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ORIGINAL ARTICLE

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Optimal antidiarrhea treatment for antitumor agent irinotecan hydrochloride (CPT-11)-induced delayed diarrhea

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Abstract *Purpose*: An antitumor camptothecin derivative CPT-11 has proven a broad spectrum of solid tumor malignancy, but its severe diarrhea has often limited its more widespread use. We have demonstrated from a rat model that intestinal β-glucuronidase may play a key role in the development of CPT-11-induced delayed diarrhea by the deconjugation of the luminal SN-38 glucuronide, and the elimination of the intestinal microflora by antibiotics or dosing of TJ-14, a Kampo medicine that contains β-glucuronidase inhibitor baicalin, exerted a protective effect. In the present study, we assessed the efficacy of several potential treatments in our rat model to clarify which is the most promising treatment for CPT-11-induced delayed diarrhea. *Methods and results*: Oral dosing (twice daily from days −1 to 4) of streptomycin 20 mg/kg and penicillin 10 mg/kg (Str/Pen), neomycin 20 mg/kg and bacitracin 10 mg/kg (Neo/Bac), both of which inhibited almost completely the fecal β-glucuronidase activity, or TJ-14 1,000 mg/kg improved the decrease in body weight and the delayed diarrhea symptoms induced by CPT-11 (60 mg/kg i.v. from days 1 to 4) to a similar extent. The efficacy was less but significant in activated charcoal (1,000 mg/kg p.o. twice daily from days −1 to 4). In a separate experiment using rats bearing breast cancer (Walker 256-TC), TJ-14, Neo/Bac, and charcoal at the same dose regimen improved CPT-11-induced intestinal toxicity without reducing CPT-11's antitumor activity. In contrast, oral dosing (twice a day) of cyclosporin A (50 mg/kg), a P-glycoprotein and cMOAT/MRP2 inhibitor or valproic acid (200 mg/kg), a UDP-glucuronosyltranferase inhibitor, exacerbated the intestinal toxicity without modifying CPT-11's antitumor activity. *Conclusions*: The result clearly demonstrated the ability of Neo/Bac, Str/Pen, and TJ-14, less but significant ability of activated charcoal, to ameliorate CPT-11-induced delayed-onset diarrhea, suggesting the treatments decreasing the exposure of the intestines to the luminal SN-38 are valuable for improvement of CPT-11-induced intestinal toxicity. In contrast, the treatments affecting the biliary excretion of CPT-11 and its metabolites might have undesirable results.

Keywords CPT-11 · Irinotecan · Diarrhea · β-Glucuronidase

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Introduction

Irinotecan hydrochloride (CPT-11), a water-soluble semisynthetic derivative of camptothecin, is an inhibitor of DNA topoisomerase I enzyme by its main active metabolite SN-38 [1, 2], and a promising antitumor agent, approved worldwide for use in patients with advanced colorectal cancer [3, 4], lung cancer [5, 6], and malignant lymphoma [7]. One of the major dose-limiting toxicities of CPT-11 therapy is unpredictable and severe diarrhea, especially delayed-onset severe diarrhea [inci-

dence of National Cancer Institute (NCI) grade 3 or 4 diarrhea is 20–40%] [8, 9]. It has limited the further evaluation of more aggressive antitumor regimens using CPT-11 [4, 5, 7, 10]. The great interpatient variations in the severity of diarrhea, the pharmacokinetics [11–16], and the efficacy of conventional antidiarrhea agents [5, 17, 18] make it difficult to understand the mechanisms of CPT-11-induced diarrhea, although preclinical and clinical studies have yielded some critical insight into the mechanisms and advances in treatment of the CPT-11-induced side effects [9].

CPT-11 is hydrolyzed by carboxylesterase to form the active metabolite SN-38 [19]. SN-38 is further conjugated to an inactive glucuronic acid conjugate (SN-38 glucuronide) by UDP-glucuronosyltransferase UGT1A1, the same isoenzyme responsible for glucuronidation of bilirubin, and excreted into the bile with other major component CPT-11 and SN-38 by P-glycoprotein (P-gp) and canalicular multispecific organic anion transporter/multidrug resistance-associated protein 2 (cMOAT/MRP2) [20–24]. SN-38 glucuronide may be deconjugated by β-glucuronidase produced by the intestinal microflora, releasing SN-38. The SN-38 deconjugated may largely be responsible for the accumulation of SN-38 in the intestine [25–27].

We have first demonstrated from a rat model that β-glucuronidase produced by microflora in the large intestine may play a key role in the development of CPT-11-induced delayed-onset diarrhea by the deconjugation of the SN-38 glucuronide, and administration of antibiotics exerted a protective effect against the diarrhea by completely inhibiting the β-glucuronidase activity, thereby decreasing the exposure of the large intestine to the luminal SN-38 [28, 29]. Furthermore, we reported that TJ-14 or TJ-114, a Chinese herbal medicine that contains β-glucuronidase inhibitor baicalin, also exerted a protective effect on the delayed-onset diarrhea in the same model [30].

Based on our findings, several nonclinical and clinical studies to alleviate CPT-11-induced diarrhea have been performed especially focusing on the attenuating antiproliferating activity of SN-38 excreted into the intestinal lumen via the bile acid. Up to date, various attractive, and promising treatments for attenuating CPT-11-induced diarrhea, including (1) inhibition of intestinal β-glucuronidase using Kampo medicine TJ-14 [31] or other antibiotics neomycin or bacitracin [32, 33], (2) prevention of intestinal transport (re-absorption) of SN-38 and/or CPT-11 by oral alkalization [34, 35] or by adsorbing of these compounds using activated charcoal [36, 37], or various nonspecific treatments for cancer chemotherapy-induced diarrhea [9], have been clinically demonstrated. However, since these studies were performed under different experimental conditions with each other, no one can expect which is the most promising and effective treatment for CPT-11-induced delayed diarrhea.

To address the question, we compared the antidiarrhea activity of the several potential treatments on CPT-11-induced diarrhea in our rat model.

Methods

Reagents

CPT-11 (Topotecin® Injection, Yakult Honsha, Tokyo, Japan); penicillin G, streptomycin, and valproic acid (Sigma, St Louis, MO, USA); neomycin, bacitracin, and cyclosporin A (Wako Pure Chemicals, Tokyo, Japan); activated charcoal (Iwaki Seiyaku, Tokyo, Japan); and TJ-14 (Hange-Shasin-To, Tsumura, Tokyo, Japan) were commercially purchased. All the potential antidiarrhea agents were dissolved and/or suspended in distilled water for oral administration (Fuso Pharmaceutical Industries, Osaka, Japan) as a volume of 10 or 20 ml/kg.

RPMI1640 medium (Invitrogen Corp.; Carlsbad, CA, USA) and fetal bovine serum (FBS; Hyclone Laboratories Inc.; Logan, UT, USA) were also commercially purchased.

Animals

The experiment was conducted using male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing about 150–180 g ($n = 4$–5). The animal room was maintained at a temperature of 23 ± 2°C and a relative humidity of 55 ± 15% with a 12-h light–dark cycle. A commercial animal chow (F-2, Funabashi Farms, Funabashi, Japan) and tap water were freely available throughout the acclimatization and experimental periods.

Experimental schedule

Animals were intravenously administered CPT-11 (60 mg/kg) from the tail vein once a day (a.m.) for four consecutive days (from days 1 to 4). In the following three antibiotic groups, 2 mg streptomycin and 1 mg penicillin, 2 mg neomycin, or 2 mg neomycin per ml of drinking water was administered from 5 days before the start of CPT-11 administration and throughout the experiment (days −5 to 4), respectively, to aspire complete individual antibiotic efficacy. Antibiotics (Str/Pen, streptomycin 20 mg/kg and penicillin 10 mg/kg; Neo, neomycin 20 mg/kg; Neo/Bac, neomycin 20 mg/kg and bacitracin 10 mg/kg), TJ-14 (1,000 mg/kg), or activated charcoal (1,000 mg/kg) were orally administered twice a day (a.m. and p.m.) from the day before (day −1) to 4 days after the start of CPT-11 injection. Under the CPT-11's regimen adopted [60 mg/kg i.v. once daily for consecutive 4 days (days 1–4)], the diarrhea monitored throughout days 5–8 was similar to human diarrhea in terms of being resistant to conventional antidiarrhea agents [38]. Diarrhea, the onset which was on or after day 5, was defined delayed diarrhea. The severity of delayed diarrhea and the daily body weight were monitored, and the results were used as an index of intestinal toxicity. The severity of delayed diarrhea was scored as follows:

- Normal (0, normal stool)
- Slight (1, slightly wet stool without staining of the coat)
- Moderate (2, wet and unformed stool with moderate perianal staining of the coat)
- Severe (3, watery stool with severe staining of the coat around the anus)

The total diarrhea score area under the score-day curve during days 5–9, and the mean score at each day were calculated. Watery diarrhea which appeared within about 2 h after the administration of CPT-11 was defined acute diarrhea. Before the start of CPT-11 administration on day 1, the fecal β-glucuronidase activity was determined by a modification of the procedure of Akao et al. [39], and the fecal pH using pH meter (HM-50G, Toa Electrics, Tokyo, Japan) after homogenization of the samples in 0.9% physiological saline.

In a separate experiment, the effects of several potential antidiarrhea treatments on CPT-11-induced antitumor activity and diarrhea were evaluated using rats bearing breast cancer. The rat breast cancer cell line Walker 256-TC cells were obtained from Cell Resource Center for Biomedical Research, Tohoku University (Miyagi, Japan) and were cultured in vitro in RPMI1640 medium supplemented with 10% (v/v) fetal bovine serum. The cultures were grown at 37°C in a 5% CO_2–95% air atmosphere, and the passages were performed twice a week. The Walker 256-TC cells (1×10^5 cells/0.1 ml) were inoculated subcutaneously into the right flank of rats. When the mean estimated tumor volume reached about 300 mm^3 on day 7 after tumor inoculation, the rats were randomly divided into experimental groups (five rats per group) to have the similar mean estimated tumor volume, and were given CPT-11 at the same regimen mentioned above with the following potential antidiarrhea compounds. (1) TJ-14 1,000 mg/kg, (2) activated charcoal 1,000 mg/kg, (3) Neo/Bac, neomycin 20 mg/kg and bacitracin 10 mg/kg—these three treatments showed an obvious antidiarrhea activity in the normal rats in the present study, (4) cyclosporin A 50 mg/kg, (5) valproic acid 200 mg/kg. Both cyclosporine A and valproic acid had been expected to enhance CPT-11's antitumor activity with reduced intestinal toxicity because both increase the area under plasma concentration–time curve of SN-38 by lowering biliary excretion of SN-38 or by inhibiting SN-38 conjugation. All these compounds were orally administered twice daily (a.m. and p.m.) for days −1 to 4, except that Neo/Bac group received 2 mg neomycin per ml of drinking water from 5 days before the start of CPT-11 administration. The estimated tumor volume was measured 4, 7, and 10 days after the start of CPT-11 administration, and the severity of delayed diarrhea and the daily body weight were also monitored.

The estimated tumor volume was calculated using the formula:

$$\text{The estimated tumor volume } (\text{mm}^3) = \frac{L \times W^2}{2},$$

where L and W represent the length and the width of the tumor mass, respectively.

All experimental procedures were performed in accordance with the in-house guidelines of the Institutional Animal Care and Use Committee of Daiichi Pharmaceutical Co., Ltd.

Results

Fecal pH and β-glucuronidase activity in normal rats

On day 1 (6 days after the start of antibiotics administration), the fecal pH and β-glucuronidase activity in the control group were 6.42 and about 190 nmol/min/mg protein, respectively. The fecal pH in the treatment groups was similar to that in the control group, except for Str/Pen group in which the fecal pH was 7.20. The fecal β-glucuronidase activities in Str/Pen and Neo/Bac treated groups were reduced to less than approximately 10% of the control group. The fecal β-glucuronidase activity of Neo group was also reduced but it remained in about 20% of the control group. The fecal β-glucuronidase activities in TJ-14 and activated charcoal groups had somewhat higher values as compared with that of the control group but it was not a statistically significant change (Fig. 1).

Effects on CPT-11-induced body weight loss and diarrhea symptoms in normal rats

Following the i.v. administration of CPT-11 (60 mg/kg once daily for the consecutive 4 days: days 1–4), body weight decreased from day 2 and reached a nadir on day 8, being about 23% decrease as compared with the initial value (day 1). No diarrhea was present during the first 2 days, but acute watery diarrhea occurred on days 3 and 4 within 1–2 h after CPT-11 injection. Thereafter, diarrhea was chronically present during days 5–8 (delayed diarrhea).

Each treatment had little or no effect on CPT-11-induced decrease in body weight during days 2–3. On or after day 4, either treatment inhibited the decrease in body weight, and improved the delayed diarrhea symptoms. Str/Pen, Neo, Neo/Bac, and activated charcoal, but not TJ-14, also inhibited the acute watery diarrhea that appeared on days 3 and 4. There was an obvious difference of the effectiveness among the treatments. In consideration of the changes of body weight and diarrhea score, the rank order for beneficial effect on CPT-11-induced intestinal toxicity was Str/Pen = TJ-14 = Neo/Bac > activated charcoal > Neo (Figs. 2, 3).

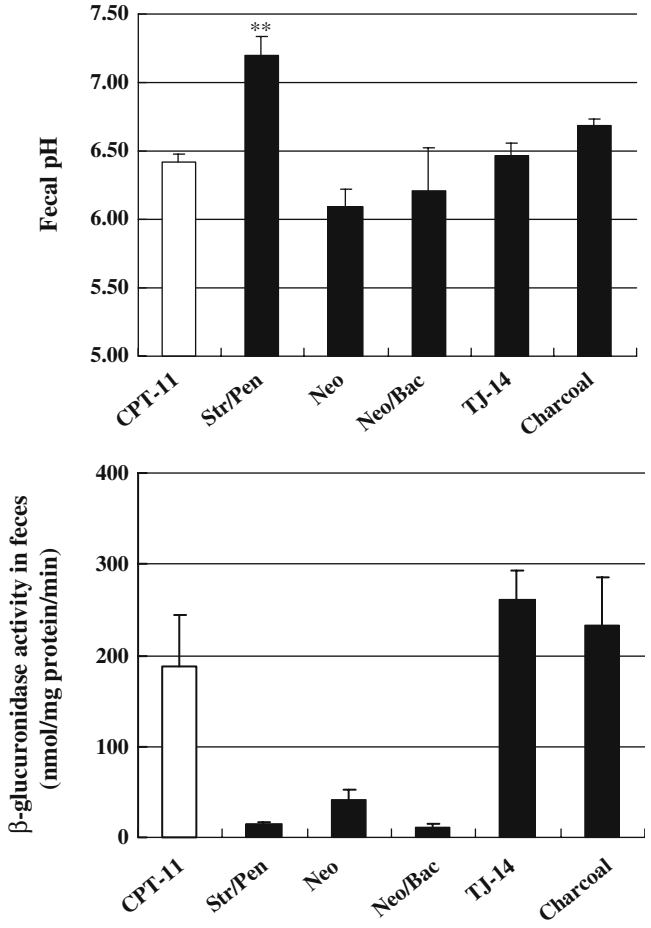

Fig. 1 The pH and *β*-glucuronidase activity of the feces on the day (day 1) of the start of CPT-11 (60 mg/kg, once daily for 4 days) injection in normal rats. Antibiotics, except for bacitracin, were administered in drinking water from 5 days (day −5) before the start of CPT-11 injection. Each data represents the mean of 4–5 animals. $^{**}P < 0.01$ versus CPT-11 group (Dunnett test). *Str*/*Pen* streptomycin (2 mg/ml) and penicillin (1 mg/ml) in drinking water + streptomycin 20 mg/kg and penicillin 10 mg/kg p.o.; *Neo* neomycin (2 mg/ml) in drinking water + neomycin 20 mg/kg p.o.; *Neo*/*Bac* neomycin (2 mg/ml) in drinking water + neomycin 20 mg/kg and bacitracin 10 mg/kg p.o.; *TJ-14* TJ-14 1,000 mg/kg p.o. *Charcoal* activated charcoal 1,000 mg/kg p.o

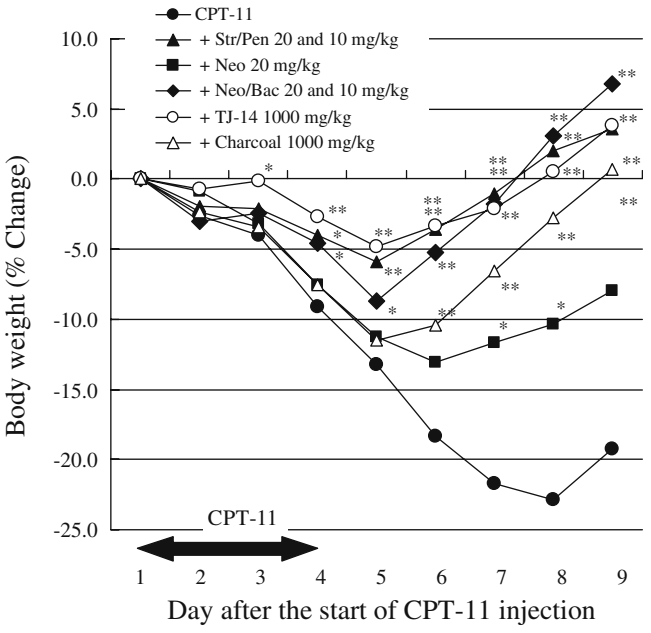

Fig. 2 Effects of several agents on CPT-11-induced body weight loss in rats. CPT-11 was given intravenously at a dose of 60 mg/kg once daily for four consecutive days (days 1–4). The agents were orally administered twice daily from the day before to 4 days after the start of CPT-11 injection. In addition, antibiotics, except for bacitracin, were administered in drinking water from 5 days before to 4 days after the start of CPT-11 injection. The change in body weight was calculated on the basis of that on day 1. Each point represents the mean of 4–5 animals. The abbreviations are referred in Fig. 1. $^{*}P < 0.05$, $^{**}P < 0.01$ versus CPT-11 group (Dunnett test)

Effects on CPT-11-induced antitumor activity and intestinal toxicity (body weight loss and diarrhea symptoms) in rats bearing breast cancer

The mean estimated tumor volume in the vehicle control group increased linearly and reached about 10,000 mm^3, which was approximately 30-fold its initial mean estimated tumor volume of 300 mm^3, on day 10. CPT-11 (60 mg/kg once daily for the consecutive 4 days: days 1–4) showed moderate but significant reduction of the mean estimated tumor volume on days 4 and 7, but its antitumor effect was no longer apparent (not significant) on day 10. The body weight decreased from day 2 and reached a nadir on day 6, being about 15% decrease as compared with the initial value (day 1). No diarrhea was present during the first 2 days, but acute watery diarrhea

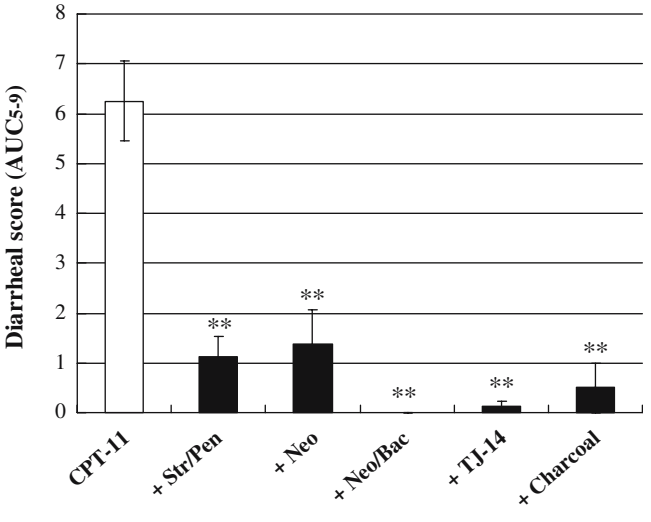

Fig. 3 Effects of several agents on CPT-11 (60 mg/kg i.v., once daily for 4 days)-induced delayed diarrhea symptoms in rats. The agents were orally administered twice daily from the day before to 4 days after the start of CPT-11 injection. In addition, antibiotics, except for bacitracin, were administered in drinking water from 5 days before to 4 days after the start of CPT-11 injection. Each data represents the mean of 4–5 animals. The abbreviations are referred in Fig. 1. $^{**}P < 0.01$ versus CPT-11 (Wilcoxon rank sum test)

occurred on days 3 and 4 within 1–2 h after CPT-11 injection. Thereafter, diarrhea was chronically present during days 6–7 (delayed diarrhea).

TJ-14, Neo/Bac, and activated charcoal inhibited the decrease in body weight, and improved the delayed diarrhea, but had no effect on the antitumor effect of CPT-11. Neo/Bac, but not TJ-14 or activated charcoal, also inhibited the acute watery diarrhea that appeared on days 3 and 4. In contrast, cyclosporin A and valproic acid augmented the loss of body weight gain and delayed diarrhea symptom score while those had no effects on CPT-11's antitumor activity (Figs. 4, 5). In addition, the acute diarrhea appeared not only on days 3 and 4 but also on day 1 in cyclosporin A and valproic acid groups.

Discussion

The clinical use of CPT-11 has been associated with early onset diarrhea that is observed immediately after CPT-11 injection (acute diarrhea) and delayed-onset diarrhea that occurs more than 24 h after CPT-11 injection and usually continues for several days (delayed diarrhea) [9, 10]. The former was usually accompanied with cholinergic symptoms such as salivation, cramps, and diaphoresis, and could be controlled with cholinergic receptor blocker atropine [40]. Therefore, its anticholinesterase activity [41, 42] is at least involved in the cholinergic symptoms including acute diarrhea. Indeed, we have confirmed that CPT-11 has not only anti-acetylcholinesterase activity but also anti-butyrylcholinesterase activity which plays a major role in the intestinal tract (unpublished data).

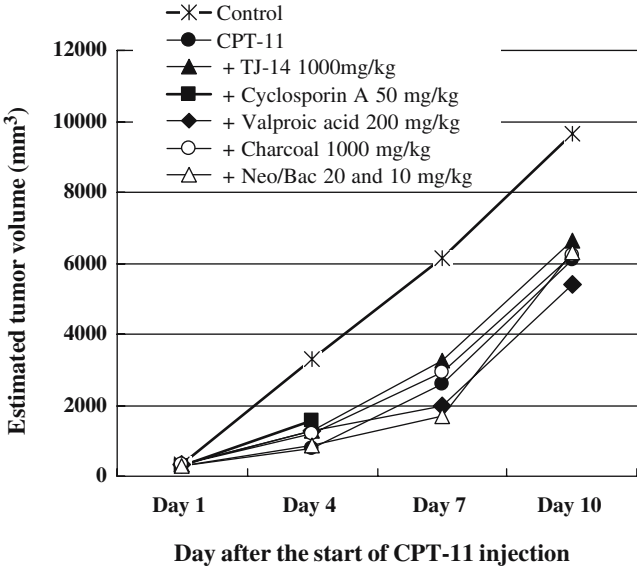

Fig. 4 Effects of several agents on antitumor activity of CPT-11 (60 mg/kg iv for 4 days) in rats bearing breast cancer (Walker 256-TC). The agents were orally administered twice daily from the day prior to the start of CPT-11 injection for total of 5 days. In addition, neomycin was administered in drinking water from 5 days prior to the start of CPT-11 injection for total of 9 days. All rats died until day 7 in cyclosporin A-treated group. One rat died on day 8 in valproic acid-treated group. *TJ-14* TJ-14 1,000 mg/kg, p.o.; *cyclosporin A* cyclosporin A 50 mg/kg, p.o.; *valproic acid* valproic acid 200 mg/kg, p.o.; *charcoal* activated charcoal 1,000 mg/kg, p.o.; *Neo/Bac* neomycin (2 mg/ml) in drinking water + neomycin 20 mg/kg and bacitracin 10 mg/kg, p.o. Each value represents the mean of 2–5 animals. There are no significant differences of the estimated tumor volumes between the CPT-11 alone and CPT-11 with agents on days 4, 7, or 10 (Student's *t* test)

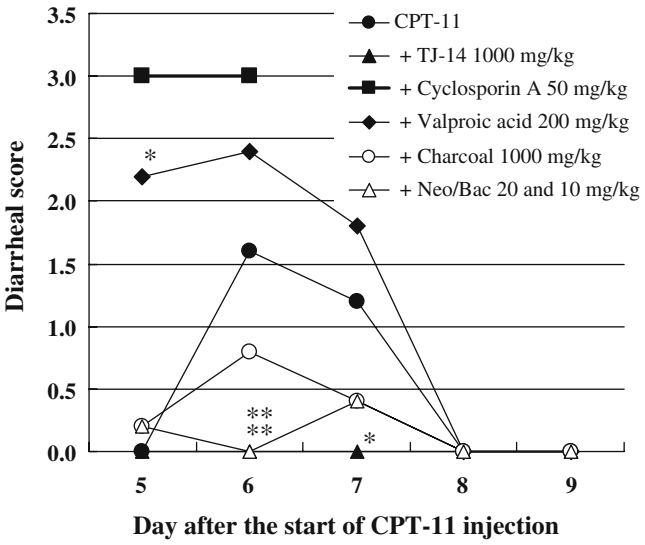

Fig. 5 Effects of several agents on delayed diarrhea symptoms caused by CPT-11 (60 mg/kg iv for 4 days) in rats bearing breast cancer (Walker 256-TC). The agents were orally administered twice daily from the day prior to the start of CPT-11 injection for total of 5 days. In addition, neomycin was administered in drinking water from 5 days prior to the start of CPT-11 injection for total of 9 days. All rats died until day 7 in cyclosporin A-treated group. One rat died on day 8 in valproic acid-treated group. The abbreviations are referred in Fig. 4. Each value represents the mean of 2–5 animals. $*P < 0.05$, $**P < 0.01$: Significantly different from the group treated with CPT-11 alone (Wilcoxon rank sum test)

In contrast, the latter is unexpected diarrhea, the severe [National Cancer Institute-Common Toxicity Criteria (NCI-CTC) grade 3 or 4] diarrhea might be a potentially life-threatening disorder, especially when concomitant with severe neutropenia. Although many pharmacokinetic analysis in humans have been made to predict the incidence or the mechanisms of delayed diarrhea, there are somewhat conflicting results [11–16]. Namely, there are no generally accepted relationship between the severity of diarrhea and any of the studied pharmacokinetic parameters.

Intensive loperamide regimens have been considered as the standard antidiarrhea treatment for CPT-11-induced diarrhea in Europe and the United States. It is one of the nonspecific treatments for cancer chemotherapy-induced diarrhea and probably reduces diarrhea by delaying intestinal transit allowing increased time for fluid absorption or reducing the fluid secretion [43], but the clinical studies could not necessarily confirm its satisfied efficacy [44]. Other potential approaches are (1) altering the metabolism [(a) inhibition of deconjugation

of SN-38 glucuronide excreted into the intestinal lumen [28, 30, 45], (b) inhibition of glucuronidation of SN-38 in the liver [20], (c) inhibition of biliary excretion of CPT-11 and its metabolites [21–24], (d) selective inhibition of intestinal SN-38 production [46], (2) prevention of intestinal re-absorption of SN-38 and/or CPT-11 by alkalization of the intestinal lumen [34, 35] or by adsorbing of CPT-11 and its metabolites using activated charcoal [36, 37], or (3) blockade of CPT-11-induced fluid secretion [43]: these are all specific measures for CPT-11-induced diarrhea (Fig. 6). In addition, other potential nonspecific treatments have also been reported (Table 1). Some of those have already been shown to improve CPT-11-induced diarrhea, but no one can expect which is the most promising and effective treatment for CPT-11-induced delayed-onset severe diarrhea.

In the present study, we assessed the efficacy of several potential antidiarrhea treatments that have been shown to be effective clinically or currently under clinical trial, in comparison with those of Str/Pen or TJ-14 treatment that has been confirmed effective in our rat model [28, 30]. The present result was well in agreement with our previous reports, namely both Str/Pen and

Table 1 Summary of nonspecific approaches and other potential approaches to prevent CPT-11-induced diarrhea

Nonspecific measures
Enkephalinase inhibitor (Tiorphan) [51]
COX_2 inhibitor (Celecoxib) [52]
IL-15 [53]
Sandostatin [44, 54, 55]
Lipopeptide JBT-3002 [56, 57]
RDP58 [58]
Radical scavenger (amifostine) [59]
Sucralfate and nifuroxazide [60]
Thalidomide [61, 62]
Glutamate [63]
Steroid (budesonide) [64]
Fish oil [65]
Modified schedule of CPT-11 dosing [66–68]
Pharmacogenetic analysis of UDP-glucuronosyltransferase [69]

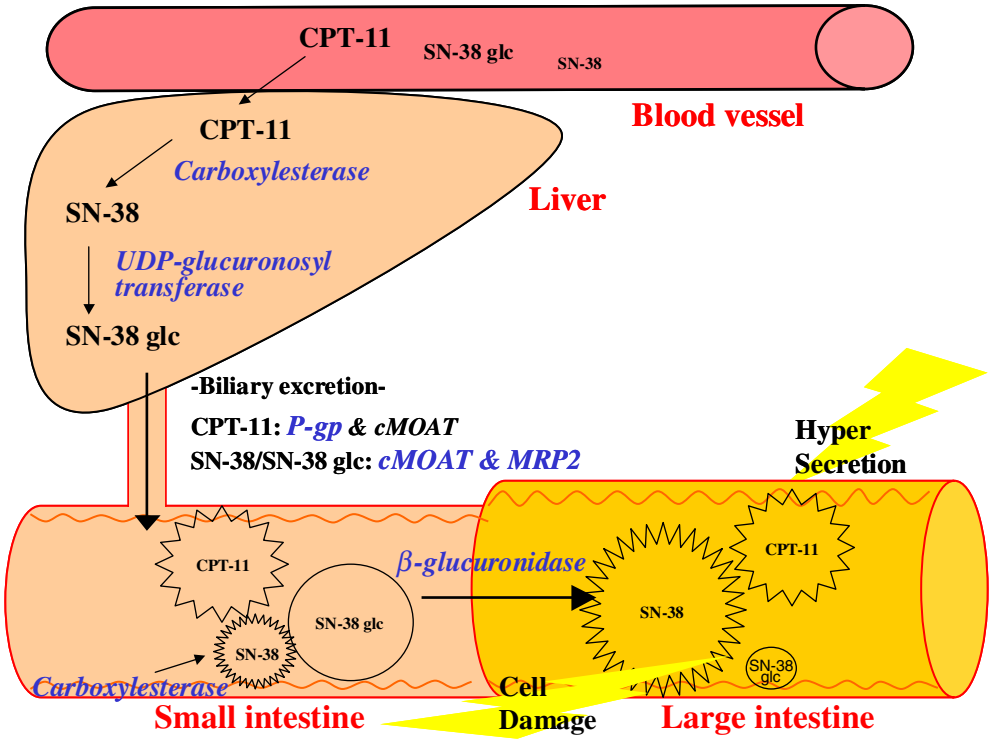

Fig. 6 Schematic representation of CPT-11 metabolism, expecting CPT-11-specific approaches to prevent intestinal toxicity of CPT-11

Inhibition of enzymes or transporter

1. *β-glucuronidase* 2. *UDP-glucuronosyl transferase*

3. P-gp, *cMOAT or MRP2* 4. *Carboxylesterase*

Inhibition of re-uptake of CPT-11 and its metabolites by

1. *Absorption*

2. *Intestinal alkalization*

Inhibition of hypersecretion

TJ-14 showed good antidiarrhea activity against CPT-11-induced delayed diarrhea, and first revealed that their efficacy was almost equivalent. The other poorly absorbed aminoglycoside antibiotics Neo/Bac also showed good antidiarrhea activity similar to that of Str/Pen or TJ-14. In contrast, the efficacy of single co-administration of neomycin was relatively low as compared with the above three treatment regimens despite inhibition (about 80%) of intestinal β-glucuronidase activity. The possible reason why neomycin could not ameliorate the CPT-11-induced intestinal toxicity as other two antibiotic regimens might be due to an incomplete inhibition of β-glucuronidase activity (< 80% in Neo vs. > 90% in Str/Pen or Neo/Bac). Another possible reason might be due to the change of fecal pH. Takeda et al. [34] and Ikegami et al. [35] have recently reported that intestinal alkalization by sodium bicarbonate supplementation ameliorated CPT-11-induced diarrhea with reduction of the histopathological damage to the mucosa of the intestine by influencing the conversion of SN-38/CPT-11 from lactone to carboxylate. In the present study, the fecal pH in Str/Pen group changed to be about pH 7.2 from the pH 6.4 (control group). The respective rates of intestinal uptake for CPT-11 and SN-38 were shown to be pH sensitive, with uptake decreasing by more than 65% at pH levels greater than 6.8 [47], suggesting that intestinal alkalization by Str/Pen might, at least in part, be involved in the ameliorating mechanism while the reason of the change in pH is not known. Since, however, there is no change in the fecal pH of Neo/Bac or TJ-14 group which exerted efficacy comparable to Str/Pen, alkalization in the intestinal lumen might not play a key role in the ameliorating efficacy of these treatments used in the present study.

An alternative measure for the inhibition of β-glucuronidase in the intestinal lumen is pharmacological inhibition using specific inhibitors including natural glucuronides [48]. Indeed, we have reported that TJ-14 or TJ-114 (0.5 and 1 g/kg twice daily), a Chinese herbal medicine that contains β-glucuronidase inhibitor baicalin, or baicalin itself (25 mg/kg) exerted a protective effect on the delayed-onset diarrhea in rats [30]. D-Glucaric acid-1,4-lactone monohydrate, a specific β-glucuronidase inhibitor, has recently been shown to reduce CPT-11-induced mucosal damage in the small intestine in rats [45]. Our preliminary study, however, did not confirm its antidiarrhea activity. The dose of glucaro-1,4-lactone used in the preliminary study (25 mg/kg orally twice daily) might be enough to inhibit β-glucuronidase because it has β-glucuronidase inhibitory activity comparable to baicalin [48]. The reason why glucaro-1,4-lactone had no efficacy in our rat model remains to be determined. We reported that CPT-11-induced delayed-onset diarrhea would be attributable to the damage to the cecum, which has the highest β-glucuronidase activity in the luminal contents, and the inhibition of β-glucuronidase by antibiotics resulted in mainly the reduction of the cecal damage, not of the small intestine [28]. Since Fittkau et al. [45] reported that glucaro-1,4-lactone reduced CPT-11-induced mucosal damage in the small intestine which almost lacks β-glucuronidase activity in the luminal contents [28], other mechanisms apart from β-glucuronidase inhibition in the intestinal lumen might be involved. Alternatively, the therapeutic effect of Kampo medicine TJ-14 on CPT-11-induced delayed diarrhea might be solely based on the inhibition of SN-38 glucuronide deconjugation but also on other mechanisms including a suppression prostaglandin E_2 production in the colon [49].

Chowbay et al. [50] reported that activated charcoal was not effective in the prevention of CPT-11-induced diarrhea as compared with inhibition of β-glucuronidase in the intestinal microflora by ceftriaxone, a third generation cephalosporin. In the present study, activated charcoal showed clearly improved CPT-11-induced intestinal toxicity though its activity was slightly weak as compared with Str/Pen, Neo/Bac, or TJ-14. Therefore, the adsorption of CPT-11 and its metabolites using activated charcoal could offer some help in reducing CPT-11-induced diarrhea as reported by Michael et al. [36] and Maeda et al. [37].

Although the pharmacokinetic or histopathologic examinations were not conducted in the present study, we have shown good correlation between the severity of indices of intestinal toxicity adopted in this study and histopathological changes in the intestine [28, 30]. Moreover, the inhibition of β-glucuronidase in the intestinal microflora by antibiotics [29, 50] or TJ-14 (unpublished data) did not affect SN-38 or CPT-11 plasma pharmacokinetics. It is suggested that the antibiotics or TJ-14 could prevent CPT-11-induced intestinal toxicity without reducing antitumor activity. Indeed, antibiotics (Neo/Bac), TJ-14, or activated charcoal ameliorated CPT-11-induced intestinal toxicity with maintenance of CPT-11's antitumor activity in rats bearing breast cancer in the present study. Since the biliary excretion of CPT-11, its active metabolite SN-38 and SN-38 glucuronide are mediated by the P-gp and cMOAT/MRP2 in the bile canalicular membrane [20–24], inhibition of the transporters, or UDP-glucuronosyl transferase has been proposed to reduce the intestinal toxicity of CPT-11 by decreasing the biliary excretion of particularly SN-38 and SN-38 glucuronide or potentially increase the CPT-11 therapeutic index by decreasing the intestinal toxicity associated with more aggressive antitumor regimens. In the present study, contrary to ones expectations, cMOAT/MRP2 inhibitor cyclosporin A or UDP-glucuronosyl transferase inhibitor valproic acid exacerbated the intestinal toxicity, and did not modify CPT-11's antitumor activity. As we do not confirm whether or not CPT-11 can show dose-dependent antitumor activity in the present rat model bearing breast cancer, we cannot conclude that co-administration of cyclosporin A or valproic acid does not enhance CPT-11's antitumor activity from the present study. However, the fact that cyclosporin A and valproic acid caused a worsening intestinal toxicity, and may produce adverse systemic reaction, probably keeping SN-38 serum and

tissue levels too high [20, 21, 23], is suggesting that it is at risk of potentiating the systemic and/or intestinal side effect of CPT-11 to control biliary excretion of CPT-11 and its metabolites by drugs for preventing CPT-11-induced intestinal toxicity in consideration of individual difference of biliary pharmacokinetics.

The present results conclude that the optimal combined use of antibiotics which completely reduces the intestinal, bacterial β-glucuronidase activity prevents CPT-11-induced intestinal toxicity to a similar extent of Kampo medicine TJ-14, and activated charcoal, a more inexpensive agent, may also be useful when antibiotics or TJ-14 could induce severe secondary complications. Moreover, the treatments affecting the biliary excretion of CPT-11 and its metabolites might have undesirable results.

To date, a lot of CPT-11 specific and nonspecific (Fig. 6, Table 1) antidiarrhea treatment designs have been proposed in animal and human studies. However, to our knowledge only Kampo medicine and antibiotics could improve CPT-11-induced delayed-onset diarrhea in both animal and human studies although our rat model might be different from human in terms of types of intestinal microflora and anatomical distribution. Therefore, we currently think that Kampo medicine, antibiotics, or both treatments if possible, are the optimal antidiarrhea treatments against CPT-11-induced diarrhea.

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