Modified irinotecan hydrochloride (CPT-11) administration schedule improves induction of delayed-onset diarrhea in rats

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Abstract Purpose: Clinically, diarrhea is the major dose-limiting toxicity of irinotecan hydrochloride (CPT-11). Using a rat model, we attempted to decrease the incidence of delayed-onset diarrhea by modifying the administration schedule of CPT-11, and studied the pharmacokinetics in this model in relation to the incidence of diarrhea. Methods: CPT-11 (total dose, 240 mg/kg) was administered intravenously (i.v.) to rats according to various schedules, and the incidence of delayed-onset diarrhea was monitored. Results: Administration of CPT-11 at a dose of 60 mg/kg once daily for four consecutive days induced severe diarrhea, while at 30 mg/kg twice daily at an interval of 9 h (daily dose 60 mg/kg) for four consecutive days alleviated the diarrheal symptoms, and at 30 or 40 mg/kg once daily for eight or six consecutive days, respectively, diarrhea was hardly induced. With the first schedule, mucosal impairment of the cecal epithelium was observed, including wall thickening, edema, decrease in crypt number and size, and formation of pseudomembrane-like substance, whereas these changes were less severe with the second schedule and were hardly observed with the other two schedules. The areas under the plasma and cecal tissue concentration-time curves (AUC$_{\text{plaq}}$ and AUC$_{\text{cec}}$), the maximum plasma concentrations ($C_{\text{max}}$) and the biliary excretions of CPT-11 and its metabolites, 7-ethyl-10-hydroxycamptothecin (SN-38) and SN-38 glucuronide (SN-38G) in rats depended on the daily dose of CPT-11. Exceptionally, CPT-11 $C_{\text{max}}$ was significantly lower and SN-38 AUC$_{\text{cec}}$ was larger in the animals treated at 30 mg/kg twice daily than in those treated at 60 mg/kg once daily. Conclusion: These results suggested that the duration of exposure to both CPT-11 and SN-38 of the intestinal epithelium and CPT-11 plasma $C_{\text{max}}$ are closely related to the incidence and severity of CPT-11-induced delayed-onset diarrhea in rats.

Key words Irinotecan hydrochloride · CPT-11 · SN-38 · Delayed-onset diarrhea · Pharmacokinetics

Introduction

Irinotecan hydrochloride (CPT-11) is a water-soluble derivative of camptothecin (CPT) [12, 25] and is used clinically to treat colorectal, gastric, lung, uterine cervical and ovarian cancers, malignant lymphoma and other malignancies [10, 32, 39, 40, 46]. However, at higher dosage, CPT-11 sometimes causes severe diarrhea, which is recognized as the dose-limiting toxicity and limits the use of more aggressive CPT-11 therapy [1, 10, 28, 31, 33, 39]. The diarrhea is of two types, acute or delayed onset, either or both of which can occur [36].

It is assumed that in the acute diarrhea the cholinergic activity of CPT-11 stimulates intestinal contractility and disturbs normal intestinal mucosal absorptive and secretory functions [13, 21, 42]. This diarrhea is of short duration and can be prevented or rapidly suppressed by atropine [13, 36]. In contrast, delayed-onset diarrhea is so severe that it can be life-threatening. Moreover, it is unpredictable despite the many pharmacokinetic studies done on it [15, 24, 31, 37]. Antidiarrheal agents such as loperamide and acetylperan may be effective in treating this diarrhea, but they are not effective in controlling the symptoms in all patients [1, 14, 36]. The great interpatient variability in both the severity of the diarrhea and the effect of the antidiarrheal agents makes it difficult to elucidate the mechanism involved in producing this diarrhea [1, 10, 28, 39].

It is possible that the mitotic activity of CPT-11 and its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), damages the gastrointestinal tract both structurally and functionally [15, 16, 42, 43, 44]. The pharmacokinetics of SN-38 are considered to be the main cause of the induction of the diarrhea, as SN-38 is much
more cytotoxic than CPT-11 [22, 23]. The major metabolic pathway of CPT-11 is shown in Fig. 1. CPT-11 is hydrolyzed to SN-38 by carboxylesterase. Most of the SN-38 is subsequently conjugated to SN-38 glucuronide (SN-38G) by UDP-glucuronosyltransferase in the liver. SN-38G is excreted in the bile with the other major components, CPT-11 and SN-38 [3, 18]. Most of the SN-38G excreted in the bile is deconjugated to SN-38 by \( \beta \)-glucuronidase of the intestinal microflora [18, 45]. Inhibition of \( \beta \)-glucuronidase by coadministration of antibiotics decreases the SN-38 concentration in the intestinal tissue and alleviates diarrheal symptoms [44, 45]. Therefore, \( \beta \)-glucuronidase is considered to be an important factor in the induction of the diarrhea [35, 43, 44, 45, 46]. Delay in SN-38 disposition might exacerbate the diarrheal symptoms [15, 16].

We investigated the mechanisms of CPT-11-induced delayed-onset diarrhea using a rat model. As in humans, CPT-11 induces two types of diarrhea, acute and delayed-onset, in rats. Takasuna et al. [42] have reported that the conventional antidiarrheal agents, atropine, ondansetron, clonidine and morphine, can prevent the acute diarrhea, but exacerbate the delayed-onset diarrheal symptoms associated with intestinal mucosal disruption. This suggests that commonly used antidiarrheal agents are unsuitable for treating CPT-11-induced delayed-onset diarrhea [42, 43].

In this study, we attempted to reduce the incidence of delayed-onset diarrhea by modifying the CPT-11 administration schedule, and studied the relationship of the pharmacokinetics of CPT-11 and its metabolites to the incidence and the severity of delayed-onset diarrhea in rats.

Materials and methods

Materials and reagents

CPT-11 (lot 115002), SN-38 (lot 30091R) and SN-38G (lot 970326) were provided by Yakult Honsha Co. (Tokyo, Japan). CPT was purchased from Sigma Chemical Co. (St. Louis, Mo.). Sodium 1-decanesulphonate was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). The water was of Milli-Q grade (Millipore Co., Bedford, Mass.) and all other chemicals were of analytical or HPLC grade obtained from commercial sources.

Animals

Male Sprague-Dawley rats were purchased from Japan SLC (Hamamatsu, Japan) and used for experiments after a 1-week acclimatization with free access to water and commercial animal chow (F-2; Funabashi Farm, Funabashi, Japan). Rats weighing 230–260 g were used in all experiments except for monitoring the incidence of diarrhea after consecutive administrations of CPT-11, for which rats weighing 165–185 g were used.

Administration schedules

Saline or CPT-11 (total dose 240 mg/kg) was administered intravenously (i.v.) to the animals via the tail vein in one of five administration schedules (control, S1, S2, S3, S4) as listed in Table 1 between 8:00 and 9:00 a.m. for the first administration and between 5:00 and 6:00 p.m. for the second administration of S2.

Monitoring of CPT-11-induced diarrhea

The severity of diarrhea and body weight changes were monitored throughout the experimental period (11 days from the first administration). Diarrhea observed after the final administration was considered to be delayed-onset diarrhea. The severity of the

![Fig. 1 Major metabolic pathway of irinotecan hydrochloride (CPT-11) in the rat](image)

Table 1 CPT-11 i.v. administration schedules. The total dose of CPT-11 in each schedule was 240 mg/kg

<table>
<thead>
<tr>
<th>Schedule</th>
<th>Compound</th>
<th>Daily dose</th>
<th>Administration period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Physiological saline</td>
<td>3 ml/kg once daily</td>
<td>1–4</td>
</tr>
<tr>
<td>S1</td>
<td>CPT-11</td>
<td>60 mg/kg once daily</td>
<td>1–4</td>
</tr>
<tr>
<td>S2</td>
<td>CPT-11</td>
<td>30 mg/kg twice daily at a 9-h interval</td>
<td>1–4</td>
</tr>
<tr>
<td>S3</td>
<td>CPT-11</td>
<td>30 mg/kg once daily</td>
<td>1–8</td>
</tr>
<tr>
<td>S4</td>
<td>CPT-11</td>
<td>40 mg/kg once daily</td>
<td>1–6</td>
</tr>
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