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Protective Effects of *Kampo* Medicines and Baicalin against Intestinal Toxicity of a New Anticancer Camptothecin Derivative, Irinotecan Hydrochloride (CPT-11), in Rats

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In clinical use, irinotecan hydrochloride (CPT-11; 7-ethyl-10-[4-(piperidino)-1-piperidino]carbonyloxycamptothecin), a novel antitumor agent, causes a relatively high incidence of severe forms of diarrhea. We investigated whether baicalin, an inhibitor of β-glucuronidase, which deconjugates the glucuronide of the active metabolite of CPT-11, SN-38 (7-ethyl-10-hydorxycamptothecin), and Japanese herbal medicines (*Kampo* medicines) which contain baicalin can ameliorate CPT-11-induced intestinal toxicity in rats. CPT-11 (60 mg/kg i.v. once daily for 4 consecutive days) induced intestinal toxicity characterized by diarrhea, loss of body weight, anorexia and disruption of intestinal epithelium. Treatment with baicalin (25 mg/kg p.o. twice daily) or *Kampo* medicines (TJ-14 and TJ-114; 1 g/kg p.o. twice daily) from the day before to 4 or 10 days after the start of CPT-11 administration resulted in significantly decreased weight loss, improved anorexia and a delayed onset of diarrheal symptoms. Histological examination revealed that *Kampo* medicine-treated animals had less damage to the intestinal epithelium and that damage was repaired more rapidly than in control rats. These results suggest that the prophylactic use of *Kampo* medicines (TJ-14 and TJ-114) may be of value against CPT-11-induced intestinal toxicity.

Key words: CPT-11 — Diarrhea — *Kampo* medicine — Baicalin — β-Glucuronidase

Camptothecin (CPT), an alkaloid isolated from *Camptotheca acuminata*, has potent antitumor activity against several experimental tumors.[1,2] However, CPT showed poor efficacy and severe toxicity, including hemorrhagic cystitis, in early clinical studies.[3,4] Studies since then, seeking CPT derivatives with greater antitumor activity and reduced toxicity, have led to the discovery of a new water-soluble CPT derivative, irinotecan hydrochloride (CPT-11).[5] CPT-11 has been found to have clinical efficacy without inducing hemorrhagic cystitis.[6–10] Moreover, preclinical studies on a major complication of cancer therapy, emesis and nausea, have shown that, compared with cisplatin, CPT-11 has mild emetic activity which is prevented by metoclopramide or ondansetron given alone.[11] However, the early clinical use of CPT-11 at higher doses was associated with an unexpected and significant incidence of diarrhea, and diarrhea is now recognized as a dose-limiting toxicity of the compound.[6–10,12] While conventional anti-diarrheal agents have some activity against mild to moderate diarrhea induced by CPT-11,[6,9,13] common anti-diarrheal drugs have limited clinical value in severe forms of the condition.[8,10] It is not presently understood why CPT-11 induces severe diarrhea as compared with other anticancer agents. One possible explanation is that CPT-11 itself has anti-cholinesterase activity,[14] which would cause diarrhea in the acute phase by stimulating intestinal contractility and impairing normal intestinal mucosal absorptive/secretory function.[15] Other possible mechanisms might include structural and functional injuries to the gastrointestinal tract owing to the mitotic inhibitory activity of its active metabolite, SN-38. However, relatively little basic research has been done on the diarrhea induced by CPT-11, which is accompanied with disruption of the intestinal epithelium. The major metabolic pathways for CPT-11 are outlined in Fig. 1. CPT-11 is hydrolyzed to form the active metabolite, SN-38. This metabolite exhibits potent antitumor activity by inhibiting DNA topoisomerase I.[16,17] Most of SN-38 undergoes conjugation to afford SN-38 glucuronide, which has only 1/100 the antitumor activity of SN-38, and is excreted into the bile with the other major components, CPT-11 and SN-38.[18,19] Approximately 34, 7, and 2% of the dose were excreted into the bile as CPT-11, SN-38 glucuronide, and SN-38, respectively, at the 40 mg/kg i.v. dose level.[18] However, since the SN-38 excretion in the feces

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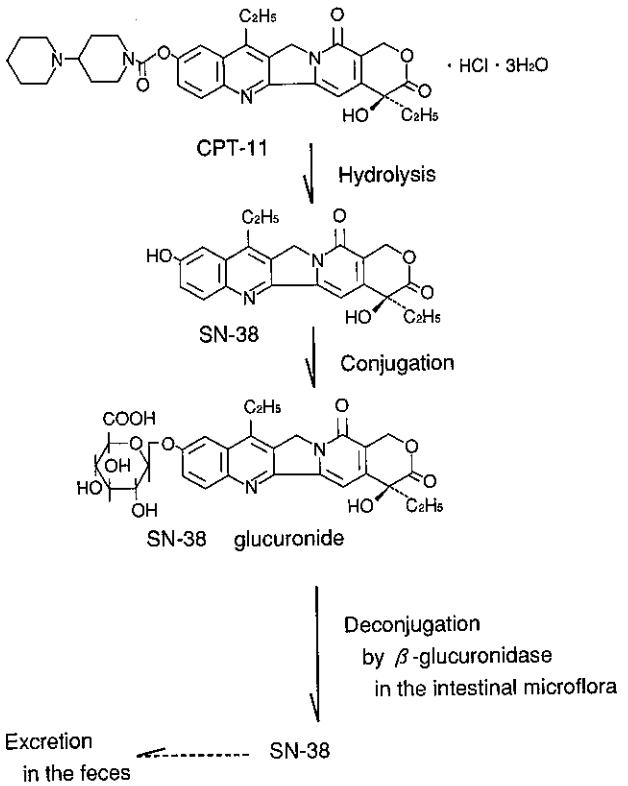

Fig. 1. Metabolic pathway of irinotecan hydrochloride (CPT-11).

was increased to about 10%, SN-38 glucuronide was considered to be deconjugated by β-glucuronidase in the intestinal microflora to regenerate SN-38.[18] If both this deconjugated SN-38 and SN-38 carried via the bloodstream injure the intestinal epithelium, the inhibition of β-glucuronidase activity may help to reduce the severity of the diarrhea. Recently, Narita *et al.*[20] showed that some naturally occurring glucuronides such as baicalin, wogonoside, luteolin-3′-glucuronide and glycyrrhizin inhibit the *in vitro* hydrolytic cleavage of SN-38 glucuronide by β-glucuronidase from *Escherichia coli*. In the present study, we therefore investigated whether clinically available *Kampo* medicines which contain baicalin as an ingredient can alleviate CPT-11-induced diarrhea *in vivo*.

MATERIALS AND METHODS

Animals The experiments were conducted using male Wistar rats (Japan SLC, Hamamatsu) weighing 150–250 g. The animal room was maintained at a temperature of 23±2°C and relative humidity of 55±15% with a 12-h light-dark cycle. A commercial animal chow (F-2, Funabashi Farm, Funabashi) and tap water were freely available throughout the acclimatization and experimental periods.

Effects of *Kampo* medicines and baicalin on CPT-11-induced intestinal toxicity Animals were given CPT-11 at an intravenous dose of 60 mg/kg/day once daily for 4 successive days (Days 1–4). Following treatment, the severity of chronic diarrhea and the daily body weight change and food intake were monitored, and the results were applied for a rapid screening method for intestinal toxicity.[21] Under the regimen adopted, diarrhea observed chronically during Days 5 to 7 had the features of the human diarrhea in terms of being resistant to conventional anti-diarrheal agents.[15] We focused on delayed-onset diarrhea and defined it as chronic diarrhea. The severity of chronic diarrhea was scored as severe (2), mild (1) or normal (absent) (0), and the mean score was calculated. *Kampo* medicine (TJ-14 or TJ-114; 0.1 and 1 g/kg) or baicalin (25 mg/kg) was orally administered twice a day at 9:00–11:00 a.m. (about 30 min before dosing of CPT-11) and 4:00–6:00 p.m. To determine the optimum treatment schedule, the following three regimens were used: (1) administration from 7 days before to 10 days after the start of CPT-11 injection (total 17 days), (2) from the day before to 10 days after the start of CPT-11 injection (total 11 days) and (3) from the day before and throughout CPT-11 injection (total 5 days). Preliminary pharmacokinetic data showed that following a single injection of CPT-11 (60 mg/kg i.v.), the concentration of SN-38 glucuronide in the small intestinal luminal content reached about 50 μg/g content. Since about 10 times higher concentration of baicalin than that of SN-38 glucuronide was needed to inhibit completely the deconjugation of SN-38 glucuronide *in vitro*,[20] we selected the 25 mg/kg dose level of baicalin (corresponding to about 1 mg/g content). Because both *Kampo* medicines contain about 25 mg baicalin per 1 g weight, the higher unit dose of each *Kampo* medicine was fixed at 1 g/kg. In separate experiments the protective effects of *Kampo* medicines were examined histologically. Moreover, atropine (1 mg/kg s.c.), which prevented watery diarrhea induced by CPT-11 in the acute phase,[15] was administered once daily throughout CPT-11 injection (30 min before CPT-11) with or without a *Kampo* medicine (TJ-114) to evaluate combination anti-diarrheal therapy.

Histological studies Intestinal tissues were fixed in 10% neutral buffered formaldehyde. Segments of jejunum, ileum, cecum and colon were embedded in paraffin wax and processed to prepare histological slides stained with hematoxylin-eosin (HE) for light microscopy.

Drugs CPT-11 was supplied by Yakult Honsha (Tokyo). *Kampo* medicines (TJ-14 and TJ-114) and baicalin were supplied by Tsumura & Co. (Tokyo). TJ-14 (Hange-Shasin-To) and TJ-114 (Sai-Rei-To) consist of spray-

dried hot-water extracts of mixtures of seven and twelve medicinal plants, respectively (Table I).

Statistical analysis The results were analyzed by ANOVA. Differences were considered significant based on multiple comparison according to Dunnett or Scheffé at $P<0.05$, except for diarrhea scores, which were analyzed using Wilcoxon's rank sum test.

Table I. Composition of *Kampo* Medicines

TJ-14 (Hange-Shasin-To)	Ratio	TJ-114 (Sai-Rei-To)	Ratio
Pinelliae tuber	5.0	Pinelliae tuber	5.0
Scutellariae radix	2.5	Scutellariae radix	3.0
Glycyrrhizae radix	2.5	Glycyrrhizae radix	2.0
Zizyphi fructus	2.5	Zizyphi fructus	3.0
Ginseng radix	2.5	Ginseng radix	3.0
Zingiberis siccatum rhizoma	2.5	Zingiberis rhizoma	1.0
		Bupleuri radix	7.0
Coptidis rhizoma	1.0	Alismatis rhizoma	5.0
		Atractylodis lanceae rhizoma	3.0
		Polyporus	3.0
		Hoelen	3.0
		Cinnamomi cortex	2.0

Kampo medicines are spray-dried hot-water extracts of a mixture of medicinal plants at the weight ratio given in the Table and contain about 25 mg of baicalin per 1 g weight.

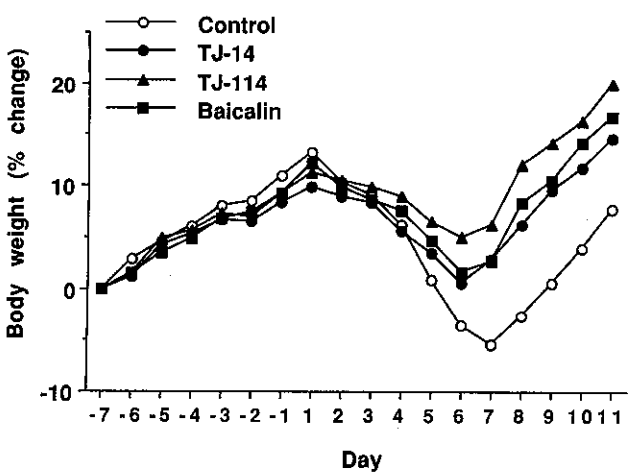

Fig. 2. Effects of *Kampo* medicines (TJ-14 and TJ-114) and baicalin on CPT-11-induced body weight loss in rats. CPT-11 was given intravenously at a dose of 60 mg/kg once daily for 4 consecutive days (Days 1 to 4). *Kampo* medicines (1 g/kg) and baicalin (25 mg/kg) were orally administered twice daily from 7 days before to 10 days after the start of CPT-11 injection. Control animals received distilled water. The change in body weight was calculated on the basis of that on Day −7. Each point represents the mean of 4–5 rats.

RESULTS

Following the i.v. administration of CPT-11 (60 mg/kg once daily for 4 consecutive days; Days 1 to 4), body weight decreased and reached a nadir on Day 6 or 7 (2–3 days after the discontinuation of CPT-11 injection (Fig. 2). No diarrhea was present during the first 2 days. However, watery diarrhea occurred 1–2 h after CPT-11 injection on Days 3 and 4 (acute diarrhea), and diarrheal

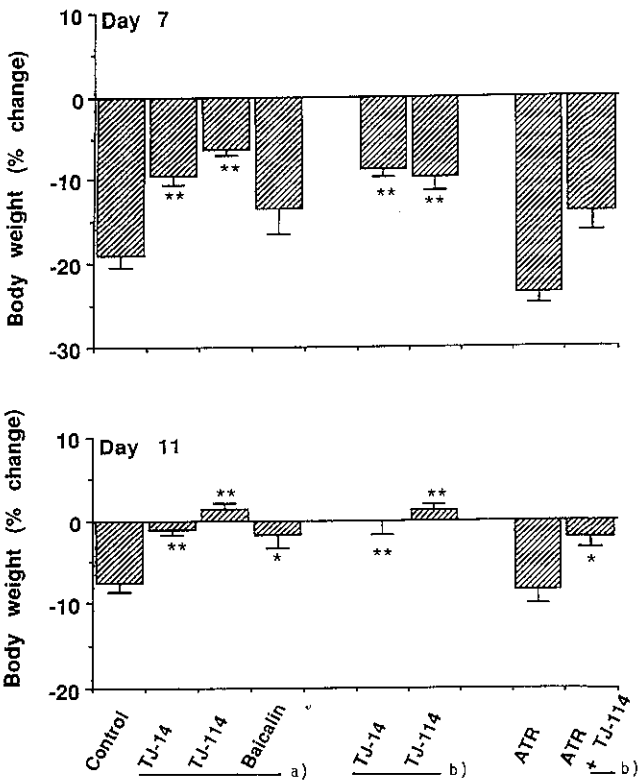

Fig. 3. Effects of *Kampo* medicines (TJ-14 and TJ-114) or baicalin on CPT-11-induced body weight loss in rats. CPT-11 was given intravenously at a dose of 60 mg/kg once daily for 4 consecutive days (Days 1 to 4). *Kampo* medicines (1 g/kg) and baicalin (25 mg/kg) were orally administered twice daily a) on the day before and for 10 days after the start of CPT-11 injection or b) on the day before and throughout the CPT-11 administration period. Atropine (ATR: 1 mg/kg) was administered once (about 30 min before CPT-11 injection) daily only during the CPT-11 administration period. Control animals received distilled water. The change in body weight was calculated on the basis of that on Day 1. Each column and vertical bar represent the mean±standard error of 5 animals on Day 7, when the decrease in body weight in the control group was at the nadir, and on Day 11, when body weight in the *Kampo* medicine-treated groups returned to initial levels. * $P<0.05$, ** $P<0.01$: Significantly different from the control group (one-way layout and multiple comparison according to Dunnett).

Table II. Effect of *Kampo* Medicines, Baicalin and Atropine on CPT-11-induced Chronic Diarrheal Symptoms in Rats

Group	Diarrheal score[a]											
	Day 5				Day 6				Day 7			
	0	1	2	(Mean)	0	1	2	(Mean)	0	1	2	(Mean)
CPT-11	1[b]	2	2	(1.2)	0	3	2	(1.4)	2	3	0	(0.6)
+ TJ-14[c] 1 g/kg	5	0	0	(0.0)*	4	0	1	(0.4)	5	0	0	(0.0)
+ TJ-114[c] 1 g/kg	5	0	0	(0.0)*	2	1	2	(1.0)	5	0	0	(0.0)
+ Baicalin[c] 25 mg/kg	3	2	0	(0.4)	3	2	0	(0.4)*	4	1	0	(0.2)
+ TJ-14[d] 1 g/kg	3	2	0	(0.4)	2	3	0	(0.6)	4	1	0	(0.2)
+ TJ-114[d] 1 g/kg	3	0	2	(0.8)	4	1	0	(0.2)*	5	0	0	(0.0)
+ Atropine 1 mg/kg	0	0	5	(2.0)	2	0	3	(1.2)	1	0	4	(1.6)
+ Atropine 1 mg/kg and TJ-114[d] 1 g/kg	3	0	2	(0.8)	3	2	0	(0.4)*	5	0	0	(0.0)

a) Chronic diarrheal score was defined as follows. 0: no diarrhea, 1: mild diarrhea, 2: severe diarrhea.
b) Number of rats showing each score.
CPT-11 was given intravenously at a dose of 60 mg/kg once daily for 4 consecutive days (Days 1 to 4). *Kampo* medicines or baicalin were orally administered twice daily *c*) from the day before to 10 days after the start of CPT-11 injection or *d*) on the day before and throughout the CPT-11 administration period. Atropine was subcutaneously administered once daily (about 30 min before CPT-11 injection) during the CPT-11 administration period.
* $P<0.05$: Significantly different from the control group (Wilcoxon's rank sum test).

symptoms were chronically present during Days 5 to 7 (chronic diarrhea). TJ-14 (1 g/kg) and TJ-114 (1 g/kg) administered from 7 days before to 10 days after the start of CPT-11 injection slightly decreased the increase in body weight throughout the pre-treatment period as compared with the control group. However, both medicines inhibited the decrease in body weight induced by CPT-11 from Days 5 to 7 (Fig. 2), and also improved the chronic diarrhea. In contrast, both failed to inhibit the acute diarrhea which appeared on Days 3 and 4. Similar effects were evident in rats co-treated with baicalin (25 mg/kg) (Fig. 2). The second regimen, involving administration of *Kampo* medicines or baicalin from the day before to 10 days after the start of CPT-11 injection, inhibited the decrease in body weight and improved the chronic diarrhea as effectively as the first regimen (Fig. 3 and Table II). Moreover, the third regimen, involving administration of *Kampo* medicines the day before injection and during the 4-day injection period, ameliorated the loss of body weight and chronic diarrhea to an extent similar to those of the first and second regimens (Fig. 3 and Table II). Both *Kampo* medicines improved the decreases in body weight and food intake and the chronic diarrhea in a dose-dependent manner at doses of 0.1 and 1 g/kg (Fig. 4). Histological examination showed that repeated dosing of CPT-11 induced the disruption of epithelial integrity, including villus fusion, inflammation or submucosal edema, from Day 3 in all areas of the intestine except the jejunum; these changes were most severe in the cecum. In terms of the degree of changes, animals co-treated with TJ-14 had less mucosal damage and that damage was repaired more rapidly than in control rats (Fig. 5). Animals pretreated with atropine (1 mg/kg s.c. once daily during the CPT-11 administration period) showed no acute diarrhea on Days 3 and 4, but had more severe body weight loss and chronic diarrhea from Days 5 to 7. Co-administration of TJ-114 and atropine produced a smaller decrease in CPT-11 toxicity than that seen with TJ-114 alone (Fig. 3 and Table II).

DISCUSSION

CPT-11 has a novel mechanism of antitumor activity, namely the inhibition of DNA topoisomerase I, and has shown interesting antitumor activity clinically.[6-10, 22] However, diarrhea has been observed at higher incidence (about 30–70%) than with other chemotherapies. The occurrence of uncontrolled severe forms of diarrhea has limited the further evaluation of more aggressive antitumor regimens using CPT-11.[8, 10, 12, 23] Conventional anti-diarrheal agents, including anti-cholinergic agents and loperamide, have some activity against CPT-11-induced diarrhea, but have shown only inconsistent success in clinical trials.[6, 8-10, 13] Indeed, we also found that conventional anti-diarrheal agents such as atropine, ondansetron, clonidine and morphine could prevent the watery diarrhea which appeared at the acute phase (within 1–2 h after a single administration of a high dose of CPT-11) in rats, but exacerbated the delayed diarrheal symptoms associated with the disruption of intestinal mucosa by repeated administration of CPT-11, suggesting that commonly used anti-diarrheal agents are unsuitable to treat CPT-11-induced diarrhea involving intestinal epithelial damage.[15]

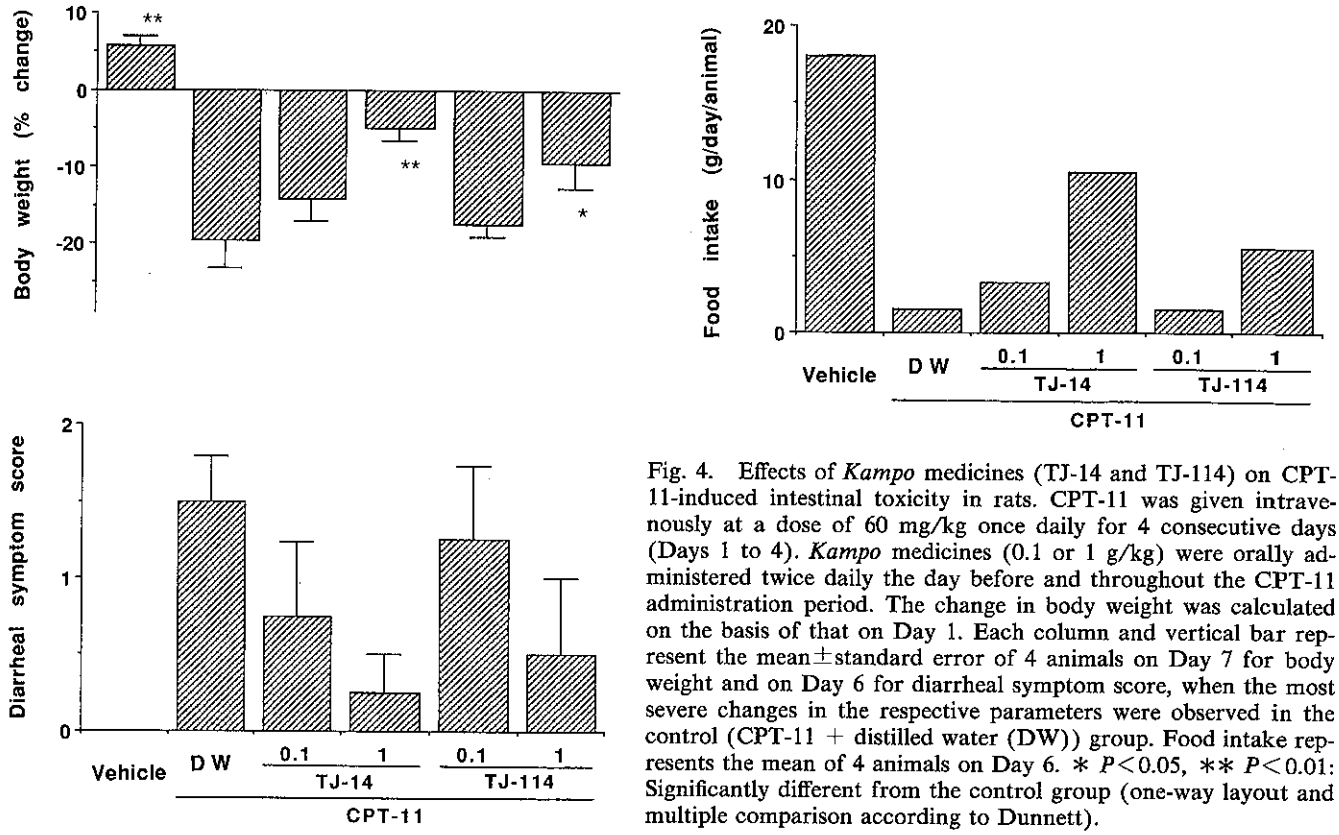

Fig. 4. Effects of *Kampo* medicines (TJ-14 and TJ-114) on CPT-11-induced intestinal toxicity in rats. CPT-11 was given intravenously at a dose of 60 mg/kg once daily for 4 consecutive days (Days 1 to 4). *Kampo* medicines (0.1 or 1 g/kg) were orally administered twice daily the day before and throughout the CPT-11 administration period. The change in body weight was calculated on the basis of that on Day 1. Each column and vertical bar represent the mean±standard error of 4 animals on Day 7 for body weight and on Day 6 for diarrheal symptom score, when the most severe changes in the respective parameters were observed in the control (CPT-11 + distilled water (DW)) group. Food intake represents the mean of 4 animals on Day 6. * $P<0.05$, ** $P<0.01$: Significantly different from the control group (one-way layout and multiple comparison according to Dunnett).

Under the regimen adopted (60 mg/kg i.v. once daily for 4 consecutive days; Days 1 to 4), marked decreases in body weight and food intake were observed, and chronic diarrhea appeared from Days 5 to 7. As anticipated, repeated administration of CPT-11 caused structural impairment of intestinal epithelium including villus fusion, epithelial regeneration of the villus region, and submucosal edema; the injuries were particularly pronounced in the cecum. In addition, watery diarrhea was also observed within about 2 h after CPT-11 injection on Days 3 and 4.

Co-administration of *Kampo* medicines (TJ-14 or TJ-114) resulted in significantly less body weight loss, improvement in the severity of anorexia and a delayed diarrhea in a dose-dependent manner, even though they could not prevent acute watery diarrhea, which has been assumed to involve the anti-cholinesterase activity of CPT-11 itself.[14, 15] The regimen in which *Kampo* medicine use was limited to the day before injection and the duration of the administration period ameliorated CPT-11-induced intestinal toxicity to a similar extent to the 2 longer regimens. It is suggested that medication with *Kampo* remedies during the CPT-11 administration period is the optimum way to ameliorate intestinal toxicity, and that *Kampo* medicines act in a protective fashion rather than through the acceleration of healing. The protective effects of *Kampo* medicine against CPT-11 were also evident on histological examination: the cecal mucosa of rats receiving *Kampo* medicine recovered more rapidly than in the control group. The mechanisms by which *Kampo* medicines provide protection are unknown. Notably, baicalin at a dose of 25 mg/kg, corresponding to that contained in *Kampo* medicines (1 g/kg), improved CPT-11-induced intestinal toxicity to a similar degree to the *Kampo* medicines. Baicalin, which has been shown to inhibit hydrolytic cleavage of SN-38 glucuronide by β-glucuronidase,[20] may play a major role in the ameliorative effects of *Kampo* medicines and it therefore seems likely that their mechanism may involve the inhibition of β-glucuronidase.

Since the acute watery diarrhea which appeared on Days 3 and 4 was not prevented by *Kampo* medicines given alone, we expected that co-administration with atropine, which has anti-diarrheal activity against CPT-11-induced acute watery diarrhea,[15] would show greater protective effects. Despite its inhibition of acute watery diarrhea, however, co-administration of atropine with TJ-114 was not beneficial against the delayed diarrhea,

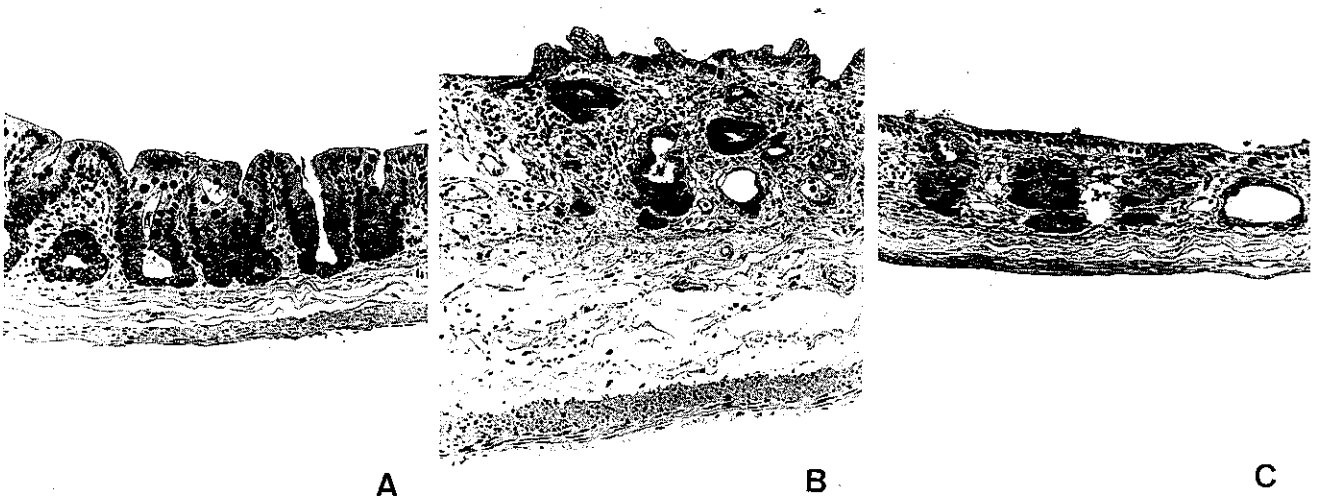

Fig. 5. Micrographs of cecal mucosa of rats which received CPT-11 with or without *Kampo* medicine. CPT-11 was given intravenously at a dose of 60 mg/kg once daily for 4 consecutive days (Days 1 to 4). TJ-14 was given orally at a dose of 1 g/kg twice daily the day before and throughout the CPT-11 administration period. A) Non-treated (physiological saline) (HE, ×20). B) CPT-11 only (Day 7) (HE, ×20); mucosal profile is destroyed. Interstitial mononuclear cell infiltration and submucosal edema are seen. C) CPT-11 + TJ-14 (Day 7) (HE, ×20); although cystic dilation of the crypt is seen, the mucosa is nearly fully repaired.

reconfirming that prophylactic treatment with atropine during CPT-11 dosing exacerbates CPT-11-induced intestinal toxicity. The mechanism by which conventional anti-diarrheal agents, including atropine, exacerbate CPT-11-induced intestinal toxicity is obscure. Because all these agents inhibit intestinal motility, they may delay excretion of CPT-11 and/or SN-38 from intestinal tissues or the intestinal lumen, thereby enhancing its cytotoxicity. Although the use of loperamide, a relatively selective agonist against peripheral opioid receptors,[24] and cholinergic antagonists has shown clinical efficacy against CPT-11-induced diarrhea,[6, 9, 13] our results do not rule out the possibility that prophylactic use of higher doses of these anti-diarrheal agents to cancer patients undergoing CPT-11 therapy would have no benefit, and might in fact worsen the delayed diarrhea associated with disruption of the intestinal epithelium, even if they do prevent the acute watery diarrhea which results at least in part from the cholinergic stimulant activity of CPT-11.[15]

In conclusion, *Kampo* medicines (TJ-14 or TJ-114) which contain baicalin, a β-glucuronidase inhibitor, alleviated CPT-11-induced intestinal toxicity. Although further investigations to determine the mechanisms of the protective effects of *Kampo* medicines are necessary, there is currently no reliable treatment for CPT-11-induced diarrhea[8, 10, 23]; the development of improved *Kampo* medicines that minimize the intestinal toxicity of CPT-11 may therefore be beneficial. The effects of *Kampo* medicine on the pharmacokinetics of CPT-11 are currently under examination.

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