Preventive Effects of *Hange-shashin-to* on Irinotecan Hydrochloride-Caused Diarrhea and Its Relevance to the Colonic Prostaglandin E\(_2\) and Water Absorption in the Rat

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ABSTRACT—The possible preventive effect of *Kampo* medicine *Hange-shashin-to* (TJ-14) on chronic diarrheal symptoms induced by the administration of the anticancer agent irinotecan hydrochloride (CPT-11) was investigated in the rat. Repeated oral administrations of TJ-14 at 125 and 500 mg/kg significantly prevented the reduction in body weight and the onset of chronic diarrheal symptoms due to CPT-11 in a dose-dependent manner, even though it failed to show a definite effect on acute diarrheal symptoms. In addition, treatment with TJ-14 accelerated the healing of the intestinal tract injured by repeated dosing of CPT-11 and inhibited significantly the increase of colonic prostaglandin E\(_2\) (PGE\(_2\)) which is closely related to the onset of diarrhea. TJ-14 also improved colonic water absorption impaired by repeated dosing of CPT-11 in rats. These results demonstrate that TJ-14 is an effective medicine for the prevention and/or treatment of CPT-11-induced chronic diarrheal symptoms.

**Keywords:** CPT-11 (irinotecan hydrochloride), Side effect, Chronic diarrheal symptom, *Hange-shashin-to*, Large intestine

Irinotecan hydrochloride (CPT-11) is a potent anticancer agent by inhibiting topoisomerase 1 (1) and is effective for treating colonic cancer and non-small cell lung cancer that are resistant to many conventional chemotherapeutic drugs (2–5). The clinical administration of CPT-11 is attended with myelosuppression and gastrointestinal toxicity that lead to severe acute diarrhea and delayed diarrhea that occur soon and 2 to 3 days after CPT-11 administration, respectively. Delayed diarrhea induced by CPT-11 has been a knotty problem clinically due to a lack of effective treatment and/or prevention strategy available to date even though attempts have been made to control these severe side effects of CPT-11 by using some conventional antidiarrheal drugs such as loperamide (6, 7).

*Hange-shashin-to* (TJ-14), a *Kampo* medicine, is composed of seven medicinal herbs including *Pinelliae tuber*, *Scutellariae radix*, *Glycyrrhizae radix*, *Zizyphi fructus*, *Ginseng radix*, *Coptidis rhizoma* and *Zingiberis siccatum rhizoma*. This drug is used to treat gastrointestinal disorders such as acute and chronic gastrointestinal catarrh, fermentative diarrhea and acute gastroenteritis.

TJ-14 is effective for castor oil-induced diarrhea, but it does not affect intestinal motility (8). Recent studies have revealed that TJ-14 suppresses the elevation in colonic prostaglandin E\(_2\) (PGE\(_2\)) level, closely associated with diarrhea, and enhances colonic water absorption (9). It is well-known that PGE\(_2\) induces diarrhea and reduces water absorption by the digestive tract. PGE\(_2\) is therefore one of major factors involved in diarrhea (10–12). The increase in colonic PGE\(_2\) and tissue injury accompanied by significantly impaired water absorption of the descending colon have been observed in chronic diarrheal symptoms in rats treated with CPT-11.

The purpose of this study was to evaluate the efficacy of TJ-14 on CPT-11-induced chronic diarrheal symptoms, focusing mainly on colonic PGE\(_2\) and water absorption in rats.

MATERIALS AND METHODS

**Animals**

Male Wistar rats, 8-weeks-old, weighing 170–200 g (Japan SLC, Hamamatsu), were used. The animals were housed in an environment of 23±2°C, 55±10% humid-
ity, 12-hr light-dark cycle (7:00–19:00). The rats were fed commercial rodent pellet chow (F-2; Funabashi Farm, Funabashi) and tap-water ad libitum.

Chemicals and reagents
CPT-11 (lot No. KO17-R) was provided by Yakult Honsha Co. (Tokyo). It was first dissolved in distilled water by moderate heating to 60°C and then diluted with 9% NaCl to the desired concentrations of CPT-11 in 0.9% NaCl. TJ-14 was a product of Tsumura & Co. and dissolved in distilled water. PGE$_2$ was quantified using a RIA kit (NEN Research Products, Boston, MA, USA). All other chemicals were of the highest grade commercially available.

Effects of TJ-14 on colonic PGE$_2$
According to the method of Takasuna et al. (13), the rats were given daily i.v. injections of CPT-11 at 60 mg/kg body weight for 4 days. Body weight and onset of diarrhea were recorded daily until the 5th day after CPT-11 administration. Acute and chronic diarrheal symptoms were defined as that occurring within 1 hr and that occurring from 6 to 24 hr after CPT-11 injection, respectively. Diarrhea was scored judging by feces as normal (−), diarrhea (+) and watery diarrhea (++). TJ-14 was orally administered once daily at 125 and 500 mg/kg body weight/day prior to CPT-11 treatment. The rats were treated with distilled water in the same way as the control. They were sacrificed 24 hr after the last dose of CPT-11. Colonic PGE$_2$ was quantified according to the method of Kobayashi et al. (14). The ileum and descending colon were removed and stained with hematoxylin and eosin for pathological examination. Each group contained 8 to 10 rats.

Effects of TJ-14 on colonic water absorption
Rats were similarly treated as detailed above and subjected to analysis of colonic water absorption 24 hr after the last dose of CPT-11 according to the method of Fedorak et al. (15) with minor modification. Briefly, a midline incision was made on the peritoneal wall of the rats anesthetized with pentobarbital (50 mg/kg, i.p.), and the large intestine was exposed and tied to the cecum. The large intestine was cleaned by infusing 0.9% NaCl into the lumen at 37°C, which was then removed via the anus. A colonic loop was made by tying one end of the colon about 0.5 cm from the cecum to the other end about 3 cm from the anus. This loop was filled with 2 ml of 0.9% NaCl at 37°C and weighed 2 hr following 0.9% NaCl injection. Water absorption was expressed as the difference between the solution injected and that retained in the lumen, by subtracting the wet weight of colonic tissue from that of the loop.

Statistical analyses
Mann-Whitney U-test was conducted in the rats with diarrhea. Other data were analyzed by Fisher's least significant difference procedure. P values less than 0.05 were considered to be statistically significant.
Table 1. Effects of TJ-14 on CPT-11-induced chronic diarrheal symptoms in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>CPT-11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>9b</td>
<td>0</td>
</tr>
<tr>
<td>TJ-14</td>
<td>125</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

*Diarrheal score was defined as follows: 0: no diarrhea, 1: diarrhea, 2: watery diarrhea. bNumber of rats for each score. CPT-11 was administered intravenously at 60 mg/kg once daily for 4 consecutive days (Days 1-4). TJ-14 was given orally once daily on the day before and throughout CPT-11 administration. * and **: significantly different from the control at P<0.05 and P<0.01, respectively (Mann-Whitney U-test).

RESULTS

Effects of TJ-14 on feed consumption and body weight gain

CPT-11 treatment for 4 consecutive days caused severe diarrhea that consequently led to substantial body weight loss in control rats. TJ-14 significantly prevented body weight loss in a dose-dependent manner (Fig. 1). Feed consumption was 7.8 and 8.9 g/rat/day in rats given TJ-14 at 125 and 500 mg/kg, respectively, compared to 5.9 g/rat/day in the controls.

Acute diarrheal symptoms were noted in some rats on the first day of CPT-11 treatment, and all control rats treated with CPT-11 alone showed watery diarrhea on the third day of CPT-11 administration. Co-administration of TJ-14 at 125 and 500 mg/kg body weight significantly inhibited chronic diarrheal symptoms (Table 1), but had no effect on acute diarrheal symptoms.

PGE2 in rat descending colon

CPT-11 administered 4 consecutive days significantly increased PGE2 in the descending colonic tissue in control rats compared with that of untreated rats (393.4±38.4 ng/g tissue vs 75.2±13.5 ng/g tissue). This increase was inhibited by TJ-14 at 125 mg/kg, and more profound inhibition was observed when the dose of TJ-14 was increased to 500 mg/kg body weight (Fig. 2). Rats that received TJ-14 at 500 mg/kg body weight did not show chronic diarrheal symptoms, and their feces were normal regardless of 4 consecutive days of CPT-11 treatment.

Fig. 2. Effects of TJ-14 on colonic PGE2 stimulated by CPT-11 in rats. CPT-11 was administered intravenously at 60 mg/kg once daily for 4 consecutive days. TJ-14 was given orally once daily the day before and throughout CPT-11 administration. Rats were sacrificed 24 hr after the last administration of CPT-11. Each column represents the mean ± S.E. of 8–10 rats. * and ***: significantly different from the control at P<0.05 and P<0.001, respectively.
Histopathological examination of rat ileum and descending colon

Significant histopathological changes were observed in the descending colon and ileum of control rats, including degeneration and necrosis of villi and cryptal cells and decrease in the goblet cells. CPT-11-caused tissue damages were much less profound in rats treated together with TJ-14 in a dose-dependent fashion (Table 2).

Effects of TJ-14 on colonic water absorption reduced by CPT-11

CPT-11 for 4 consecutive days significantly impaired colonic water absorption in control rats that showed an absorpational rate one third the level seen in the untreated rats (Fig. 3). Treatment with TJ-14 at 125 and 500 mg/kg body weight significantly improved colonic water absorption in a dose-dependent manner. Water absorption at high doses of TJ-14 was essentially the same as in the untreated rats (Fig. 3).

DISCUSSION

CPT-11 is an effective anticancer agent for non-small cell lung cancer (2-4), but this agent causes severe dian-

Table 2. Effects of TJ-14 on CPT-11-induced damage of intestinal mucosa in rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CPT-11 (mg/kg)</th>
<th>TJ-14 (mg/kg)</th>
<th>N</th>
<th>Necrosis</th>
<th>Decrease in the number of goblet cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ileum</td>
<td>60</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>125</td>
<td>10</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>500</td>
<td>10</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Colon</td>
<td>60</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>125</td>
<td>10</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>500</td>
<td>10</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

CPT-11 was administered intravenously at 60 mg/kg, once daily for 4 consecutive days. TJ-14 was given orally once daily the day before and throughout CPT-11 administration. Rats were sacrificed 24 hr after the final injection of CPT-11. Grade sign: - : no lesion, + : slight, ++ : moderate, +++ : severe. Each value represents the number of animals in each grade of histopathological findings.

**Fig. 3.** Effects of TJ-14 on colonic water absorption impaired by CPT-11 in rats. CPT-11 was administered intravenously at a dose of 60 mg/kg once daily for 4 consecutive days. TJ-14 was given orally once daily the day before and throughout CPT-11 administration. Rats were sacrificed 24 hr after the last administration of CPT-11. Each column represents the mean ± S.E. of 9 rats. * and ***: significantly different from the control at P < 0.05 and P < 0.001, respectively.
rhea, thus limiting its clinical application (2–5). CPT-11 is known to produce acute and delayed diarrhea; the former is well-responsive to the treatment of anticholinergic agents such as atropine, while the latter has been a knotty problem clinically since the symptoms are usually severe and there is no effective treatment available to date. The present study was carried out to elucidate the pharmacological effectiveness of Kampo medicine for delayed diarrhea due to CPT-11.

CPT-11 is metabolized by carboxylesterase in the liver to the active intermediate SN-38 (7-ethyl-10-hydroxy camptothecin), which is then conjugated to glucuronic acid through catalysis by glucuronyltransferase and excreted into the intestine (16, 17). It was proposed that SN-38 produced by the deconjugation by β-glucuronidase of the microflora in the intestine was responsible for the gastrointestinal toxicity and delayed diarrhea (18). PGE₂ and poor colonic water absorption were two important factors closely associated with the development of diarrhea (10–12). We have shown previously that the development of CPT-11-induced chronic diarrheal symptoms was accompanied by gastrointestinal injury which caused significant increase in colonic PGE₂ and impaired colonic water absorption (19). In order to prevent and/or to treat chronic diarrheal symptoms induced by CPT-11, it was assumed to be effective to inhibit the increased colonic PGE₂ and to improve colonic water absorption.

Recently, Takasuna et al. investigated the possible inhibitory effect of Kampo medicine on CPT-11-induced chronic diarrheal symptoms and reported that TJ-14 was the most effective one (20). The mechanism of this inhibition was postulated by Narita et al. to be a competitive inhibition of bacterial β-glucuronidase by baicalin present in TJ-14, leading to reduction in the active metabolite SN-38 in the gut (21).

Our results demonstrate that TJ-14 inhibits effectively chronic diarrheal symptoms even though it has little effect on acute diarrheal symptoms caused by CPT-11, which is consistent with the observations of Takasuna et al. CPT-11-induced acute diarrheal symptoms result from the inhibition of cholinesterase by this agent (22) and can be effectively treated by anticholinergic drugs.

Even though therapy for acute diarrhea has been established, there has not been any effective drug and therapy for delayed diarrhea caused by CPT-11. Treatment with CPT-11 for 4 consecutive days resulted in significant gastrointestinal toxicity including decrease in the body weight and tissue injury in the gut. The onset of severe chronic diarrheal symptoms was correlated with a significant increase in PGE₂ in descending colon and impaired colonic water absorption. Co-administration of TJ-14 with CPT-11 inhibited chronic diarrheal symptoms in a dose-dependent manner. This inhibition was correlated with significantly decreased PGE₂, diminished intestinal tissue injury and improved colonic water absorption. PGE₂ is secreted by the mucosa and smooth muscle of the small intestine (23) and is known to induce diarrhea by stimulating colonic secretion and hyperperistalsis of the gut (24, 25). In addition, PGE₂ inhibits Na⁺, K⁺-ATPase, whereby it affected the absorption of electrolytes (26, 27). Thus, the improvement of colonic water absorption in rats in the present study can be attributed to the decrease in PGE₂ in the gut by TJ-14. On the other hand, baicalin, an active substance from Scutellariae radix comprised in TJ-14, was reported to inhibit β-glucuronidase, leading to a decrease in the deconjugation of the glucuronide of SN-38 responsible for the gastrointestinal toxicity caused by CPT-11 (21). It is therefore reasonable to assume that the inhibitory effect of TJ-14 on chronic diarrheal symptoms is mediated through the diminished tissue injury in the intestine. We have previously reported that acute diarrheal symptoms following CPT-11 treatment probably resulted from enhanced intestinal motility caused by the inhibition of cholinesterase (19). It has also been shown that TJ-14 possesses neither anti-cholinergic action nor an action to inhibit intestinal motility (8), and that the lack of these actions explain why TJ-14 does not markedly suppress CPT-11-induced acute diarrheal symptoms. On the other hand, since chronic diarrheal symptoms are caused by tissue damage, TJ-14 which suppresses tissue damage and PGE₂ production can inhibit chronic diarrheal symptoms induced by CPT-11.

TJ-14 was reported to suppress the elevation in colonic PGE₂ levels and to enhance colonic water absorption (9). Some reports revealed that PGE₂ production was suppressed by Zingiberis siccata radix and Glycyrrhizae radix (28, 29). In a previous study, we have found that TJ-14 selectively inhibits cyclooxygenase-2 (COX-2) activity, although this action of TJ-14 is weaker than that of existing anti-inflammatory agents (Y. Kase et al., submitted for publication). Furthermore, Ginsenosides contained in Ginseng radix were reported to increase plasma corticosterone levels by the stimulation of adrenocorticotropic (30). Therefore, the effects of TJ-14 in suppressing PGE₂ production and promoting colonic water absorption seem to contribute to its effect in suppressing CPT-11-induced chronic diarrheal symptoms. In addition, berberine contained in Coptidis rhizoma improved the intestinal hypersecretion induced by cholera toxin (31). Although further investigations are necessary to elucidate the mechanisms by which TJ-14 suppressed CPT-11-induced chronic diarrheal symptoms, these lines of evidence lead to the conclusion that the therapeutic effects of TJ-14 on chronic diarrheal symptoms due to CPT-11 are not solely based on the inhibition of the SN-38-glucuronide deconjugation.
In summary, TJ-14 revealed a therapeutic effect on chronic diarrheal symptoms due to CPT-11 in rats, and this effect was mediated through significant decrease in PGE2 in the descending colon, improvement of colonic water absorption and relieving intestinal toxicity by CPT-11. TJ-14 is thus expected to provide an effective therapy for delayed diarrhea in cancer patients on CPT-11 treatment.

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REFERENCES


