Interleukin 15 Offers Selective Protection from Irinotecan-induced Intestinal Toxicity in a Preclinical Animal Model

Shousong Cao, Jennifer D. Black, Anthony B. Troutt, and Youcef M. Kustuni


Abstract

Irinotecan (CPT-11) is a chemotherapeutic agent that is active in the treatment of a variety of solid tumor malignancies. Diarrhea represents the most common dose-limiting toxicity that is independent of the schedule of administration. A rat model with dose-limiting toxicity profiles that are similar to those observed in patients treated with CPT-11 was developed and used to evaluate the role of interleukin 15 (IL-15) in the modulation of the therapeutic selectivity of CPT-11 in normal rats and rats bearing advanced colorectal cancer. The maximum tolerated dose and lethal dose (LD) of CPT-11 by i.v. push daily x 3 were 150 and 200 mg/kg/day, respectively. CPT-11 at the LD induced a 93–100% incidence of severe diarrhea and an 86–100% incidence of lethality in treated animals. IL-15, a cytokine with multiple mechanisms of action, was used at a 100 or 400 µg/kg/dose with different schedules of administration (3, 8, and 11 doses, i.p.) to protect against CPT-11-induced toxicity. IL-15 offered complete and sustained selective protection against CPT-11-induced delayed diarrhea and lethality. IL-15 also moderately potentiated the antitumor activity of CPT-11 in rats bearing advanced colorectal cancer. Morphological examination of rat intestinal tissues after treatment with LD of CPT-11 revealed dramatic protection of duodenal and colonic tissue architecture by IL-15. CPT-11 alone produced serious damage to duodenal villi and colonic crypts.

The results clearly demonstrated the ability of IL-15 to provide significant protection from CPT-11-induced intestinal toxicity with maintenance of antitumor activity, resulting in an increase in the therapeutic index of CPT-11. The clinical relevance of the results obtained in this model system needs to be confirmed.

Introduction

CPT-113 (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin) is a water-soluble, semisynthetic derivative of camptothecin that is active in gastrointestinal malignancies, with no cross-resistance to prior therapy with FUra, with or without LV (1–4). The mechanism of action of CPT-11 is inhibition of nuclear DNA topoisomerase I, leading to an accumulation of single-strand DNA breaks and, ultimately, to cell death (5). The major dose-limiting toxicities of CPT-11 are myelosuppression and gastrointestinal toxicity, especially severe diarrhea. Although CPT-11-induced myelosuppression, manifested as neutropenia (1), is of little clinical significance because it is of short duration and can be readily managed by colony-stimulating factors (6), the diarrhea may be extremely severe, and it does not respond well to conventional antidiarreal agents. Although loperamide, acetorphan (Tiorfan, an inhibitor of enkephalinase). and Tj-14 (a Kampo, Chinese herb medicine) could offer some help in reducing CPT-11-induced diarrhea (1, 7–9), the dose-limiting toxicity remains a clinical problem for outpatients.

IL-15 is a cytokine that shares many biological activities with IL-2 (10). Recent studies have shown that the IEC-18 rat intestinal crypt cell line expresses high-affinity IL-15 receptors and that the cytokine can promote modest proliferation of the Caco-2 human intestinal epithelial cell line. Furthermore, we have previously demonstrated the ability of IL-15 to protect against mucosal toxicity induced by FUra with or without LV in rats bearing colorectal cancer with potentiation of antitumor activity (11). These findings suggest that administration of IL-15 might alter the response of the gastrointestinal tract to chemotherapy-induced damage (12). Here, we describe the development of an animal model that exhibits a profile of CPT-11-induced mucosal toxicity that is similar to that observed clinically and its use in evaluating the potential of IL-15 to protect from CPT-11-induced diarrhea without reversing its antitumor activity.

Materials and Methods

Animals and Tumor. Eight- to 12-week-old female Fisher 344/N rats (body weight, 150–180 g) were obtained from Harlan Sprague Dawley (Indianapolis, IN) and kept at four rats per cage with water and food ad libitum, according to an institutionally approved animal protocol. The chemically induced Ward colorectal carcinoma was used in this study (13). Nonnecrotic tumor pieces (0.1 g) were transplanted s.c. via trocar under slight ether anesthesia.

Materials. CPT-11 was supplied by Pharmacia & Upjohn (Kalamazoo, MI) as a ready-to-use solution, in 2- and 5-ml vials that contained 40 and 100 mg of drug (20 mg/ml), respectively. Purified human recombinant IL-15 was provided by Immunex (Seattle, WA). The stock solution of IL-15 was diluted in PBS containing 1 mg/ml BSA (Sigma Chemical Co., St. Louis, MO) prior to administration. The stocks were stored at 4°C and used within 2 weeks.

Drug Doses and Schedules. CPT-11 was administered by i.v. push daily for 3 days at 150 and 200 mg/kg/day. IL-15 (100 or 400 µg/kg/dose) was administered i.p. according to three schedules: (a) at −24, −12, and −2 h relative to the first dose of CPT-11, for a total of 3 doses; (b) three doses prior to initiation of CPT-11 treatment and twice daily during CPT-11 administration, for a total of 8 doses; and (c) three doses prior to initiation of CPT-11 treatment and 3 times a day during CPT-11 administration for a total of 11 doses.

MTD and Toxicity Evaluation. The MTD was defined as the maximum dose that caused no drug-related lethality and that produced animal body weight loss of <20% of original weight. The kinetics of drug-induced toxicities (body weight loss, diarrhea, and lethality) were determined daily for a minimum of 3 weeks and observed at least twice a week thereafter.

Antitumor Activity. Drug treatments were initiated 12–14 days after s.c. tumor transplantation, when tumor weight was ~3.0 g, as described previously (13). Each group had four rats per experiment, and each experiment was repeated at least three times. Tumor response was expressed as partial tumor regression when tumor weight was temporarily reduced by at least 50% and as CR when tumor was undetectable by palpation.

Morphological Analysis. Animals were treated with CPT-11 at 200 mg/kg for 3 days in the absence or presence of IL-15 (100 µg/kg, 11 doses in total),
IL-15 PROTECTS FROM CPT-11-INDUCED INTESTINAL TOXICITY

Table 1: Modulation of the toxicity of CPT-11 by IL-15 in normal rats

CPT-11 was administered by i.v. push daily × 3. IL-15 was administered by i.p. injection 24, 12, and 2 h prior to CPT-11 (3 doses); 24, 12, and 2 h prior to CPT-11, then twice daily during CPT-11 administration (8 doses); or 24, 12, and 2 h prior to CPT-11, then three times a day during CPT-11 administration (11 doses). The data are combined from four to seven independent experiments, with 16–28 rats in total for each experimental group.

<table>
<thead>
<tr>
<th>CPT-11 (mg/kg/day)</th>
<th>IL-15 (µg/kg/dose)</th>
<th>Maximum weight loss</th>
<th>Diarrhea (%)</th>
<th>Death (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>4.5 ± 1.2</td>
<td>0/16</td>
<td>0/16</td>
</tr>
<tr>
<td>150</td>
<td>None</td>
<td>14.2 ± 3.2</td>
<td>50/12(24)</td>
<td>0/3(24)</td>
</tr>
<tr>
<td>200</td>
<td>400 × 3</td>
<td>23.5 ± 3.2</td>
<td>93/26(28)</td>
<td>86/24(28)</td>
</tr>
<tr>
<td>200</td>
<td>400 × 8</td>
<td>17.0 ± 4.6</td>
<td>50/8(16)</td>
<td>50/8(16)</td>
</tr>
<tr>
<td>200</td>
<td>400 × 11</td>
<td>16.3 ± 4.0</td>
<td>50/8(16)</td>
<td>44/7(16)</td>
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<tr>
<td>200</td>
<td>100 × 3</td>
<td>13.2 ± 4.5</td>
<td>50/8(16)</td>
<td>44/7(16)</td>
</tr>
<tr>
<td>200</td>
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<td>13.0 ± 5.2</td>
<td>50/8(16)</td>
<td>35/6(16)</td>
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<tr>
<td>200</td>
<td>100 × 11</td>
<td>8.4 ± 2.4</td>
<td>8/2(2/2)</td>
<td>0/2(2/2)</td>
</tr>
</tbody>
</table>

a Mean ± SD.
b MTD.
c,d,e Significantly different from same dose of CPT-11 alone:
   c P < 0.05.
d P < 0.01.
e P < 0.001.

Statistical Analysis. The differences between the mean values were analyzed for significance using the unpaired two-tailed Student’s t test for independent samples; P ≤ 0.05 was considered to be statistically significant.

Results

Diarrhea Incidence Induced by CPT-11 in Normal Rats. At the MTD of CPT-11 (150 mg/kg/day × 3), 50% of treated animals developed mild and reversible diarrhea. Following i.v. push of CPT-11 at 200 mg/kg/day for 3 days, no diarrhea was observed after the first two doses. However, watery diarrhea occurred on day 3 or 4 and lasted for 2–3 days in 93% (26 of 28) of treated animals (acute diarrhea). Subsequently, the diarrhea was associated with moderate bleeding (chronic or delayed diarrhea) and significant body weight loss at 6–10 days after treatment. Eighty-six % (24 of 28) of animals died by day 12 (7% of animals recovered from diarrhea and survived). The kinetics of acute and chronic diarrhea observed in this model system are consistent with those normally observed in patients treated with CPT-11.

Toxicity Protection in Normal Rats. IL-15 alone produced no observable toxicity in rats at the tested doses (Table 1). Administration of three doses of IL-15 at 100 or 400 µg/kg/dose offered partial protection from CPT-11-induced toxicity; the incidence of diarrhea and lethality was reduced to 44–50%. Little improvement was observed with longer duration of higher doses of IL-15 (8 or 11 doses). In contrast, significant protection was observed with 11 doses of IL-15.
IL-15 protects from CPT-11-induced intestinal toxicity

Fig. 2. Structural effects of CPT-11 alone or in combination with IL-15 on the duodenal (A) and colonic (B) mucosa. A, a, duodenum from untreated animals. b, duodenum from two animals treated with CPT-11 for 3 days. Note the shortened, irregular villi in the micrograph on the left (arrow) and the absence of villi in the micrograph on the right (arrow). c, duodenum from two animals treated with CPT-11 in combination with IL-15. Note the dramatic protection of duodenal structure in these samples. B, a, proximal colon from an untreated animal. b, colonic mucosa from a rat treated with CPT-11 (200 mg/kg) for 3 days. Note the shortened glands and the reduction in goblet cells. c, colonic tissue from an animal treated with CPT-11 (200 mg/kg) in combination with IL-15 (100 µg/kg). The crypt architecture appears normal.

Toxicity and Antitumor Activity in Rats Bearing Colorectal Carcinoma. Studies were performed to determine whether IL-15 is able to provide selective protection against CPT-11-induced toxicity and maintain its antitumor activity in rats bearing advanced colorectal cancer. The toxicity profile induced by CPT-11 at LD was more severe than in normal rats, with 100% incidence of severe diarrhea (20 of 20) and death of all treated animals within 12 days. Significant protection against CPT-11-induced diarrhea and lethality was also provided by IL-15 administration in tumor-bearing animals. The degree of IL-15 protection was also found to be dose and schedule dependent; the 11 low doses of IL-15 administration (100 µg/kg/dose) offered better protection than did 3 higher doses (Fig. 1).

The kinetics of treatment-induced tumor growth inhibition and toxicities with CPT-11 with or without IL-15 are shown in Fig. 1. Greater tumor growth inhibition was apparent with both schedules of IL-15 combined with CPT-11 over CPT-11 alone. However, no CR was observed with either group. These data clearly demonstrated that higher doses of CPT-11, under conditions of complete protection from treatment-induced toxicity by IL-15, did not translate into significant improvement in the tumor response of CPT-11. The results have clinical relevance.

Morphological Analysis. Paraffin sections of rat duodenum and colon were examined to determine the mucosal effects of treatment with a lethal dose of CPT-11 (200 mg/kg) daily for 3 days, with collection of tissues 24 h after the final treatment (Fig. 2). CPT-11 produced marked shortening, if not complete destruction, of duodenal villi. When present, villi appeared irregular and stunted (Fig. 2a). CPT-11 also produced shortening of colonic crypts and a marked reduction in the goblet cell population (Fig. 2b). In contrast, animals treated with 11 doses of IL-15 (100 µg/kg/dose) with CPT-11 showed villi with almost normal architecture and normal colonic structure (as well 100% survival from drug treatment; Table 1). IL-15 alone produced no obvious changes in mucosal architecture (data not shown).

Discussion

Studies were performed to develop an animal model mimicking the profile of clinical toxicities observed with CPT-11 (1–4) and to evaluate the ability of IL-15 to selectively modulate the toxicity and antitumor activity of CPT-11 in rats bearing advanced colorectal cancer. The results presented here demonstrate that IL-15 provides

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dramatic protection against CPT-11-induced toxicity in a dose- and schedule-dependent manner in this model. Although IL-15 was effective in protection of CPT-11-induced toxicities with all of doses and schedules used in this study, 11 doses of IL-15 at 100 μg/kg/dose were found to be the best schedule (Table 1).

CPT-11 is a promising new anticancer agent with a novel and unique mechanism of action. It is active against a variety of cancers and is increasingly being used in patients with colorectal cancer, particularly in those previously treated with FUra/LV protocol (2–4). Diarrhea represents a major dose-limiting toxicity (1–4). Several approaches were used in this study to develop an animal model of CPT-11-induced diarrhea similar to that observed in the clinic. Attempts to produce diarrhea in Balb/c and athymic nude mice were unsuccessful, even with lethal doses of CPT-11 (up to 200 mg/kg/day × 5). In contrast, administration of CPT-11 to Fisher rats by i.v. push daily for 3 or 5 days produced significant and reproducible diarrhea that allowed evaluation of the role of IL-15 in the reversal/protection from CPT-11-induced toxicity. The diarrhea profile exhibited by the model developed in this study was similar to that observed in the Wistar rat model reported by Takasuna et al. (14). It is noteworthy that the method of CPT-11 administration was found to be of critical importance if immediate death from convulsion and respiratory depression was to be avoided. This acute toxicity, possibly resulting from the solvent rather than drug itself, could be controlled by slow injection (2 min or longer), with rest periods when the animal had an abnormal reaction. Of interest, although administration of CPT-11 by continuous infusion resulted in a significant reduction of diarrhea in rats, the antitumor activity was also diminished (data not shown).

The precise mechanism(s) underlying CPT-11-induced diarrhea have yet to be fully defined. Histological studies from our laboratory and other groups suggest that structural and functional damage to the gastrointestinal tract by CPT-11 and/or its active metabolite, SN-38, may cause changes in absorption and/or other intestinal functions with resulting toxicity (Fig. 2; Refs. 15 and 16). Other reported mechanisms that have been suggested include: (a) the anticholinesterase activity of CPT-11, which may cause diarrhea by stimulating intestinalcontractility and impairing intestinal mucosal absorptive and secretory functions (17); and (b) an increase in β-glucuronidase activity resulting from drug-mediated disturbance of the intestine bacterial flora (18). Although the mechanism(s) by which IL-15 provided protection against CPT-11-induced diarrhea in the rat model remain to be determined, possible explanations include IL-15-mediated inhibition of drug-induced intestinal epithelial cell apoptosis and/or stimulation of intestinal epithelial cell growth (16). IL-15 may also contribute to self-defense mechanisms to reduce bacteria in intestines by promoting immunity, including induction of proliferation of natural killer cells, T cells, and B cells, production of IPN-γ, and generation of lymphokine-activated killer cell activity (10). Takasuna et al. (18) demonstrated that antibiotics could protect against CPT-11-induced diarrhea via inhibition of glucuronidase production. Furthermore, Kampo medicines (TJ-14 and TJ-114) and their active component, baikalin, were effective in protecting CPT-11-induced delayed diarrhea via inhibition of β-glucuronidase. However, these agents could not protect from CPT-11-induced acute watery diarrhea (14). The data reported herein demonstrate that IL-15 can prevent both acute and chronic diarrhea induced by CPT-11 and, therefore, appears to be more effective than Kampo medicines and baikalin.

Another advantage of using IL-15 in combination with CPT-11 is that IL-15 appears not only to provide protection from CPT-11-induced gastrointestinal toxicity but to also, perhaps, reduce drug-mediated myelosuppression. Histopathological and hematological studies demonstrated that IL-15 significantly stimulated rapid recovery of bone marrow and peripheral blood platelets, WBCs, and RBCs from FUra (with or without LV)-induced myelosuppression. Thus, both dose-limiting toxicities of CPT-11, i.e., diarrhea and neutropenia, may be alleviated by the administration of this cytokine.

We have previously demonstrated that IL-15 can significantly increase CR rate in rats bearing advanced colorectal cancer treated with FUra/LV (11). Here, although IL-15 potentiated CPT-11-mediated tumor growth inhibition (Fig. 1), this did not translate into an increase of CR rate in this model system. These data suggest that IL-15 may produce different effects when combined with CPT-11 or with FUra/LV. However, with IL-15, the MTD of CPT-11 could be increased from 150 to 200 mg/kg without an increase in toxicity but with no improvement in the tumor response. Thus, higher doses of CPT-11 do not necessarily translate into higher tumor response with this agent, even under conditions of optimal protection from drug-induced toxicity. Therefore, unlike other chemotherapeutic agents, application of high drug intensity with CPT-11 is not warranted, a result consistent with previous findings by Houghton et al. (19, 20).

In summary, although the mechanisms of interaction of IL-15 and CPT-11 treatment must be investigated further, the findings that IL-15 can provide effective protection against CPT-11-induced toxicities, particularly diarrhea, and can increase antitumor activity by allowing the delivery of higher drug dose intensity are, potentially, of significant therapeutic interest clinically. The generality of the observed effects in this model system needs to be confirmed in other tumor models, with parallel understanding of underlying mechanism(s) of action. Clinical verification of this concept is warranted.

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