

PERSPECTIVE

Pharmacogenomics of Human UDP-Glucuronosyltransferases and Irinotecan Toxicity

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The human UDP-glucuronosyltransferases (UGTs) represent a superfamily of proteins (Mackenzie et al., 1997) that are attracting much interest because of their extensive polymorphic patterns of gene and tissue specific expression (Mackenzie et al., 2000; Tukey and Strassburg, 2000). Because glucuronidation comprises a significant pathway for drug detoxification and elimination, polymorphic patterns of UGT expression are increasingly recognized as having an important role in the pharmacokinetics of drug disposition and elimination. In this issue of *Molecular Pharmacology*, Gagné et al. (2002) report on the contribution of human UGTs toward the metabolism of a carboxylesterase-formed metabolite of irinotecan (Slatter et al., 1997) [CPT-11 or 7-ethyl-10-[4-(1-piperidino)-1-piperidino (Camptosar) Pharmacia and Upjohn, Kalamazoo, MI] called 7-ethyl-10-hydroxycamptothecin (SN-38). Irinotecan is a camptothecin derivative (Iyer and Ratain, 1998; Garcia-Carbonero and Supko, 2002) anticancer agent (Firvida et al., 2001; Kakolyris et al., 2001; Ando et al., 2002; Vamvakas et al., 2002) that inhibits topoisomerase I activity (Creemers et al., 1994). Irinotecan has been approved for the standard therapy of colorectal cancer. It has shown favorable response rates as first and second line therapy of this common gastrointestinal type cancer. Although irinotecan possesses some topoisomerase I inhibitory activity, it must be considered a pro-drug because metabolism by tissue and serum carboxylesterases (Satoh et al., 1994) are required to generate the more active topoisomerase I inhibitor, SN-38 (Kawato et al., 1991).

Metabolism of SN-38 by glucuronidation is the primary route of detoxification leading to its elimination through biliary excretion (Atsumi et al., 1991). The presence of SN-38 as a β -D-glucopyranosiduronic acid in the bile renders the metabolite susceptible to subsequent metabolism by intestinal

β -glucuronidase, which removes the glucuronic acid from the aglycone allowing for reabsorption of SN-38 from the intestine (Atsumi et al., 1991) (Fig. 1). Although irinotecan is a promising chemotherapeutic agent, the most common unwanted side effects are bone marrow toxicity leading to abnormal blood counts, in particular leukopenia, and ileocolitis, which leads to diarrhea (Sasaki et al., 1995) (Fig. 2). Adverse effects are an important issue because they may limit therapeutic efficacy and may require discontinuation of an otherwise effective anticancer treatment. Glucuronidation of SN-38 has been theorized to lessen adverse effects of irinotecan. Higher plasma ratios of SN-38:SN-38G (SN-38 glucuronide) have been correlated with increased levels of both gastrointestinal and hematological toxicities (Atsumi et al., 1991), suggesting that the efficiency of SN-38 glucuronidation is an important determinant of toxicity. Because most, if not all of the human UGTs have been cloned and a good appreciation of their tissue specific distribution is now available, understanding the factors that lead to efficient SN-38 glucuronidation and clearance is now possible.

There are 16 genes that encode full-length UGT proteins (Mackenzie, 1995; Tukey and Strassburg, 2000). Eight are encoded by the *UGT1A* locus (1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10) (Ritter et al., 1992; Gong et al., 2001) and eight are encoded by *UGT2* genes (2A1, 2B4, 2B7, 2B10, 2B11, 2B15, 2B17, and 2B28). Gagné et al. (2002) demonstrate that stable expression of the majority of these gene products in tissue culture confirm that UGT1A1, UGT1A7, and UGT1A9 were the most efficient in glucuronidating SN-38, a finding that supports previous observations (Iyer et al., 1998; Ciotti et al., 1999). What distinguishes the work published by Gagné et al. (2002) in this issue of *Molecular Pharmacology* from previous reports is the important addition of expression experiments carried out with allelic variants of these UGTs. The authors carefully compare the catalytic efficiencies of

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expressed UGTs and conclude that selective allelic variants associated with *UGT1A1* and *UGT1A7* have a significant impact on the ability of these enzymes to contribute to SN-38 glucuronidation. The relevance of these findings to the pharmacokinetics of irinotecan in a clinical setting becomes apparent knowing that *UGT1A1* and *UGT1A7* are highly polymorphic. Importantly, allelic variation and reductions in overall glucuronidation capacity have been speculated to be a major determinant of the severity of the adverse reactions brought on by irinotecan therapy. At question is how this information will be useful in relating genetic predisposition to the risk for drug-mediated toxicity.

More than 50 genetic lesions in *UGT1A1* have been reported (Kadacol et al., 2000; Tukey and Strassburg, 2000), many of which are found in patients with Gilbert's syndrome. Gilbert's syndrome is characterized by mild nonhemolytic, unconjugated hyperbilirubinemia. One of the most common genotypes leading to Gilbert's syndrome is the inheritance of the promoter containing [A(TA)₇TAA] (*UGT1A1**28) which leads to approximately a 70% reduction in transcriptional activity compared with wild type *UGT1A1* as represented by a [A(TA)₆TAA] sequence. Although heterozygous carriers of *UGT1A1**28 polymorphisms do not display clinical signs of hyperbilirubinemia when other genetic alterations of the *UGT1A1* gene are absent (Bosma et al., 1995; Lampe et al., 1999), patients with a genotype either heterozygous or homozygous for the *UGT1A1**28 allele do exhibit attenuated expression of UGT1A1 and are predisposed to SN-38 initiated diarrhea (Ando et al., 2000). Hematological disorders associated with SN-38 have also been found to be irinotecan

dose-dependent, an observation that helps link blood toxicities with reduced levels of SN-38 glucuronidation as observed in those with Gilbert's syndrome. Standard dosing regimens given to patients with Gilbert's syndrome with mild hyperbilirubinemia display an increased area under the curve (AUC) of SN-38:SN-38G (Ando et al., 2002), a factor that is linked to leukopenia (Ando et al., 2000).

To further explore the impact of several *UGT1A1* allelic variants on SN-38 glucuronidation, Gagné et al. (2002) demonstrate that the expression of variants *UGT1A1**6, *UGT1A1**7, *UGT1A1**27, and *UGT1A1**35 elicit reduced SN-38 glucuronidation capacity. *UGT1A1**6 and *UGT1A1**27 are rare mutations that have been reported to lead to unconjugated hyperbilirubinemia compatible with the Gilbert syndrome phenotype, whereas inheritance of the homozygous *UGT1A1**7 predisposes persons to more severe hyperbilirubinemia, which represents the clinical picture of Crigler-Najjar's disease. In lieu of observations that patients with Gilbert's syndrome are predisposed to SN-38 initiated toxicity, persons who inherit genotypes leading to poor UGT1A1 activity may be highly susceptible to the toxic actions of irinotecan therapy. However, it is important to note that functional UGT1A1 protein is still synthesized in persons that are heterozygous for *UGT1A1* polymorphisms, and although a degree of unconjugated hyperbilirubinemia may be present this does not lead to a jaundiced phenotype in heterozygous persons. The *UGT1A1**28 polymorphism is present in about 40% of white persons, of whom only about 8% suffer from Gilbert's disease (Bosma et al., 1995; Lampe et al., 1999). When the high frequency of *UGT1A1* polymorphisms is taken into account, the real question is whether persons with compound

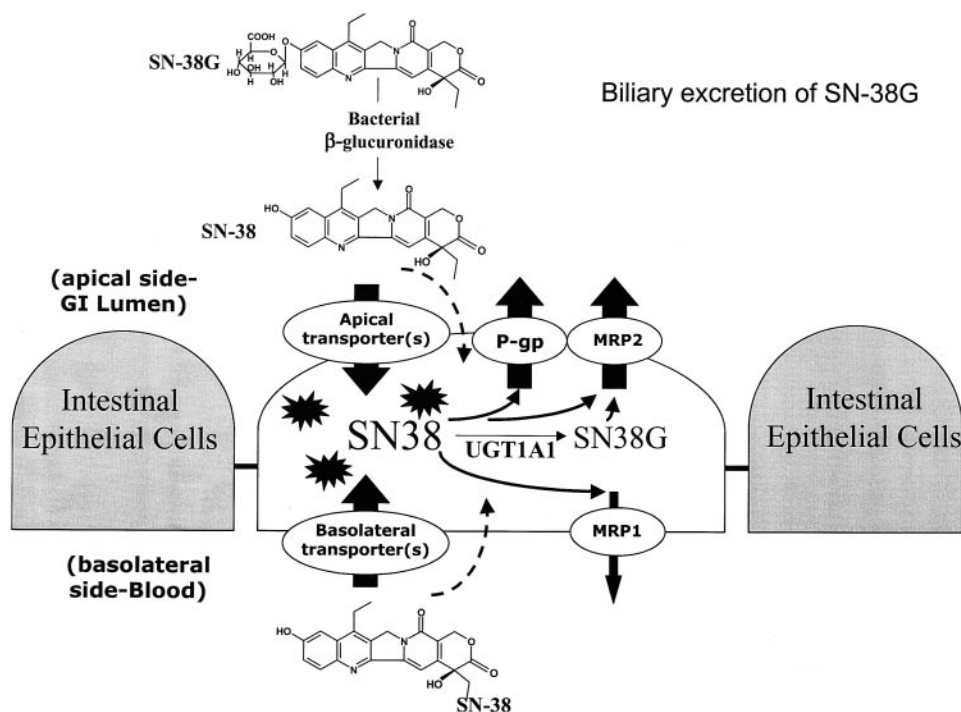


Fig. 1. Potential routes of SN38 exposure to intestinal epithelial cells. The routes of SN-38 exposure are shown to occur from both the brush border or apical side of the gastrointestinal tract as well as from the basolateral or blood side. Apical exposure of SN-38 is generated from cleavage of SN-38 glucuronide (SN-38G) by bacterial β-glucuronidase. Transport of SN-38 into epithelial cells has been shown in part to occur by passive diffusion (Kobayashi et al., 1999) (indicated by the dashed arrows entering the cell) in addition to active transport processes, which are indicated by the apical and basolateral transporters (Yamamoto et al., 2001). Efflux of SN-38 into the lumen occurs through P-glycoprotein and MRP2 and movement into the blood is facilitated by MRP1. Removal of SN-38 glucuronide (SN38G) from epithelial cells into the lumen is processed by MRP2. Any reduction in UGT1A1-initiated glucuronidation will lead to an accumulation of unconjugated SN-38, an event that may underlie the toxicity associated with SN-38 exposure.

heterozygote are frequent and are at a higher risk for SN-38–associated toxicity.

Whereas an elevated plasma SN-38 level resulting from a reduced glucuronidation capacity may explain SN-38–mediated hematological toxicity, the role of a reduced SN-38 glucuronidation capacity in SN-38 mediated intestinal toxicity is less clear. The majority of SN-38 in the intestine accrues through enterohepatic circulation, after cleavage of biliary-transported SN-38G by intestinal β -glucuronidases (Fig. 1). Only small amounts of SN-38 are directly excreted through the biliary systems (Slatter et al., 2000). Thus, elevated levels of SN-38 glucuronidation in the liver and other extrahepatic tissues are likely to lead to elevated SN-38 levels in the gastrointestinal tract. As the toxic actions of SN-38 result from contact of unconjugated drug with the intestinal mucosa, one would predict that reduced glucuronidation capacity in the liver and other tissues would lead to reduced levels of intestinal SN-38 and thus reduced toxicity. Reduced levels of hepatic glucuronidation carried out by UGT1A1 in patients with Gilbert's syndrome would naturally lead to lower than normal levels of SN-38G, followed by reduced formation of intraluminal SN-38. If gastrointestinal toxicity were to result from a graded dose response effect, patients with Gilbert's syndrome might be predicted to have less toxicity, because they would not accumulate high levels of SN-38G in bile. However, if anything, a heightened sensitivity to irinotecan induced toxicity is observed in patients with reduced UGT1A1 activity. This finding, that low UGT1A1 expression is associated with SN-38 initiated toxicity may be explained if the capacity of the gastrointestinal tract to glucuronidate SN-38 is also taken into account. Because UGT1A1 is present in the gastrointestinal tract (Strassburg et al., 2000), patients with Gilbert's syndrome would also have a reduced intestinal capacity to decrease SN-38 levels by glucuronidation.

Understanding the mechanism of SN-38 initiated gastrointestinal toxicity may be advanced in future experiments when complete analysis of UGT expression patterns and

drug transport in the human gastrointestinal tract are available and better understood. In cellular models developed with Caco-2 colon cancer cells, flavonoids have been shown to undergo basolateral and apical uptake followed by apical efflux of the flavonoid glucuronide by MRP2 (Walle et al., 1999). Recent evidence has demonstrated that SN-38G in Caco-2 cells is transported to the apical side by the canalicular multispecific organic anion transporter cMOAT (MRP2) (Yamamoto et al., 2001) and that SN-38 and irinotecan can be taken up into intestinal cells by both passive diffusion and active transport processes (Kobayashi et al., 1999). Translating this result into physiological events occurring in vivo, SN-38 can be absorbed into intestinal epithelial cells from the basolateral (blood) or apical surface (luminal side), eventually resulting in glucuronidation and efflux of SN-38G into the lumen by MRP2 (Fig. 1). It has also been shown that SN-38 can also be transported into the lumen by MRP2 as well as by P-glycoprotein and shuttled toward the basolateral side by MRP1. This result gives credence to the idea that drugs such as SN-38 may be absorbed from both the intraluminal space as well as the blood into epithelial cells, targeted for glucuronidation, and effluxed back into the lumen. Whereas some of the SN-38 accumulating in the cells will efflux in an apical-to-basolateral direction (Yamamoto et al., 2001), such a recycling mechanism involving glucuronidation serves to protect the epithelial cells from SN-38 directed toxicity. Because recent evidence suggests that the capacity of the epithelial cells to form glucuronides can be compromised, an event that occurs in patients with Gilbert's syndrome, exposure of epithelial cells from the basolateral or apical surfaces may lead to a dosing burden of SN-38 that cannot be adequately handled because of a compromise in the efficiency of glucuronidation. Based upon the detection of *UGT1A1* gene transcripts in human intestine (Strassburg et al., 1998a, 2000), we could predict that reduced mucosa associated UGT activity would render the small and large intestine susceptible to the toxic actions of SN-38. Thus, appreciating the functionality and expression patterns of UGT1A1 in human intestine would be important in elucidating the cellular mechanisms associated with toxicity of SN-38 in the gastrointestinal tract.

Recent molecular studies examining *UGT* gene expression patterns have demonstrated that UGT1A1 along with UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, and UGT1A10 gene transcripts are present in human small and large intestine (Strassburg et al., 1998a). Confirmation of the existence of these proteins in colon is still lacking. However, indirect immunofluorescence analysis using a specific UGT1A antibody shows an abundance of UGT protein in colon epithelial cells (Strassburg et al., 1999). In intestinal tissue, UGT1A1 is differentially regulated and displays expression polymorphism among persons as shown through RNA expression and protein patterns (Strassburg et al., 2000). Thus, normal patterns of interindividual variation resulting in reduced UGT1A1 expression in small intestine may in part predispose those persons to a potential toxic episode.

Guillemette et al. (2000) recently identified three variant *UGT1A7* alleles, each represented by missense mutations. UGT1A7*3 (N¹²⁹K/R¹³¹K/W²⁰⁸R) and UGT1A7*4 (W²⁰⁸R), which share the W²⁰⁸R mutation, are shown by Gagné et al. (2002) to metabolize SN-38 poorly. In a control population,

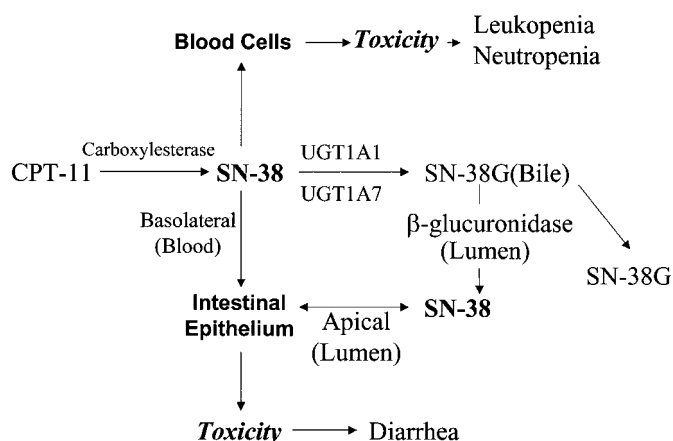


Fig. 2. Distribution of SN-38 in blood and intestinal tissue. The flow chart illustrates the cellular targets of SN-38 in the blood and intestinal tissues. After cleavage of the pro-drug irinotecan by carboxylesterases, free SN-38 elicits a toxic reaction on blood cells as well as intestinal tissue. SN-38 distribution to the intestinal epithelium can occur by direct exposure and uptake from the blood as well as by luminal exposure after cleavage of the glucuronide by bacterial β -glucuronidase. Accumulation of SN-38 in the blood as well as in the intestinal cells is believed to lead to cellular damage and toxicity in both of those tissues.

*UGT1A7*3* is homozygous in 15% of the population whereas homozygosity for *UGT1A7*4* is rare (0.7%). Genotypes represented with a *UGT1A7*3* haplotype were found in approximately 36% of the samples. However, the frequency of inheriting the *UGT1A7*3* allele in subjects with liver (Vogel et al., 2001) and colon (Strassburg et al., 2002) cancers is much higher than that observed in normal populations, indicating that irinotecan therapy may predispose persons with a genetic predisposition for hepatocellular cancer and those suffering from colorectal cancer to altered SN-38 pharmacokinetics. Thus, changes in the systemic concentrations of SN-38/SN-38-glucuronide resulting from differences in the *UGT1A7* genotype may influence the adverse effects attributed to irinotecan therapy, but not necessarily in a pattern concordant with those observed with inheritance of Gilbert's syndrome. The difficulty in rationalizing a linkage between the inheritance of *UGT1A7*3* or *UGT1A7*4* and an adverse reaction in the gastrointestinal tract (diarrhea) is not easily made because unlike *UGT1A1*, several studies have demonstrated that *UGT1A7* is not expressed in the small or large intestine (Strassburg et al., 1998a, 1999). Expression of *UGT1A7* is most abundant in the proximal regions of the gastrointestinal tract such as the esophagus (Strassburg et al., 1998b; Tukey and Strassburg, 2001) and gene transcripts have been found in other tissues associated with the throat and nasal passages (Zheng et al., 2001). The volume of distribution of irinotecan is large and its ability to associate with plasma proteins is not as great as that observed for SN-38 (99%), indicating that irinotecan enjoys extensive tissue distribution. Because Gagné et al. (2002) have demonstrated that *UGT1A7* is the most efficient of the UGTs in metabolizing SN-38, systemic distribution of irinotecan as well as SN-38 to these tissues might lead to an overall increase in SN-38 glucuronide formation. This may be relevant because toxicities associated with irinotecan therapy and enhancement of gastrointestinal side effects is a result of accumulating SN-38 glucuronide. Patients with elevated biliary indices (SN-38:SN-38G) have experienced more severe diarrhea than those with low biliary indices (Ratain, 2000). Thus, patients with a *UGT1A7*1* or *UGT1A7*2* genotype might be considered extensive metabolizers, a phenotype that could predispose persons to SN-38-initiated gastrointestinal toxicity as a result of an increased efflux of SN-38 glucuronide into the bile.

SN-38 concentrations in plasma are elevated with sequential increases in irinotecan administration. Increases in AUC of SN-38 have been described to correlate with neutropenia (Mathijssen et al., 2001), and inheritance of *UGT1A1*28* was described to be a risk factor for irinotecan toxicity. However, *UGT1A7* poor metabolizers (*UGT1A7*3* and *UGT1A7*4*) might also be at heightened risk for developing leukopenia and other blood-related toxicities, because a reduction in SN-38 glucuronidation capacity could lead to increases in SN-38/SN-38G AUCs relative to those patients that are extensive metabolizers (*UGT1A7*1* and *UGT1A7*2*). It would be of considerable interest to examine the correlation between irinotecan-induced myelosuppression and *UGT1A7* genotype.

The pharmacogenomics of *UGT1A7*-initiated SN-38 metabolism potentially pose an interesting dilemma regarding the toxicity associated with SN-38. An increase in SN-38 glucuronidation from tissue stores that express *UGT1A7*1*

or *UGT1A7*2* may contribute to a total reduction of plasma SN-38 yet enhance gastrointestinal toxicity because of increased fecal SN-38 after deconjugation of SN-38 glucuronide. Simultaneously, it might be expected that a reduction in plasma SN-38 concentrations resulting from favorable *UGT1A7* dependent glucuronidation would provide some degree of cellular protection toward the adverse actions of SN-38 on bone marrow. Concomitantly, poor *UGT1A7* dependent metabolizers would be at risk for blood toxicities yet favorably positioned to escape gastrointestinal discomfort.

Because it seems that the expression patterns associated with *UGT1A1* are related to irinotecan-induced gastrointestinal toxicities and *UGT1A7* expression may also play a role, identifying the allelic variants of these two genes may improve clinical therapy. For example, recent clinical studies have shown that combined treatment of irinotecan with the nonresorbable aminoglycoside antibiotic neomycin reduced fecal bacterial β -glucuronidase, increased the molar ratio of SN-38G/SN-38 and eliminated diarrhea in the majority of the patients (Kehrer et al., 2001). Such clinical intervention in combination with genotyping *UGT1A7* to adjust for systemic irinotecan therapy may decrease the side effects of irinotecan. In addition, a recent report has shown that levels of *UGT1A1* in colon epithelial Caco-2 cells are significantly induced after exposure to dietary flavonoids such as chrysin (Galijatovic et al., 2001). It is known that fasting in persons with Gilbert's syndrome leads to elevated serum bilirubin levels (Ishihara et al., 2001); in cancer patients chemotherapy tends to lead to a reduction in appetite. Thus, a diet rich in flavonoids may counter this problem by promoting transcriptional activation of *UGT1A1* in intestinal tissue and diminishing the toxic actions of SN-38.

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