Life Sciences 139 (2015) 132–138

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Impact of obesity on accumulation of the toxic irinotecan metabolite, SN-38, in mice

Pankajini Mallick [a], Pranav Shah [b], Adarsh Gandhi [c], Romi Ghose [a,*]

[a] Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX, USA
[b] Division of Pre-Clinical Innovation, National Center for Advancing Translational Sciences, Rockville, MD, USA
[c] Department of Bioanalysis and Physiology, Lundbeck Research Inc. USA, Paramus, NJ, USA

ARTICLE INFO

Article history:
Received 27 April 2015
Received in revised form 19 August 2015
Accepted 22 August 2015
Available online 1 September 2015

Keywords:
Obesity
Ugt1a1
SN-38
Irinotecan
Colorectal cancer
Chemotherapy
Pharmacokinetics

ABSTRACT

Aim: Our aim is to investigate the impact of high fat diet-induced obesity on plasma concentrations of the toxic irinotecan metabolite, SN-38, in mice.
Main methods: Diet-induced obese (DIO, 60% kcal fed) and lean mice (10% kcal fed) were treated orally with a single dose of 10 mg/kg irinotecan to determine pharmacokinetic (PK) parameters. Feces and livers were collected for quantification of irinotecan and its metabolites (SN-38 & SN-38G). SN-38G formation by Ugt1a1 enzyme was analyzed in liver S9 fractions. Expression of the pro-inflammatory cytokine, TNF-α was determined in liver and plasma. Hepatic β-glucuronidase and carboxylesterase enzymes (CES) were also determined.
Key findings: AUC_{0-8} and C_{max} of SN-38 increased by 2-fold in DIO mice compared to their lean controls. This was accompanied by a ~2-fold reduction in AUC_{0-8} and C_{max} of SN-38G in DIO mice. There were no differences in the PK parameters of irinotecan in DIO or lean mice. Conversion of SN-38 to SN-38G by Ugt1a1 enzyme was reduced by ~2-fold in liver S9 fractions in DIO mice. Furthermore, in DIO mice, β-glucuronidase activity increased by 2-fold, whereas there was no change in CES activity. TNF-α mRNA expression was 3 fold higher in DIO mice.
Significance: Our study demonstrates that reduced hepatic Ugt1a activity during obesity likely contributes to reduced glucuronidation, and results in higher levels of the toxic metabolite, SN-38. Thus, irinotecan dosage should be closely monitored for effective and safe chemotherapy in obese cancer patients who are at a higher risk of developing liver toxicity.

1. Introduction

Approximately 136,830 Americans are diagnosed with colon or rectal cancer annually as reported by American Cancer Society. Colorectal liver metastasis (CLM) develops in 40–50% of these patients and is one of the major causes of death [1]. Irinotecan is a topoisomerase-I inhibitor used to treat cancer of the colon or rectum. Irinotecan-based preoperative chemotherapy in CLM patients with initial unresectable tumors has improved five-year survival rates by ~58% [8,14].

Irinotecan biotransformation involves two pathways (Fig. 1): (i) bioactivation by carboxylesterases (CES) to form the toxic metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38) [60] which is detoxified to inactive SN-38 glucuronide (SN-38G) predominantly by uridine diphosphate glucuronosyltransferases (UGT) 1A1 enzyme; (ii) Oxidation by cytochrome P450 (CYP) 3A enzymes to form two inactive metabolites: APC ({7-ethyl-10[4-N-(5-aminopentanoicacid)-1-piperidino] carbonyloxycamptothecin}) and NPC ({7-ethyl-10[4-amino-1 piperidino]carbonyloxycamptothecin}). NPC is converted to SN-38 by the CES. Irinotecan, SN-38 and SN-38G are subsequently eliminated in bile or urine. SN-38G is deconjugated to SN-38 by intestinal bacterial β-glucuronidase [44,48,51].

Irinotecan causes life-threatening diarrhea which accounts for its dose-limiting toxicity. UGT1A1 polymorphism has been primarily linked to increased risk for diarrhea with irinotecan, due to accumulation of SN-38 in the intestine [6,11,22,34,54]. Hydrolysis of SN-38G to SN-38 by enteric bacterial β-glucuronidases is also indicated to potentially contribute to higher levels of SN-38 [61].

Clinical investigation shows irinotecan-based pre-operative chemotherapy causes non-alcoholic fatty liver disease (NAFLD). NAFLD encompasses a spectrum of liver injuries including steatosis (fat accumulation), which progresses to non-alcoholic steatohepatitis (NASH; steatosis accompanied with inflammation) that eventually cause fibrosis (scar tissue) disrupting structure and function of the liver, and end-stage cirrhosis [64].

CLM patients treated with irinotecan had 27% steatosis compared to only 3% of patients without chemotherapy [39,40,66]. In CLM patients, preoperative chemotherapy with irinotecan was associated with NASH (20%) compared to patients without any chemotherapy (4.4%) and subsequent higher 90 day postoperative mortality after hepatic

* Corresponding author at: College of Pharmacy, Department of Pharmacological & Pharmaceutical Sciences, University of Houston, 1441 Moursund Street, Houston, TX 77030, USA. Tel.: +1 832 842 8343; fax: +1 832 842 8305.
E-mail address: rghose@uh.edu (R. Ghose).

http://dx.doi.org/10.1016/j.lfs.2015.08.017
0024-3205/

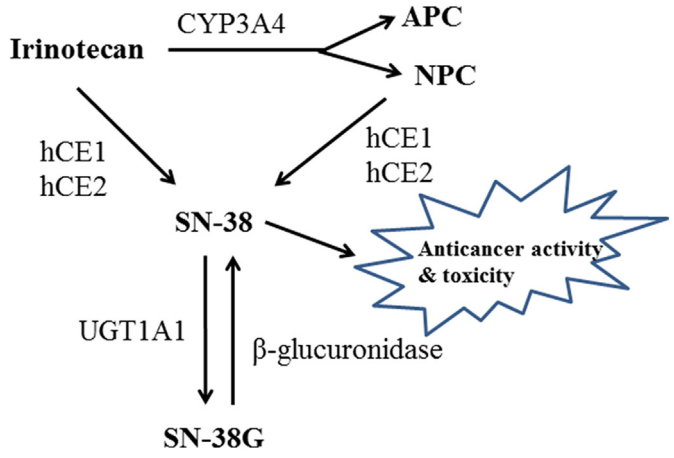

Fig. 1. Schematic representation of the metabolic pathway of irinotecan and its metabolites.

surgery. NASH was more pronounced in patients with a higher BMI (BMI < 25 kg/m^2, 12.1% vs BMI >25 kg/m^2, 24.6%) [15,56]. Obesity encompasses a chronic low-grade inflammation state and leads to increased levels in expression of TNF-α [55]. Evidence supports the central role of cytokine imbalance, and, in particular, an increase in TNF-α in the development/progression of NASH [31].

In this study, we aim to determine the impact of obesity on plasma concentrations of SN-38 and SN-38G in DIO and lean mice. DIO mouse model is clinically relevant as it closely mimics consumption of diets high in fat which are main contributors to the obesity trend in humans and subsequently lead to increased weight gain and elevated plasma concentrations of free fatty acids (FFAs) [10,59].

Accumulation of SN-38, in obesity, owing to altered activity of enzymes involved in its disposition can lead to increased liver toxicity Thus, we determined: (1) SN-38 glucuronidation by hepatic Ugt1a1 in vitro; (2) plasma concentrations of irinotecan, SN-38 & SN-38G; (3) activity of β-glucuronidase and CES enzymes; (4) mRNA and plasma levels of TNF-α.

2. Materials & methods

2.1. Chemicals

Camptothecin (CPT; internal standard (I.S.)), p-nitrophenyl-β-D-glucuronide (Cat # N1627) and 4-Nitrophenyl acetate (PNPA; Cat # N8130) were purchased from Sigma-Aldrich, St. Louis, MO. Irinotecan hydrochloride for injections was purchased from APP Pharmaceuticals, Schaumburg, IL (Cat # NDC-63323–19305). SN-38 and SN-38G were a kind gift from Dr. Ming Hu's lab at the University of Houston, Houston, TX. TNF-α Elisa Max™ Deluxe assay kit (Cat # 430904) was purchased from Biolegend, San Diego, CA. Solvents purchased for chromatography were LC-MS grade and were purchased from VWR international, LLC (Suwanee, GA, USA). Unless specified, all other materials were purchased from Sigma-Aldrich (St Louis, MO, USA.).

2.2. Animals

Male, 12-weeks old C57BL/6 J 60 kcal% fat diet (DIO) mice and age-matched controls 10 kcal% fat diet (lean) mice were purchased from Jackson Labs, Bar Harbor, ME. The animals were maintained in a 12 h dark/light cycle and in a temperature-and-humidity-controlled environment. The DIO and lean mice had access to 60% fat (D12492, Research Diets, Inc.) and 10% fat (D12450B, Research Diets, Inc.) diet, respectively ad libitum. All the animal care and use protocols were approved by the Institutional Animal Care and Use Committee guidelines. All experiments were performed in triplicate and repeated at least three times.

2.3. Treatments

Mice (n = 4–5) were treated orally with single dose of vehicle or irinotecan hydrochloride solution (10 mg/kg). Blood was collected from 0 to 8 h from the tail vein. Livers were harvested at 8 h, cryopreserved and stored in −80 °C until further use. At the end of the study, feces samples were collected, combined and stored at −80 °C to prevent loss of enzyme activity.

2.4. Preparation of S9 fractions and Ugta1 activity assay

Mouse liver S9 fractions were prepared using a procedure adopted from the literature with minor modifications as described below [7]. Livers from untreated DIO and lean mice were perfused with sodium phosphate buffer (pH 7.4) and homogenized in ice-cold homogenization buffer (50 mM potassium phosphate buffer (pH 7.4), 250 mM sucrose, 1 mM EDTA). This homogenate was centrifuged at 15,400 rpm for 15 min at 4 °C. The supernatant fractions were collected after discarding the fat layer and stored at −80 °C. Protein concentration was determined using the bicinchoninic acid (BCA) assay according to the manufacturer's protocol (Pierce, Rockford, IL, U.S.A).

For Ugt1a1 activity assay, each reaction contained 2 mg/ml hepatic S9 fractions, 15 μM of SN-38, solution A (3.6 mM UDPGA triammonium salt) and solution B (4 mM saccharolactone, 0.88 mM $MgCl_2$ and 0.022 mg/ml alamethacin) in 0.05 M Potassium phosphate (KPi, pH 7.4) buffer (total volume 170 μl) for 90 min. The reaction was quenched by adding 50 μl of 94% acetonitrile (ACN) and 6% glacial acetic acid containing 1 μg/ml of CPT (I.S.). Standards were prepared using S9 fractions and different concentrations of SN-38 ranging from 0 to 15 μM without the cofactors. The samples were then analyzed for SN-38 and SN-38G using LC-MS/MS as described below.

2.5. Real-Time PCR

Total RNA was isolated from mouse liver using TRIzol reagent (Sigma-Aldrich, St. Louis, MO, U.S.A) according to the manufacturer's protocol. cDNA was synthesized from 5 μg of total RNA using the High Capacity Reverse Transcription Kit from Applied Biosystems. Real-time PCR was performed using an ABI PRISM 7300 Sequence Detection System instrument and software (Applied Biosystems) as described previously [18,19,20]. In short, each reaction mixture (total of 25 μl) contained 50–100 ng of cDNA, 300 nM forward primer, 300 nM reverse primer, 200 nM fluorogenic probe, and 15 μl of TaqMan Universal PCR Master Mix. Quantitative expression values were normalized to cyclophilin (Table 1). The PCR thermocycling parameters were 50 °C for 2 min (stage 1), 95 °C for 10 min (stage 2), 95 °C for 15 s, and 60 °C for 1 min (stage 3, 50 cycles). The threshold cycle (Ct) was calculated by the instrument. Quantitative expression values were normalized to the "housekeeping" gene, Cyclophilin.

Table 1
Primers and probes used for RT-PCR analysis of target mRNAs.

mRNA	Sequence
Cyclophilin	
Forward primer	5′-GGCCGATGACGAGCCC
Reverse primer	5′-TGTCTTTGGAACTTTGTCTGCA
Probe	5′-6TGGGCCGCGTCTCCTTCGA0
TNF-α	
Forward primer	5′-CATCTTCTCAAAATTCGAGTGACAA
Reverse primer	5′-TGGGAGTAGACAAGGTACAACCG
Probe	5′-6 CACGTCGTAGCAAACCACCAAGTGGA0