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Irinotecan-induced bile acid malabsorption is associated with down-regulation of ileal Asbt (*Slc10a2*) in mice

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ABSTRACT

Irinotecan, (CPT-11), an antitumor agent primarily used for the treatment of solid tumors, has often compromised clinical application due to the inducement of severe delay-onset diarrhea. Bile acid malabsorption (BAM) is widely accepted as the common cause of diarrhea. However, whether CPT-11-induced diarrhea has correlation with BAM is unknown. The aim of this study was to investigate the effect of CPT-11 on the bile acid homeostasis in mice. The mice were administrated with CPT-11 intravenously for four consecutive days. The total bile acids (TBAs) levels in the small intestine, colon, feces, liver, serum and gallbladder were evaluated by automatic biochemical analyzer, and the individual bile acids were also measured by LC-MS/MS. Real-time qPCR and Western blot techniques were used to evaluate the mRNA and protein expressions of Cyp7a1, Cyp27a1, Asbt, Ostα/β. In situ loop method was carried out to evaluate the function of apical Na^+-dependent bile salt transporter (Asbt). Results showed that the bile acid pool size was significantly reduced by 17%, 25%, and 40% respectively at 2, 3, and 4 days post CPT-11 treatment. The fecal excretions of TBAs were significantly increased by 2.1-fold at 3 and 4 days post CPT-11 treatment. The ileal expression of Asbt was significantly decreased at mRNA and protein levels, and the transport ability of Asbt was also attenuated after CPT-11 treatment. Moreover, the incidence of CPT-11-induced delay-onset diarrhea was also decreased after cholestyramine administration in CPT-11-treated mice. These results indicated that BAM may be partially responsible for CPT-11-induced delay-onset diarrhea, and the underlying mechanism may have correlation with down-regulation of the Asbt in the ileum of mice.

1. Introduction

Camptothecin (CPT)-11 (irinotecan), a semi synthetic camptothecin analog with topoisomerase-I inhibitory action (Tanizawa et al., 1994; Smith et al., 2006), is an antitumor agent mainly used for the treatment of solid tumors. CPT-11 has been approved as the first- and second-line treatment of metastatic colon cancer (CRC) either alone or in combination with other agents (Rougier et al., 1997). CPT-11 showed good activity in many cancers, such as oesophagus/gaster, lung, pancreas, ovary, leukemia, lymphoma, cervix, breast, head and neck and brain tumors (Rosen, 1998; Prados et al., 2004; Swami et al., 2013).

However, the efficacy and safety of CPT-11 are compromised because of the severe and unpredictable delay-onset diarrhea. As one of the major dose-limiting toxicities of CPT-11 therapy, delay-onset diarrhea is generally occurring >24 h after CPT-11 administration (Mathijssen et al., 2001; Takasuna et al., 2006). CPT-11-indcued diarrhea often lasts for many days and leads to dehydration and electrolyte imbalance (Chen et al., 2013). Currently, the mechanisms underlying CPT-11-indcued diarrhea is still not clear, but it is considered to have correlation with direct intestinal mucosal damage caused by SN-38, the main metabolite of CPT-11 (Hecht, 1998). It is found that the concentration of SN-38 is influenced by intestinal carboxylesterase (CES) (Ahmed et al., 1999), bacterial β-glucuronidase (Kehrer et al., 2001), and enterohepatic recycling (Rothenberg et al., 1993; Chen et al., 2013). In addition, it is reported that CPT-11 may injure tight junction proteins claudin-1 and occludin, and thus damaged intestinal barrier and induced bacterial translocation.

Recent studies have showed that bile acid malabsorption (BAM) is a common cause of diarrhea (Barkun et al., 2013). Enterohepatic circulation (EHC) is an important mechanism which responsible for the movement of bile acid molecules from the liver to the small intestine and back to the liver. It is reported that bile acid storage capacity in healthy adult is about 3–4 g, whereas the daily bile acid synthesis is only about

Abbreviations: CPT-11, irinotecan; TBAs, total bile acids; BAM, bile acid malabsorption; ASBT, apical Na^+-dependent bile salt transporter; EHC, enterohepatic circulation; IS, internal standard; NDCA, nor-desoxycholic acid; d_4-CDCA, d_4-chenodeoxycholic; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; β-MCA, β-muricholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid; HDCA, hyodexycholic acid; T-CA, tauro-cholic acid; T-CDCA, tauro-chenodeoxycholic acid; T-DCA, tauro-deoxycholic acid; T-β-MCA, tauro-β-muricholic acid; T-LCA, tauro-lithocholic acid; T-UDCA, tauro-ursodeoxycholic acid; T-HDCA, tauro-hyodexycholic acid; RIPA lysis buffer, radio immunoprecipitation assay lysis buffer; PMSF, phenylmethanesulfonyl fluoride.

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0.4–0.6 g. Thus, the reabsorption of bile acids (BAs) in the intestine plays a vital role in maintaining bile acid homeostasis. It is well known that about 95% of the bile acids secreted into the small intestine are reclaimed (Ferrebee and Dawson, 2015), and the fraction of BAs leaving in the lumen is in part passively absorbed by colon, which is a process facilitated by bacterial deconjugated and in part transformed and extruded with feces (Hofmann, 1994). In contrast, ileal uptake is predominantly an active process carried out by the apical sodium-dependent BA transporter (ASBT gene symbol *SLC10A2*), also called ileal BA transporter. ASBT is abundantly expressed in the terminal ileum and this sodium- and potential-driven transporter reabsorbs most of BAs from the ileal lumen through the apical brush border membrane (Dawson, 2002). Dawson and colleagues found that targeting deletion of the Asbt led to eliminate EHC of BAs in mice (Dawson et al., 2003), and mutations in the ileal bile acid transporter gene (*SLC10A2*) were found in the patients with primary BAM who had congenital diarrhea, steatorrhea, and reduced plasma cholesterol levels (Oelkers et al., 1997). These studies indicated that ASBT plays a critical role in reabsorption of BAs. Shih DQ, et al. reported that obvious BAM was found in hepatocyte nuclear factor (HNF)-1α knock-out mice, and the ileum ASBT expression was also markedly reduced(Shih et al., 2001; Shneider, 2001). Evidence from clinical study showed that defect of ileal ASBT gene in children has close correlation with intractable diarrhea. (Oelkers et al., 1997; Shneider, 2001). These studies indicated that the deficiency of ASBT expression or function play an important role in BAM pathogenesis. Impaired absorption of BAs in the terminal ileum is the main cause of BAM. The excess BAs in the colon is associated with diarrhea (Johnston et al., 2011), and the mechanisms involves increased mucosal permeability, mucus secretion and colonic motility, as well as inhibition of apical Cl/OH exchange (Chadwick et al., 1979; Barcelo et al., 2001; Alrefai et al., 2007; Alemi et al., 2013; Ao et al., 2013; Camilleri, 2015).

Previous study reported that CPT-11 could notably increase the expressions of CYP7A1 and CYP27A1, the key hepatic enzymes involved in bile acid synthesis in rats (Sawano et al., 2015), Zhong-Ze Fang et al. found that CPT-11 caused metabolic changes in the composition of BAs in mice (Fang et al., 2016). It is found that CPT-11-induced diarrhea in mice could be attenuated by octreotide via reducing bile acid secretion (Zidan et al., 2001). These evidences implicated that CPT-11 might have effect on the bile acid homeostasis. Besides, it is reported that antibacterial drugs, such as bacitracin, neomycin, and streptomycin, had therapeutic effect on CPT-11-induced diarrhea via increasing the expression of Asbt in mice (Miyata et al., 2015). Based on above findings, we speculated the reduced expression or function of ASBT may be one of the reasons that responsible for the delay-onset diarrhea caused by CPT-11.

In this present study, we investigated the effect of CPT-11 on bile acid pool, total bile acids (TBAs), and individual bile acids in different compartments in mice. Moreover, the expressions of Cyp7a1, Cyp27a1, Asbt, and Ostα/β as well as the function of Asbt were also evaluated to clarify the underlying mechanism of CPT-11-induced diarrhea in mice.

2. Materials and methods

2.1. Materials

CPT-11 (95%), SN-38 (95%) and CPT (95%, internal standard (IS)), were purchased from Bolon Pharmachen ltd., Taizhou, China. Cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), β-muricholic acid (β-MCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA), hyodexycholic acid (HDCA), tauro-cholic acid (T-CA), tauro-chenodeoxycholic acid (T-CDCA), tauro-deoxycholic acid (T-DCA), tauro-β-muricholic acid (T-β-MCA), tauro-lithocholic acid (T-LCA), tauro-ursodeoxycholic acid (T-UDCA), tauro-hyodexycholic acid (T-HDCA), d_4-chenodeoxycholic (d_4-CDCA) and nor-desoxycholic acid (NDCA) were purchased from Sigma-Aldrich Inc. The anti-CYP7A1 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-ASBT and anti-CYP27A1 were purchased from Abcam Inc. (MA, USA). The anti-GAPDH was purchased from Goodhere Biotechnology Co. (Hangzhou, China). The secondary antibodies were obtained from Abcam Inc. Other reagents, unless mentioned, were of analytical grade and were commercially available.

2.2. Animals

Male Kunming mice (22 ± 3 g) were obtained from the Laboratory Animal Center of Lanzhou University. Animals were housed in an air-conditioned room (25 °C) under a 12-h light/dark cycle for one week before experiments and allowed water and standard rodent chow ad libitum. Animal experimental protocols were reviewed and approved by the Institutional Animal Experimentation Committee of Lanzhou University (LDYYLL2015-0021).

2.3. Experimental design

The first series of experiments aimed to investigate the effect of CPT-11 on bile acid homeostasis. Mice were randomly divided into control group and CPT-11 group, sixteen animals in each group. CPT-11(40 mg/kg/d) or vehicles were administered intravenously (i.v.) once a day for one day, two days, three days, four days. After 24 h of the last administration, eight mice fasted for 6 h were sacrificed by collecting blood from the eyeball. Subsequently, the gallbladder, entire liver, entire small intestine and its contents, entire colon of eight mice in each group were harvested and stored at −80 °C for TBAs analysis. The bile acid pool size was analyzed by measuring TBAs in the whole enterohepatic system, including the liver, gallbladder and the entire small intestine and its contents. As for the rest eight mice in each group, the gallbladder, liver, distal ileum (10 cm from cecum), colon (3 cm from cecum) were harvested and stored at −80 °C for individual bile acids analysis. Fecal excretions of BAs were measured in stools collected over 12 h in metabolic cages.

The second series of experiments aimed to measure the capacity of ileal bile acids absorption. Mice were randomly divided into control group and CPT-11 group, six animals in each group. CPT-11 (40 mg/kg/d) or vehicles were administered intravenously once a day for one day, two days, three days and four days to the animals. In situ loop method was performed as previously described by Masaaki Miyata et al. (2015). Mice were anesthetized with 4% chloral hydrate (0.1 mL/10 g, i.p.). An ileal loop of approximately 10 cm in length was isolated using ligatures at both ends, and 500 μL of dosing solution [5 mM taurochenodeoxycholic acid (T-CDCA) in phosphate-buffered saline] was injected into the loop with a syringe. The portal blood was collected once from each mouse at 10 min after the injection. T-CDCA concentration in the portal blood was analyzed by LC-MS/MS. The portal blood was suspended in three volumes (v/v) of ethanol and was centrifuged at 12000 × *g* for 10 min. Supernatants were subjected to LC-MS/MS analysis.

The third series of experiments aimed to further determine the BAM caused by CPT-11. Firstly, two groups of five mice were used to evaluate whether the bile acid sequestrant cholestyramine had effect on the plasma concentrations of CPT-11 and SN-38 by LC-MS/MS, the active metabolite of CPT-11. Both groups administered 40 mg/kg CPT-11 i.v. once a day for three consecutive days at noon. On the morning of the third day after first administration, CPT-11 group treated with water i.g. whereas CPT-11 + Seq group (CPT-11 co-administration of sequestrant group) treated with cholestyramine 0.8 g/kg/d i.g. Then, after 3 h of the last CPT-11 administration, mice were sacrificed by collecting blood from the eyeball. Secondly, three groups of mice were used to investigate the effect of cholestyramine on delay-onset diarrhea induced by CPT-11: the control group of 10 mice was administered vehicle i.v. for four consecutive days at noon and treated with water i.g. from the third day after first administration to sixth day in the morning.