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Pharmacokinetic modulation of irinotecan metabolites by sulphobromophthalein in rats

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Abstract

The purpose of this study was to modulate the pharmacokinetics of irinotecan metabolites, SN-38 and SN-38-glucuronide, by possibly reducing biliary excretion, which in turn could lower irinotecan toxicity. SN-38-glucuronide is associated with severe diarrhoea that occurs after irinotecan therapy as a result of enteric injury caused by SN-38. Sulphobromophthalein is used clinically as a drug for testing liver function and is considered to be a safe drug. We investigated the effect of sulphobromophthalein on the disposition of irinotecan metabolites after CPT-11 (7-ethyl-10-[10-4-(1-piperidino)-1-piperidino]-carbonyloxy-camptothecin) administration. Wistar rats were administered CPT-11 (500 μg/body) in saline as a bolus injection into the femoral vein through a catheter. The volume of drug solution injected into each animal was 1 mL. Rats were either administered CPT-11 alone or simultaneously with sulphobromophthalein (20 mg/body). After administration, blood and bile samples were taken at appropriate intervals and analysed by HPLC. Co-administration of sulphobromophthalein partially inhibited the biliary excretion of SN-38-glucuronide with a concomitant increase in its area under the plasma concentration–time curve (AUC) but did not significantly alter the biliary excretion and AUC of the active metabolite SN-38. These results suggested that cotreatment of CPT-11 with sulphobromophthalein might decrease intraluminal SN-38 concentrations without altering the pharmacokinetics of SN-38.

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Introduction

Irinotecan, 7-ethyl-10-[10-4-(1-piperidino)-1-piperidino]-carbonyloxy-camptothecin (CPT-11) exhibits a potent antitumour effect by inhibiting topoisomerase I (Houghton et al 1993). As a prodrug, CPT-11 is converted into its primary active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin), by the enzyme carboxylesterase (Rivory et al 1996). SN-38 subsequently undergoes glucuronic acid conjugation to form the corresponding glucuronide, SN-38 glucuronide (SN-38-Glu) (Atsumi et al 1991). Biliary excretion is a major elimination pathway for CPT-11 and its metabolites (Kaneda & Yokokura 1990).

The major dose-limiting toxicity after irinotecan administration is severe diarrhoea and neutropenia (Abigerges et al 1994) The SN-38-Glu excreted in the gut via bile is hydrolysed to SN-38 by β-glucuronidase, and consequently it impairs the gut (Takasuna et al 1996). Hange-Shashin-To (Tsumura Co. Ltd; Tokyo, Japan), a herbal medicine that contains the β-glucuronidase inhibitor baicalin, effectively prevents CPT-11-induced diarrhoea (Takasuna et al 1995). However, Hange-Shashin-To worked intraluminally.

In the light of these hypotheses, it is likely that inhibition of the biliary excretion of SN-38-Glu and SN-38 could reduce the severity of this adverse effect. Sulphobromophthalein is widely used clinically as a drug for testing liver function because of its high biliary excretion clearance (Winkler 1961). Moreover, sulphobromophthalein is regarded as a particularly safe drug. Therefore, in this study, we have examined the effect of sulphobromophthalein on the disposition of irinotecan metabolites, with the aim of finding a means to reduce the late-onset diarrhoea observed following CPT-11 administration.

Materials and Methods

Chemicals

All chemicals and reagents used were of analytical grade. Sulphobromophthalein was obtained from Sigma (St Louis, MO). CPT-11 was kindly donated by Daiichi Pharmaceutical (Tokyo, Japan).

Animals

The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the "Guide for the Care and Use of Laboratory Animals". Male Wistar rats (6–7-weeks old, 230–320 g) were obtained from Japan SLC (Hamamatsu Japan). Eight rats were used in this study. The rats were housed for at least one week at $23 \pm 1°C$ and $60 \pm 10\%$ relative humidity, with a 12-h light/dark cycle. During the acclimatization the rats were allowed free access to food and water. Non-fasted animals were used for the experiments.

Drug administration and sample collection

The surgical operation was performed according to Itagaki et al (2003) with some modifications. Wistar rats were kept under light ether anaesthesia. The femoral artery and vein of each rat were cannulated with polyethylene tubing (PE50), and the common bile duct cannulated with PE10 tubing. The animals were kept on a warm operating table, and CPT-11 (500 μg/body) in saline was injected as a bolus into the femoral vein through a catheter. The volume of drug solution injected into each animal was 1 mL. To determine the inhibitory effect of sulphobromophthalein on the disposition of SN-38 and SN-38-Glu, the rats were divided into two groups. The first group received CPT-11 without sulphobromophthalein. The second group received a simultaneous administration of CPT-11 and sulphobromophthalein (20 mg/body). The catheter was flushed with 500 μL saline after injection. Blood samples (300 μL) were collected into heparinized microtubes at 1, 5, 10, 15, 30, 60, 120, 180 min after injection. Plasma was prepared by centrifugation (3000 *g* for 10 min) of blood samples. Bile was collected into microtubes at 0–15, 15–30, 30–45, 45–60, 60–75, 75–90, 90–105, 105–120, 120–150, 150–180 min after injection.

Preparation of samples

We determined the level of lactone plus carboxylate (total) form of SN-38 as the lactone form of SN-38 (Itoh et al 2000). For the analysis of plasma samples, plasma (100 μL) was mixed with 10 μL 50% dimethylsulfoxide (DMSO) in water (v/v), 100 μL 1 M aqueous zinc sulphate/methanol/ethylene glycol (1/1/2, v/v) and 10 μL 2 μg mL^{-1} camptothecin as an internal standard. After vortexing briefly, samples were centrifuged at 5000 *g* for 3 min at 4°C. A 100-μL sample of the supernatant was transferred to a fresh tube, and 100 μL 50 mM monobasic potassium phosphate (pH 2.5) was added. After vortexing briefly, the sample was left overnight at 37°C.

For the analysis of bile samples, bile (40 μL) was mixed with 40 μL 50% DMSO in water (v/v), 400 μL 1 M aqueous zinc sulphate/methanol/ethylene glycol (1/1/2, v/v) and 40 μL 2 μg mL^{-1} camptothecin and then vortexed for 30 s. After centrifugation of the mixture (5000 *g* for 2 min), 100 μL of the upper aqueous layer was transferred to a fresh tube, and 100 μL 50 mM monobasic potassium phosphate (pH 2.5) was added. After vortexing briefly, the sample was left overnight at 37°C. SN-38-Glu was determined in the same manner as SN-38, except for the addition of 50% DMSO in water (v/v) containing 5000 U mL^{-1} β-glucuronidase, and the sample was left for 3 h at 37°C, to ensure complete deconjugation of SN-38-Glu (van Groeningen et al 2000).

Analytical procedures

The samples were analysed by high-performance liquid chromatography using a C8 column (250×4.5 mm, 5 μm; GL Sciences) as described by Itoh et al (2004). A mobile phase consisting of (50 mM monobasic potassium phosphate (pH 2.5), 7 mM tetrabutylammonium bromide):acetonitrile (70:30, v/v) was used. Column temperature and flow rate were 40°C and 0.8 mL min^{-1}, respectively. The fluorescence detector (F1000; Hitachi) was operated at excitation and emission wavelengths of 355 nm and 515 nm, respectively. The lower limit of quantitation for SN-38 was 50 pmol mL^{-1}.

Data analysis

The area under the plasma concentration–time curve (AUC) was estimated by the trapezoidal rule. The clearance value of bile (CL_{bile}) was determined by dividing the amount of compounds excreted into bile from 0 to 180 min by the AUC from 0 to 180 min. The non-parametric Mann-Whitney test was used for statistical analysis, and a two-sided significance level of < 0.05 was considered significant.

Results

As shown in Figure 1, the cumulative amount of SN-38-Glu excreted into bile was significantly reduced by the co-administration of sulphobromophthalein, whereas that of SN-38 was not significantly affected.

The plasma concentration profiles for SN-38-Glu and SN-38 are shown in Figure 2. The AUC of SN-38-Glu was 43.0 ± 10.5 and 24.4 ± 6.94 μg min mL^{-1} in the presence or absence of sulphobromophthalein, respectively. The AUC of SN-38 was 27.2 ± 11.8 and 22.9 ± 5.11 μg min mL^{-1} in the presence or absence of sulphobromophthalein, respectively. The AUC of SN-38-Glu in the presence of sulphobromophthalein was significantly higher compared with in the absence of sulphobromophthalein. Little change was seen for SN-38.

The co-administration of sulphobromophthalein reduced the CL_{bile} of SN-38-Glu (Figure 3A). Little difference was seen in the CL_{bile} of SN-38 in the presence of sulphobromophthalein (Figure 3B).

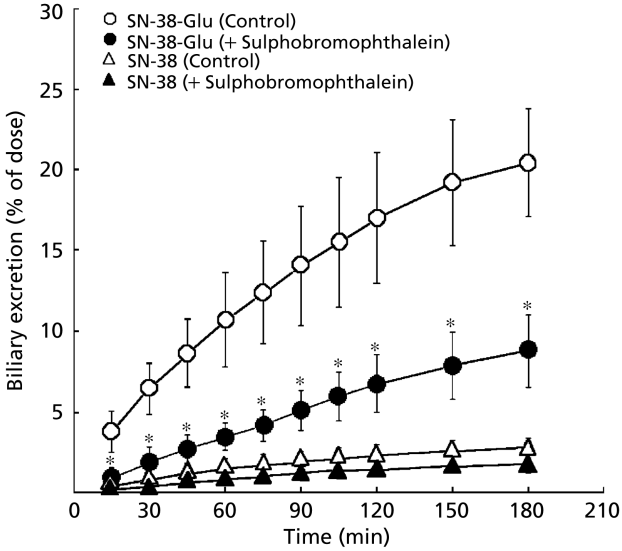

Figure 1 Effect of sulphobromophthalein on the biliary excretion of SN-38-Glu and SN-38 after CPT-11 administration. CPT-11 (500 μg/body) was injected through the femoral vein in the presence or absence of sulphobromophthalein (20 mg/body). Each point represents the mean with s.d. of four determinations. $^*P < 0.05$ compared with absence of sulphobromophthalein.

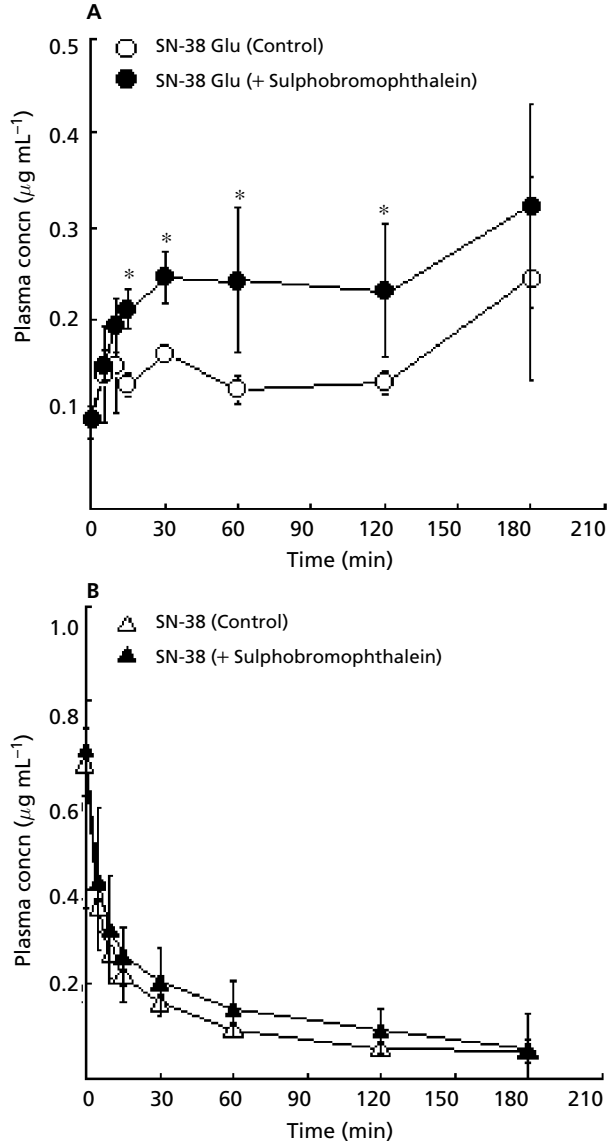

Figure 2 Effect of sulphobromophthalein on the plasma concentration of SN-38-Glu (A) and SN-38 (B) after CPT-11 administration. CPT-11 (500 μg/body) was injected through the femoral vein in the presence or absence of sulphobromophthalein (20 mg/body). Each point represents the mean with s.d. of four determinations. $^*P < 0.05$ compared with absence of sulphobromophthalein.

Discussion

Due to the unpredictable severe diarrhoea observed in patients treated with CPT-11, the clinical use of this anticancer agent has remained limited (Abigerges et al 1994). It has been proposed that hydrolysis of SN-38-Glu to form active SN-38 may play a major role in the development of CPT-11-induced diarrhoea (Takasuna et al 1996; Kehrer et al 2001). Intestinal toxicity may arise from biliary excretion of SN-38-Glu and SN-38. In this study, we tested a potential strategy to inhibit biliary excretion of SN-38-Glu and SN-38 to reduce exposure of the intestine to SN-38.

Sulphobromophthalein is widely used clinically as a drug for testing liver function (Winkler 1961). With this in mind, we examined the effect of sulphobromophthalein on the disposition of SN-38-Glu and SN-38 after CPT-11 administration. It was found that sulphobromophthalein significantly inhibited the biliary excretion of SN-38-Glu. The inhibitory effect of sulphobromophthalein on SN-38-Glu excretion resulted in an increase in the blood level of SN-38-Glu. As a result of this inhibition, the AUC and CL_{bile} of SN-38-Glu were increased and decreased, respectively. Unlike ciclosporin, little difference was seen in the biliary excretion and plasma concentration of the active compound SN-38 in the presence of sulphobromophthalein (Gupta et al 1996). Co-administration of sulphobromophthalein did not significantly alter the AUC and CL_{bile} of SN-38. These results suggested that sulphobromophthalein partially inhibited the secretion of SN-38-Glu into the gastrointestinal lumen via the biliary route. The disposition of the active metabolite SN-38 in the presence of sulphobromophthalein was similar with that in the absence of sulphobromophthalein. Co-administration of sulphobromophthalein with CPT-11 may lower the late-onset gastrointestinal toxicity observed during treatment with CPT-11 by inhibiting the biliary excretion and subsequent exposure of intestinal tissues to SN-38 without lowering anticancer efficiency. It has been reported that co-treatment of CPT-11 with Hange-Shashin-To and neomycin decreased CPT-11-induced chronic diarrhoeal symptoms by inhibition of β-glucuronidase (Takasuna et al 1996; Kehrer et al 2001). It is possible that our method enhanced the effects of these intraluminal

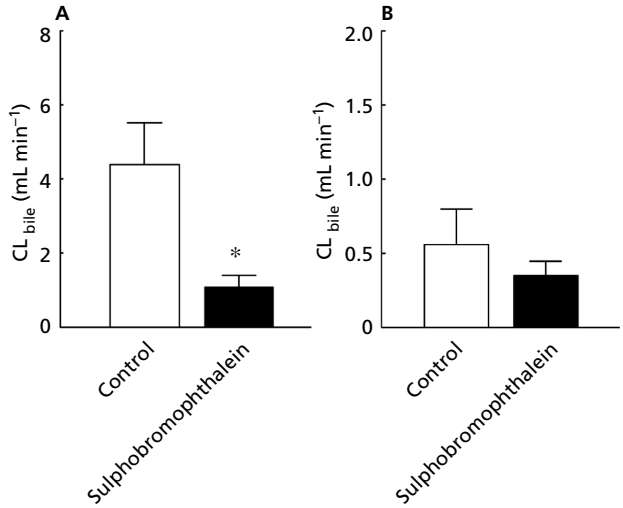

Figure 3 Biliary excretion clearance of SN-38-Glu (A) and SN-38 (B) after CPT-11 administration. CPT-11 (500 μg/body) was injected through the femoral vein in the presence or absence of sulphobromophthalein (20 mg/body). The CL_{bile} values were calculated from the values shown in Figures 1 and 2. Each value represents the mean with s.d. of four determinations. $*P < 0.05$ compared with absence of sulphobromophthalein.

treatments. Further studies with this or other modulations of biliary secretion are required to determine whether CPT-11 chemotherapy with sulphobromophthalein could decrease intestinal toxicity.

Conclusion

Sulphobromophthalein partially inhibited the secretion of SN-38-Glu into the gastrointestinal lumen, whereas little change was seen in that of active metabolite SN-38. Co-administration of sulphobromophthalein with CPT-11 might lower the late-onset gastrointestinal toxicity observed during treatment with CPT-11 without lowering anticancer activity.

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