INFLUENCE OF MULTIPLE DOSE ACTIVATED CHARCOAL ON THE DISPOSITION KINETICS OF IRINOTECAN IN RATS

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SUMMARY

***Introduction*:** The main clinical adverse effect of irinotecan (CPT-11) therapy is diarrhoea. Using a rat model, we attempted to study the effects of activated charcoal on the prevention of diarrhoea after a bolus dose of CPT-11, and also investigated the disposition kinetics of CPT-11 and its metabolite, SN-38, as well as SN-38 glucuronide (SN-38G) in the presence and absence of charcoal.

***Materials and Methods*:** Male Sprague-Dawley rats were given a daily oral dose of activated charcoal (Ultracarbon, 2.5 g/kg daily for 5 days) 10 min before an i.v. bolus injection of CPT-11 (60 mg/kg daily for 5 days; total dose 300 mg/kg); the control group was given CPT-11 alone. The pharmacokinetics of CPT-11, SN-38 and SN-38G were determined in both groups of rats on day 1. The incidence of diarrhoea was monitored throughout the course of the study.

***Results*:** There were no differences in the mean (± SD) C_{max} (15.8 ± 7.5 vs 12.1 ± 3.3 μg/ml), $t_{½}$ (2.2 ± 0.7 vs 2.2 ± 0.5 h), CL (5.7 ± 2.1 vs

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6.8 ± 1.2 l/h/kg), V_d (1798 ± 958 vs 2280 ± 731 l/kg) or $AUC_{0-\infty}$ (11.8 ± 3.9 vs 9.1 ± 1.7 μg·h/ml) of CPT-11 after dosing with or without activated charcoal. Similarly, charcoal treatment had no effect on the disposition kinetics of SN-38 and SN-38G. A higher frequency of grade 3 diarrhoea was observed in the control group compared to the charcoal treatment group (log OR: -1.06; 95% CI: -2.25, 0.13) but this was only marginally statistically significant (p = 0.08).

***Conclusion*:** These results suggest that multiple oral doses of activated charcoal do not modulate the clearance of CPT-11 and SN-38 in rats. The implication is that activated charcoal alone may not be very effective in preventing CPT-11-induced diarrhoea.

KEY WORDS

irinotecan, CPT-11, SN-38, charcoal, diarrhoea

INTRODUCTION

Irinotecan hydrochloride (CPT-11) is a water-soluble derivative of camptothecin (CPT) /1,2/. CPT-11 acts as a prodrug *in vivo* and is converted to SN-38 by the enzyme carboxylesterase. SN-38 is the active metabolite and is mainly responsible for the antitumour activity by inhibiting topoisomerase I and causing single-strand breaks /3/. SN-38 undergoes glucuronidation by uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) to form the inactive metabolite, SN-38 glucuronide (SN-38G). SN-38G is eliminated from the systemic circulation by excretion into bile along with the other major components, CPT-11 and SN-38. The SN-38G excreted in bile is deconjugated to SN-38 by β-glucuronidase-producing intestinal bacteria such as *Escherichia coli* and *Clostridium perfringes*.

The major dose-limiting toxicities of CPT-11 are myelosuppression and diarrhoea /4/. The diarrhoea presents in two forms, acute-onset and delayed-onset diarrhoea /5/. Acute-onset diarrhoea is thought to be due to the cholinergic activity of CPT-11 that stimulates intestinal contractility and can often be reversed by treatment with anticholinergics such as atropine /6/. Delayed-onset diarrhoea is often severe with a median onset time of about 5 days and a median duration of 5 days. It has been postulated that delayed-onset diarrhoea

is due to accumulation of SN-38 in the intestine /7/. Enterohepatic cycling can prolong the retention time and exposure of SN-38 to intestinal mucosa, resulting in local damage.

Several investigators have attempted to use different antibiotics to modulate CPT-11-induced diarrhoea. Preclinical data in rats utilising penicillin combined with streptomycin was effective in inhibiting β-glucuronidase activity from the intestinal microflora, thereby reducing caecal damage and ameliorating diarrhoea. Antibiotic treatment did not alter the pharmacokinetics of CPT-11, SN-38 or SN-38G in the blood, or in the tissues or contents of the small intestine /8/. Neomycin had also been used to control CPT-11-induced delayed diarrhoea in cancer patients and found to be effective in six of seven patients tested /9/.

Owing to its effective adsorbent property, activated charcoal (AC) has been used to prevent the absorption of many orally administered xenobiotics, especially in instances of overdose /10,11/. Several studies have also demonstrated the usefulness of AC in enhancing the elimination of certain drugs from the body. The latter mechanism has been called 'gastrointestinal dialysis' /11/. AC is also known to disrupt the enterohepatic cycling of drugs and xenobiotics, thus enhancing their elimination by the faecal route. We hypothesised that adsorption of SN-38 and SN-38G by AC might decrease the frequency and grade of CPT-11-induced diarrhoea by decreasing the exposure of the gut wall to SN-38. We also examined the influence of oral AC on the disposition of CPT-11 and its metabolites.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (92-118 g) (Animal Resource Centre, Western Australia) were fed standard laboratory chow and were allowed to acclimate to their environment for 1 week before starting the study. The rats were administered water *ad libitum* and were housed and treated in accordance with the recommendations and policies of the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals.

Materials and reagents

CPT (lot 95), CPT-11 (lot 115122), SN-38 (lot 3L 931001 WS) and SN-38G (lot 970326) were kind gifts from Yakult Honsha Co. (Tokyo, Japan). A purity of >96% for each compound was confirmed by HPLC analysis. Ultracarbon charcoal was purchased from Merck, Germany.

Drug administration and pharmacokinetic studies

Two groups of Sprague-Dawley rats (n = 7 per group) were used for the study. Rats in the control group received CPT-11 60 mg/kg by slow bolus injection via the tail vein. In the charcoal-treated group, the rats were administered charcoal (2.5 g/kg) orally as a slurry in 2 ml of distilled water by gastric intubation. Charcoal was administered 10 min before CPT-11 dosing and continued for 5 days. The total dose of CPT-11 administered to each rat was 300 mg/kg over a period of 5 consecutive days.

Blood samples were collected through in-dwelling jugular venous catheters before and at 5, 15, 30 min and 1, 2, 4, 6, 9, 12 and 24 hours after administration of CPT-11 on day 1. Approximately 0.2 ml of blood was obtained at each sampling time, after 0.1 ml of fluid had been withdrawn (which was subsequently reinjected and followed by 0.2 ml of saline) to avoid an artifact caused by samples contaminated by saline or blood trapped in the cannula. Blood samples were processed to serum by centrifuging at 3,000 rpm for 10 min at 4°C, and stored immediately at -20°C until analysis by high-performance liquid chromatography (HPLC).

Diarrhoea assessment

The time of onset and duration of diarrhoea were recorded by the same person daily. A scoring system (grades 0-3) was used to assess the severity of diarrhoea as described by Takasuna *et al.* /12/. Daily changes in body weight were also recorded.

HPLC analysis of CPT-11, SN-38 and SN-38G

For the determination of CPT-11 and SN-38 levels in serum, 50 µl of serum sample was mixed with 130 µl ice-cold methanol and 20 µl

of internal standard (camptothecin 500 μg/ml) and vortexed for 30 s. The mixture was then centrifuged for 5 min at 9,000 rpm at 4°C. An aliquot of the supernatant was then analysed by HPLC.

SN-38G concentrations were determined as the increase in SN-38 concentration following incubation with β-glucuronidase (Type HA-4, Sigma Chemical Co., St. Louis, MO, USA). To 50 μl of serum, a solution of 200 units β-glucuronidase in 10 μl of water was added. After incubation for 2 h at 37°C, 140 μl of methanol was added. The sample was vortexed briefly, followed by centrifugation and analysis by HPLC.

A reversed-phase HPLC method with fluorescence detection was used for the determination of CPT-11, SN-38 and SN-38G. The HPLC system consisted of a Waters 2690 separation module (Waters Associates, Milford, MA, USA), a Waters 474 fluorescence detector, and a C18 analytical column (Symmetry®, 5 μm, 150 x 3.9 mm i.d.; Waters Associates, Milford, MA, USA) preceeded by a C18 Symmetry® guard column. An isocratic elution mode was employed at flow rate of 1 ml/min at ambient temperature. Sample volumes of 20 μl were injected into a mobile phase consisting of 30% acetonitrile: 70% 45 mM potassium phosphate buffer (pH 3.5) containing 10 mM heptanesulphonate. The detector was set at 373 and 428 nm (excitation and emission wavelengths, respectively) from 6 to 10 min for the determination of CPT (the internal standard) and CPT-11 and 380 and 540 nm from 0 to 6 min for the determination of SN-38. Analysis of the chromatograms was performed with the Millenium software program (version 3.20, Waters Associates, Milford, MA, USA).

The calibration curves for CPT-11, SN-38 and SN-38G were linear over their respective concentration ranges (3.2-10,000 ng/ml, 0.64-2,000 ng/ml and 1.28-4,000 ng/ml for CPT-11, SN-38 and SN-38G, respectively). The lower limit of quantification (LLOQ) was 1.0, 0.5 and 1.0 ng/ml for CPT-11, SN-38 and SN-38G, respectively. The within-day coefficient of variation (CV) values (n = 5) at high (10,000, 2,000 and 4,000 ng/ml for CPT-11, SN-38 and SN-38G, respectively) and low (3.2, 0.64 and 1.28 ng/ml for CPT-11, SN-38 and SN-38G, respectively) concentrations of the compounds were all below 6%. For the between-day variation (n = 5), the CV values at high and low concentrations were all below 7%.

Pharmacokinetic analysis

Pharmacokinetic parameters for CPT-11, its metabolites, SN-38 and SN-38G were determined by non-compartmental methods using a non-linear regression program, WinNonLin version 2.1 (Pharsight Inc., Montain View, CA). Peak plasma concentrations (C_{max}) and time to peak concentration (t_{max}) were identified from individual subject concentration-time curves. Area under the plasma concentration-time curve from time zero to the time (t) of the last detectable concentration (AUC_{0-t}) was calculated using the trapezoidal rule. The area was extrapolated to infinity ($AUC_{0-\infty}$) by adding $C_t/\lambda z$ to AUC_{0-t}, where C_t was the last detectable plasma concentration. Apparent elimination rate constants (λ_z) were estimated by least-squares regression of values in the terminal log-linear region of the plasma CPT-11, SN-38 and SN-38G concentration-time curves, whereas half-life ($t_{½z}$) was calculated as $\ln(2)/\lambda_z$. Total clearance of CPT-11 was calculated as the ratio of dose to $AUC_{0-\infty}$.

Statistical analysis

ANOVA was used to analyse differences in pharmokinetic parameters between the two groups. The proportional odds model was used to analyse the diarrhoea grade data.

RESULTS

Pharmacokinetics of CPT-11 and its metabolites

CPT-11 plasma concentrations decreased biexponentially in both groups (Fig. 1), although the decline was more rapid in the charcoal-treated group compared with the control group. C_{max} and $AUC_{0-\infty}$ were 1.3-fold higher in the control group compared with the charcoal-treated group but these were statistically not significant ($p > 0.05$, Table 1). All other pharmacokinetic parameters ($t_{½}$, V_d, V_{ss} and CL) were similar between control and charcoal-treated groups (Table 1).

The plasma concentration-time profiles of SN-38 and SN-38G in both groups of rats are shown in Figure 1. C_{max} and $t_{½}$ were similar in the two groups ($p > 0.05$, Table 1). As expected, a transient increase in SN-38 concentrations were observed in both groups between 2 and 4 hours, probably due to enterohepatic circulation (Fig. 1). This was

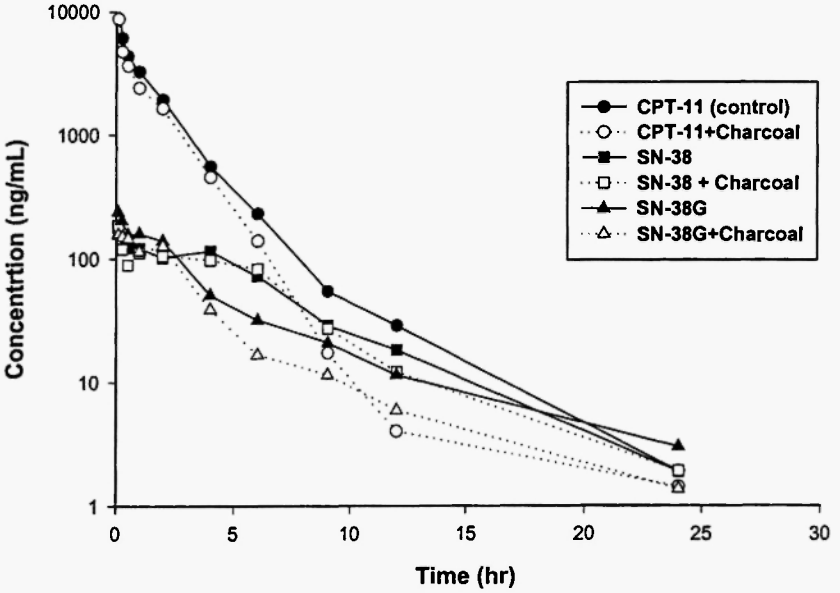

Fig. 1: Effects of oral charcoal (2.5 mg/kg) on the serum concentration-time curves of CPT-11 and its metabolites after a single i.v. dose of CPT-11 (60 mg/kg). Each point represents the mean concentration from seven rats.

followed by a parallel decline in SN-38 concentration in both groups. There was no difference in $AUC_{0-\infty}$ between the two groups ($p > 0.05$, Table 1), although a marginally statistically significant increase in $AUC_{0-\infty}$ was observed for SN-38G in the charcoal-treated group.

Diarrhoea grade in control vs charcoal-treated rats

Table 2 shows the frequency distribution of diarrhoea grade between the two groups of rats. The rats in the control group had at least grade 2 diarrhoea and an obviously higher frequency of grade 3 diarrhoea. Treatment with charcoal resulted in a lower odds ratio of -1.06 (95% CI: -2.25, 0.13), suggesting that charcoal had some effect in reducing diarrhoea grade, although it was only marginally statistically significant ($p = 0.08$).

TABLE 1

Pharmacokinetic parameters of CPT-11 and its metabolites after i.v. bolus injection of CPT-11 (60 mg/kg) to rats in the absence and presence of activated charcoal (AC)

		CPT-11	CPT-11 + AC	P-value
CPT-11	**$AUC_{0-\infty}$** (μg·h/ml)	11.8 (3.9)	9.1 (1.7)	0.12
	C_{max} (μg/ml)	15.8 (7.5)	12.1 (3.3)	0.25
	$t_{½}$ (h)	2.2 (0.7)	2.2 (0.5)	0.96
	Vd (l/kg)	1798 (958)	2280 (731)	0.31
	Vss (l/kg)	911 (411)	1086 (168)	0.32
	CL (l/h/kg)	5.7 (2.1)	6.8 (1.2)	0.25
SN-38	**$AUC_{0-\infty}$** (μg·h/ml)	0.9 (0.4)	0.9 (0.2)	0.62
	C_{max} (μg/ml)	0.2 (0.03)	0.2 (0.1)	0.71
	$t_{½}$ (h)	3.8 (0.8)	3.8 (0.8)	0.93
SN-38G	**$AUC_{0-\infty}$** (μg·h/ml)	0.8 (0.2)	0.6 (0.2)	0.05
	C_{max} (μg/ml)	0.4 (0.1)	0.2 (0.1)	0.09
	$t_{½}$ (h)	4.9 (1.2)	4.6 (1.3)	0.66

P-values based on ANOVA.
Results are means (standard deviations are given in parentheses).
n = 7.
$AUC_{0-\infty}$ = area under the concentration-time curve from t = 0 h to t = ∞;
C_{max} = maximum concentration;
$t_{½}$ = elimination half-life;
Vd = volume of distribution;
Vss = volume of distribution at steady state;
CL = clearance.

TABLE 2

Frequency distribution of diarrhoea grade between rats given CPT-11 alone (60 mg/kg) and rats also treated with activated charcoal (AC)

Treatment	Frequency of diarrhoea grade			Proportional odds model	
	Grade 1	Grade 2	Grade 3	Log odds ratio	95% CI
CPT-11	0	23	12	0.00	(Reference group)
CPT-11 + AC	2	27	6	-1.06	(-2.25, 0.13)

Left panel shows frequency distribution of diarrhoea grade by treatment groups (14 rats; 70 daily observations in all); right panel shows results from a proportional odds model with charcoal as indicator variable.
95% confidence intervals (CI) based on Huber-White method to adjust for repeated measures within each rat.

DISCUSSION

CPT-11 and its metabolites have very complex disposition characteristics and the use of AC in enhancing the elimination of CPT-11 and its metabolites and preventing CPT-11-induced diarrhoea could be of great therapeutic benefit to cancer patients. AC is a readily available, cheap and relatively safe drug with minimal side effects. Its excellent adsorbent properties have resulted in it being routinely used in the treatment of poisoning. Adsorption of drugs to AC increases with the increase in surface area of AC. Drug adsorption onto AC varies for different drugs, being greater for aromatic than aliphatic compounds, and for unionised than ionised drugs /10/.

AC can enhance the elimination of drugs by two mechanisms. Firstly, through the process of 'gastrointestinal dialysis', it facilitates the adsorption of non-protein bound, nonionic drugs from the systemic circulation into the gastrointestinal lumen and prevents reabsorption /10,11/. Because the concentration of drug in the gastrointestinal lumen is kept near zero, a concentration gradient is established that allows continual diffusion of drug from the systemic circulation into the gastrointestinal lumen. Secondly, AC can interrupt the entero-hepatic circulation of drugs that are secreted into bile before being reabsorbed into the body. The drugs may be in an unchanged form or may be present in the form of a conjugated metabolite which may be subsequently enzymatically hydrolysed in the gastrointestinal lumen /11/.

There are three possible routes of entry of SN-38 into the gut lumen: firstly, via biliary excretion along with CPT-11 and SN-38G; secondly, by the process of 'gastrointestinal dialysis' whereby free SN-38 present in plasma diffuses from the basolateral to the apical region in the gut; and thirdly, through conversion of SN-38G to SN-38 by the action of bacterial and enteric β-glucuronidases. Which of these processes predominates is not clear. Furthermore, *in vitro* studies using Caco-2 cell monolayers showed that the transepithelial flux of CPT-11 and SN-38 occurred in a concentration-dependent manner, being four- to nine-fold greater from the basolateral to the apical side than from the apical to the basolateral side /13/. Nevertheless, all three processes above can certainly contribute to the local increase in SN-38 concentration in the intestinal lumen and cause intestinal mucosal damage leading to diarrhoea.

The present study did not show AC to have any substantial effect on the pharmacokinetics of CPT-11, SN-38 and SN-38G. The principal findings were that oral administration of multiple doses of high-surface area AC had no effect on the serum $t_{1/2}$ or systemic clearance of SN-38. It is possible that recycling of SN-38 may add a distributional compartment by way of the enteric circuit and prevent it from being effectively adsorbed by AC. SN-38G, on the other hand, does not undergo enterohepatic cycling (Fig. 1), consistent with its increased aqueous solubility resulting from the attached glucuronic acid group which is highly polar in nature.

AC-treated rats had a marginally statistically significant reduction in the incidence of grade 3 diarrhoea compared with controls (Table 2), showing a lack of significant contribution by AC in prevention of CPT-11-induced diarrhoea. We employed multiple doses of AC to enhance the rate of elimination of CPT-11 and its metabolite, SN-38. Multiple dose therapy, as opposed to single-dose AC therapy, has been shown to be effective in enhancing the rate of elimination of absorbed drugs in the treatment of poisoning. Although no studies were done to determine the optimal dose of AC, previous studies have shown lower doses to be effective in the treatment of poisoning in preclinical models.

CONCLUSION

This study showed that multiple dose therapy with AC is not very effective in preventing CPT-11-induced diarrhoea. Since several possible routes are available for SN-38 to gain entry into the intestinal lumen and cause damage to intestinal mucosal walls, other therapeutic strategies should be attempted, such as inhibiting the liberation of active metabolite, SN-38, in the intestinal lumen by inhibiting β-glucuronidase.

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