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Loperamide inhibits the biliary excretion of irinotecan (CPT-11) in the rat isolated perfused liver

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Abstract

Patients treated with irinotecan (CPT-11) occasionally suffer from severe diarrhoea and aggressive treatment with loperamide at the first signs of loose stools is recommended. We have examined the effect of loperamide on the hepatic metabolism and biliary excretion of CPT-11 in the isolated perfused rat liver (IPRL). CPT-11 (0.5 μmol) was injected as a bolus into the IPRL reservoir, and perfusate and bile samples were collected over 3 h. Experiments were conducted using loperamide-free perfusate (n = 5) or perfusate containing 10 μM loperamide (n = 6). Perfusate and bile concentrations of total CPT-11 and the major metabolites SN-38 (7-ethyl-10-hydroxy-camptothecin), SN-38G (7-ethyl-10-hydroxy-camptothecin glucuronide) and APC (7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidine] carbonyloxycamptothecin) were determined by HPLC. The unchanged parent drug was the predominant species in bile, with approximately 4% of the dose recovered over 180 min as compared with only 1% for the metabolites. Loperamide significantly reduced the biliary excretion of CPT-11 by approximately 50% (2.0 ± 0.9% dose compared with 3.8 ± 1.0% in the control group, $P = 0.019$) over the same period. In contrast, the biliary excretion of SN-38, SN-38G and APC was not significantly affected by loperamide ($P > 0.05$). Furthermore, bile flow rate was not affected by loperamide. Loperamide appeared to selectively inhibit the biliary excretion of CPT-11, although the extent to which loperamide altered the disposition of CPT-11 in the clinical setting remains to be determined.

Introduction

Irinotecan (CPT-11) is a water-soluble analogue of 20(*S*)-camptothecin used in the treatment of metastatic colorectal cancer and increasingly in the management of advanced lung cancer. CPT-11 is considered to be a prodrug that is activated by carboxylesterases to produce the cytotoxic metabolite SN-38 (7-ethyl-10-hydroxy-camptothecin) (Gupta et al 1994; Satoh et al 1994; Rivory et al 1996a; Haaz et al 1997). SN-38 is further metabolized by UDP-glucuronosyltransferase 1A1 (UGT1A1) to the inactive glucuronide product SN-38 glucuronide (SN-38G) (Iyer et al 1998). CPT-11 can also be metabolized to the inactive compound APC (7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidine] carbonyloxycamptothecin), predominately by CYP3A4 (Figure 1) (Rivory et al 1996b).

CPT-11 and SN-38 exist as lactone (closed-ring) and carboxylate (open-ring) forms. The interconversion between these two forms is pH- and temperature-dependent (Fassberg & Stella 1992). CPT-11 lactone is converted to SN-38 at a greater rate than CPT-11 carboxylate, and this may contribute to the predominance of SN-38 lactone in-vivo (Haaz et al 1997). This is significant as SN-38 lactone is a much more potent topoisomerase I inhibitor than SN-38 carboxylate (Wani et al 1987; Jaxel et al 1989; Kawato et al 1991; Slichenmyer et al 1993).

The reactivation of SN-38 from SN-38G in the gut can also occur, and this is believed to contribute to the severe dose-limiting diarrhoea that occurs 4–7 days post-therapy (Araki et al 1993; Takasuna et al 1998; Slatter et al 2000). Prompt and aggressive loperamide therapy is routinely given to patients at the first sign of loose stools and this has reduced the morbidity associated with this side-effect (Abigerges et al 1994).

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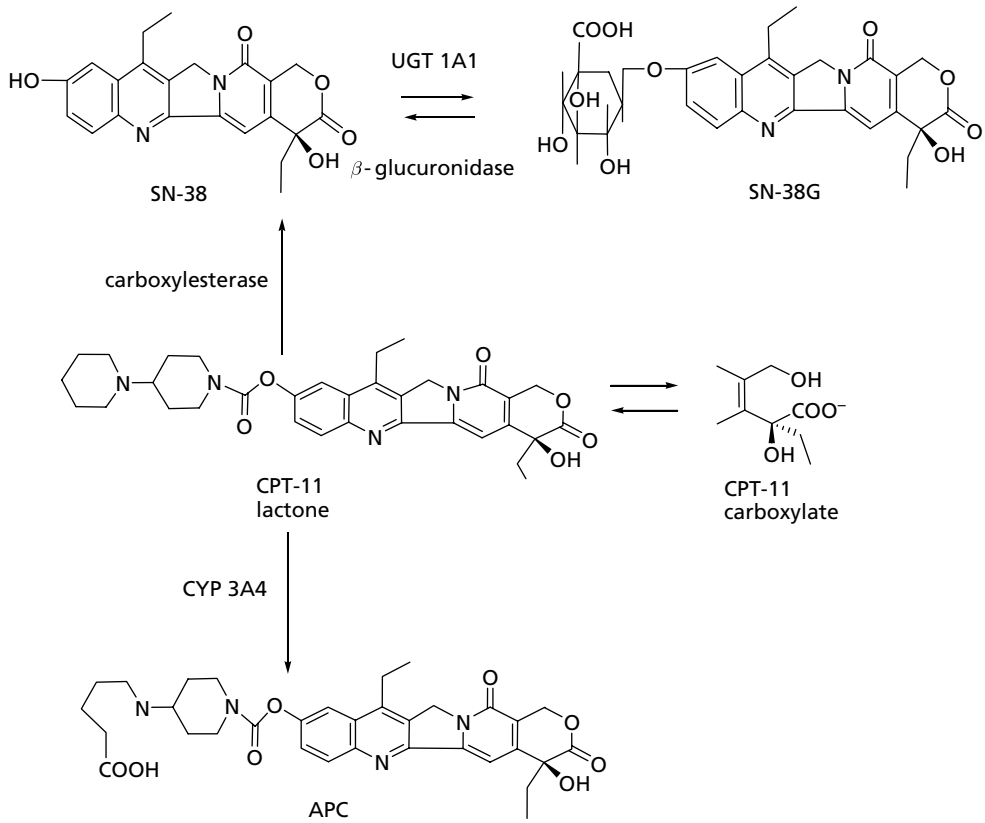

Figure 1 Structures of CPT-11 (lactone and carboxylate forms) and its main metabolites SN-38, SN-38G and APC.

Loperamide is a peripheral μ opiate receptor agonist that inhibits gastrointestinal motility and also reduces fluid and electrolyte secretion (Sandhu et al 1981; Press et al 1991). CPT-11 (Chu et al 1999a, b; Yamamoto et al 2001; Iyer et al 2002) and loperamide (Schinkel et al 1996; Sadeque et al 2000) are substrates of P-glycoprotein (P-gp/ABCB1). P-gp is a member of the adenosine triphosphate-binding cassette (ABC) transporter superfamily and is involved in the transmembrane flux of drugs at sites of importance to the pharmacokinetics of drugs, such as the liver, the intestine and the blood–brain barrier.

It has been shown in mouse fibroblast cells transfected with human MDR1 that loperamide inhibits P-gp-mediated transport of CPT-11 (Tobin et al 2003). As P-gp is expressed on bile canaliculi (Booth et al 1998), we proposed that loperamide might inhibit the biliary excretion of CPT-11. Iyer et al (2002) observed a significant decrease in the biliary excretion of CPT-11 in mice exposed to the potent, P-gp-specific inhibitor PSC833. Furthermore, biliary transport of CPT-11 was significantly lower in mice lacking mdr1-type P-gp compared with wild-type mice (Iyer et al 2002).

The ABC transporters MRP2/ABCC2 and BCRP/ABCG2 are also involved in the transport of CPT-11 and SN-38, and like P-gp, are expressed in the hepatic canaliculi (Brangi et al 1999; Chu et al 1999b; Schellens et al 2000; Horikawa et al 2002). However, even at high concentrations, loperamide had no effect on MRP2 or BCRP function in cells overexpressing these two transporters (Tobin et al 2003).

In man and rat CPT-11 is extensively excreted in bile. In rats given 1–40 mg kg^{-1} CPT-11 intravenously, 33–57% of the parent compound was excreted into bile (Kaneda et al 1990). In one patient with a biliary T-tube, $\sim$20% of the parent compound was recovered in bile following intravenous infusion of 125 mg m^{-2} CPT-11 (Slatter et al 2000). Therefore, inhibition of the biliary excretion of CPT-11 may have a significant impact on gastrointestinal exposure to CPT-11 and help explain why loperamide is effective in managing diarrhoea in patients receiving CPT-11. Localized conversion of CPT-11 to SN-38 has been observed in intestinal tissue (Ahmed et al 1999), thus reduced gastrointestinal exposure to CPT-11 may lead to a subsequent reduction in gut exposure to SN-38, thereby reducing gastrointestinal damage. The aim of this study was to examine whether loperamide affected the biliary excretion of CPT-11 or its metabolites in the isolated perfused rat liver (IPRL).

Materials and Methods

Chemicals

SN-38 was prepared by Yakult (Tokyo, Japan) and provided by Rhone-Poulenc Rorer laboratories (Neuilly, France).

CPT-11 lactone was prepared by diluting clinical stock (Sydney Cancer Centre, Australia) in 0.05 M citric acid. APC was provided by Rhone-Poulenc Rorer. 20(*S*)-Camptothecin and loperamide were purchased from Sigma-Aldrich (St Louis, MO). All other reagents were of analytical grade or above.

Liver perfusion

Male Sprague-Dawley rats (200–300 g) from the Animal Resources Centre (Perth, Western Australia) were used at 6–10 weeks of age. The rats had free access to standard alimentation and water. Ethics approval was obtained from the University of Sydney Animal Ethics Committee.

The rats were anaesthetized by intraperitoneal injection of 60 mg kg^{-1} pentobarbitone sodium. The bile duct and the portal vein were cannulated and the liver isolated and then perfused with 100 mL of recirculating Krebs–Henseleit buffer (mM: 118 NaCl, 4.69 KCl, 2.52 $CaCl_2$, 1.18 $MgSO_4$, 1.33 NaH_2PO_4, 24.9 $NaHCO_3$, pH 7.4) containing 2% (w/v) bovine serum albumin and 0.2% (w/v) glucose. The perfusate was oxygenated with O_2/CO_2 (95%/5%). Taurocholate was infused at 0.5 μmol min^{-1} into the reservoir throughout the 3-h perfusion to maintain bile flow. Temperature (37°C), pH (7.4) and perfusion pressure (10 cm water) were continuously monitored during the perfusion and remained constant. Bile flow ($= 5\,\mu L\,min^{-1}$) and oxygen consumption ($> 1.8\,\mu mol\,min^{-1}$ (g liver)$^{-1}$) were monitored to ensure the liver remained viable throughout the perfusion.

Study design

The liver was allowed to equilibrate for 20 min, during which time bile and 200 μL perfusate was collected. Then 0.5 μmol CPT-11 lactone (final concentration = 5 μM) and the loperamide vehicle (20 μL ethanol) were added as a bolus injection to the perfusate in the reservoir. Using mouse fibroblast cells transfected with human MDR1 we have shown previously that CPT-11 lactone was a better substrate of P-gp than the carboxylate form (Tobin et al 2003). Furthermore, CPT-11 is administered to patients as the lactone form. Hence, in this study the biliary excretion of CPT-11 lactone was studied in the presence of loperamide or the loperamide vehicle.

Bile was collected in 20 min fractions over a total period of 180 min. Samples (200 μL) of circulating perfusate were collected at 2, 5, 10, 30, 70, 90, 105, 120, 150 and 180 min after injection. The total amount sampled accounted for a total of 2% of the reservoir volume. Bile flow was determined gravimetrically, assuming a bile density of 1 g mL^{-1}. A total of five control experiments were conducted under these conditions. Another six experiments were conducted in a similar manner, except that loperamide was added to the perfusate (final concentration 10 μM). Animals were randomly assigned to the control or treatment group.

Drug and metabolite analysis

Perfusate and bile concentrations of total (lactone and carboxylate) CPT-11 and metabolites were determined using a variation of a previously validated method (Rivory & Robert 1994). Standards were prepared in a similar manner to the perfusate and bile samples by being mixed with 100 μL ice-cold acetonitrile:methanol (50:50, v/v) containing 5 ng camptothecin as an internal standard. The standards and samples were then centrifuged (15 000 *g*, 10 min) and the supernatant was acidified with 2.5 μL 2 M HCl to ensure that total (lactone and carboxylate) forms of CPT-11 and metabolites were measured.

For SN-38G quantification, 50 μL perfusate or 1.5 μL bile was incubated at 37°C with 25 U β-glucuronidase for 1 h. The incubated samples were then treated in the same manner as described above.

Separation was performed at ambient temperature using a Waters Nova-Pak Radial-Pak C18 reversed-phase column (5 × 250 mm, 4 μm) with 7.5 mM ammonium formate buffer (pH 4.0):acetonitrile (78:22) as the mobile phase at a flow rate of 1.2 mL min^{-1}. Fluorescence detection (RF-10AXL; Shimadzu, Sydney, Australia) was optimized for detection of SN-38 with excitation and emission wavelengths set at 380 and 530 nm, respectively. Samples (10 μL) were injected onto the chromatograph. Standard curves were constructed for each batch of samples and were linear from 0.5 to 250 ng mL^{-1} SN-38, 10–5000 ng mL^{-1} CPT-11 and 5–2500 ng mL^{-1} APC ($r^2 > 0.99$ for each standard in the range quoted). The lower limit of quantitation for the analytes ranged from 0.5 to 5 ng mL^{-1}. Total accuracy and imprecision was assessed from back-calculated standards prepared on different days (n = 12). The accuracy ranged from 92 to 109% for SN-38, 97–107% for CPT-11 and 99–105% for APC. The imprecision (CV) ranged from 3 to 19% for SN-38, 5–20% for CPT-11 and 4–19% for APC. Recovery was examined by comparing measured peak areas for each compound over the respective analytical ranges with those when the plasma was replaced by an equal volume of phosphate buffer (10 mM, pH 7.4, n = 3). Recovery ranged from 88 to 116% and the standard deviation (s.d.) was less than 9% for all compounds at all concentrations tested.

Data analysis

Pharmacokinetic analysis was performed by non-compartmental methods. Specifically, the area-under-the-perfusate concentration–time data from 0 to 180 min (AUC_{0-180}) was determined by the linear trapezoidal method for each of the compounds of interest. Perfusate metabolic ratios were calculated by dividing the AUC_{0-180} of the metabolite by that of CPT-11.

The percent of the CPT-11 dose excreted was calculated by dividing the total molar amount of CPT-11 or metabolite excreted by the molar dose of CPT-11. Biliary clearance was calculated as the amount excreted in bile over 180 min divided by the AUC_{0-180}. The cumulative biliary excretion of CPT-11 in the loperamide and control groups was compared using an unpaired two-tailed *t*-test. Exploratory statistical analysis was also performed for the secondary pharmacokinetic end-points also using unpaired two-tailed *t*-tests. However, in the latter case, the Dunn–Sidak correction was applied to account for multiple comparisons. In all

cases, data are presented as mean ± s.d., and $P < 0.05$ was considered statistically significant.

Results

The concentration–time profiles for CPT-11, SN-38, SN-38G and APC in the bile for the two treatment groups are shown in Figure 2 and were qualitatively similar. However, the biliary excretion of CPT-11 decreased by approximately 50% in the presence of 10 μM loperamide (2.0 ± 0.9% dose, compared with 3.8 ± 1.0% in the control group, $P = 0.019$, Table 1). This change was also reflected in a significant reduction in biliary clearance of CPT-11 in the presence of loperamide ($P = 0.036$; Table 2). In contrast, the biliary excretion of the metabolites SN-38, SN-38G and APC were not significantly different between the two groups ($P > 0.05$). Likewise, the biliary clearance of SN-38 and SN-38G was not significantly different between the two groups ($P > 0.05$). Bile flow was not significantly different in the presence of 10 μM loperamide (5.8 ± 2.5 μL min^{-1}, compared with 7.1 ± 2.2 μL min^{-1} in the control group, $P > 0.05$). In the control group, the unchanged parent drug was the predominant species in bile, with approximately 4% of the dose excreted into bile as CPT-11 compared with approximately 1% of the dose excreted in the form of metabolites (SN-38, SN-38G and APC).

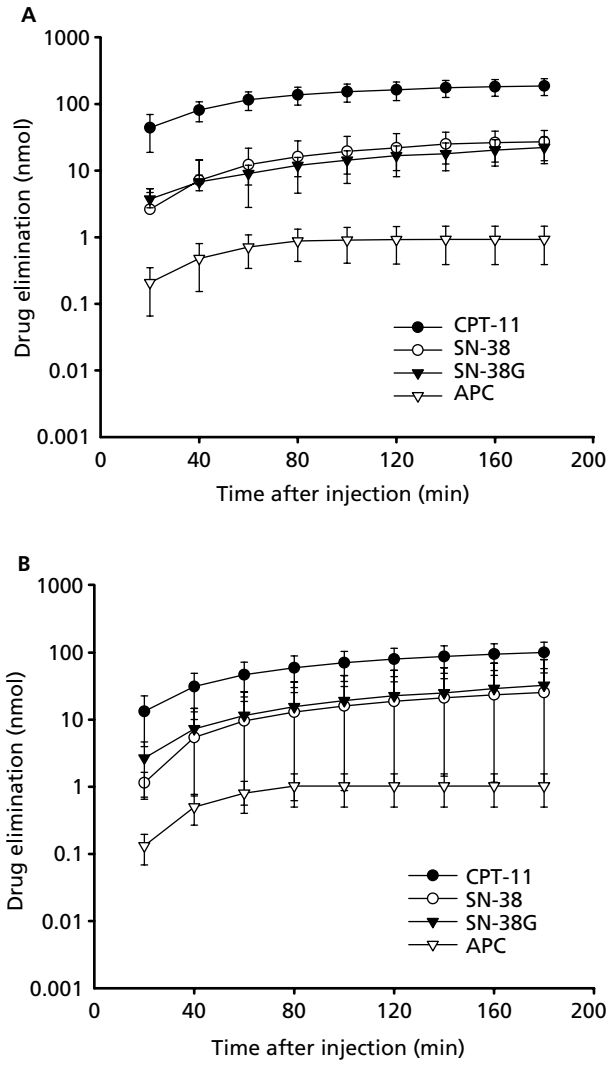

Figure 2 Cumulative biliary excretion of CPT-11 and metabolites. Results for (A) control IPRL (n = 5) and (B) experiments with loperamide (10 μM) in IPRL perfusate (n = 6) are presented. Data are presented as mean ± s.d.

Table 1 Comparison of the biliary excretion of CPT-11 and metabolites in the presence and absence of 10 μM loperamide. The percent of the CPT-11 dose excreted was calculated by dividing the total amount of CPT-11 or metabolite by the dose of CPT-11

Compound	Control (%)	Loperamide treatment (%)
CPT-11	3.8 ± 1.0	2.0 ± 0.9*
SN-38	0.6 ± 0.2	0.3 ± 0.2
SN-38G	04 ± 0.2	0.3 ± 0.2
APC	0.2 ± 0.01	0.02 ± 0.01

*$P < 0.05$, control vs loperamide treatment.

Table 2 Comparison of the biliary clearance (CL_{bile}) of CPT-11 and metabolites in the presence and absence of 10 μM loperamide treatment

Compound	Control CL_{bile} (mL h^{-1})	Loperamide CL_{bile} (mL h^{-1})
CPT-11	6.6 ± 3.7	2.5 ± 1.6*
SN-38	9.7 ± 6.5	5.5 ± 2.6
SN-38G	20.2 ± 11.5	12.1 ± 4.1

*$P < 0.05$, control vs loperamide treatment.

The perfusate concentration of CPT-11 decreased slowly over the 3-h perfusion, consistent with low hepatic extraction. As expected the perfusate concentrations of SN-38 and SN-38G increased over the time course of the IPRL perfusion (Figure 3), but APC, the cytochrome P450 metabolite, was not detected in the perfusate (limit of detection = 5 ng mL^{-1}). The AUC_{0-180} for CPT-11, SN-38 and SN-38G were not significantly different in the control and the loperamide perfusions (Table 3, $P > 0.05$ in each instance), and the concentration–time profiles were very similar also. The SN-38 and SN-38G metabolic ratios were not significantly different in the two groups ($P > 0.05$ in each case).

Discussion

The primary objective of this study was to examine the effect of loperamide on the biliary excretion of CPT-11. Loperamide (10 μM) in the perfusate inhibited the biliary

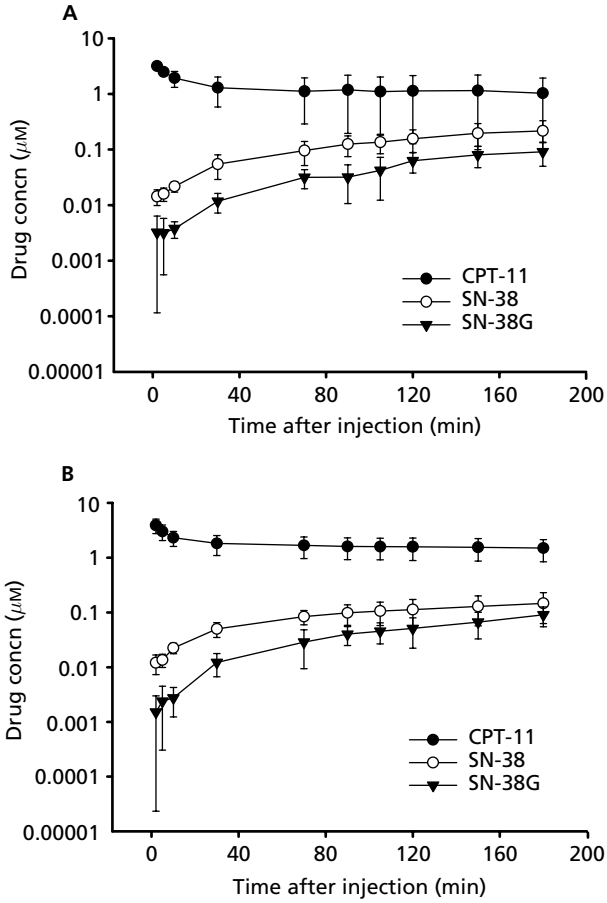

Figure 3 Concentrations of CPT-11 and metabolites in perfusate. Results for (A) control IPRL (n = 5) and (B) experiments with loperamide (10 μM) in IPRL perfusate (n = 6) are presented. Data are presented as mean ± s.d.

Table 3 Comparison of the perfusate concentrations and metabolism of CPT-11 in the presence (treatment) and absence (control) of 10 μM loperamide. AUC_{0-180} (μM h) values were calculated using the trapezoidal method. Metabolic ratios were calculated by dividing the AUC_{0-180} of the metabolite by that of CPT-11

Parameter	Control	Loperamide treatment
CPT-11 AUC_{0-180}	3.80 ± 2.50	5.21 ± 2.10
SN-38 AUC_{0-180}	0.34 ± 0.12	0.26 ± 0.12
SN-38G AUC_{0-180}	0.13 ± 0.05	0.12 ± 0.05
SN-38 metabolic ratio	0.11 ± 0.06	0.06 ± 0.04
SN-38G metabolic ratio	0.04 ± 0.03	0.03 ± 0.02

excretion of CPT-11 in the isolated perfused rat liver by approximately 50% (2.0 ± 0.90 vs 3.8 ± 1.0% of dose, $P = 0.019$). Consistent with this finding, the biliary clearance of CPT-11, which takes into account the perfusate AUC, was also found to be significantly lower in the presence of loperamide (2.5 ± 1.6 vs 6.6 ± 3.7, $P = 0.036$). Loperamide (10 μM) has been shown to selectively inhibit P-gp-mediated transport of CPT-11 in-vitro (Tobin et al 2003). Furthermore, bile flow was not affected by loperamide ($P > 0.05$), suggesting that inhibition of P-gp might have been responsible for the decrease in biliary excretion of CPT-11. That the inhibition was not greater, may suggest that other transporters were involved in the biliary excretion of CPT-11. A similar decrease in the biliary excretion of CPT-11 has been observed using the potent P-gp-specific inhibitor PSC833 in mice (Iyer et al 2002).

The biliary excretion of SN-38G and APC were not affected by the presence of loperamide in the perfusate ($P > 0.05$). This suggested that inhibition of P-gp was unlikely to affect the biliary excretion of SN-38G or APC. Although the excretion of SN-38 appeared to be lower in the presence of loperamide (0.6 ± 0.2% in controls and 0.3 ± 0.2% in the presence of loperamide) this difference was not found to be statistically significant ($P > 0.05$). These findings were consistent with those of Iyer et al (2002), who observed that the excretion of SN-38 and SN-38G was not significantly different in wild-type mice compared with mice lacking mdr1-type P-gp. Interestingly, however, PSC833 decreased the biliary recovery of SN-38 and SN-38G in the wild-type and P-gp knockout mice. Iyer et al (2002) proposed that the high dose of PSC833 used might have resulted in a loss of specificity for P-gp inhibition.

CPT-11 was preferentially excreted into bile as the parent drug, with approximately 4% of the dose excreted as CPT-11 compared with ~1% of the dose excreted in the form of metabolites. Although the biliary excretion of CPT-11 was lower than previously reported in human studies, this might have been due to intrinsic species differences and because the perfusion was only conducted for 3 h. The concentration of CPT-11 chosen (5 μM) in this experiment was within the range of concentrations typically reached in patients following intravenous administration. Furthermore, it has been shown to be well below the threshold at which saturation of CPT-11 metabolism or biliary excretion occurred in the IPRL (Farabos et al 2001). Therefore CPT-11 appeared to be poorly extracted by the liver, in accordance with the observations of Farabos et al (2001) who also used the IPRL model to examine the hepatic disposition of CPT-11. The SN-38 and SN-38G metabolic ratios in perfusate were quite low. This suggested that CPT-11 was slowly converted to SN-38, and this was also consistent with previous findings (Gupta et al 1994; Satoh et al 1994; Rivory et al 1996a; Haaz et al 1997; Ahmed et al 1999).

APC appeared to be efficiently excreted into the bile as it was detectable in bile but not in perfusate. However, this may have been merely due to a concentrating effect in the bile. The proportion of the dose recovered in bile as SN-38 and SN-38G paralleled the concentrations of these two metabolites in perfusate, suggesting that the efficiency of biliary excretion of these compounds was comparable.

The principal reason for the disparity between the low clearance of CPT-11 observed in the IPRL as compared with the total clearance in rats in-vivo was the absence of rodent plasma in the IPRL. Rodent plasma contains a very

high level of CPT-11 carboxylesterase activity (Kaneda et al 1990; Tsuji et al 1991). In contrast to rodents, human plasma carboxylesterase activity is negligible (Guemei et al 2001). Nevertheless, the absence of significant rat plasma in the IPRL was an advantage as it enabled an unambiguous evaluation of the biliary excretion of CPT-11 and the possible impact of loperamide. Furthermore, the absence of erythrocytes in this study eliminated the influence of erythrocyte uptake of the drug and metabolites, thereby allowing a clearer evaluation of the impact of loperamide on the biliary excretion of CPT-11. In an in-vitro study of the binding and partitioning of CPT-11 and SN-38, approximately 33% of CPT-11 and 13% of SN-38 was bound to erythrocytes (Combes et al 2000).

Loperamide had no statistically significant effect on perfusate concentrations of CPT-11 or metabolites (Figure 3). Consistent with the decreased biliary excretion of CPT-11 in the presence of loperamide, the perfusate AUC_{0-180} of CPT-11 appeared to be slightly higher in the presence of loperamide (3.8 ± 2.5 μM.h in control and 5.2 ± 2.1 μM.h in the presence of loperamide), however this difference did not reach significance ($P > 0.05$). Loperamide had no detectable effect on the perfusate AUC values of the metabolites ($P > 0.05$). Earlier studies showed loperamide to be a weak inhibitor of the conversion of CPT-11 to SN-38, but only at high concentrations (Rivory et al 1996a; Haaz et al 1997).

Conclusions

Loperamide inhibited the biliary excretion of CPT-11 in the rat perfused liver. It had been assumed that loperamide provided relief from CPT-11-induced gastrointestinal toxicity through symptomatic means, via its direct inhibitory effects on gut motility. The results from this study raised the possibility that a component of the beneficial effects of loperamide occurred through reduced biliary excretion of CPT-11 into the gastrointestinal tract. No prospective clinical studies of the effect of loperamide on CPT-11 pharmacokinetics have been reported and these should be considered.

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