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SHORT COMMUNICATION

Ugt1a is required for the protective effect of selenium against irinotecan-induced toxicity

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Abstract
Purpose Irinotecan (CPT-11) is widely used for the treatment of patients with colorectal cancer. However, the adverse effects associated with the treatment have hindered the efficacies of irinotecan. We have shown that organic selenium compounds could significantly attenuate irinotecan-associated toxicity and enhance antitumor activity in xenograft tumor models. The objective of this study is to determine the role of a specific group of uridine diphosphate glucuronosyltransferases, which is coded by *UGT1A*, in detoxification process of irinotecan as well as selenium-associated protective effect against irinotecan-induced toxicity.
Methods In this study, the toxicities of irinotecan, docetaxel and cisplatin in the *Ugta1* mutant rats and their wild-type controls were compared. The plasma concentrations of irinotecan and SN-38 were measured. The modulatory effect of a selenium compound on irinotecan-induced toxicity was analyzed in these rats.
Results We demonstrated that the maximum tolerated doses of irinotecan in the homozygous mutant rats were significantly lower than those in wild-type rats, 25 mg/kg × 1 versus 200 mg/kg × 1 and 3 mg/kg/day × 3 versus 100 mg/kg/day × 3, respectively. The enhanced sensitivity was specific to irinotecan and was not observed with other chemotherapeutic agents, such as docetaxel and cisplatin, where *Ugt1a* is not required for their metabolism. Our results also showed that selective protection against irinotecan-induced toxicity by 5-methylselenocysteine was achieved in the wild-type rats but not in the *Ugt1a* null rats.
Conclusion These data support the hypothesis that expression of *UGT1A* is critical for 5-methylselenocysteine to exert its protective effect against irinotecan-induced toxicity.

Keywords Ugt1a · Irinotecan-induced toxicity · Selenium · Gunn rats

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Introduction

As a chemotherapeutic agent, irinotecan is used extensively in the treatment for cancers, particularly colorectal cancer [1]. Irinotecan is converted by carboxylesterases to its active metabolite, SN-38, which can stabilize ternary complexes between topoisomerase I and DNA and thereby cause replication fork arrest and double-stranded DNA breaks [2–6]. In combination therapy, irinotecan together with 5-fluorouracil and leucovorin can lead to positive responses in about half the patients with advanced colorectal cancer, but 20–30% of these patients develop severe adverse responses, such as diarrhea and neutropenia [7, 8]. This dose-limiting toxicity may affect the therapeutic potential of irinotecan in cancer treatment. SN-38 is the

active metabolite of irinotecan responsible for its toxicity and antitumor activity [9–11]. Irinotecan is detoxified in part by converting SN-38 to SN-38G through glucuronidation catalyzed by a specific group of uridine diphosphate glucuronosyltransferases (UDPGT) in the liver and intestines, which is coded by *UGT1A* [12, 13]. A portion of irinotecan is degraded in vivo to APC, NPC, M1, 2, 3, 4, catalyzed by CYP3A4 and CYP3A5, which are far less toxic than SN-38 [1, 14]. We have been exploring approaches to enhancing the therapeutic selectivity of irinotecan by focusing on the selenium-containing compounds. Selenium is an essential trace element, and dietary selenium is predominantly in the form of organic compounds, such as 5-methylselenocysteine (MSC), a known antioxidant. It was confirmed that therapeutic doses of selenium effectively protect against irinotecan-induced toxicity in rodent models with the wild-type *Ugt1a* allele [15, 16]. Our results showed that MSC administrated prior and concurrently with irinotecan could significantly reduce the toxic effects of irinotecan, allowing the administration of higher dosages, which resulted in improved therapeutic efficacies and cures in tumor xenograft models with the wild-type *Ugt1a* allele [15, 16]. In contrast, using *Ugt1a* mutant rats, MSC did not offer any significant protection from irinotecan-induced toxicity. These data provide further evidence for the role of Ugt1a in the detoxification of irinotecan.

Results and discussion

In this study, we set out to study the factors associated with the toxicity and detoxification of irinotecan by comparing *Ugta1* mutant rats and the wild-type controls. We first wanted to confirm the role of the rat ortholog of *UGT1A*, *Ugt1a*, in irinotecan detoxification by comparing the toxic effects of irinotecan on homozygous Gunn rats (j/j), heterozygous Gunn rats (j/+) and wild-type controls. The Gunn rats carry a frameshift mutation, which causes a complete loss of the enzymatic activities of UDPGT coded by the *Ugt1a* locus [17–19]. In the experiments, we used 8-to-12-week-old female j/j and j/+ rats and their wild-type littermates (+/+) as well as Fischer 344/N rats with body weights of 150–180 g from Harlan Sprague–Dawley Inc. (Indianapolis, IN, USA). Four rats were maintained per cage with water and food ad libitum. The experimental procedures were approved by the Institutional Animal Care and Use Committee of Roswell Park Cancer Institute. Irinotecan (CPT-11) was purchased from Pfizer Inc. (Kalamazoo, MI, USA) as a ready-to-use clinical formulation solution in 5-ml vials containing 100 mg drug (20 mg/ml). Irinotecan was given by i.v. injection via the animals' tail veins. We established the maximum tolerated dose (MTD) for the different groups of the rats, which was defined as the maximum dose that could be administered to animals, which resulted in no drug-related lethality and body weight loss of less than 20%. After drug administration, the rats were monitored daily for drug-induced toxic effects, including body weight loss, diarrhea, stomatitis and lethality, for a minimum of 2 weeks, and 3–4 times a week thereafter. The differences between the mean values in the different treatment groups were analyzed for significance using one-way analysis of variances (ANOVA), with Tukey–Kramer multiple comparison tests. A P value of less than 0.05 was considered statistically significant. The analysis of our experimental data showed that, following a single i.v. injection of irinotecan, the MTD were 200 mg/kg and 25 mg/kg for +/+ and j/j rats, respectively. Using the daily × 3 schedule, the MTD were 100 mg/kg

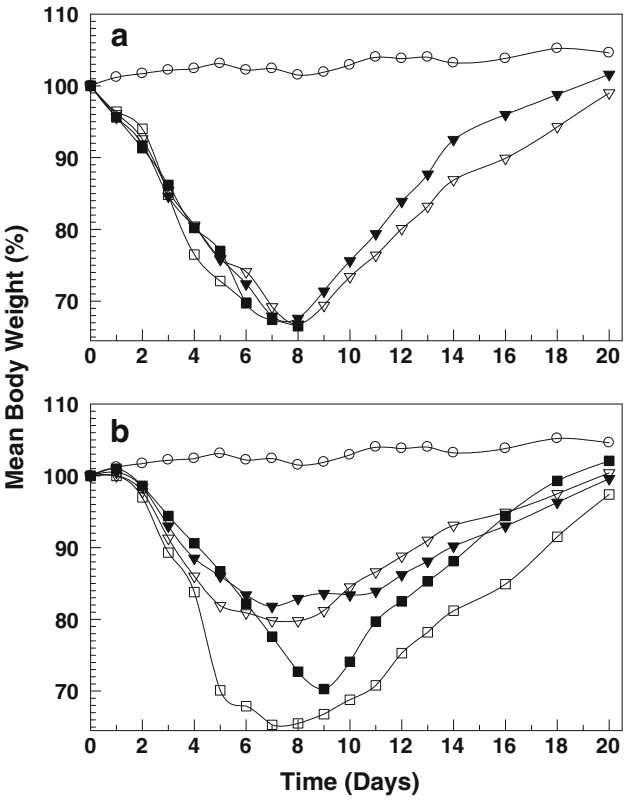

Fig. 1 Toxicity of irinotecan ± MSC in j/j rats with a single i.v. injection (**a**) and i.v. injection once a day for three days (i.v. daily × 3, b) schedules. **a** *Circle* control; *inverted triangle* irinotecan 35 mg/kg (50% death); *filled inverted triangle* irinotecan 35 mg/kg + MSC 0.5 mg/rat/day (50% death), *square* irinotecan 40 mg/kg (100% death); *filled inverted triangle* irinotecan 40 mg/kg + MSC 0.5 mg/rat/day (100% death). **b** *Circle* control; *inverted triangle* irinotecan 4 mg/kg/day (50% death); *filled inverted triangle* irinotecan 4 mg/kg/day + MSC 0.5 mg/rat/day (43% death); *square* irinotecan 5 mg/kg/day (83% death); *filled square* irinotecan 5 mg/kg/day + MSC 0.5 mg/rat/day (66% death). Additional information on irinotecan and MSC administrations was described in the text. Four rats were used for each experimental group, and the experiments were repeated at least once

Table 1 Effect of MSC on the toxicity of irinotecan in j/j and j/+ rats

Genotype of rats	Drug (dose)	Irinotecan schedule	Toxicities		
			MWL	Diarrhea	Death
j/j	Irinotecan (40 mg/kg)	i.v. × 1	29.2 ± 1.2	100	100
j/j	Irinotecan (40 mg/kg) + MSC (0.5 mg/rat/d)	i.v. × 1	31.2 ± 0.8	100	100
j/j	Irinotecan (35 mg/kg)	i.v. × 1	26.5 ± 1.4	100	50
j/j	Irinotecan (35 mg/kg) + MSC (0.5 mg/rat/d)	i.v. × 1	22.2 ± 2.8	50	50
j/j	Irinotecan (5 mg/kg)	i.v. daily × 3	26.6 ± 1.9	100	83
j/j	Irinotecan (5 mg/kg) + MSC (0.5 mg/kg/d)	i.v. daily × 3	29.0 ± 3.6	66	66
j/j	Irinotecan (4 mg/kg)	i.v. daily × 3	22.8 ± 2.6	50	50
j/j	Irinotecan (4 mg/kg) + MSC (0.5 mg/rat/d)	i.v. daily × 3	19.3 ± 2.2	43	43
j/+	Irinotecan (100 mg/kg)	i.v. daily × 3	29.5 ± 5.2	100	100
j/+	Irinotecan (100 mg/kg) + MSC (0.5 mg/rat/d)	i.v. daily × 3	26.1 ± 6.0	50	50
j/+	Irinotecan (75 mg/kg)	i.v. daily × 3	32.0 ± 3.5	100	25
j/+	Irinotecan (75 mg/kg) + MSC (0.5 mg/rat/d)	i.v. daily × 3	12.4 ± 3.3	25	0

MSC was administered by p.o. once a day, 7 days before and 7 days after irinotecan, for a total of 14 (i.v. × 1) or 16 (i.v. daily × 3) days. Four rats were used for each experimental group, and the experiments were repeated at least once

MWL maximum body weight loss (%)

and 3 mg/kg for +/+ and j/j rats, respectively (Fig. 1; Table 1; *Online Resource* Figs. S2 and S3 and Tables S1 and S3). Our results showed that severe diarrhea is a dosage-limiting condition associated with lethality in the treated rats (Table 1; *Online Resource* Table S3). The plasma concentrations of irinotecan and SN-38 were measured 1 h after the administration of irinotecan to j/j and +/+ rats using a validated high-performance liquid chromatography method, as previously described [20]. The separation method was carried out on a Waters Nova-Pak C18 column equipped with a μ Bondapak C18 guard column, with the mobile phase consisting of 20% acetonitrile and 80% triethylamine acetate. Detection was by fluorescence with excitation at 370 nm and emission at 510 nm. The limit of quantitation for both was 2.5 ng/ml. Our results showed that the plasma concentrations of irinotecan and SN-38 in j/j rats were higher than those of the wild-type controls (*Online Resource* Table S2), which is consistent with the observations that mutations in the *UGT1A* locus caused the elevated levels of SN-38 in the patients treated with irinotecan [21–23]. The severity of the toxic response in j/+ rats falls between that of j/j and +/+ rats (Table 1; *Online Resource* Fig. S4 and Table S3), reflecting the consequence of copy-number variations of the *Ugt1a* gene. These data confirm the critical role of *Ugt1a* in the detoxification of irinotecan.

To determine the drug specificity for the role of the *Ugt1a* mutation in the detoxifications of irinotecan, we evaluated the toxic effects of different chemotherapeutic agents in rats with different genotypes. The results shown in *Online Resource* Fig. S5 and Table S4 indicated that the *Ugt1a* mutation did not adversely affect the MTD or toxic profiles of docetaxel and cisplatin, suggesting that toxicity and detoxification of irinotecan are specifically dependent on *Ugt1a* expression.

To examine the potential role of *Ugt1a* on the observed protection of selenium against irinotecan-associated toxicity in mice and rats with the wild-type *Ugt1a* [15, Cao et al. unpublished data], we evaluated the MTDs and toxic effects of irinotecan alone and in combination with MSC in Gunn rats. MSC was purchased from Sigma (St. Louis, MO, USA) and dissolved in sterile saline (0.9% NaCl) at a concentration of 1 mg/ml. MSC was administered orally (p.o.) once a day at 0.5 mg/rat/day, 7 days before and during and 7 days after irinotecan treatment. Our results indicate that, unlike the animals with the wild-type *Ugt1a* in which MSC offers strong protection against lethal doses of irinotecan and prevents irinotecan-induced diarrhea, bone marrow suppression and lethality [15, Cao et al. unpublished data] MSC did not yield any significant protection in j/j rats (Fig. 1; Table 1). It is interesting to observe that MSC-associated protection was also evident for j/+ rats (*Online Resource* Fig. S6; Table 1), suggesting MSC still played an essential role in alleviating the toxic effects of irinotecan, even when only one copy of *Ugt1a* was functional.

Besides being converted to SN-38 by carboxylesterases, irinotecan can also be metabolized to compounds that have fewer cytotoxic effects than SN-38, which is catalyzed by CYP3A4 and CYP3A5 in rats [1, 14, 24]. Thus, the protective effect of selenium against irinotecan-induced host toxicities could be due to the enhanced activities of carboxylesterases/UDPGT encoded by *Ugt1a* and/or *Cyp3a4/Cyp3a5* by selenium. Although it has been reported that

selenium could significantly increase expression of *Cyp3a5* [25], it is presently unknown whether the expression of *Cyp3a4* is elevated. Promoting expression of *Ugt1a* by selenium has also been reported [26, 27]. If selenium-induced elevation of expression of *Cyp3a4*/*Cyp3a5* plays a major role in the detoxification of irinotecan, the significant protection of selenium against irinotecan-associated toxicity should have remained even when *Ugt1a* is inactivated. Since our result showed that selenium-induced protection largely disappeared in Gunn rats, it suggests that the *Cyp3a4*/*Cyp3a5*-mediated metabolic pathway basically did not play a significant role in selenium-mediated protection. Therefore, we have established conclusively for the first time that *Ugt1a* is essential for selenium to exert its protective role against irinotecan-induced toxicity. Although it is possible that the selenium-induced elevated expression of *Ugt1a* may be a key reason, our results provide a strong impetus to fully explore the mechanistic details related to this phenomenon. Such an effort may lead to a better strategy in using selenium and/or novel modulators to increase the therapeutic efficacies of irinotecan in cancer treatment.

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Conflict of interest None of the authors has any conflict of interest.

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