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Short communication

P-glycoprotein-dependent pharmacokinetics of irinotecan and its active metabolite, SN-38 in rats: Effect of verapamil

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Abstract

We have recently demonstrated that the oral bioavailability of irinotecan (80 mg/kg) can be increased at least 7-fold by co-administration of the P-gp blocker verapamil (25 mg/kg, Oral). As a result, co-treatment with P-gp inhibitor could be a useful strategy for bioavailability enhancement. However, in view of narrow therapeutic index, the co-administration of irinotecan and verapamil may result in unanticipated toxicities. Therefore, dose optimisation studies of irinotecan were performed when it is given in conjunction with a P-gp inhibitor. For dose optimization study, the bioavailability and pharmacokinetic parameters were studied in rats after oral administration of irinotecan at three doses (i.e. 20, 40 and 80 mg/kg) alone and in combination with verapamil (25 mg/kg, oral). The area under the plasma-concentration time curve (AUC) of irinotecan at 20, 40 and 80 mg/kg was 3.51 ± 1.20, 8.81 ± 1.93 and 14.03 ± 2.18 h µg/ml, respectively which after treatment with verapamil, increased dose dependently to 7.84 ± 1.20, 19.94 ± 2.39 and 61.71 ± 15.0 h µg/ml, respectively. In addition to irinotecan, plasma concentrations of SN-38, one of the major active metabolite of irinotecan, were also monitored. The less than proportional increase in SN-38 AUC from 20 to 80 mg/kg is consistent with the saturation of carboxylesterase. Our results indicate that oral drug treatment of irinotecan in presence of temporary P-gp inhibition could be as equally safe and effective as intravenous administration. Nevertheless, safe P-gp inhibitors need to be identified as alternatives to verapamil for development of efficacious oral irinotecan formulations.

Keywords

Irinotecan, bioavailability, verapamil, P-glycoprotein, pharmacokinetics

Introduction

Irinotecan is a worldwide approved anti-cancer agent for the treatment of colorectal cancers and other malignancies. It is currently marketed for intravenous use although few reports of oral irinotecan administration exist which demonstrate its low and highly variable oral bioavailability [1-4]. As a result novel formulations of irinotecan exhibiting better oral absorption and bioavailability need to be developed.

In our previous studies, irinotecan at 1 and 10 µM showed much higher basal-to-apical transport than apical-to-basal transport in Caco-2 cells saturable at 100 µM concentrations. This could be due to the active carrier mediated transport of irinotecan by the intestinal drug efflux pump, P-glycoprotein (P-gp). Keeping this in view, pharmacokinetic studies of irinotecan were conducted with verapamil in Wistar rats. Verapamil increased the absolute bioavailability (F) of irinotecan by 4.3 fold and decreased its biliary excretion. It appears that the concomitant and synergistic inhibition of P-gp present in rat intestine and liver is a plausible explanation for prominent increase in oral bioavailability of irinotecan [5]. However, in view of narrow therapeutic index, the co-administration of irinotecan and verapamil may result in unanticipated toxicities.

Co-administration of P-gp inhibitor would maintain irinotecan plasma concentrations in the same range even at lower doses as compared to when it is administered alone at high dose. We propose that oral drug treatment of irinotecan in presence of temporary P-gp inhibition could be as equally safe and effective as intravenous administration. In the current investigation, the bioavailability and pharmacokinetic parameters were studied in female Wistar rats after oral administration of irinotecan alone and in combination with verapamil (25 mg/kg, oral) at three doses (i.e. 20, 40 and 80 mg/kg). The main objective of the study is to evaluate the magnitude of improvement in oral delivery of irinotecan via co-treatment with oral P-gp inhibitor.

Experimental

Chemicals and animals

Irinotecan (>99 %), SN-38 (>96 %), topotecan (>98 %) and irinotecan hydrochloride injection (20 mg ml^{-1}) originated from Dabur Pharma Limited (U.P, India). All chemical and reagents were of analytical or HPLC grade as appropriate and procured locally. Healthy Female Wistar rats (180-200 g, n=5) used for pharmacokinetic studies were obtained from breeding stock of Dabur Research Foundation (Ghaziabad, U.P, India). Rats housed in cages were kept in a room under controlled temperature (20-22 °C) and 12 h day-night cycle. Animals were used for pharmacokinetic studies after one-week acclimatization with free access to water and feed. All animal procedures were approved by Institutional Animal Ethics Committee (Dabur Research Foundation, U.P, India). Verapamil (Sigma-Aldrich, St. Louis, MO, USA) solutions were prepared in distilled water containing 5 % DMSO. The purity of both irinotecan and verapamil solutions was >96 % and was checked before administration into rats by HPLC [5].

In-vivo studies

The animals were divided into three different dose levels of 20, 40 and 80 mg/kg. Within each dose level, the animals were further sub-divided into a control group and a pre-treated group. Rats in control group received irinotecan orally via gavage using a ball-tipped needle. The pre-treatment group received oral verapamil (25 mg/kg), 2 h prior to irinotecan administration. In the 20 mg/kg-dose group, three additional rats received the irinotecan only as single dose injection via the lateral tail vein. Blood samples were withdrawn prior to dosing and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post-dosing from retro-orbital plexus into microtubes containing the anti-coagulant [5]. Plasma was obtained immediately and processed by protein precipitation. Samples were stored at -80 °C until HPLC analysis.

Analytical Assay Procedures

The concentrations of irinotecan and SN-38 in plasma were determined using high performance liquid chromatographic (HPLC) method using ultraviolet detection (λ_{max} = 254 nm, 380 nm) as described previously [6]. The sample pre-treatment from plasma involved a single protein precipitation step with cold acetonitrile. Topotecan, a structurally related camptothecin, was used as an internal standard. Method was found to be selective, linear ($R^2 \approx 0.999$), accurate (recovery ± 15 %) and precise (<5 % C.V.) in the selected concentration ranges for both the analytes. The quantification limit for irinotecan was 40 ng ml^{-1} and for SN-38 was 25 ng ml^{-1} [6].

Pharmacokinetic data analysis

Pharmacokinetic parameters were calculated by non-compartment model using WinNon-Lin 5.0 programme (Pharsight, Mountain View, CA, USA). The plasma irinotecan and SN-38 concentration-versus-time curves were used to determine maximum plasma concentration (C_{max}), time to achieve maximum plasma concentration (t_{max}), mean residence time (MRT), area under the concentration time curve to the respective sampling point (AUC0-t), volume of distribution (V_d), elimination rate constant (K_{el}), half-life ($t_{1/2}$) and total body clearance. C_0 was the initial plasma concentration of drugs obtained by back-extrapolation to y-axis. The absolute bioavailability (F) of irinotecan after the oral administration (80 mg/kg) compared to the intravenous (I.V) administration (20 mg/kg) was calculated as follows:

$$F = \frac{AUC_{oral}\ \text{I.V Dose x 100}}{AUC_{IV}\ \text{Oral Dose}} \quad (1)$$

Statistical analysis

Data of five different experiments/animals was reported as mean ± standard error of means (S.E.M) unless otherwise noted. Statistical analysis was performed using GraphPad prism software version 4.0 (San Diego, CA, USA). Student's unpaired t-test was used to test the significance of differences between the controls and treated groups. Differences between the concentration time profiles over the entire range tested were analyzed by two-way ANOVA (Bonferoni post test). The differences were considered to be significant at $P < 0.05$.

Results

Plasma concentration versus time curves of irinotecan following oral administration at three dose levels (20, 40 and 80 mg/kg) in the absence and presence of a concomitant verapamil oral dose (25 mg/kg) are depicted in Figure 1. Tables 1, 2 and 3 show the pharmacokinetic parameters for each dose and administration route. The plasma concentrations of irinotecan following oral administration reached peak values at the 2-3 h, indicating that the absorption of irinotecan is from the intestine. Irinotecan was absorbed rapidly after oral administration and the observed time to peak irinotecan and SN-38 levels was within 2 h of administration. After an initial absorption phase, plasma concentrations of irinotecan declined

monoexponentially. Verapamil administration was associated with an increase in irinotecan and SN-38 plasma concentrations following both i.v and oral administration. Oral irinotecan concentrations increased significantly leading to pronounced alteration in the pharmacokinetics. The overall AUC was raised paralleled with a reduction in irinotecan CL and increase in half-life ($P < 0.05$). The oral bioavailability of irinotecan was 30-40 % without verapamil, which was increased significantly when given with verapamil.

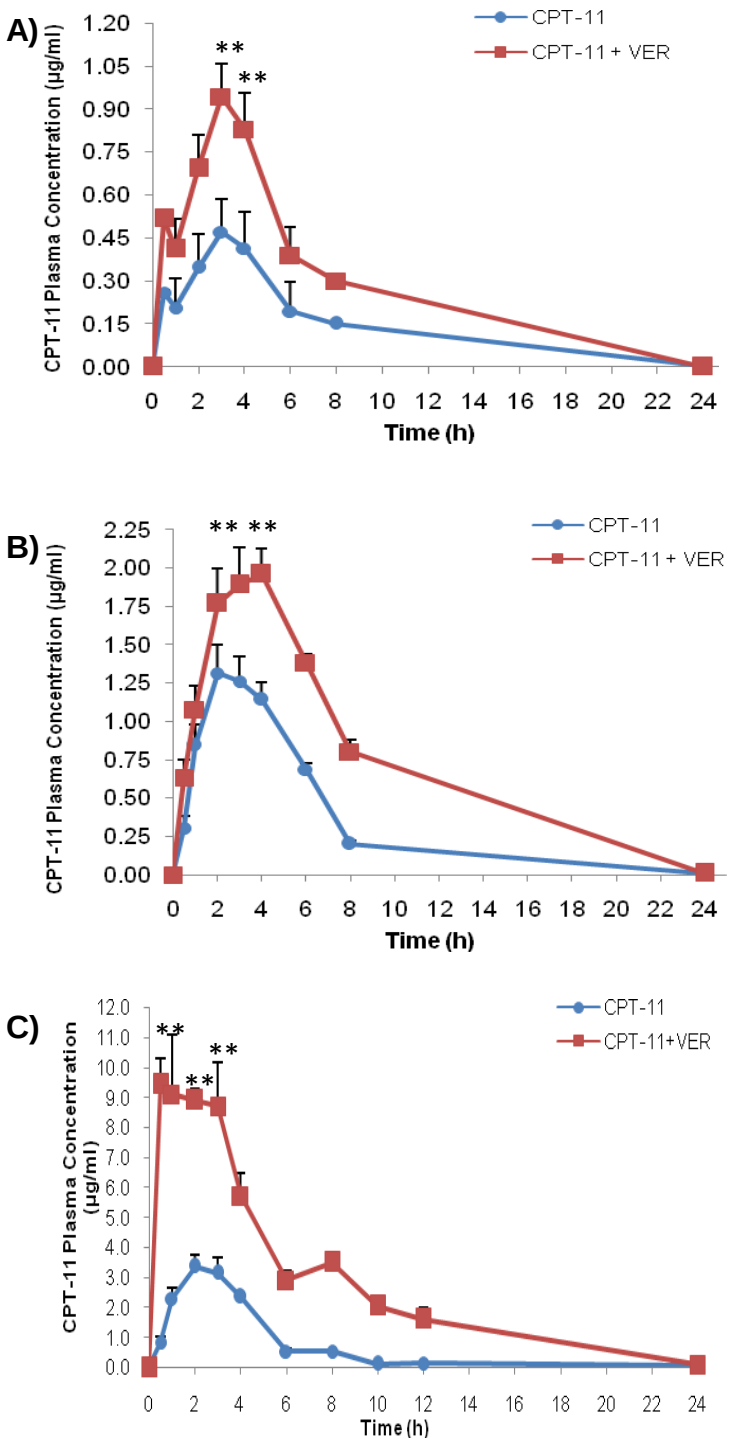

Figure 1. Mean plasma irinotecan (CPT-11) concentration-time plot for A) 20, B) 40 and C) 80 mg/kg after oral administration in control (-●-) and in verapamil (VER) treated (-■-) rats. Verapamil at 25 mg/kg was administered orally 2 h prior to irinotecan dosing. Each data point represents the mean ± SEM of five different rats. **Statistical significant difference between control and verapamil treated rats, $p<0.01$

Table 1. Pharmacokinetic parameters of irinotecan with and without pre-treatment with oral verapamil (25 mg/kg), after oral and intravenous (20 mg/kg) administration of irinotecan in rats [5].

PK parameters of Irinotecan	Irinotecan Dose			
	20 mg/kg, i.v	20 mg/kg, Oral		
	Control	Control	Treated	Change
$AUC_{0\text{-last}}$ (h µg/ml)	10.76 ± 2.0	3.51 ± 1.20	7.84 ± 1.20*	+220 %
C_0 (µg/ml)	9.51 ± 1.4	--	--	
C_{max} (µg/ml)	2.47 ± 0.41	0.63 ± 0.27	1.05 ± 0.23*	+66 %
t_{max} (h)	--	3.21 ± 0.95	2.94 ± 1.17	
MRT (h)	--	5.52 ± 0.20	5.49 ± 0.11	
$t_{1/2}$ (h)	3.1 ± 0.60	3.24 ± 0.22	3.11 ± 0.06	
CL_{obs} (ml/h/kg)	1206.4 ± 159.7	5664.8 ± 363.9	2573.8 ± 498.4*	-54 %
$V_{ss,obs}$ (ml/kg	4852.2 ± 703.8	27368.4 ± 2440.9	12115.4 ± 3105.5*	-56 %
K_{el} (h^{-1})	0.232 ± 0.05	0.214 ± 0.0.02	0.214 ± 0.0.02	
F (%)		32	72	

Each value represents the mean ± SEM of five rats.
*Statistical significant difference between control and verapamil treated rats, *P*<0.05

Table 2. Pharmacokinetic parameters of irinotecan with and without pre-treatment with oral verapamil (25 mg/kg), after oral (40 mg/kg) administration of irinotecan in rats.

PK parameters of Irinotecan	Irinotecan Dose		
	40 mg/kg, Oral		
	Control	Treated	Change
$AUC_{0\text{-last}}$ (h*µg/ml)	8.81 ± 1.93	19.94 ± 2.39*	+126%
C_{max} (µg/ml)	1.48 ± 0.57	2.24 ± 0.54	+51%
t_{max} (h)	2.33 ± 0.58	4.67 ± 1.15	
MRT (h)	5.21 ± 0.63	6.19 ± 1.10	
$t_{1/2}$ (h)	4.25 ± 2.08	3.59 ± 1.84	
CL_{obs} (ml/h/kg)	4578.7 ± 1073.02	1981.9 ± 299.7*	-56%
$V_{ss,obs}$ (ml/kg	26088.9 ± 6232.1	9761.5 ± 3385.9*	-62%
K_{el} (h^{-1})	0.186 ± 0.07	0.223 ± 0.09	
F (%)	41	93	

Each value represents the mean ± SEM of five rats.
*Statistical significant difference between control and verapamil treated rats, P<0.05

Table 3. Pharmacokinetic parameters of irinotecan with and without pre-treatment with oral verapamil (25 mg/kg), after oral (80 mg/kg) administration of irinotecan in rats [5].

PK parameters of Irinotecan	Irinotecan Dose		
	80 mg/kg, Oral		
	Control	Treated	Change
$AUC_{0\text{-last}}$ (h*µg/ml)	14.03 ± 2.18	61.71 ± 15.0**	+440%
C_{max} (µg/ml)	2.93 ± 0.37	10.75 ± 1.0*	+266%
t_{max} (h)	2.6 ± 0.89	1.75 ± 1.8	
MRT (h)	3.6 ± 0.69	5.13 ± 1.48	
$t_{1/2}$ (h)	2.24 ± 0.51	4.18 ± 1.2*	
CL_{obs} (ml/h/kg)	5613.8 ± 1126.3	897.09 ± 177.9*	-84%
$V_{ss,obs}$ (ml/kg	32197.0 ± 8067.1	7571.1 ± 151.3*	-76%
K_{el} (h^{-1})	0.293 ± 0.09	0.17 ± 0.05*	
F (%)	33	143	

Each value represents the mean ± SEM of five rats.
*Statistical significant difference between control and verapamil treated rats, P<0.05

The plasma concentrations versus time curves of SN-38, after oral administration of irinotecan at three doses are shown in Figure 2. Oral pharmacokinetic parameters of SN-38 were significantly modified by verapamil ($P < 0.05$) (Table 4). SN-38 showed biphasic plasma disposition after oral administration, with a terminal half-life of about 1-2 h.

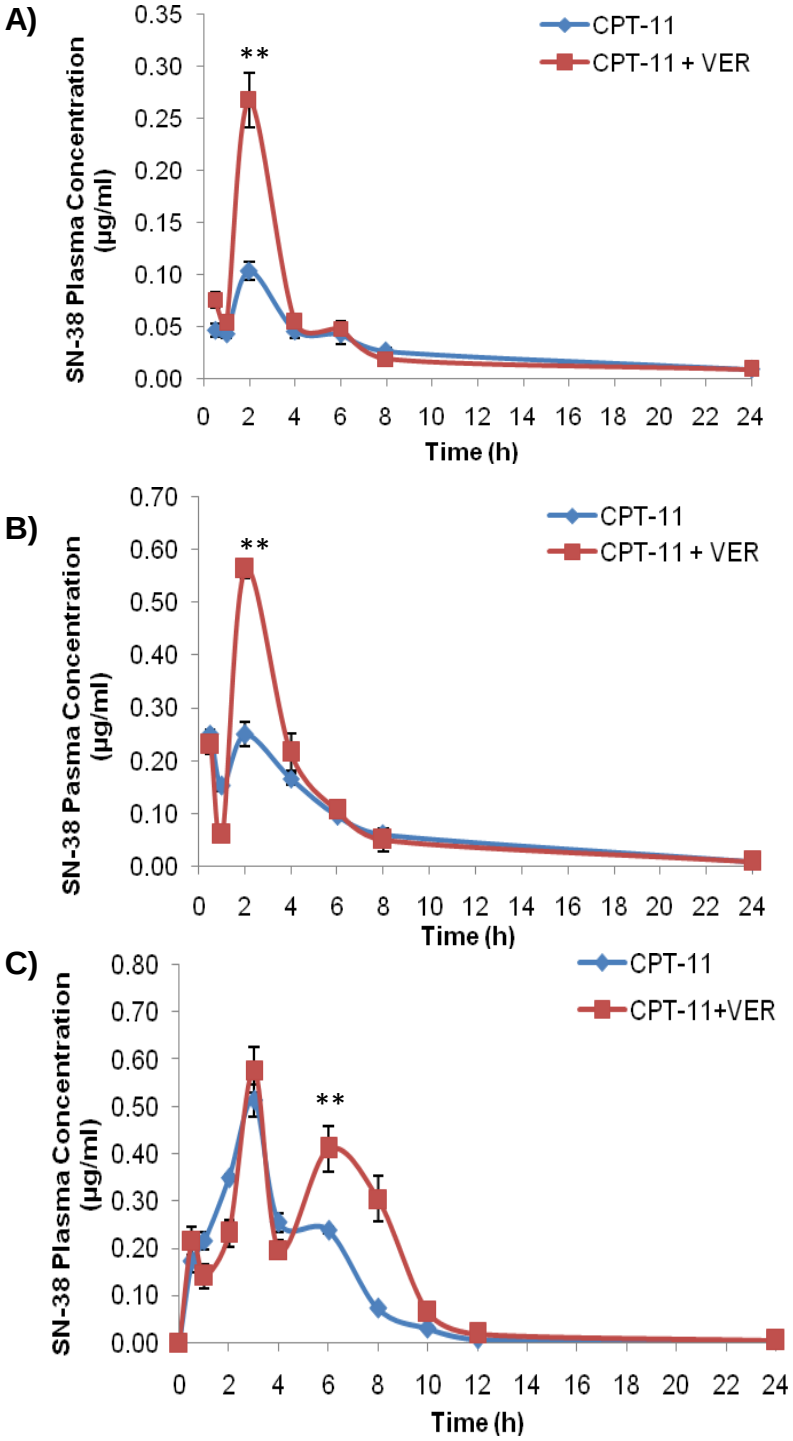

Figure 2. Plasma concentrations of SN-38 following irinotecan (CPT-11) administration in control and verapamil (VER) treated female Wistar rats. The control group was given an oral dose of A) 20, B) 40 and C) 80 mg/kg irinotecan only (-♦-). The treated (-■-) were administered verapamil at 25 mg/kg orally 2 h prior to irinotecan dosing. Each data point represents the mean ± SEM of five different rats. **Statistical significant difference between control and verapamil treated rats, $p<0.01$

Table 4. Pharmacokinetic parameters of SN-38, major metabolite of irinotecan, with and without pre-treatment with oral verapamil (25 mg/kg), after oral (20, 40 and 80 mg/kg) and intravenous (20 mg/kg) administration of irinotecan in rats

Irinotecan Dose	Groups	PK parameter of SN-38				
		$AUC_{0\text{-last}}$ (h µg/mL)	**C_0 (µg/ml)**	**C_{max} (µg/ml)**	**t_{max} (h)**	**$t_{1/2}$ (h)**
20 mg/kg, i.v	Control	8.7 ± 0.49	2.10 ± 0.61	--	--	1.1 ± 0.16
20 mg/kg, Oral	Control	0.70 ± 0.026	--	0.10 ± 0.01	2	2.18 ± 0.69
	Treated	0.94 ± 0.08*	--	0.27 ± 0.03*	2	2.83 ± 1.11
	Change	**+34 %**		**+170 %**		
40 mg/kg, Oral	Control	1.76 ± 0.01	--	0.26 ± 0.01	1.0 ± 0.87	2.56 ± 0.19
	Treated	2.30 ± 0.30*	--	0.56 ± 0.02*	2	2.35 ± 0.73
	Change	**+30 %**		**+115 %**		
80 mg/kg, Oral	Control	1.81 ± 0.30	--	0.48 ± 0.07	2.8 ± 0.45	1.47 ± 0.35
	Treated	2.99 ± 0.34*	--	0.62 ± 0.06*	4.2 ± 1.64	1.28 ± 0.26
	Change	**+65 %**		**+29 %**		

Discussion

Oral anti-cancer chemotherapy has gained wide acceptance and became standard approach for the treatment of cancer due to various advantages such as greater safety and flexibility, more convenient and cost-effectiveness. Unfortunately, the majority of anticancer drugs have a low and highly variable oral bioavailability making intravenous route as the only alternative. Data available on irinotecan absorption and disposition showed discouraging results with variable absorption, poor efficacy and toxicity profiles [7]. As a result novel formulations of irinotecan exhibiting better oral absorption and bioavailability need to be developed. Verapamil is the most extensively characterized P-gp inhibitor and multi-drug resistance (MDR) reversal agent that has entered clinical trials [8]. Therefore, the effect of co-administration of verapamil on the oral bioavailability and pharmacokinetics of irinotecan at various doses was the subject of current investigation.

The irinotecan AUC increased linearly with dose from 20 to 80 mg/kg indicating linear pharmacokinetics. After treatment with verapamil, irinotecan increased linearly with dose from 20 to 40 mg/kg, but from 40 to 80 mg/kg the irinotecan AUC increased non-linearly (i.e. twofold increase in dose, sevenfold increase in AUC). Overall, the oral combination of irinotecan with verapamil was well tolerated in rat without acute toxicities. A disproportionate increase in oral bioavailability was seen at 80 mg/kg with the same dose of P-gp inhibitor (four fold increase in irinotecan dose resulted in sevenfold increase in AUC).

The increase in systemic exposure to irinotecan in combination with verapamil is of the same order of magnitude as that with high dose of irinotecan. P-gp inhibition may enable chronic oral therapy with irinotecan. However, safe P-gp inhibitors need to be identified as alternatives to verapamil. This was tested by decreasing the dose in the same proportion as clearance of irinotecan is decreased by verapamil administration.

Irinotecan has been reported to undergo metabolic saturation (non-linear clearance) at higher concentrations which were achievable in this study, by verapamil pre-treatment [9]. The irinotecan

clearance in the control groups was unaffected by the increase in dose and remained almost constant. The decrease in irinotecan clearance following verapamil pre-treatment is ~50 % at both 20 and 40 mg/kg. However, ~80 % decrease in irinotecan clearance is observed at 80 mg/kg.

Hydrolysis of irinotecan by carboxylesterase enzyme has been reported to be responsible for the conversion of irinotecan to SN-38 [7]. The detecachievabletion of SN-38 at early time points may be a consequence of the presence of carboxylesterase in rat plasma, first pass metabolism of irinotecan or metabolism of irinotecan by intestinal carboxylesterase, and subsequent absorption of SN-38. The less than proportional increase in SN-38 AUC from 20 to 80 mg/kg is consistent with the saturation of carboxylesterase [9].

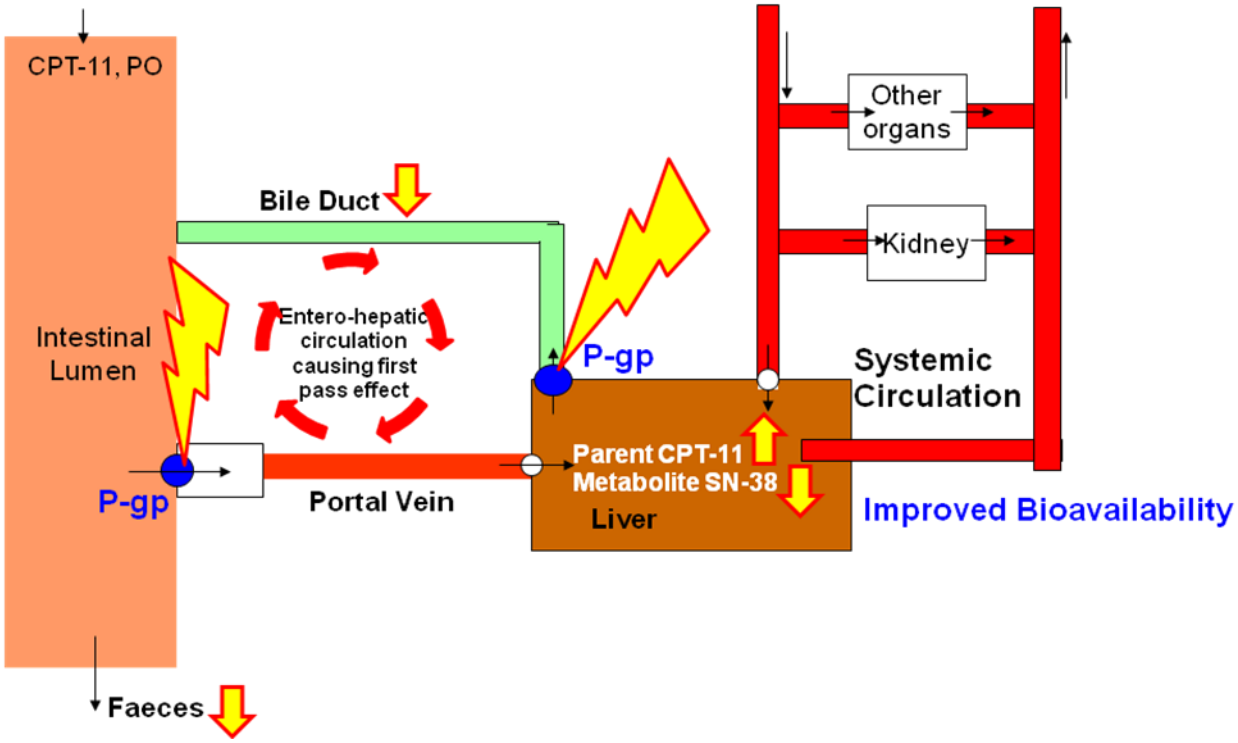

Figure 3. Role of P-gp in determining pharmacokinetics of irinotecan (CPT-11) after oral and intravenous (I.V) administration. P-gp is present in intestinal epithelium affecting absorption; in hepatocytes affecting metabolism and at biliary canalicular surface affecting excretion. In addition, gastro-intestinal toxicity of irinotecan is proposed to be caused by biliary excretion of its major active (100-1000 times) metabolite, SN-38; and its glucuronide (SN-38G). P-gp inhibition at both intestine and bile eliminates first pass effect, resulting in increased oral absorption and systemic exposure. Diarrhea could also be ameliorated due to inhibition of biliary excretion of metabolite causing decreased accumulation

In conclusion, P-gp in the gastro-intestinal mucosa limits the absorption of orally administered xenobiotics and at least in part, due to its high affinity for P-gp efflux pump. The observed effect may be beneficial in a way to develop oral irinotecan dosage forms using safe P-gp inhibitors to improve its oral bioavailability. Clinically, concomitant verapamil and irinotecan treatment would allow dose reduction (about 50-80 %) and still achieve comparable exposure of irinotecan. More importantly, the risk of intestinal toxicity could be substantially reduced because of reduced dose and lowered biliary secretion and accumulation (Figure 3). The results of this study could be utilized to evaluate different dosing strategies and methods of administration for irinotecan in humans. Oral formulations of irinotecan having better efficacy and less toxicity could be developed using appropriate P-gp inhibitor. However, further

studies are required to compare the efficacy of verapamil with other novel P-gp inhibitors to enable pharmacokinetic modulation of irinotecan and its metabolites.

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