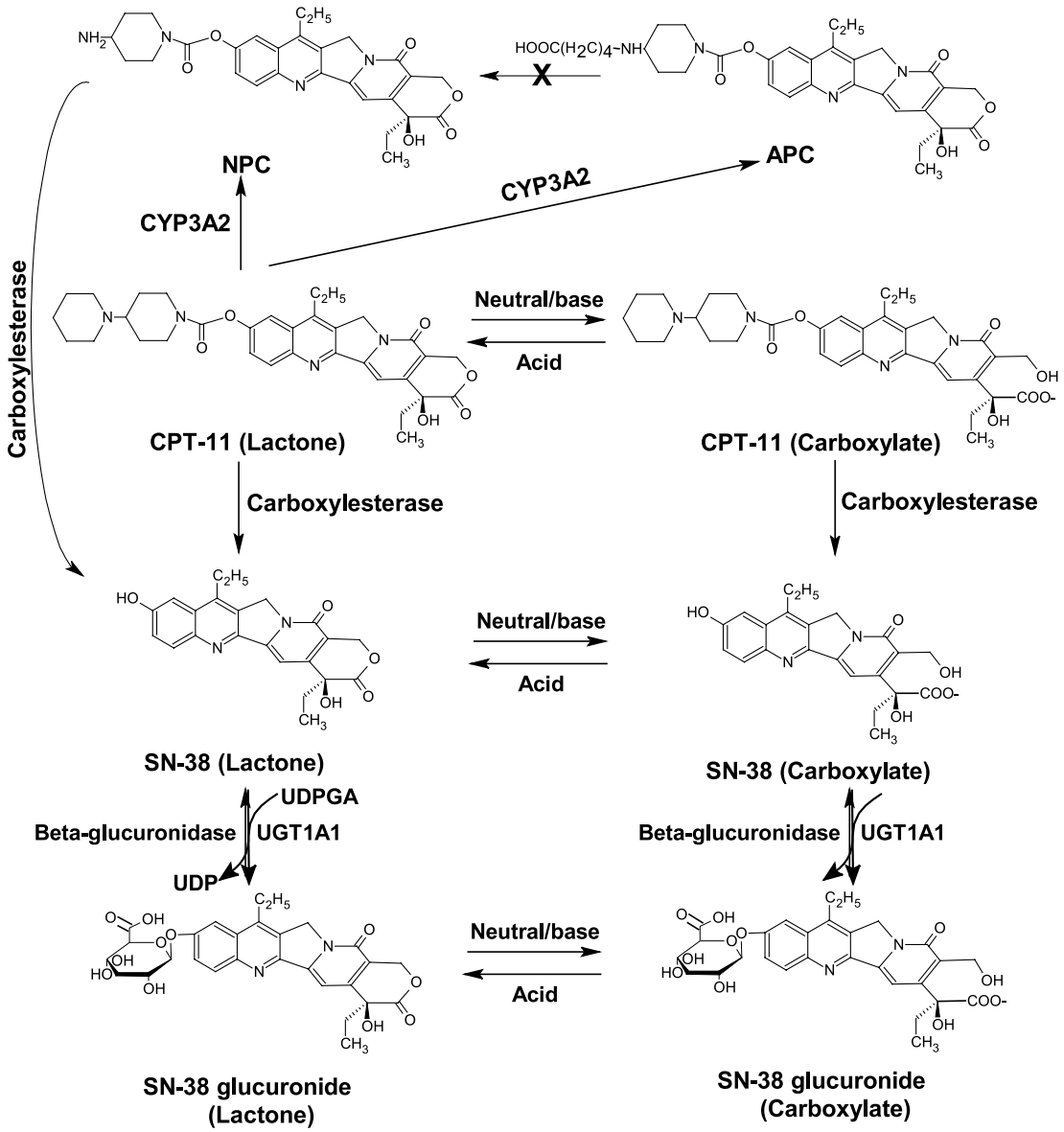

Fig. 1. The metabolic scheme of CPT-11 in rats.

early or late onset, that is, occurring <24 h or ≥24 h after administration, respectively. Early-onset diarrhea is observed immediately after CPT-11 administration and probably due to acetylcholinesterase inhibition, as it can be abolished by the use of atropine (30). By contrast, severe late-onset diarrhea at 3 (severe) or 4 (life-threatening) grade occurs in up to 40% patients treated with CPT-11 after an average period of 6 days (31). The biochemical mechanisms for CPT-11-induced late-onset diarrhea are not fully identified, but it appears to be associated with intestinal exposure to SN-38. As the major active metabolite of CPT-11, SN-38 may bind to intestinal epithelial Topo I and induce cellular apoptosis (32,33). CPT-11 and SN-38 also induced the secretion of Na^+ and Cl^- (34) and stimulated the production of pro-inflammatory cytokines and prostaglandins (35,36). Early treatment of severe late-onset diarrhea with high-dose loperamide (a synthetic opiate derivative) resulted in a decreased diarrhea and patient morbidity (37). Extensive studies have been carried out to identify other potential compounds to ameliorate late-onset diarrhea. These included alkali (e.g., sodium bicarbonate) (38), oral antibiotics (e.g., neomycin) (39–41), enzyme inducers (e.g., phenobarbital) (42), P-glycoprotein (PgP) inhibitors (e.g., cyclosporine) (43,44), cyclooxygenase 2 inhibitors (e.g., celecoxib) (45), herbal components (e.g. baicalin) and blockers of biliary SN-38 (e.g., probenecid and valproic acid) (46,47). Many of these agents have been shown to decrease CPT-11-induced diarrhea in preclinical studies.

St. John's wort (*Hypericum perforatum*, SJW) is one of the most commonly used herbal medicines for the treatment of mild to moderate depression (48–50). SJW contains over two dozen constituents, among which hyperforin and hypericin are the major active components. Extensive preclinical and clinical studies have reported on the inducing effect of SJW (mainly via hyperforin) on CYP2B6/CYP3A4 and PgP (51–61). More importantly, a number of pharmacokinetic

and/or pharmacodynamic interactions of SJW with other clinically important drugs (e.g., cyclosporine, amitriptyline, digoxin, and methadone) have been reported (62–64). Furthermore, *Hypericin* is reported (64) to be the major component for the inhibition of CYP2C9, 2D6 and 3A4. In a recent unblinded, randomized crossover study in 5 cancer patients, it was found that treatment of SJW (900 mg/day, oral) for 18 days decreased the plasma levels of the active metabolite SN-38 by 42%, which was accompanied by a decreased diarrhea and myelosuppression (65). Surprisingly, it appeared that the antitumor activity of CPT-11 was potentiated. The mechanisms by which coadministered SJW resulted in attenuated CPT-11 toxicity and enhanced anticancer effect are unknown, but both pharmacokinetic and pharmacodynamic components have been implicated. As such, this study aimed to establish a suitable animal model for the combination of SJW with CPT-11 using rats by examining whether SJW modulated the toxicity and pharmacokinetics of CPT-11.

MATERIALS AND METHODS

Chemicals

CPT-11 lactone form (diethyl-4,11 hydroxy-4 (piperidino-4 piperidino-carbonyloxy)-9 1H-pyrano (3′,4′,6,7) indolizino (1,2-b) quinolein-(4H,12H) dione-3,14 hydrochloride trihydrate, MW = 677.20) and SN-38 (7-ethyl-10-hydroxy-camptothecin, MW = 392) were supplied by Sinochem Ningbo Import and Export Co. (Ningbo, China). Both compounds have a purity of 99.8% as determined by high performance liquid chromatography (HPLC). An injectable formulation of CPT-11 was prepared by dissolving CPT-11 (20 mg/ml), D-sorbitol (45 mg/ml) and D-lactic acid (0.9 mg/ml) in Milli-Q water heated to 70–90°C for 5–10 min. The pH of this clear solution was adjusted to 3.5 by 1 M NaOH. The resulting solution was then sterile-filtered (0.22 μm, Millipore, MA, USA) and stored at 4°C under dark condition (45). The SJW sugar-coated tablets [LI-160, 300 mg St. John's wort dry extract, DER 3-6:1, solvent methanol 80% (v/v)) were purchased from local pharmacy of Singapore, which were manufactured by Lichtwer Pharma GmbH (Berlin, Germany), and the SJW-free control vehicle (also formulated as sugar-coated tablets) was kindly supplied by Lichtwer Pharma GmbH (Berlin, Germany). The contents of hypericin and hyperforin in the SJW tablets have been standardized to 0.3% and 5%, respectively, by manufacturer. Analysis using HPLC methods at our laboratory found similar contents of both compounds in the preparations. Prior to use, both tablets were peeled off the sugar-coat, grinded to powder by mortar and pestle and stored in the dryer protected from light. The administration form of SJW or vehicle was prepared by suspending SJW or vehicle powder in physiological saline for oral gavage administration. CPT (camptothecin), DMSO (dimethyl sulfoxide), D-sorbitol, D-lactic acid, the ion-pairing reagent sodium 1-heptane-sulfonate, lyophilized type IX-A β-glucuronidase (from *Escherichia coli*, activity 1,724,400 units per gram solid), hypericin and hyperforin were all purchased from Sigma Chemical Co. (St. Louis, MO, USA). The water used was of Milli-Q grade (Millipore, Bedford, MA, USA) and all other chemicals were of analytical grade or HPLC grade obtained from commercial sources.

Animals

Healthy male Sprague–Dawley rats (200–220 g) were purchased from the Laboratory Animals Centre, National University of Singapore. Rats housed in cages were kept in a room under controlled temperature (23–24°C) and 12-h day-night cycle. Animals were used for toxicity and pharmacokinetic studies after one-week acclimatization with free access to tap water and regular diet *ad libitum*. All animal procedures were approved by the Animal Ethical Committee of the National University of Singapore.

Toxicity Study

Studies were conducted to investigate whether SJW modulated the toxicities induced by CPT-11 in rats. The CPT-11 induced diarrhea model in male Sprague–Dawley rats (n = 6 per group) was constructed as previously described (66). Rats were treated with CPT-11 at a dose of 60 mg/kg body weight/day by i.v. injection (3 ml/kg body weight/day) via tail vein for 4 consecutive days (days 1–4), or in combination with SJW (400 mg/kg body weight by oral gavage for 8 consecutive days, 6 ml/kg) starting one day before the first CPT-11 injection. The rats in control groups (CPT-11 only) received SJW-free vehicle (400 mg/kg body weight by oral gavage for 8 consecutive days, 6 ml/kg).

Additional rats (n = 6) were included to receive CPT-11 for 4 consecutive days with or without SJW (400 mg/kg body weight by p.o. for 5–8 consecutive days, 6 ml/kg) combination and sacrificed by day 5, 7, 9, or 11 to monitor gastrointestinal damages. Physiological saline was given (3 ml/kg body weight per day) by i.v. injection through the tail vein for 4 consecutive days for the blank groups (n = 6).

The effects of co-administered SJW on the body weight and severity of CPT-11-induced diarrhea in the rats were monitored twice a day throughout the study (total 12 days from the first administration of SJW). Diarrhea observed after the final injection of CPT-11 (beginning from day 5) in rats was defined as late-onset diarrhea. The severity of diarrhea was assigned a score according to the following rating scale as described previously (45,67):

- 0: normal, normal stool or absent;
- 1: slight, slightly wet and soft stool;
- 2: moderate, wet and unformed stool with moderate perianal staining of the coat;
- 3: severe, watery stool with severe perianal staining of the coat.

Rat blood samples (about 300 μl) were collected into the tubes with K_2-EDTA (Microtainer, Becton Dickison and Company, Franklin Lakes, NJ, USA) prior to drug administration and 5, 7, 9, 11 days after first CPT-11 dosage. The numbers of erythrocyte, lymphocyte, neutrophil, and platelet were counted using a Sysmex XE-2100 Automated Hematology Analyzer (Sysmex, Mundelein, IL, USA). In some cases, Wright's-stained blood smears were prepared for verifying the counting.

The effects of co-administered SJW on CPT-11-induced intestinal epithelial injuries in rats were evaluated by examining the histological changes at macroscopic level. The experimental rats were sacrificed on days 5, 7, 9, or 11 after

the first CPT-11 injection, and the intestinal tissues (ileum, cecum and colon) collected and examined. The scoring for macroscopic and microscopic evaluation of intestinal damage were based on changes in epithelial tissues as previously described (66,68–70). Normal tissues from healthy rats were also examined for the purpose of comparison.

Pharmacokinetic Studies

The pharmacokinetic interaction studies included experiments with short-term (3 days) or long-term (14 days) administration of SJW to rats. Rats were randomized to 4 groups (n = 6 per group) receiving SJW at 400 mg kg^{-1} day^{-1} (6 ml/mg) or SJW-free control vehicle for 3 or 14 consecutive days by oral gavage before CPT-11 injection. On day 4 or 15, CPT-11 (60 mg/kg, i.v.) was administered via tail vein. Blood samples (~200 μl) were collected into heparinized tubes from tail vein 0.25, 0.5, 1, 2, 4, 6, 8 and 10 h after CPT-11 injection. Plasma was obtained by immediate centrifugation at 2500 × g for 10 min at 4°C, and stored at −80°C until analysis. After deproteinization, plasma drug concentrations were analysed by HPLC.

The rat plasma was divided into 2 aliquots (50 μL each): one for CPT-11 and SN-38 analysis and the other for SN-38G determination using β-glucuronidase. For CPT-11 and SN-38 concentration determination, each sample was allowed to thaw at room temperature, and 50 μl of a solution of CPT (I.S.) (4 μg/ml) and 100 μl of a mixture of acetonitrile-1 mM orthophosphoric acid (90:10, v/v) was added to 50 μl of plasma. The tube was vortex-mixed for 10 s, and centrifuged at 6000 × g for 10 min. An aliquot of 150 μl of the resultant supernatant was added to 175 μl of 50 mM disodium hydrogen phosphate (pH 3.0) buffer. After vortex-mixing, 100 μl of this mixture was injected into the HPLC system.

For SN-38G analysis, the β-glucuronidase was then dissolved in the 0.1 M sodium phosphate buffer (pH 6.4) to obtain a concentration of 20,000 units/ml. An aliquot of rat plasma sample (50 μl) was incubated in water bath with 50 μl of the solution of β-glucuronidase (1000 units) for 2 h at 37°C. Then the samples were processed by the same procedures as for CPT-11 and SN-38, except that the volumes of all the added solutions were doubled.

High-Performance Liquid Chromatography Method

The rat plasma concentrations of CPT-11, SN-38 and SN-38G were quantitated by validated HPLC methods. The chromatographic system consisted of a Shimadzu SCL-$10A_{VP}$ system controller, a LC-$10AT_{VP}$ pump, a DGU-14A degasser, a RF-10A XL fluorescence detector and a SIL-$10AD_{VP}$ autoinjector. Data were monitored and analyzed using CLASS VP software. A stainless steel (200 mm × 4.6 mm i.d.) analytical column packed with 5 μm Hyperclon ODS (C18) material (Phenomenex, Torrance, CA, USA) preceded by a Phenomenex C18 guard cartridge was used for separation of compounds. The mobile phase was composed of acetonitrile-50 mM disodium hydrogen phosphate buffer containing 10 mM sodium 1-heptane-sulfonate, with the pH adjusted to 3.0 with 85% (w/v) orthophosphoric acid (27/73, v/v). Prior to use, the mobile phase was filtered through a 0.45 μm NYLON Membrane filter (Whatman, Maidstone, UK). The mobile phase was delivered at a flow-rate of 1.0 ml/min, and the column effluent was monitored at 540 nm (with an excitation wavelength of 380 nm).

Standards were prepared from normal rat blank plasma spiked with different amounts of CPT-11 and SN-38, both in acetonitrile-1 mM orthophosphoric acid (90:10, v/v), together with the corresponding I.S. solution: 100 μl of each solution (CPT-11, SN-38 and CPT) were added to 100 μl of plasma. The standards were then analyzed as rat samples collected from kinetic studies. The calibration curves were constructed by plotting the peak area ratio of the analyte to I.S. (Y values) *vs.* the concentrations spiked (X values). The linearity of the assay procedure was assessed by means of visual inspection of scatter plots of Y *vs.* X and of residuals *vs.* fitted Y values. The slope and intercept of the best-fit linear regression line were determined using the method of least squares analysis. Concentrations in unknown samples were calculated from the resulting peak area ratios and the regression equation of the calibration curve. The limit of quantification (LOQ) was defined as the lowest drug concentration that could be determined with a coefficient of variation (CV) ≤ 20% and a recovery of 100 ± 20% on a day-to-day basis. All validation runs were performed on three consecutive days and all samples used for validation were prepared as standard samples. The recovery was determined by comparing the peak areas of plasma samples with those replaced by an equal volume of phosphate-buffered saline at pH 7.4 after the same sample handling. Within-day and between-day precision and the mean accuracy were determined by repeated analysis at different concentrations on a single day and on 3 consecutive days respectively.

Pharmacokinetic Calculation

Plasma concentration *vs.* time profiles were obtained by plotting the mean concentrations of each analyte at each time point *vs.* time on a semi-logarithmic scale for each rat studied. Pharmacokinetics parameters were calculated by non-compartmental model using WinNonlin program version 1.0 (Scientific Consulting Inc., Cary, NC, USA). The total areas under plasma concentration-time curve from time zero to the last quantifiable time point (AUC_{0-t}) and from time zero to infinity ($AUC_{0-\infty}$) (ng·h/ml) were estimated using the log-linear trapezoidal rule, while the apparent volume of distribution (V_d) and plasma clearance (CL) were determined using the standard formulae. The elimination half-life ($t_{1/2,z}$) was calculated as 0.693/λz where λz is the elimination rate constant calculated from the terminal linear portion of the log plasma concentration-time curve.

Statistical Analysis

Data are expressed as mean ± SD. Diarrhea scores were analyzed using Wilcoxons rank sum test. Differences between groups for continuous variables on more than one occasion were evaluated with repeated measures analysis of variance (ANOVA). Statistical comparison for macroscopic intestinal damage was performed using a one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test. Differences between two groups were analysed using unpaired Student's *t* test. Statistical significance was set as $p < 0.05$.

RESULTS

Validation of HPLC Method for Determination of CPT-11, SN-38, and SN-38G in Rat Plasma

Under the chromatographic conditions used for the analysis of the analytes, the retention times for SN-38, CPT and CPT-11 were 6.15, 7.80 and 10.04 min (n = 100) respectively. The peaks for all analytes were slightly skewed to the right. We evaluated peak skew using the asymmetry coefficient = d_a/d_b, where d_a is the distance after the peak maximum and d_b is the distance before the peak maximum, both d_a and d_b being measured at 10% of the total peak height. The asymmetry coefficients were between 1.05–1.40 for SN-38, CPT-11 and CPT. These methods employed a simple protein precipitation step, with a recovery of 90–110% at concentrations of 5–25,000 ng/ml (0.0074–36.88 μM) for CPT-11 and 2–1,600 ng/ml (0.0051–4.08 μM) for SN-38. The recovery of the I.S. determined at the concentration used was 97.2 ± 5.1% (n = 6). Matrix-specific interfering peaks that required modification of the mobile phase composition were not observed in any cases, including in the presence of drugs such as St. John's wort.

Both the compounds gave linear response as a function of concentration over 5–25,000 ng/ml (0.0074–36.88 μM) for CPT-11 and 2–1600 ng/mL (0.0051–4.08 μM) for SN-38. The mean coefficients of determination (r^2 values) for the daily calibration curves were all >0.999 (n = 6) and the within- and between-run coefficients of variation (CVs) of the response factors for each concentration assayed were below 10%. The mean y intercepts were 0.007–0.05 (n = 6) for both analytes. For each data point on the calibration curves for two analytes, the concentrations back-calculated from the equation of the regression analysis were within acceptable limits for accuracy and precision of 20%. A linear regression of the back-calculated concentrations *vs.* the nominal values provided a unit slope and an intercept not statistically significantly different from zero. The distribution of the residuals showed random variation, was normally distributed and centered on zero. The bias was not statistically different from zero, and the 95% confidence intervals included zero (data not shown).

The LOQ in rat plasma (100-μl aliquot) was 2 ng/ml for CPT-11 and 1 ng/ml for SN-38 respectively. The differences between theoretical and actual concentration and the CVs were less than 15% at any quality control sample concen-

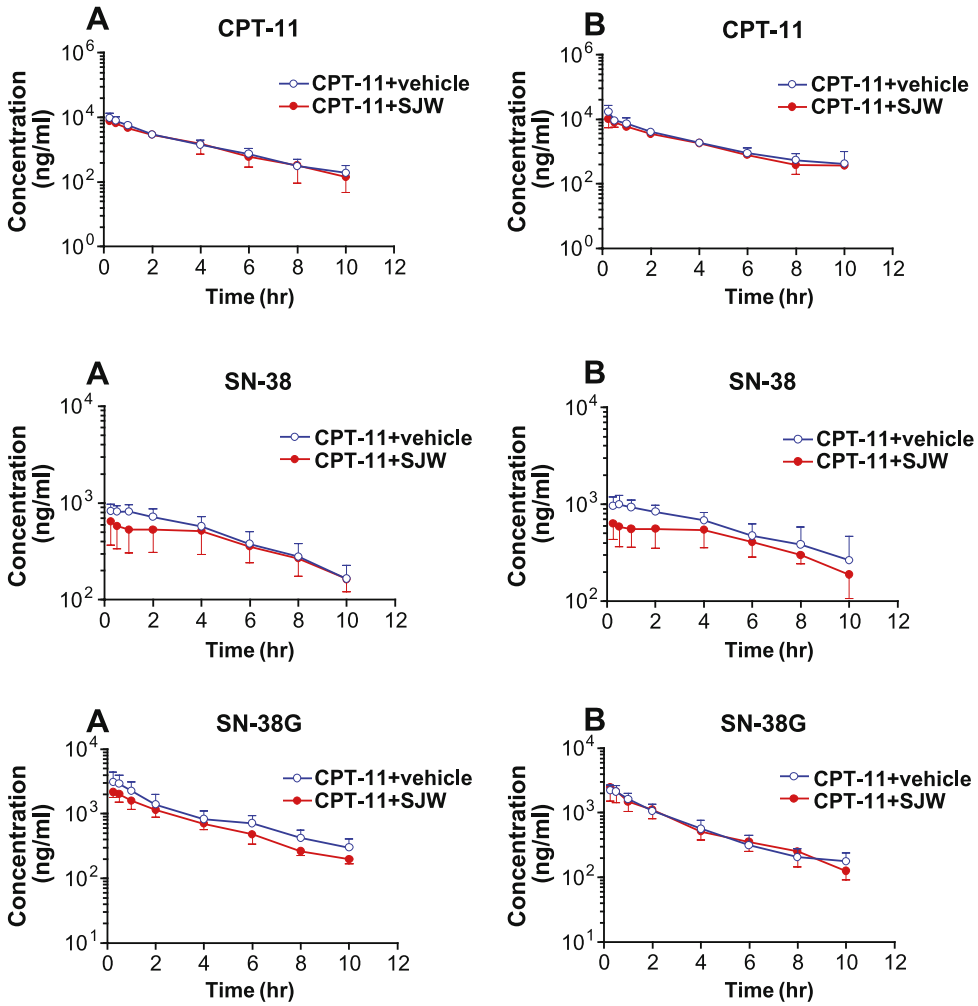

Fig. 2. Plasma concentration-time profiles of CPT-11 and SN-38 in rats receiving CPT-11 alone and in combination with St. John's wort. (A) Short-term (3-days) kinetics interaction study; (B) Long-term (14-days) kinetics interaction study.

Table I. Comparison of Pharmacokinetic Parameters Between Two Groups of Rats Receiving CPT-11 Alone or Pretreated with St. John's Wort for 3 Days

Parameters	Treatment groups		Change (%)	p value[a]
	CPT-11 + SJW	CPT-11 + vehicle		
CPT-11				
C_0 (ng/ml)[b]	8966.8 ± 1967.2	12140.0 ± 6695.3	−26.1	0.212
$t_{1/2,z}$ (h)	1.68 ± 0.34	1.85 ± 0.32	−9.2	0.229
$AUC_{0-10\ hr}$ (ng · h/ml)	18523.3 ± 3265.2	20959.8 ± 3429.0	−11.6	0.152
$AUC_{0-\infty}$ (ng · h/ml)	18903.4 ± 3497.0	21472.7 ± 3532.1	−12.0	0.148
V_d (ml/kg)	7799.7 ± 1382.0	7533.6 ± 1264.6	3.5	0.381
CL (ml/h/kg)	3263.0 ± 579.2	2856.8 ± 510.9	14.2	0.141
SN-38				
C_0 (ng/ml)	665.9 ± 277.3	846.9 ± 136.1	−21.4	0.116
$AUC_{0-10\ hr}$ (ng · h/ml)	4158.7 ± 1544.5	4824.6 ± 1079.6	−13.8	0.222
$t_{1/2,z}$ (h)	3.70 ± 0.73	3.63 ± 0.72	1.9	0.442
$AUC_{0-\infty}$ (ng · h/ml)	5073.9 ± 1600.5	5759.6 ± 1360.9	−11.9	0.235
SN-38G				
C_0 (ng/ml)	2220.4 ± 463.3	3124.5 ± 1236.3	−28.9	0.072
$AUC_{0-10\ hr}$ (ng · h/ml)	7332.9 ± 1056.5	9943.7 ± 2936.8	−26.3	**0.043**
$t_{1/2,z}$ (h)	2.88 ± 0.93	3.88 ± 0.71	−25.8	**0.033**
$AUC_{0-\infty}$ (ng · h/ml)	8074.6 ± 1063.7	11733.8 ± 3754.7	−31.2	**0.031**

[a] Compared with the controls using Students' unpaired t test.
[b] Obtained by back-extrapolation to the zero time using WinNonlin program.

trations. Dilution of CPT-11 and SN-38 at 1:10 gave acceptable precision (CVs < 7%) and accuracy (86.13–97.42%).

Effect of SJW Pretreatment on Plasma Pharmacokinetics of CPT-11, SN-38 and SN-38G in Rats

Figure 2 shows the representative plasma concentration-time profiles for all analytes studied in rats receiving CPT-11 alone and in combination with SJW. For the short-term (3 days) study, pretreatment of rats with SJW (p.o. 400 mg/kg for consecutive 3 days) did not significantly alter the pharmacokinetic parameters for CPT-11 and SN-38. Interestingly, $AUC_{0-10\ hr}$, $AUC_{0-\infty}$ and $t_{1/2,z}$ for SN-38G were significantly decreased in rats pretreated with SJW compared to control rats (Table I).

For the long-term (14 days) study, pretreatment of rats with SJW (p.o. 400 mg/kg for consecutive 14 days) significantly decreased the peak plasma concentration C_{max} of CPT-11 by 74.1%, and increased $t_{1/2,z}$ and V_d by 16.2% and 33.4%, respectively (Table II). However, the $AUC_{0-\infty}$ and CL of CPT-11 were not significantly altered. Compared to the control group, the C_{max} and $AUC_{0-10\ hr}$ of SN-38 in SJW-

Table II. Comparison of Pharmacokinetic Parameters Between Two Groups of Rats Receiving CPT-11 Alone or Pretreated with St. John's Wort for 14 Days

Parameters	Treatment groups		Change (%)	p value[a]
	CPT-11 + SJW	CPT-11 + vehicle		
CPT-11				
C_0 (ng/ml)[b]	9358.4 ± 1971.4	15469.9 ± 6206.0	−65.3	**0.031**
$t_{1/2\beta}$ (h)	2.01 ± 0.31	1.73 ± 0.15	16.2	**0.043**
$AUC_{0-10\ hr}$ (ng · h/ml)	23227.6 ± 4847.1	28678.5 ± 9284.8	−19.0	0.140
$AUC_{0-\infty}$ (ng · h/ml)	23967.5 ± 5389.2	29104.7 ± 9368.2	−17.7	0.160
V_d (ml/kg)	7393.9 ± 1077.5	5541.5 ± 1636.8	33.4	**0.033**
CL (ml/h/kg)	2597.1 ± 509.3	2242.4 ± 723.5	15.8	0.194
SN-38				
C_0 (ng/ml)	639.4 ± 200.3	1046.9 ± 232.2	−38.9	**0.004**
$AUC_{0-10\ hr}$ (ng · hr/ml)	4342.7 ± 1250.0	5895.3 ± 1372.7	−26.3	**0.034**
$t_{1/2\beta}$ (h)	4.25 ± 1.63	4.82 ± 3.81	−11.8	0.374
$AUC_{0-\infty}$ (ng · h/ml)	5688.7 ± 1409.9	8644.4 ± 5543.2	−34.2	0.128
SN-38G				
C_0 (ng/ml)	2681.8 ± 963.1	2320.8 ± 544.8	15.6	0.224
$AUC_{0-10\ hr}$ (ng · h/ml)	7038.2 ± 1837.7	6523.4 ± 1577.1	7.9	0.307
$t_{1/2\beta}$ (h)	2.61 ± 0.50	2.79 ± 0.56	−6.5	0.277
$AUC_{0-\infty}$ (ng · h/ml)	7649.9 ± 2293.1	7073.1 ± 1688.3	8.2	0.316

[a] Compared with the controls using Students' unpaired t test.
[b] Obtained by back-extrapolation to the zero time using WinNonlin program.

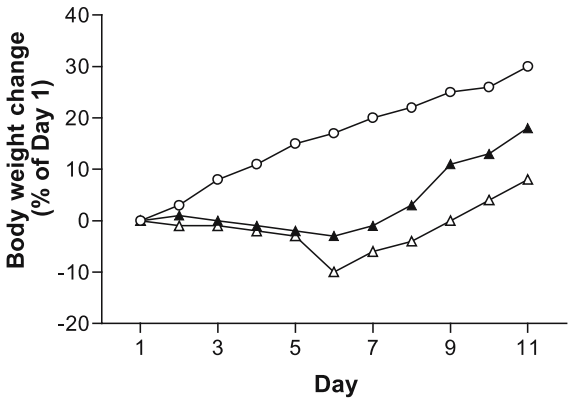

Fig. 3. Body weight changes (% compared to day 1) in two groups receiving CPT-11 alone or in combination with St. John's wort. ○, blank (physiological saline 3 ml/kg); ▲, CPT-11 + St. John's wort; △, CPT-11 + control vehicle.

pretreated rats were significantly reduced by 38.9% and 26.3%, respectively. However, $t_{1/2,z}$ of SN-38 was not significantly changed. Moreover, pretreatment of rats with SJW for 14 days did not significantly alter the pharmacokinetic parameters of SN-38G.

Effect of SJW Coadministration on the Toxicity Induced by CPT-11

Rats treated with CPT-11 alone experienced rapid decrease in body weight, reached a nadir by day 6 with a decrease of 10% compared to the baseline (day 1), and recovered to 108% of the baseline by day 11 (Fig. 3). Coadministration of SJW with CPT-11 resulted in significantly lesser ($p = 0.0022$, by repeated measures ANOVA test) body weight loss compared to rats receiving CPT-11 alone, with a decrease of 3% by day 6 and recovery to 118% of the baseline by day 11 (Fig. 3). Rats received physiological saline only had a gradual increase of body weight over 11 days.

Administration of CPT-11 alone at 60 mg/kg by i.v. for 4 consecutive days induced severe early-(days 1–4) and late-onset (days 5–8) diarrhea, with mean severity scores of 0.14, 0.14, 0.43, 1.43, 1.71, 2.07, 1.25 and 0.75 by days 1–8, respectively (Fig. 4 and Table III). The severity scores for both early- and late-onset diarrhea were significantly ($p < 0.05$, Wilcoxon rank sum test) brought down in rats treated with CPT-11 in combination of SJW (400 mg/kg, i.p.) (Table III). Rats receiving physiological saline had no diarrhea.

The counts of neutrophils, lymphocytes and platelets were significantly decreased and reached a minimum by day 5 or day 7 in rats treated with CPT-11 only (Fig. 5). The combination of CPT-11 with SJW resulted in lesser decrease ($p < 0.05$, by unpaired *t*-test) in the numbers of these blood cells. Rats receiving either CPT-11 alone or combination therapy had significantly increased blood cell number after day 7.

Marked macroscopic pathological differences were observed in the gastrointestinal tissues between the rats receiving CPT-11 injection with or without SJW pretreatment. In the control rats receiving CPT-11 with SJW-free vehicle only, wide macroscopic wall thickening, hyperemia, hemorrhage, ulceration and adhesion were observed in the intestinal tissues at days 5 and 7, though these symptoms were relieved after day 9. Surprisingly, most of these rats experienced severe stomach swell at the same time. By contrast, gastrointestinal tissues from rats pretreated with SJW were significantly ($p < 0.05$) lesser impaired in the intestinal tissues (Fig. 6). No tissue damage was observed in the rats treated with physiological saline.

DISCUSSION

A simple and reliable HPLC method for the determination of CPT-11, SN-38 and SN-38G in rat plasma was developed and validated. The presented method was fast and efficient, with simple sample preparation procedure and total running time of analytes and I.S. less than 12 min which was much shorter than those reported previously (about 30–35 min) (71–73). We measure all analytes in lactone forms by acidifying the samples to pH 3.0. In the present study, we have chosen a mobile phase consisting of acetonitrile-50 mM disodium hydrogen phosphate buffer containing 10 mM ion-pairing reagent, sodium 1-heptane-sulfonate, at pH 3.0,

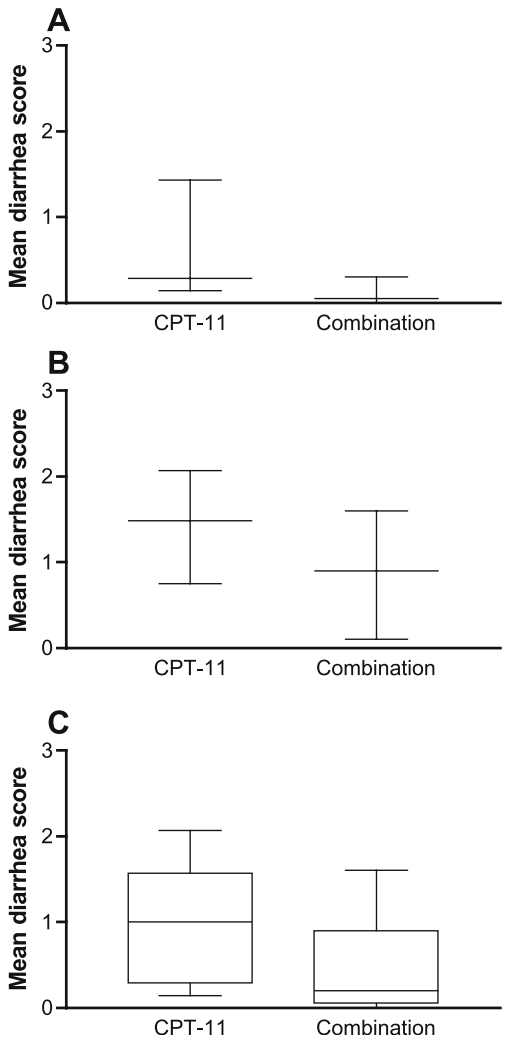

Fig. 4. Effect of coadministered St. John's wort on the early-onset (A) and late-onset (B) diarrhea induced by CPT-11 in the rat; (C) represents the combined results of both early- and late-onset diarrhea.

Table III. Incidence of Early-onset and Late-onset Diarrhea in Rats Treated with CPT-11 Alone or in Combination with St. John's Wort

		Diarrhea score[a]																			
		Day 1					Day 2					Day 3					Day 4				
Treatment group	n	0	1	2	3	Mean	0	1	2	3	Mean	0	1	2	3	Mean	0	1	2	3	Mean
Early-onset Diarrhea																					
Blank	5	10				0	10				0	10				0	10				0
CPT-11 + SJW	5	9	1			0.10	10				0	10				0	8	1	1		0.30*
CPT-11 + vehicle	7	12	2			0.14	12	2			0.14	11	0	3	0	0.43	6	1	2	5	1.43

		Diarrhea score[a]																			
		Day 5					Day 6					Day 7					Day 8				
Treatment group	n	0	1	2	3	Mean	0	1	2	3	Mean	0	1	2	3	Mean	0	1	2	3	Mean
Late-onset Diarrhea																					
Blank	5	10				0	10				0	10				0	10				0
CPT-11 + SJW	5	3	4	3	0	1.00*	3	1	3	3	1.60*	5	2	3	0	0.80*	9	1	0	0	0.10*
CPT-11 + Vehicle	7[b]	2	3	6	3	1.71	2	3	1	8	2.07	1	4	3	0	1.25	2	6	0	0	0.75

The values are the number of animals with each score.
* $p < 0.05$, CPT-11 alone *vs.* CPT-11 + SJW.
[a] Two readings at a.m. and p.m. everyday.
[b] Three rats died of severe diarrhea on day 7.

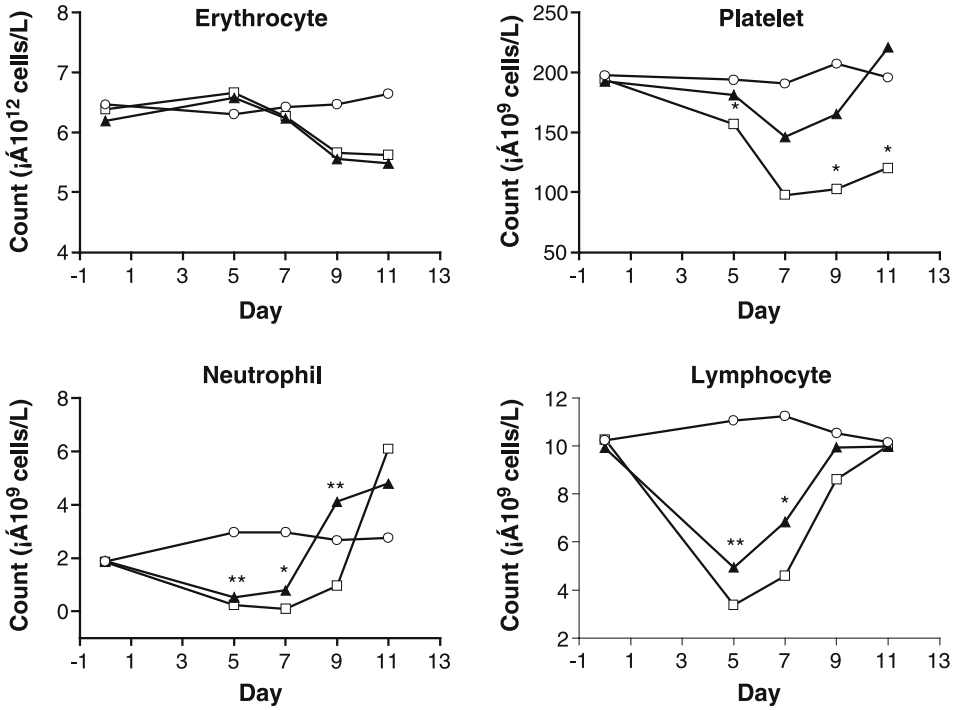

Fig. 5. Change of blood cell counts in rats treated with CPT-11 alone or in combination with St. John's wort. ○, blank (physiological saline 3 ml/kg); ▲, CPT-11 + St. John's wort; □, CPT-11 + control vehicle. Asterisks ($*p < 0.05$, $**p < 0.01$) denote significant differences between rats co-treated with St. John's wort and vehicle.

resulting in efficient separation between compounds with suitable retention times. As to the wavelengths, we selected an excitation wavelength at 380 nm and set the emission wavelength at 540 nm in favor of SN-38 given that the plasma concentrations of CPT-11 are often higher than SN-38. The validated HPLC method has been applied to pharmacokinetic studies in rats receiving CPT-11 in the absence and presence of SJW.

The choice of the doses of SJW and CPT-11 in the present study was based on the pharmacokinetic, pharmacodynamic and toxicological properties of both compounds and the nature of the study. For the dose of SJW, we have chosen 400 mg/kg body weight by oral gavage for 8 or 14 days consecutive days for the toxicity and kinetic study, respectively. Such dose is higher than those used in humans (900–1050 mg/day), but it is widely used in rat studies and significant drug metabolizing enzyme and transporter inducing effects resulting in drug interactions have been observed (74–76). This dose also showed no toxicities in rats. The dose of CPT-11 (60 mg/kg body weight per day by i.v. injection via tail vein for 4 consecutive days for toxicity studies) used in our studies is close to the maximum tolerated dose (21,77–80), leading to significant blood and intestinal toxicities, but not fatal. A lower dose of CPT-11 such as 30 mg/kg i.v. for 6 days did not induce or just produced minor early- or late-onset diarrhea in a pilot study. Due to the study nature and special properties of SJW, we used different regimens of SJW for kinetic and toxicological studies. In the kinetic study, we examined the time-dependent effect of SJW pretreatment on CPT-11 pharmacokinetic behaviors probably via modulation of drug metabolizing enzymes and transporters, whereas we concomitantly administered CPT-11 (for 4 days) with SJW (for 8 days) to observe the toxicological interactions while incorporating the kinetic factor.

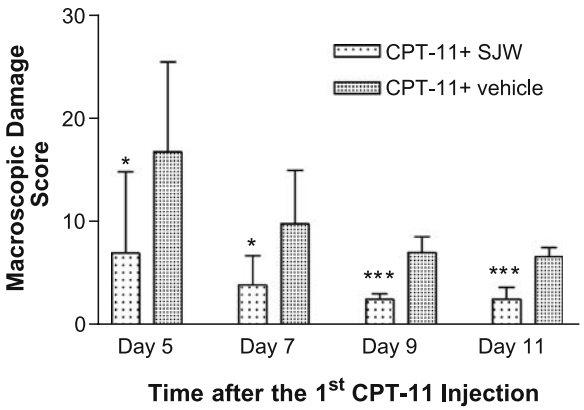

Fig. 6. Scores of macroscopic intestinal (including ileum, colon, and cecum) damages by days 5, 7, 9 and 11 induced by CPT-11 in rats pretreated with St. John's wort or vehicle. Asterisks ($*p < 0.05$, $***p < 0.001$) denote significant differences between rats pretreated with St. John's wort and vehicle.

The standard and FDA-approved use of CPT-11 in clinical setting is at 350 mg/m^2 via intravenous injection over 90 min every 3 weeks (81). Thus, we also gave CPT-11 to the rats by i.v. injection whereas St John's wort was administered via gavage to mimic delivery route of the two compounds in humans, As such, the significantly variable oral absorption of CPT-11 that has been encountered in preclinical studies and difficulties in development of acceptable oral formulations of CPT-11 was sidestepped. However, oral anticancer chemotherapy is becoming an accepted and standard approach for

the treatment of cancer, due to several advantages such as greater safety and flexibility, reduced financial cost, improved quality of life, and the potential for improved efficacy (82,83). To obtain maximal efficacy and minimal toxicity, appropriate intestinal absorption of oral anti-cancer agents are required (84). As for CPT-11, a number of preclinical studies with oral administration have been conducted (85–90), but variable absorption, poor efficacy and considerable toxicity have been observed. Therefore, novel oral formulations of CPT-11 with good oral absorption and high bioavailability and acceptable efficacy and toxicity profiles are needed.

We examined the pharmacokinetic interaction of SJW with CPT-11 using a rat model. The present study revealed that long-term exposure to SJW significantly decreases the C_{max} for both CPT-11 and SN-38, and $AUC_{0-10\ hr}$ of SN-38 as well, whereas short-term (3 days) SJW coadministration did not significantly alter the pharmacokinetics of CPT-11 and SN-38. Because SN-38 is the active metabolite of CPT-11, it may partially explain the finding that coadministered SJW reduced the gastrointestinal toxicity of CPT-11.

The reason for the reduced plasma SN-38 levels by SJW may be due to the induced CYP3A expression. SJW is a potential inducer of CYP3A4 in humans (60,91,92). In a rat study, the administration of SJW to rats for 14 days resulted in a 2.5-fold increase in hepatic CYP3A2 expression (74). This may subsequently lead to the increased metabolism from CPT-11 to APC and NPC, though the latter one can be partially converted into SN-38 by carboxylesterases. In addition, the modulation of glucuronidation of SN-38 by SJW and its metabolites is also likely, as the rat plasma level of SN-38G was significantly increased in the short-term study. This could be due to UGT1A induction by SJW components, resulting in accelerated SN-38 glucuronidation. Hyperforin, hypericin and other flavonoids in SJW may be able to upregulate UGT1A by stabilizing mRNA and increasing protein expression. Surprisingly, in the long-term study, the SN-38G levels were not significantly influenced by SJW, suggesting the inducing effect of SJW on multiple drug metabolizing enzymes and transporters resulting in negating effect. The induction of PgP (MDR1) and MRP1-2 by SJW may contribute to the altered CPT-11's and SN-38's pharmacokinetics, given that CPT-11, SN-38 and SN-38G are known substrates for PgP, MRP1 and MRP2 (29,93–97). Further studies are needed to explore the effects of SJW on the metabolism and transport of CPT-11 and SN-38.

The V_d of CPT-11 was significantly increased in rats receiving combination therapy compared to those receiving CPT-11 alone. It is possible that SJW may influence the binding of CPT-11 to plasma and tissue proteins.

The results showed that coadministered SJW reduced the dose-limiting toxicities induced by CPT-11 in rats. This was indicated by alleviated body weight loss, lower early-and late-onset diarrhea scores, decrease in neutrophil, lymphocyte and platelet numbers, lower macroscopic intestinal damage. Compared to the control rats without SJW treatment, the body weight loss induced by CPT-11 was much lesser and recovered more quickly in rats pretreated with SJW. Both early- (at day 4) and late-onset diarrhea (days 5–8) were attenuated by the coadministration of SJW. The numbers of neutrophil, lymphocyte and platelet were lesser decreased and recovered more rapidly in rats receiving CPT-11 in combination with SJW. The macroscopic histological damages in intestinal tissues were also alleviated, and subsequently the stomach damage was reduced as well. The swollen stomach induced by CPT-11 may be due to the direct effect of toxic SN-38 and/or decreased gastrointestinal peristalsis arising from tissue damage.

One possible mechanism for the protective effect of SJW on CPT-11-induced diarrhea is a pharmacokinetic interaction between SJW and CPT-11. However, pharmacodynamic component may also play an important role. SJW has been reported to show antiinflammatory effect (98,99) through the inhibition of NF-κB activation (100), protein kinase C (101), cytokine production (102), and cytokine-induced expression of cyclooxygenase-2 (103,104). SJW also reduced tyrosine phosphorylation of the STAT-1α protein (105).

In summary, coadministered SJW ameliorated the gastrointestinal and hematological toxicities of CPT-11 in rats, as indicated by alleviation of diarrhea and suppression of decreased leukocyte counts. The data obtained from the pharmacokinetic interaction studies in rats partially explained the findings that coadministered SJW reduced the dose-limiting toxicity of CPT-11. Further studies are warranted to explore the underlying mechanisms for the observed kinetic and dynamic interactions between CPT-11 and SJW.

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REFERENCES

1. M. R. Redinbo, L. Stewart, P. Kuhn, J. J. Champoux, and W. G. Hol. Crystal structures of human topoisomerase I in covalent and noncovalent complexes with DNA. *Science* **279**:1504–1513 (1998).
2. L. Stewart, M. R. Redinbo, X. Qiu, W. Hol, and J. J. Champoux. A model for the mechanism of human topoisomerase I. *Science* **279**:1534–1541 (1998).
3. F. Goldwasser, T. Shimizu, J. Jackman, Y. Hoki, P. M. O'Connor, K. W. Kohn, and Y. Pommier. Correlations between S and G2 arrest and the cytotoxicity of camptothecin in human colon carcinoma cells. *Cancer Res.* **56**:4430–4437 (1996).
4. T. Kunimoto, K. Nitta, T. Tanaka, N. Uehara, H. Baba, M. Takeuchi, T. Yokokura, S. Sawada, T. Miyasaka, and M. Mutai. Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothec in, a novel water-soluble derivative of camptothecin, against murine tumors. *Cancer Res.* **47**:5944–5947 (1987).
5. P. J. Houghton, P. J. Cheshire, J. C. Hallman, M. C. Bissery, A. Mathieu-Boue, and J. A. Houghton. Therapeutic efficacy of the topoisomerase I inhibitor 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy-camptothecin against human tumor xenografts: lack of cross-resistance *in vivo* in tumors with acquired resistance to the topoisomerase I inhibitor 9-dimethylaminomethyl-10-hydroxycamptothecin. *Cancer Res.* **53**: 2823–2829 (1993).
6. M. Potmesil. Camptothecins: from bench research to hospital wards. *Cancer Res.* **54**:1431–1439 (1994).
7. K. Ota, R. Ohno, S. Shirakawa, T. Masaoka, K. Okada, Y. Ohashi, and T. Taguchi. Late phase II clinical study of irinotecan hydrochloride (CPT-11) in the treatment of malignant lymphoma and acute leukemia. The CPT-11 Research

Group for Hematological Malignancies. *Gan To Kagaku Ryoho* **21**:1047–1055 (1994).
8. Y. Shimizu, S. Umezawa, and K. Hasumi. Successful treatment of clear cell adenocarcinoma of the ovary (OCCA) with a combination of CPT-11 and mitomycin C. *Gan To Kagaku Ryoho* **23**:587–593 (1996).
9. C. F. Verschraegen, T. Levy, A. P. Kudelka, E. Llerena, K. Ende, R. S. Freedman, C. L. Edwards, M. Hord, M. Steger, and A. L. Kaplan. Phase II study of irinotecan in prior chemotherapy-treated squamous cell carcinoma of the cervix. *J. Clin. Oncol.* **15**:625–631 (1997).
10. W. P. Irvin, F. V. Price, H. Bailey, M. Gelder, R. Rosenbluth, H. J. Durivage, and R. K. Potkul. A phase II study of irinotecan (CPT-11) in patients with advanced squamous cell carcinoma of the cervix. *Cancer* **82**:328–333 (1998).
11. S. Kudoh, Y. Fujiwara, Y. Takada, H. Yamamoto, A. Kinoshita, Y. Ariyoshi, K. Furuse, and M. Fukuoka. Phase II study of irinotecan combined with cisplatin in patients with previously untreated small-cell lung cancer. West Japan Lung Cancer Group. *J. Clin. Oncol.* **16**:1068–1074 (1998).
12. T. Tsuji, N. Kaneda, K. Kado, T. Yokokura, T. Yoshimoto, and D. Tsuru. CPT-11 converting enzyme from rat serum: purification and some properties. *J. Pharmacobio-dyn.* **14**:341–349 (1991).
13. T. Satoh, M. Hosokawa, R. Atsumi, W. Suzuki, H. Hakusui, and E. Nagai. Metabolic activation of CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, a novel antitumor agent, by carboxylesterase. *Biol. Pharm. Bull.* **17**:662–664 (1994).
14. L. P. Rivory, M. R. Bowles, J. Robert, and S. M. Pond. Conversion of irinotecan (CPT-11) to its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), by human liver carboxylesterase. *Biochem. Pharmacol.* **52**:1103–1111 (1996).
15. R. Humerickhouse, K. Lohrbach, L. Li, W. F. Bosron, and M. E. Dolan. Characterization of CPT-11 hydrolysis by human liver carboxylesterase isoforms hCE-1 and hCE-2. *Cancer Res.* **60**:1189–1192 (2000).
16. S. Bencharit, C. L. Morton, E. L. Howard-Williams, M. K. Danks, P. M. Potter, and M. R. Redinbo. Structural insights into CPT-11 activation by mammalian carboxylesterases. *Nat. Struct. Biol.* **9**:337–342 (2002).
17. T. Itoh, I. Takemoto, S. Itagaki, K. Sasaki, T. Hirano, and K. Iseki. Biliary excretion of irinotecan and its metabolites. *J. Pharm. Pharm. Sci.* **7**:13–18 (2004).
18. Y. Kawato, M. Aonuma, Y. Hirota, H. Kuga, and K. Sato. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res.* **51**:4187–4191 (1991).
19. R. H. Mathijssen, R. J. van Alphen, J. Verweij, W. J. Loos, K. Nooter, G. Stoter, and A. Sparreboom. Clinical pharmacokinetics and metabolism of irinotecan (CPT-11). *Clin. Cancer Res.* **7**:2182–2194 (2001).
20. A. Santos, S. Zanetta, T. Cresteil, A. Deroussent, F. Pein, E. Raymond, L. Vernillet, M. L. Risse, V. Boige, and A. Gouyette. Metabolism of irinotecan (CPT-11) by CYP3A4 and CYP3A5 in humans. *Clin. Cancer Res.* **6**:2012–2020 (2000).
21. K. Arimori, N. Kuroki, A. Kumamoto, N. Tanoue, M. Nakano, E. Kumazawa, A. Tohgo, and M. Kikuchi. Excretion into gastrointestinal tract of irinotecan lactone and carboxylate forms and their pharmacodynamics in rodents. *Pharm. Res.* **18**:814–822 (2001).
22. N. Hanioka, S. Ozawa, H. Jinno, M. Ando, Y. Saito, and J. Sawada. Human liver UDP-glucuronosyltransferase isoforms involved in the glucuronidation of 7-ethyl-10-hydroxycamptothecin. *Xenobiotica* **31**:687–699 (2001).
23. L. P. Rivory, J. F. Riou, M. C. Haaz, S. Sable, M. Vuilhorgne, A. Commercon, S. M. Pond, and J. Robert. Identification and properties of a major plasma metabolite of irinotecan (CPT-11) isolated from the plasma of patients. *Cancer Res.* **56**:3689–3694 (1996).
24. L. P. Rivory, M. C. Haaz, P. Canal, F. Lokiec, J. P. Armand, and J. Robert. Pharmacokinetic interrelationships of irinotecan (CPT-11) and its three major plasma metabolites in patients enrolled in phase I/II trials. *Clin. Cancer Res.* **3**:1261–1266 (1997).
25. M. C. Haaz, L. Rivory, C. Riche, L. Vernillet, and J. Robert. Metabolism of irinotecan (CPT-11) by human hepatic microsomes: participation of cytochrome P-450 3A and drug interactions. *Cancer Res.* **58**:468–472 (1998).
26. A. Santos, S. Zannetta, and T. Cresteil. Metabolism of action of camptothecin. *Ann. N.Y. Acad. Sci.* **922**:1–10 (2000).
27. C. Farabos, M. C. Haaz, P. Gires, and J. Robert. Hepatic extraction, metabolism, and biliary excretion of irinotecan in the isolated perfused rat liver. *J. Pharm. Sci.* **90**:722–731 (2001).
28. E. Gupta, T. M. Lestingi, R. Mick, J. Ramirez, E. E. Vokes, and M. J. Ratain. Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. *Cancer Res.* **54**:3723–3725 (1994).
29. Y. Sugiyama, Y. Kato, and X. Chu. Multiplicity of biliary excretion mechanisms for the camptothecin derivative irinotecan (CPT-11), its metabolite SN-38, and its glucuronide: role of canalicular multispecific organic anion transporter and P-glycoprotein. *Cancer Chemother. Pharmacol.* **42**: S44–S49 (1998).
30. D. Gandia, D. Abigerges, J. P. Armand, G. Chabot, L. Da Costa, M. De Forni, A. Mathieu-Boue, and P. Herait. CPT-11-induced cholinergic effects in cancer patients. *J. Clin. Oncol.* **11**:196–197 (1993).
31. J. R. Hecht. Gastrointestinal toxicity or irinotecan. *Oncology (Huntingt)* **12**:72–78 (1998).
32. L. F. Liu, S. D. Desai, and T. K. Li. Mechanism of action of camptothecin. *Ann. N.Y. Acad. Sci.* **922**:1–10 (2000).
33. Y. Xu and M. A. Villalona-Calero. Irinotecan: mechanisms of tumor resistance and novel strategies for modulating its activity. *Ann. Oncol.* **13**:1841–1851 (2002).
34. S. Kornblau, A. B. Benson, R. Catalano, R. E. Champlin, C. Engelking, M. Field, C. Ippoliti, H. M. Lazarus, E. Mitchell, and J. Rubin. Management of cancer treatment-related diarrhea. Issues and therapeutic strategies. *J. Pain Symptom Manag.* **19**:118–129 (2000).
35. H. Sakai, T. Sato, N. Hamada, M. Yasue, A. Ikari, B. Kakinoki, and N. Takeguchi. Thromboxane A2, released by the antitumour drug irinotecan, is a novel stimulator of Cl-secretion in isolated rat colon. *J. Physiol.* **505**(Pt. 1):133–144 (1997).
36. T. Suzuki, H. Sakai, and A. Ikari. Inhibition of thromboxane A2-induced Cl-secretion by antidiarrhea drug loperamide in isolated rat colon. *J. Pharmacol. Exp. Ther.* **295**:233–238 (2000).
37. F. Saliba, R. Hagipantelli, J. L. Misset, G. Bastian, G. Vassal, M. Bonnay, P. Herait, C. Cote, M. Mahjoubi, and D. Mignard. Pathophysiology and therapy of irinotecan-induced delayed-onset diarrhea in patients with advanced colorectal cancer: a prospective assessment. *J. Clin. Oncol.* **16**:2745–2751 (1998).
38. T. Ikegami, L. Ha, K. Arimori, P. Latham, K. Kobayashi, S. Ceryak, Y. Matsuzaki, and B. Bouscarel. Intestinal alkalization as a possible preventive mechanism in irinotecan (CPT-11)-induced diarrhea. *Cancer Res.* **62**:179–187 (2002).
39. H. Shinohara, J. J. Killion, H. Kuniyasu, R. Kumar, and I. J. Fidler. Prevention of intestinal toxic effects and intensification of irinotecan's therapeutic efficacy against murine colon cancer liver metastases by oral administration of the lipopeptide JBT 3002. *Clin. Cancer Res.* **4**:2053–2063 (1998).
40. K. Takasuna, T. Hagiwara, M. Hirohashi, M. Kato, M. Nomura, E. Nagai, T. Yokoi, and T. Kamataki. Inhibition of intestinal microflora beta-glucuronidase modifies the distribution of the active metabolite of the antitumor agent, irinotecan hydrochloride (CPT-11) in rats. *Cancer Chemother. Pharmacol.* **42**:280–286 (1998).
41. D. F. Kehrer, A. Sparreboom, J. Verweij, P. de Bruijn, C. A. Nierop, J. van de Schraaf, E. J. Ruijgrok, and M. J. de Jonge. Modulation of irinotecan-induced diarrhea by cotreatment with neomycin in cancer patients. *Clin. Cancer Res.* **7**:1136–1141 (2001).
42. M. Prados, J. Kuhn, and W. Yung. A phase-I study of CPT-11 given every 3 weeks to patients with recurrent malignant glioma. A North American Brain Tumor Consortium (NABTC) study. *Proc. -Am. Soc. Clin. Oncol.* **19:**162 (2001).
43. E. Gupta, A. Safa, X. Wang, and M. Ratain. Pharmacokinetic modulation of irinotecan and metabolites by cyclosporin A. *Cancer Res.* **56**:1309–1314 (1996).

44. M. Ratain. Insights into the pharmacokinetics and pharmacodynamics of irinotecan. *Clin. Cancer Res.* **6**:3393–3394 (2000).
45. O. C. Trifan, W. F. Durham, V. S. Salazar, J. Horton, B. D. Levine, B. S. Zweifel, T. W. Davis, and J. L. Masferrer. Cyclooxygenase-2 inhibition with celecoxib enhances antitumor efficacy and reduces diarrhea side effect of CPT-11. *Cancer Res.* **62**:5778–5784 (2002).
46. M. Horikawa, Y. Kato, and Y. Sugiyama. Reduced gastrointestinal toxicity following inhibition of the biliary excretion of irinotecan and its metabolites by probenecid in rats. *Pharm. Res.* **19**:1345–1353 (2002).
47. M. Horikawa, Y. Kato, and Y. Sugiyama. Reduced gastrointestinal toxicity following inhibition of the biliary excretion of irinotecan and its metabolites by probenecid in rats. *Pharm. Res.* **19**:1345–1353 (2002).
48. G. Di Carlo, F. Borrelli, E. Ernst, and A. A. Izzo. St. John's wort: prozac from the plant kingdom. *Trends Pharmacol. Sci.* **22**:292–297 (2001).
49. A. R. Bilia, S. Gallori, and F. F. Vincieri. St. John's wort and depression: efficacy, safety and tolerability-an update. *Life Sci.* **70**:3077–3096 (2002).
50. V. Schulz. Clinical trials with hypericum extracts in patients with depression-results, comparisons, conclusions for therapy with antidepressant drugs. *Phytomedicine* **9**:468–474 (2002).
51. E. Ernst. Second thoughts about safety of St. John's wort. *Lancet* **354**:2014–2016 (1999).
52. A. Johne, J. Brockmoller, S. Bauer, A. Maurer, M. Langheinrich, and I. Roots. Pharmacokinetic interaction of digoxin with an herbal extract from St. John's wort (Hypericum perforatum). *Clin. Pharmacol. Ther.* **66**:338–345 (1999).
53. D. Durr, B. Stieger, G. A. Kullak-Ublick, K. M. Rentsch, H. C. Steinert, P. J. Meier and K. Fattinger. St. John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. *Clin. Pharmacol. Ther.* **68**:598–604 (2000).
54. L. B. Moore, B. Goodwin, S. A. Jones, G. B. Wisely, C. J. Serabjit-Singh, and T. M. Willson. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proc. Natl. Acad. Sci. USA* **97**:7500–7502 (2000).
55. S. C. Piscitelli, A. H. Burstein, D. Chaitt, R. M. Alfaro, and J. Falloon. Indinavir concentrations and St. John's wort. *Lancet* **355**:547–548 (2000).
56. C. A. Roby, G. D. Anderson, E. Kantor, D. A. Dryer, and A. H. Burstein. St. John's wort: effect on CYP3A4 activity. *Clin. Pharmacol. Ther.* **67**:451–457 (2000).
57. F. Ruschitzka, P. J. Meier, M. Turina, T. F. Luscher, and G. Noll. Acute heart transplant rejection due to Saint John's wort. *Lancet* **355**:548–549 (2000).
58. J. M. Wentworth, M. Agostini, J. Love, J. W. Schwabe, and V. K. Chatterjee. St. John's wort, a herbal antidepressant, activates the steroid X receptor. *J. Endocrinol.* **166**:R11–R16 (2000).
59. B. Goodwin, L. B. Moore, C. M. Stoltz, D. D. McKee, and S. A. Kliewer. Regulation of the human CYP2B6 gene by the nuclear pregnane X receptor. *Mol. Pharmacol.* **60**:427–431 (2001).
60. Z. Q. Wang, C. Gorski, M. A. Hamman, S. M. Huang, L. J. Lesko, and S. D. Hall. The effects of St. John's wort (*Hypericum Perforatum*) on human cytochrome P450 activity. *Clin. Pharmacol. Ther.* **70**:317–326 (2001).
61. A. Johne, J. Schmider, J. Brockmoller, A. M. Stadelmann, E. Stormer, S. Bauer, G. Scholler, M. Langheinrich, and I. Roots. Decreased plasma levels of amitriptyline and its metabolites on comedication with an extract from St. John's wort (Hypericum perforatum). *J. Clin. Psychopharmacol.* **22**:46–54 (2002).
62. Z. Wang, M. A. Hamman, S. M. Huang, L. J. Lesko, and S. D. Hall. Effect of St. John's wort on the pharmacokinetics of fexofenadine. *Clin. Pharmacol. Ther.* **71**:414–420 (2002).
63. A. A. Izzo. Drug interactions with St. John's Wort (Hypericum perforatum): a review of the clinical evidence. *Int. J. Clin. Pharmacol. Ther.* **42**:139–148 (2004).
64. S. Zhou, E. Chan, S. Q. Pan, M. Huang, and E. J. Lee. Pharmacokinetic interactions of drugs with St. John's wort. *J. Psychopharmacol.* **18**:262–276 (2004).
65. R. H. Mathijssen, J. Verweij, P. de Bruijn, W. J. Loos, and A. Sparreboom. Effects of St. John's wort on irinotecan metabolism. *J. Natl. Cancer Inst.* **94**:1247–1249 (2002).
66. A. Kurita, S. Kado, N. Kaneda, M. Onoue, S. Hashimoto, and T. Yokokura. Alleviation of side effects induced by irinotecan hydrochloride (CPT-11) in rats by intravenous infusion. *Cancer Chemother. Pharmacol.* **52**:349–360 (2003).
67. A. Kurita, S. Kado, N. Kaneda, M. Onoue, S. Hashimoto, and T. Yokokura. Modified irinotecan hydrochloride (CPT-11) administration schedule improves induction of delayed-onset diarrhea in rats. *Cancer Chemother. Pharmacol.* **46**:211–220 (2000).
68. M. Miampamba, E. J. Parr, D. M. McCafferty, J. L. Wallace and K. A. Sharkey. Effect of intracolonic benzalkonium chloride on trinitrobenzene sulphonic acid-induced colitis in the rat. *Aliment Pharmacol. Ther.* **12**:219–228 (1998).
69. M. Kruschewski, T. Foitzik, A. Perez-Canto, A. Hubotter, and H. J. Buhr. Changes of colonic mucosal microcirculation and histology in two colitis models: an experimental study using intravital microscopy and a new histological scoring system. *Dig. Dis. Sci.* **46**:2336–2343 (2001).
70. I. Maric, L. Poljak, S. Zoricic, D. Bobinac, D. Bosukonda, K. T. Sampath, and S. Vukicevic. Bone morphogenetic protein-7 reduces the severity of colon tissue damage and accelerates the healing of inflammatory bowel disease in rats. *J. Cell. Physiol.* **196**:258–264 (2003).
71. A. Sparreboom, P. de Bruijn, M. J. de Jonge, W J. Loos, G. Stoter, J. Verweij and K. Nooter. Liquid chromatographic determination of irinotecan and three major metabolites in human plasma, urine and feces. *J. Chromatogr., B, Biomed. Sci. Appl.* **712**:225–235 (1998).
72. J. Escoriaza, A. Aldaz, C. Castellanos, E. Calvo, and J. Giraldez. Simple and rapid determination of irinotecan and its metabolite SN-38 in plasma by high-performance liquid-chromatography: application to clinical pharmacokinetic studies. *J. Chromatogr., B, Biomed. Sci. Appl.* **740**:159–168 (2000).
73. S. Poujol, F. Pinguet, F. Malosse, C. Astre, M. Ychou, S. Culine, and F. Bressolle. Sensitive HPLC-fluorescence method for irinotecan and four major metabolites in human plasma and saliva: application to pharmacokinetic studies. *Clin. Chem.* **49**:1900–1908 (2003).
74. D. Durr, B. Stieger, G. A. Kullak-Ublick, K. M. Rentsch, H. C. Steinert, P. J. Meier and K. Fattinger. St. John's wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. *Clin. Pharmacol. Ther.* **68**:598–604 (2000).
75. Y. Shibayama, R. Ikeda, T. Motoya, and K. Yamada. St. John's Wort (Hypericum perforatum) induces overexpression of multidrug resistance protein 2 (MRP2) in rats: a 30-day ingestion study. *Food Chem. Toxicol.* **42**:995–1002 (2004).
76. M. L. Wong, F. O'Kirwan, J. P. Hannestad, K. J. Irizarry, D. Elashoff, and J. Licinio. St. John's wort and imipramine-induced gene expression profiles identify cellular functions relevant to antidepressant action and novel pharmacogenetic candidates for the phenotype of antidepressant treatment response. *Mol Psychiatry* in press (2004).
77. O. C. Trifan, W. F. Durham, V. S. Salazar, J. Horton, B. D. Levine, B. S. Zweifel, T. W. Davis, and J. L. Masferrer. Cyclooxygenase-2 inhibition with celecoxib enhances antitumor efficacy and reduces diarrhea side effect of CPT-11. *Cancer Res.* **62**:5778–5784 (2002).
78. K. Arimori, N. Kuroki, M. Hidaka, T. Iwakiri, K. Yamsaki, M. Okumura, H. Ono, N. Takamura, M. Kikuchi, and M. Nakano. Effect of P-glycoprotein modulator, cyclosporin A, on the gastrointestinal excretion of irinotecan and its metabolite SN-38 in rats. *Pharm. Res.* **20**:910–917 (2003).
79. B. Chowbay, A. Sharma, Q. Y. Zhou, Y. B. Cheung, and E. J. Lee. The modulation of irinotecan-induced diarrhoea and pharmacokinetics by three different classes of pharmacologic agents. *Oncol. Rep.* **10**:745–751 (2003).
80. A. Kurita, S. Kado, N. Kaneda, M. Onoue, S. Hashimoto, and T. Yokokura. Alleviation of side effects induced by irinotecan hydrochloride (CPT-11) in rats by intravenous infusion. *Cancer Chemother. Pharmacol.* **52**:349–356 (2003).
81. J. F. Pizzolato and L. B. Saltz. The camptothecins. *Lancet* **361**:2235–2242 (2003).
82. M. D. DeMario and M. J. Ratain. Oral chemotherapy-Rationale and future directions. *J. Clin. Oncol.* **16**:2557–2567 (1998).

83. F. A. Greco. Evolving role of oral chemotherapy for the treatment of patients with neoplasms. *Oncology* **12**:43–50 (1998).
84. J. M. M. Terwogt, J. H. M. Schellens, W. W. T. Huinink, and J. H. Beijnen. Clinical pharmacology of anticancer agents in relation to formulations and administration routes. *Cancer Treat. Rev.* **25**:83–101 (1999).
85. C. F. Stewart, W. C. Zamboni, W. R. Crom, and P. J. Houghton. Disposition of irinotecan and SN-38 following oral and intravenous irinotecan dosing in mice. *Cancer Chemother. Pharmacol.* **40**:259–265 (1997).
86. W. C. Zamboni, P. J. Houghton, J. Thompson, P. J. Cheshire, S. K. Hanna, L. B. Richmond, X. Lou, and C. F. Stewart. Altered irinotecan and SN-38 disposition after intravenous and oral administration of irinotecan in mice bearing human neuroblastoma xenografts. *Clin. Cancer Res.* **4**:455–462 (1998).
87. R. L. Drengler, J. G. Kuhn, L. J. Schaaf, G. I. Rodriguez, M. A. Villalona-Calero, L. A. Hammond, J. A. Stephenson Jr, S. Hodges, M. A. Kraynak, and B. A. Staton. Phase I and pharmacokinetic trial of oral irinotecan administered daily for 5 days every 3 weeks in patients with solid tumors. *J. Clin. Oncol.* **17**:685–696 (1999).
88. C. F. Stewart, M. Leggas, J. D. Schuetz, J. C. Panetta, P. J. Cheshire, J. Peterson, N. Daw, J. J. Jenkins III, R. Gilbertson, and G. S. Germain. Gefitinib enhances the antitumor activity and oral bioavailability of irinotecan in mice. *Cancer Res.* **64**:7491–7499 (2004).
89. N. E. Schoemaker, I. E. Kuppens, W. W. Huinink, P. Lefebvre, J. H. Beijnen, S. Assadourian, G. J. Sanderink, and J. H. Schellens. Phase I study of an oral formulation of irinotecan administered daily for 14 days every 3 weeks in patients with advanced solid tumours. *Cancer Chemother. Pharmacol.* **55**:263–270 (2005).
90. O. Soepenberg, H. Dumez, J. Verweij, D. Semiond, M. J. Dejonge, F. A. Eskens, J. T. Steeg, J. Selleslach, S. Assadourian, and G. J. Sanderink. Phase I and pharmacokinetic study of oral irinotecan given once daily for 5 days every 3 weeks in combination with capecitabine in patients with solid tumors. *J. Clin. Oncol.* **23**:889–898 (2005).
91. J. S. Markowitz, C. L. DeVane, D. W. Boulton, S. W. Carson, Z. Nahas, and S. C. Risch. Effect of St. John's wort (Hypericum perforatum) on cytochrome P-450 2D6 and 3A4 activity in healthy volunteers. *Life Sci.* **66**:PL133–PL139 (2000).
92. J. S. Markowitz, J. L. Donovan, C. L. DeVane, R. M. Taylor, Y. Ruan, J. S. Wang, and K. D. Chavin. Effect of St. John's wort on drug metabolism by induction of cytochrome P450 3A4 enzyme. *JAMA* **290**:1500–1504 (2003).
93. X. Y. Chu, Y. Kato, K. Niinuma, K. I. Sudo, H. Hakusui, and Y. Sugiyama. Multispecific organic anion transporter is responsible for the biliary excretion of the camptothecin derivative irinotecan and its metabolites in rats. *J. Pharmacol. Exp. Ther.* **281**:304–314 (1997).
94. X. Y. Chu, Y. Kato, and Y. Sugiyama. Multiplicity of biliary excretion mechanisms for irinotecan, CPT-11, and its metabolites in rats. *Cancer Res.* **57**:1934–1938 (1997).
95. X. Y. Chu, H. Suzuki, K. Ueda, Y. Kato, S. Akiyama, and Y. Sugiyama. Active efflux of CPT-11 and its metabolites in human KB-derived cell lines. *J. Pharmacol. Exp. Ther.* **288**:735–741 (1999).
96. M. Yoshikawa, Y. Ikegami, S. Hayasaka, K. Ishii, A. Ito, K. Sano, T. Suzuki, T. Togawa, H. Yoshida, and H. Soda. Novel camptothecin analogues that circumvent ABCG2-associated drug resistance in human tumor cells. *Int. J. Cancer* **110**:921–927 (2004).
97. M. Yoshikawa, Y. Ikegami, K. Sano, H. Yoshida, H. Mitomo, S. Sawada, and T. Ishikawa. Transport of SN-38 by the wild type of human ABC transporter ABCG2 and its inhibition by quercetin, a natural flavonoid. *J. Exp. Ther. Oncol.* **4**:25–35 (2004).
98. J. Barnes, L. A. Anderson, and J. D. Phillipson. St. John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. *J. Pharm. Pharmacol.* **53**:583–600 (2001).
99. E. Tedeschi, M. Menegazzi, D. Margotto, H. Suzuki, U. Forstermann, and H. Kleinert. Anti-inflammatory actions of St. John's wort: inhibition of human inducible nitric-oxide synthase expression by down-regulating signal transducer and activator of transcription-1alpha (STAT-1alpha) activation. *J. Pharmacol. Exp. Ther.* **307**:254–261 (2003).
100. P. M. Bork, S. Bacher, M. L. Schmitz, U. Kaspers, and M. Heinrich. Hypericin as a non-antioxidant inhibitor of NF-kappa B. *Planta Med.* **65**:297–300 (1999).
101. P. Agostinis, A. Donella-Deana, J. Cuveele, A. Vandenbogaerde, S. Sarno, W. Merlevede, and P. de Witte. A comparative analysis of the photosensitized inhibition of growth-factor regulated protein kinases by hypericin-derivatives. *Biochem. Biophys. Res. Commun.* **220**:613–617 (1996).
102. B. L. Fiebich, A. Hollig, and K. Lieb. Inhibition of substance P-induced cytokine synthesis by St. John's wort extracts. *Pharmacopsychiatry* **34**:S26–S28 (2001).
103. G. M. Raso, R. Meli, G. Di Carlo, M. Pacilio, and R. Di Carlo. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A. 1. *Life Sci.* **68**:921–931 (2001).
104. G. M. Raso, M. Pacilio, G. Di Carlo, E. Esposito, L. Pinto, and R. Meli. *In-vivo* and *in-vitro* anti-inflammatory effect of *Echinacea purpurea* and *Hypericum perforatum*. *J. Pharm. Pharmacol.* **54**:1379–1383 (2002).
105. E. Tedeschi, M. Menegazzi, D. Margotto, H. Suzuki, U. Forstermann, and H. Kleinert. Anti-inflammatory actions of St. John's wort: inhibition of human inducible nitric-oxide synthase expression by down-regulating signal transducer and activator of transcription-1alpha (STAT-1alpha) activation. *J. Pharmacol. Exp. Ther.* **307**:254–261 (2003).