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ORIGINAL ARTICLE

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# Modulation of glucuronidation of SN-38, the active metabolite of irinotecan, by valproic acid and phenobarbital

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**Abstract** *Purpose*: Irinotecan (CPT-11) is hydrolyzed to its active metabolite SN-38 which is subsequently conjugated by uridine diphosphate glucuronosyl transferase (UDP-GT) to the glucuronide (SN-38G). Both preclinical and clinical data indicate that conjugation is a primary clearance mechanism for SN-38 with the plasma glucuronide levels being substantially higher than those of SN-38. This investigation was designed to determine the possibility of modulation of glucuronidation of SN-38 and its effect on the disposition of the parent drug and metabolites. *Methods*: Female Wistar rats were pretreated with 200 mg/kg valproic acid (VPA), an inhibitor of glucuronidation, 5 min prior to the administration of 20 mg/kg irinotecan. The control rats were given 20 mg/kg irinotecan only. To study the effect of inducers of UDP-GT activity, rats were pretreated with phenobarbital (PB) before irinotecan administration. *Results*: Pretreatment with VPA caused a 99% inhibition in the formation of SN-38G leading to a 270% increase in the area under plasma concentration-time curve (AUC) of SN-38 compared with the control rats. The irinotecan estimations were unchanged in the two groups. PB pretreatment caused a 1.7-fold increase in the AUC of SN-38G and a concomitant 31% and 59% reduction in the AUCs of SN-38 and irinotecan, respectively. *Conclusions*: The most plausible explanation for the alterations in SN-38G disposition is inhibition of SN-38 conjugation by VPA and induction of the conjugation by PB.

**Key words** SN-38 · Glucuronidation · Inhibition · Induction · Pharmacokinetics

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## Introduction

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy camptothecin, CPT-11) is a topoisomerase I inhibitor that has been approved in the US, Japan and France. The drug undergoes hydrolysis by the enzyme carboxyl esterase to form the primary metabolite SN-38 (7-ethyl-10-hydroxy camptothecin) [22] which is more cytotoxic than irinotecan and is considered responsible for both the activity and the toxicity of the drug [12]. SN-38 is conjugated by uridine diphosphate glucuronosyl transferase (UDP-GT) at the $C_{10}$ position to form the glucuronide, SN-38G [2]. Thus SN-38 concentrations in the plasma are a resultant, in part, of the rate of formation from irinotecan and the rate of elimination as the glucuronide. In humans, SN-38G plasma concentrations exceed those of SN-38 following irinotecan infusion, and in some patients the ratio of SN-38G to SN-38 is greater than ten, indicating that conjugation is a primary clearance mechanism for SN-38 [6, 15, 18]. A wide range in areas under the curve (AUC) of SN-38G and in the ratios of the AUCs of SN-38 to SN-38G is suggestive of interpatient variability in the rate of conjugation of SN-38.

Renal excretion of irinotecan and metabolites accounts, on average, for $<20\%$ of the dose. On the other hand, concentrations of irinotecan, SN-38 and SN-38G in the bile have been reported to be severalfold higher than plasma concentrations, and about 26% and 53% of an irinotecan dose has been recovered in the bile of two patients [5, 18, 19]. These observations

are in agreement with preclinical studies which show substantially higher biliary elimination compared with renal excretion, in the rank order irinotecan > SN-38G > SN-38 [2, 11]. In a previous report we have suggested that increased SN-38 concentrations in the gut, a potential consequence of low glucuronidation and therefore high biliary excretion, is related to the incidence of diarrhea, the major dose-limiting toxicity of irinotecan therapy [6]. Recently, we validated the applicability of a biliary index in a total of 40 patients receiving irinotecan at a dose of 145 mg/m$^2$ [7]. Thus overall, glucuronidation is an important variable in the determination of the activity (plasma SN-38 concentrations) as well as the toxicity (biliary SN-38 concentrations) attributed to irinotecan. This investigation was designed to determine the influence of an inhibitor and an inducer of UDP-GT conjugation on the disposition of irinotecan and its metabolites.

Valproic acid (VPA, 2-propylpentanoic acid, Depakene) is extensively conjugated in humans and inhibits glucuronidation of other substrates when administered concomitantly. VPA inhibits parahydroxyphenobarbital glucuronidation by rat liver microsomes [21]. Administration of VPA decreases the formation clearance of lorazepam glucuronide, the primary metabolite of lorazepam [1] and increases the oral bioavailability of zidovudine by inhibiting its glucuronidation [14]. Sodium valproate acutely inhibits lamotrigine conjugation leading to reduced clearance [25]. The initial portion of our investigation was to determine the effect of VPA on SN-38 glucuronidation.

Phenobarbital (PB) induces UDP-GT in rat livers and UDP-GT isozymes have been delineated based on differential induction by PB [3, 24]. Human livers treated with PB display significantly higher UDP-GT activities towards 1-naphthol, 4-methylumbelliferone and bilirubin compared with untreated livers [4]. PB-inducible UDP-GT has been shown to be responsible for the glucuronidation of zidoverdine in human and rat liver microsomes [8]. A recombinant PB-inducible rat liver UDP-GT 2B1 has been reported to catalyze the formation of both ester and ether glucuronides [17]. The next objective of our investigation was to determine the inducibility of SN-38 UDP-GT activity by PB.

## Materials and methods

### Materials

Irinotecan solution was a gift from the Yakult Honsha Co., Tokyo, Japan. VPA (sodium salt) was purchased from Sigma Chemical Co. (St. Louis, Mo.), and PB was obtained from Elkins-Sinn (Cherry Hill, N.J.). All other chemicals were of analytical grade and purchased from Fisher Scientific Co. (Itasca, Ill.).

### Animal protocol

Experimental procedures were in accordance with the guidelines of The University of Chicago Manual on Laboratory Animals prepared by the Animal Care Committee. Female Wistar rats (about 200 g) with a permanent catheter implanted in the right jugular vein were purchased from Charles River Breeding Laboratories (Wilmington, Mass.). The rats were placed in metabolism cages for a period of at least 24 h before the experiments and food and water were supplied *ad libitum*. For the investigations, the animals were divided into two groups: control and pretreated. The control animals were given an intravenous bolus dose of irinotecan at a dose of 20 mg/kg through the catheter. To test the effect of VPA, the pretreated animals were given an intravenous bolus dose of 200 mg/kg VPA 5 min prior to a 20 mg/kg dose of irinotecan. For the induction experiments, the pretreated rats were given 100 mg/kg PB intraperitoneally on day 1 and received 0.1% w/v in drinking water for 4 days, as described by Bock et al. [4]. The catheter was flushed with physiologic saline after each administration of dosing solution to prevent contamination. Blood samples of about 200 µl were withdrawn through the catheter at predose and at 3, 5, 10, 15, 30, 60, 120, 240 and 360 min following irinotecan administration. After each withdrawal, an equal volume of physiologic saline was injected through the catheter. Plasma was immediately separated following centrifugation of the samples at 2500*g* for 10 min and stored at −70 °C until analysis. Urine samples from control, VPA- and PB-pretreated groups were collected for 24 h after the administration of irinotecan. Based on a mean terminal half-life of 1.7 h observed in rats [11], >99% of the dose would be eliminated during this time period. Total irinotecan, SN-38 and SN-38G concentrations in plasma samples and in 1:5-diluted urine samples were quantitated as described previously [6]. The limit of detection was approximately 5 ng/ml for irinotecan and SN-38. The concentrations of SN-38G were expressed in SN-38 equivalents. The assay was reproducible with <10% intra- and interday coefficients of variation.

### Data analysis

The plasma concentration-time profile was analyzed using noncompartmental methods by PCNONLIN (v 4.2, SCI, Lexington, Ky.). Maximal plasma concentrations ($C_{max}$) were obtained by visual estimation of the plasma concentration-time profiles. Terminal half-life ($T_{1/2}$) was calculated by dividing 0.693 by $\lambda_z$, the slope obtained by log-linear regression of the terminal phase of the plasma concentration-time profile. Clearance was determined as the ratio of the dose and the AUC. $F_e$ was the amount of unchanged irinotecan, SN-38 or SN-38G in the urine.

The nonparametric Mann Whitney test was used to test for differences in the pharmacokinetic parameter estimates between the control and the pretreated groups with a two-sided significance level of $<0.05$.

## Results

### Effect of VPA pretreatment

Comparison of plasma profiles in control and VPA-pretreated rats is shown in Fig. 1. The plasma concentrations of irinotecan and SN-38 were similar to those of a previously reported rodent study by Kaneda and Yokokura [11]. In the control and pretreated groups, the irinotecan disposition curves were almost superimposable. On the other hand, VPA caused dramatic

changes in the plasma concentrations of the two metabolites. The effect on SN-38 appeared to be almost instantaneous with significantly elevated concentrations being achieved within 3 min (first sampling time-point) and sustained throughout the 6-h sampling period. In sharp contrast, the glucuronide concentrations reduced dramatically and were undetectable within 15 min of irinotecan administration, resulting in an overall 99% reduction in plasma availability.

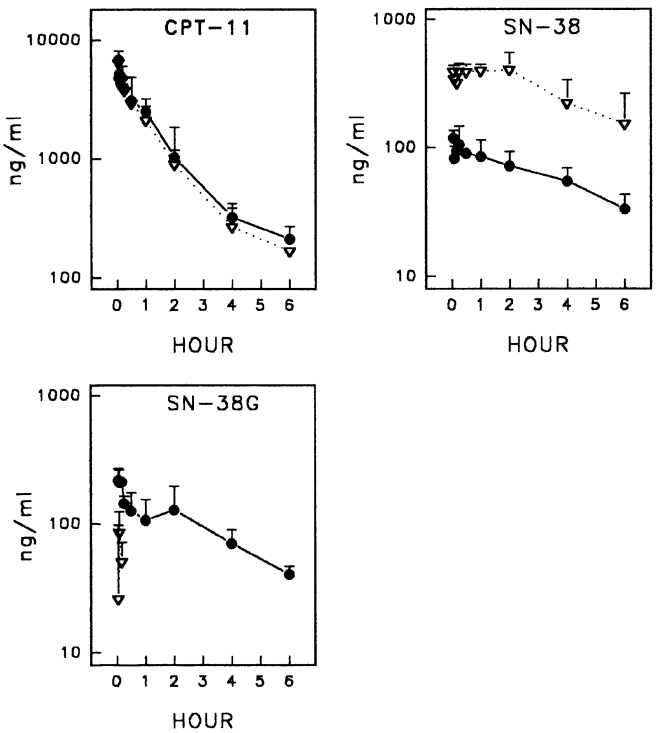


**Fig. 1** Plasma concentrations of irinotecan, SN-38 and SN-38G in 20 mg/kg irinotecan-treated control rats (*circles*), and in 200 mg/kg VPA-pretreated rats (*triangles*). Values are means + SD

**Table 1** Effect of VPA and PB on the disposition of irinotecan, SN-38 and SN-38G. Control rats were given an intravenous bolus dose of 20 mg/kg irinotecan. VPA-pretreated rats were given 200 mg/kg VPA 5 min prior to 20 mg/kg irinotecan. PB-pretreated rats were given an intraperitoneal dose of 100 mg/kg PB followed by 0.1% w/v in drinking water for 4 days before 20 mg/kg irinotecan. Values are means ± SE

| | Control ($n = 4$) | VPA-pretreated ($n = 3$) | PB-pretreated ($n = 3$) |
|---|---|---|---|
| Irinotecan | | | |
| 1. AUC (ng.hr/ml) | 7960 ± 1372 | 6028 ± 1556 | 3298 ± 237* |
| 2. CL (l/h/kg) | 2.62 ± 0.43 | 3.45 ± 0.71 | 6.12 ± 0.43* |
| 3. $T_{1/2}$ (h) | 1.18 ± 0.06 | 0.99 ± 0.21 | 1.23 ± 0.40* |
| 4. $F_e$ (% dose) | 27.0 ± 2.11 | 29.6 ± 9.61 | 22.5 ± 4.09 |
| SN-38 | | | |
| 1. $T_{1/2}$ (h) | 3.36 ± 0.37 | 3.06 ± 1.35 | 3.66 ± 1.11 |
| 2. AUC (ng.h/ml) | 532 ± 58.0 | 1971 ± 540* | 365 ± 102* |
| 3. $F_e$ (% dose) | 1.13 ± 0.15 | 2.03 ± 0.24* | 1.41 ± 0.96 |
| SN-38G | | | |
| 1. $T_{1/2}$ (h) | 3.26 ± 0.69 | 0.05 ± 0.03* | 2.04 ± 0.19 |
| 2. AUC (ng.h/ml) | 754 ± 37.9 | 8.75 ± 6.76* | 1298 ± 339* |
| 3. $F_e$ (% dose) | 2.13 ± 0.05 | 0.70 ± 0.18* | 2.86 ± 0.97 |

* $P < 0.05$ vs control group

As evident from the plasma disposition curves, there were no changes in the pharmacokinetic parameters of irinotecan in the pretreated group compared with the control group (Table 1). While the elimination rate of SN-38 did not change, there was a 3.4- and 3.7-fold enhancement in the $C_{max}$ and AUC, respectively, in the pretreated group compared with the controls. On the other hand, both the elimination half-life and the plasma AUC were significantly reduced in the pretreated SN-38G profiles. The urinary concentrations reflected, to a certain extent, the plasma AUC of the drug and metabolites. In the pretreated group, the amount of irinotecan in the urine remained unchanged, while SN-38 increased by 80% and SN-38G decreased by 67%.

## Effect of PB pretreatment

PB pretreatment caused a 72% enhancement in the SN-38G AUC (Fig. 2). Concurrently there was a 31% and a 59% reduction in the AUCs of SN-38 and irinotecan, respectively (Table 1). The effect of PB pretreatment was also evident from the initial sampling time-point. The change in irinotecan concentrations

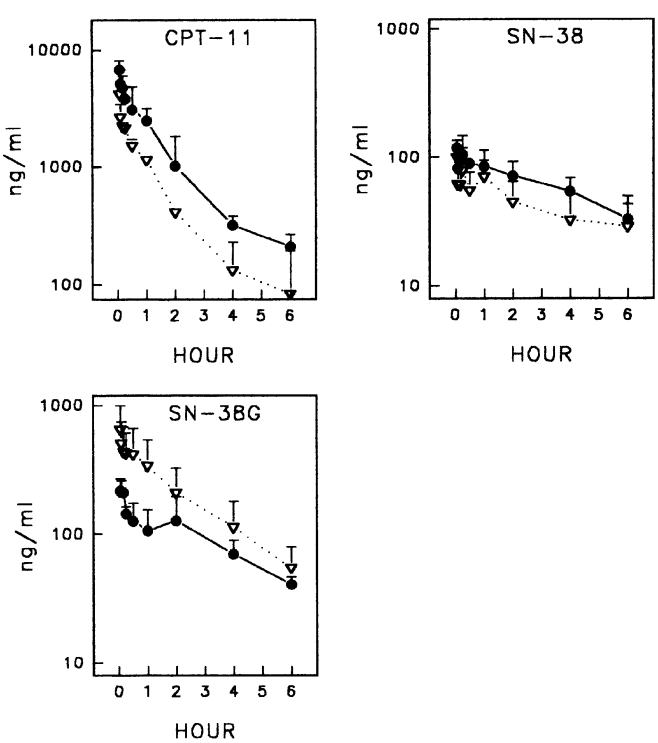


**Fig. 2** Plasma concentrations of irinotecan and metabolites in PB-pretreated rats (*triangles*) compared to control rats (*circles*). Values are means + SD

was sustained over the 6-h sampling interval while that of SN-38 and SN-38G approached control concentrations by the end of the sampling interval. In a similar way to the parent drug, the elimination half-lives of the two metabolites remained unchanged. There were no significant differences in the urinary recovery of irinotecan and metabolites between the control and pretreated groups.

## Discussion

This investigation showed the altered disposition of irinotecan and its metabolites by probable modulation of the intrinsic clearance of SN-38. The possible mechanisms involved were the inhibition and the induction of UDP-GT conjugation of SN-38 by VPA and PB, respectively.

VPA has been shown to inhibit hepatic UDP-GT conjugation both by reversible, noncompetitive and by competitive mechanisms [10, 21]. In mice, VPA causes about 90% depletion in UDP-glucuronic acid within 7 to 15 min of drug administration [10]. However, this noncompetitive inhibition is quickly reversible, with UDP-glucuronic acid levels approaching pretreatment levels by 1 h. Understanding of the exact mechanisms involving inhibition of SN-38 conjugation requires further investigation.

Apart from the inhibition of UDP-GT conjugation, VPA and its metabolites exert a prominent choleretic effect in rats [23]. The effect is dependent on the osmotic activity of the drug and metabolites following biliary excretion. This would also mean that VPA and its metabolites could possibly compete with irinotecan and metabolites for canalicular transport. If this were the case, the biliary excretion of irinotecan, SN-38 and SN-38G would be reduced and the corresponding plasma concentrations would be elevated. This phenomenon may explain the increment in SN-38 concentrations but would not account for the unchanged and decreased concentrations of irinotecan and SN-38G, respectively. Thus VPA-induced alteration in biliary transport of irinotecan and metabolites would not completely explain our findings.

In addition to induction of UDP-GT activity, PB pretreatment in rats causes a two fold increase in the bile secretion rate [20] which is mediated by an increase in the bile salt-dependent flow [13]. Furthermore, PB pretreatment in rats also significantly induces carboxyl esterase activity in plasma and liver [9, 16]. The diminished irinotecan concentrations could be a consequence of augmented biliary excretion coupled with enhanced esterase activity following PB pretreatment. Decreased SN-38 AUC, in spite of induced esterase activity, could be due to a faster rate of conjugation compared to de-esterification and increased elimination in the bile. However, SN-38G is also substantially excreted in the bile and PB-induced increased biliary excretion would not cause the significant enhancement of the glucuronide concentrations. Thus the most plausible explanation for the PB-related SN-38G alterations was induction of UDP-GT activity which markedly increased the conjugation of SN-38. The changes in plasma concentrations were, however, not accurately reflected in the urinary concentrations owing to the low renal elimination rates of the drug and metabolites. Overall, it appears that PB-inducible forms of UDP-GT are responsible for the glucuronidation of SN-38. However the effect of other inducers such as 3-methylcholanthrene, clofibrate, and phenytoin remain to be investigated.

In conclusion, SN-38 UDP-GT activity can be modulated by VPA and PB which could have important clinical implications. VPA coadministration with irinotecan would result in increased bioavailability of the active metabolite SN-38 but would also substantially increase the risk of dose-limiting intestinal toxicity. Therefore when coadministered with potential inhibitors of UDP-GT, the dosing of irinotecan would need to be cautiously monitored. On the other hand, concomitant administration of PB may be beneficial to patients with compromised liver function and could probably reduce toxicity as a result of the increased detoxification of SN-38. Futher investigation of the influence of other potent modulators of UDP-GT activity on the pharmacokinetics of irinotecan is warranted.

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