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ORIGINAL ARTICLE—ALIMENTARY TRACT

Daikenchuto (TU-100) ameliorates colon microvascular dysfunction via endogenous adrenomedullin in Crohn's disease rat model

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Abstract
Background Daikenchuto (TU-100), a traditional Japanese medicine, has been reported to up-regulate the adrenomedullin (ADM)/calcitonin gene-related peptide (CGRP) system, which is involved in intestinal vasodilatation. The microvascular dysfunction of the intestine in Crohn's disease (CD), due to down-regulation of the ADM/CGRP system, is etiologically related to the recurrence of CD. Therefore, we investigated the vasodilatory effect of TU-100 in a CD rat model.
Methods Colitis was induced by the rectal instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS) in rats. Laser Doppler blood flowmetry was used to measure colonic blood flow. ADM, CGRP, and their receptors in the ischemic colon were measured by reverse transcription polymerase chain reaction (RT-PCR) and enzyme immunoassays. Additionally, we determined whether the intestinal epithelial cell line IEC-6 released ADM in response to TU-100.
Results TU-100 increased blood flow in ischemic segments of the colon but not in hyperemic segments. Pretreatment with an antibody to ADM abolished the vasodilatory effect of TU-100. CGRP levels and βCGRP mRNA expression were decreased in the ischemic colon, while protein and mRNA levels of ADM were unchanged. Hydroxy α-sanshool, the main constituent of TU-100, was the most active component in improving blood flow. Additionally, both TU-100 and hydroxy α-sanshool enhanced the release of ADM from IEC-6 cells.
Conclusions In the ischemic colon, endogenous βCGRP, but not ADM, was decreased. Thus, it was concluded that TU-100 ameliorated microvascular dysfunction by the up-regulation of endogenous ADM in the CD rat model. TU-100 may be a possible therapeutic agent for gastrointestinal ischemia-related diseases including CD.

Keywords Daikenchuto (TU-100) · Adrenomedullin · Crohn's disease · Ischemia · Colonic blood flow

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Introduction

Postoperative recurrences of Crohn's disease (CD) at the anastomotic site are common, often requiring further abdominal surgery for anastomotic strictures. Indeed, within 1 week after operation, histopathological findings of recurrent CD may be seen [1], and the incidence of endoscopically detected recurrence reaches 70–90% at 1 year postoperatively [2, 3], thereby predisposing such patients to develop symptomatic stenosis. It has been reported that blood flow is decreased by more than 50% in the terminal ileum and colon of CD patients [4, 5]. Decreased blood flow is one of the factors involved in recurrence at the anastomotic site [6]. A potent endogenous

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microvascular dilator, calcitonin gene-related peptide (CGRP), a neuropeptide produced by the nervous system of the gut, is decreased in animal models of CD and in CD patients [7]. Moreover, it has been reported that ischemia plays a key role in the pathogenesis of CD. In fact, several clinicians and researchers who have investigated the vascular contribution to the pathogenesis of CD have suggested the possibility of vasodilators as therapeutic agents [8, 9].

Daikenchuto (TU-100), a traditional Japanese medicine (such medicines are known as Kampo), is manufactured as a recognized prescription drug with standardized quality and quantities of ingredients [10]. In Japan, TU-100 is prescribed to improve gastrointestinal motility and prevent postoperative adhesion and paralytic ileus after abdominal surgery, and its clinical efficacy has been defined [11–15]. Moreover, TU-100 had a significant promotility effect in the small bowel and in ascending colon transit in healthy subjects compared with placebo in a randomized, double-blind study in the United States [16]. Recently, we reported that TU-100 exerted therapeutic effects in a CD mouse model via the enhancement of endogenous adrenomedullin (ADM) in the intestine [17]. TU-100 directly stimulated intestinal epithelial cells, resulting in increased ADM production. We also reported that the administration of TU-100 to normal rats increased small and large intestinal blood flow via CGRP systems [18, 19].

ADM, like CGRP, is a peptide of the calcitonin family and a potent endogenous vasodilator [20, 21]. ADM is ubiquitous in the intestinal tract and plays physiologically important roles in the microcirculation of the intestine [22, 23]. Therefore, up-regulation of endogenous ADM may attenuate the microvascular dysfunction in the CD intestine.

CGRP has two different isoforms, αCGRP and βCGRP. The former is mainly expressed in the sensory afferent neurons of the extrinsic nervous system and the latter in the intrinsic primary afferent neurons [24–26]. A decrease in CGRP has been reported in CD and this decrease might contribute to microvascular dysfunction in the CD intestine [7, 27]. However, there is no information on which types of CGRP are decreased in the CD intestine.

It is our hypothesis that TU-100 enhances endogenous ADM, which increases intestinal blood flow to compensate for the decrease due to decreased CGRP in the CD intestine. In this study, we examined the vasodilatory effects of TU-100 in a rat model of CD produced using 2,4,6-trinitrobenzenesulfonic acid (TNBS), identified the active constituents of TU-100, and explored potential mechanisms related to the ADM/CGRP system, using antibodies and an intestinal epithelial cell line. Moreover, we investigated which isoform of CGRP was decreased in the CD intestine.

Materials and methods

Animals

Male Sprague–Dawley rats (Japan SLC, Shizuoka, Japan) weighing 300–400 g were used. The animals were allowed free access to water and standard laboratory food and were housed at a temperature of 23 ± 2°C, relative humidity of 55 ± 10%, and a 12-h light: 12-h dark cycle with lights on from 0700 to 1900 hours daily. All experimental procedures were performed according to the "Guidelines for the care and use of laboratory animals" of Asahikawa Medical University and Tsumura Research Laboratories. All animal procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Reagents

TU-100, obtained from Tsumura (Tokyo, Japan), is manufactured as a water-soluble extract (containing processed ginger, ginseng radix, Japanese pepper, and maltose syrup [88.9%]) under strictly controlled conditions. The qualities of these raw materials were tested according to the standards of the Japanese Pharmacopoeia and Tsumura, and a routine chemical analysis was performed (Fig. 1). Maltose and 6-shogaol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Hydroxy α-sanshool (HAS) was extracted from Japanese pepper at Tsumura. Ginsenoside Rb1 was purchased from Extrasynthese (Genay, France), rabbit anti-ADM polyclonal IgG was purchased from Peninsula Laboratories (Belmont, CA, USA), and rabbit IgG was purchased from Abcam (Cambridge, UK) as an isotype-matched control.

Colonic blood flow

Total transmural colonic blood flow was measured using a laser Doppler flowmeter (ALF21N; Advance, Tokyo, Japan) as described previously [19]. Briefly, rats were anesthetized with urethane (900 mg/kg i.v.), α-chloralose (45 mg/kg i.v.), and butorphanol (Bristol-Myers Squibb, New York, NY, USA; 1 mg/kg i.m.). A tracheotomy was performed, and rats were ventilated artificially (respiratory rate 60 breaths/min) and placed on a heating pad. The left cervical artery was cannulated and connected to a transducer (P23XL; Nihon Kohden, Tokyo, Japan) to monitor systemic arterial blood pressure (AP) and heart rate (HR). An oral thermometer was placed in the mouth and body temperature was maintained at 37 ± 0.5°C. After exposing the colon by a midline laparotomy, a cannula (18G) was inserted via the cecum into the proximal colon to facilitate injection of the test substances. The distal colon was exposed and covered with plastic wrap to prevent the tissue

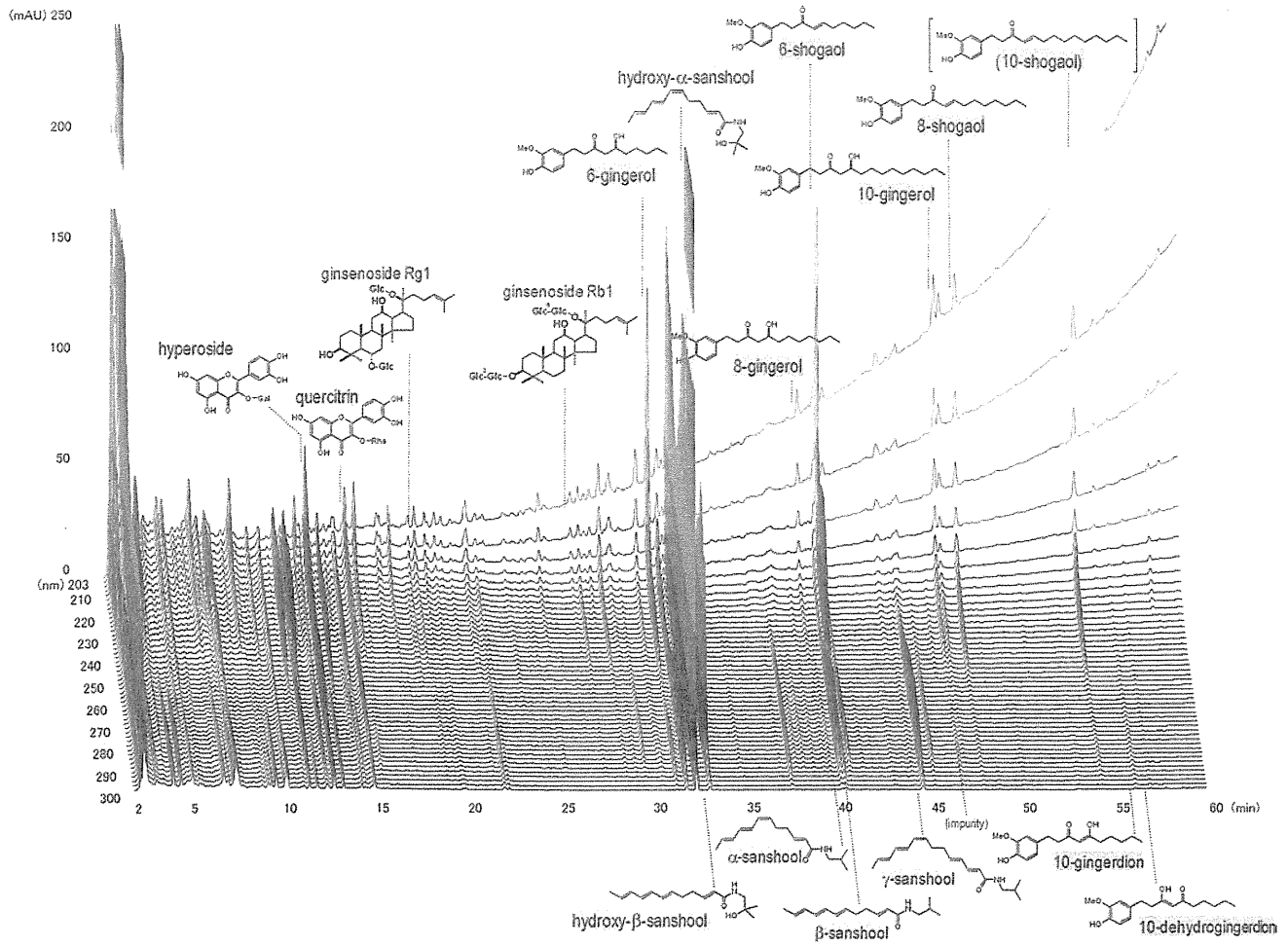

Fig. 1 Three-dimensional high-performance liquid chromatograph of TU-100

from becoming dehydrated. A fiberoptic probe was positioned 4 mm above the surface of the distal colon. Blood flow, AP, and HR were monitored continuously by a PowerLab data-sampling unit (PowerLab 8/30; AD Instruments, Tokyo, Japan) and recorded. Colonic vascular conductance (CVC) was measured as an index of total transmural colonic blood flow [28]. CVC was calculated as the quotient of mean blood flow divided by mean AP.

Effects of antibody and constituents of TU-100 on CVC

TU-100 (900 mg/5 mL/kg) or distilled water was injected into the lumen of the colon after confirming a stable blood flow. The ADM antibody (50 μg/kg) was injected through an intravenous polyethylene tube in the right jugular vein. Fifteen minutes later, TU-100 (900 mg/5 mL/kg) was injected. In another set of experiments, the effects of specific constituents of TU-100 on CVC were examined. The CVC responses to HAS, ginsenoside Rb1, 6-shogaol, and maltose were examined using doses of 0.3, 1, 0.2, and

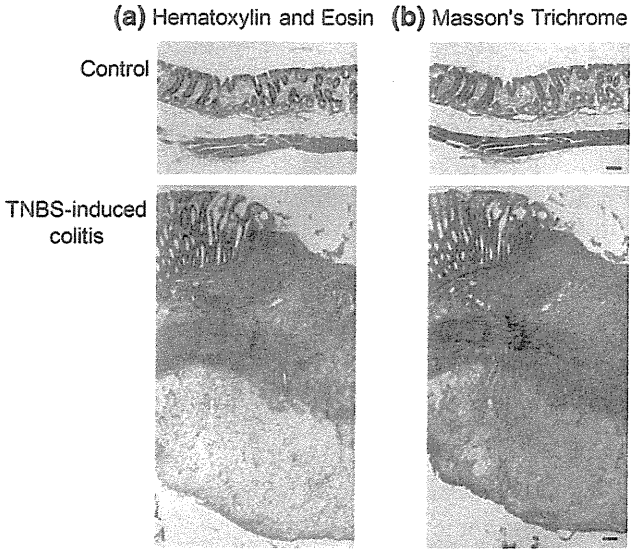

Fig. 2 Histology of segments of ischemic colon in rats with colitis. Sections of the colon 10 days after instillation of 2,4,6-trinitrobenzenesulfonic acid (*TNBS*) or 0.9% NaCl (control) were stained with hematoxylin and eosin (**a**) or Masson's trichrome (**b**). *Scale bar* indicates 100 μm

800 mg/kg, respectively. The test substances were dissolved in 1% Tween 80 and administered intraluminally. The blood flow and AP were measured at 15-min intervals after intracolonic administration.

ADM production assay

ADM enhancing activity was assessed as described previously. Namely, the rat intestinal epithelial cell line IEC-6 was obtained from Dainippon Sumitomo Pharma (Osaka, Japan) and grown in DMEM supplemented with 10% fetal bovine serum (FBS), 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10 mmol/L hydroxyethylpiperazine ethanesulfonic acid (HEPES). IEC-6 cells between the 30th and 37th passages were plated in 96-well flat-bottom microtiter plates at 1×10^4 cells/well in DMEM supplemented with the same additives, allowed to settle overnight, and then culture fluids were replaced with fresh DMEM containing 3% FBS and the test sample. Extract samples were suspended in dimethyl sulfoxide (DMSO) at 100 mg/mL, diluted in DMEM, passed through a 0.45-μm filter, and then added to the cultures. Cells were incubated for an additional 24 h, and ADM in the supernatants was assessed by using an enzyme immunoassay (EIA) kit specific for rat ADM according to the procedure provided by the manufacturer (Phoenix Pharmaceuticals, Burlingame, CA, USA). The lowest level of detection was 10 pg/mL.

Induction of colitis

Colitis was induced by a procedure described previously, with a slight modification [29]. In brief, with the rat under ether anesthesia, 25 mg TNBS (Tokyo Chemical Industries, Tokyo, Japan) in 0.5 mL of 50% ethanol was instilled transanally into the lumen of the distal colon using a flexible feeding tube (RZ-2; CLEA Japan, Suita, Japan). All experiments were performed 10 days after TNBS or 0.9% NaCl (control) administration. The colonic segments that exhibited less than 0.06 CVC showed prominent fibrous thickening histologically, without exception. We selected these segments as ischemic colons, and studied them histologically and biochemically as described below.

Histologic examination

The colonic tissues from control rats and TNBS-induced colitis rats were excised, opened by a longitudinal incision, rinsed with 0.9% NaCl, and weighed. Representative colonic segments were fixed in 4% buffered paraformaldehyde and embedded in paraffin. The histology was evaluated using hematoxylin and eosin and Masson's trichrome stains.

Immunohistochemistry for ADM

Small and large intestinal tissues obtained from normal rats were fixed overnight in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) and cut into 5-μm sections. Subsequently, serial sections were stained with rabbit polyclonal IgG antibody against ADM (Peninsula Laboratories) as the primary antibody, and peroxidase-conjugated goat anti-rabbit IgG (DAKO, Glostrup, Denmark) was used as a secondary antibody. The reaction was developed by adding 3,3′ diaminobenzidine (Sigma-Aldrich, St. Louis, MO, USA) solution. All incubations were done for 20 min, with saturated antibody concentrations and followed by two washes.

Immunoreactive CGRP and ADM in colon

Isolated segments of colon were homogenized in 2 N acetic acid at a concentration of 10 mg/mL, heated for 15 min at 90°C, and centrifuged at 10,000×g for 20 min at 4°C. The supernatant was applied to an activated C18 Sep column (Phoenix Pharmaceuticals) and eluted with 60% acetonitrile in 1% trifluoroacetic acid. The eluate was vacuum-dried and stored at −80°C until assay. The CGRP level was measured using a rat CGRP EIA Kit (Peninsula Laboratories). The lowest level of detectability for CGRP was 2 pg/mL. The intraassay coefficient of variation was 2.7%, and the interassay coefficient of variation was 9.3%. Cross-reactivity between rat αCGRP and βCGRP was 100%. ADM was quantified by using the EIA kit as described above.

Real-time reverse transcription polymerase chain reaction (RT-PCR)

Segments of normal or ischemic colon were homogenized in QIAzol Lysis Reagent (Qiagen, Valencia, CA, USA), and total RNA was isolated using an RNeasy kit (Qiagen) according to the manufacturer's instructions. Messenger RNA expressions of αCGRP, βCGRP, ADM, ADM2, calcitonin receptor-like receptor (CRLR), receptor activity-modifying protein (RAMP)1, RAMP2, and RAMP3 were measured by real-time quantitative RT-PCR (TaqMan gene expression assays) and an ABI Prism 7900 sequence detection system (Applied Biosystems, Warrington, UK). Sample-to-sample variation in RNA loading was controlled by comparison with glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Relative quantitation of gene expression was performed using the ΔΔ CT (change in the cycle threshold) method.

Statistical analysis

All values are expressed as means ± SEM. Statistical significance was evaluated by two-way analysis of variance

(ANOVA) followed by Dunnett's test or Student's *t*-test. For all tests, significance was accepted at $p < 0.05$.

Results

Histology of ischemic colon

The disease states of our model were confirmed by histological observation. Figure 2 shows the histology of representative segments of the ischemic colon on day 10 after instillation of TNBS or saline (control). In rats with TNBS-induced colitis, transmural inflammation was present in all layers of the bowel wall, with a marked increase in the thickness of the mucosal and muscular layers. The affected colon wall consisted of granulomatous tissue in which fibroblasts and fibrosis were present in the submucosa together with regenerative changes in the overlying epithelium. Masson's trichrome stain showed extensive fibrosis in the ischemic colon.

TU-100 had vasodilatory effect on the ischemic segment of TNBS-induced colitis, suggesting the possible involvement of endogenous ADM.

Colonic blood flow after intraluminal administration of TU-100 (900 mg/5 mL/kg) to TNBS-induced colitis rats is shown in Fig. 3a. The involved segments of the ischemic colon (basal CVC $\leq$ 0.06) and the hyperemic colon (basal CVC $\geq$ 0.20) in colitis rats were identified by measurement of blood flow; in comparison, that of normal rats was 0.10 $\pm$ 0.003 (basal CVC). Colonic blood flow in the ischemic site was increased to the normal CVC range within 15 min after administration of TU-100, and the effect lasted throughout the data acquisition period. By contrast, blood flow in the hyperemic site was not changed by TU-100 administration. Mean AP and HR were unchanged after administration of TU-100 (data not shown). As shown in Fig. 3b, the TU-100-induced vasodilatation in the ischemic segments was substantially abolished by pretreatment with the antibody against ADM, except for the initial increase in blood flow for 15–30 min after TU-100 administration. Figure 4 shows the vascularity of an ischemic colon segment before and after TU-100 treatment. Microvascular blood flow was evidently increased by TU-100 administration.

CGRP and ADM in ischemic colon

As shown in Fig. 5, the CGRP level in TNBS-induced colitis rats was lower than that in control rats (23.9 $\pm$ 6.7 vs. 80.2 $\pm$ 12.4 ng/g tissue, $p < 0.05$). In contrast, the ADM level in colitis rats was unchanged compared with that in control rats (1.2 $\pm$ 0.1 ng/g tissue).

Expressions of CGRP and ADM mRNA in the colon are shown in Table 1. Judging from the difference in cycle threshold values, the mRNA expression of βCGRP was up to 23 times greater than that of αCGRP in the colons of control rats. The level of βCGRP mRNA in the colons of colitis rats was lower than that in control rats (0.62 $\pm$ 0.12 vs. 1.00 $\pm$ 0.09, $p < 0.05$). In contrast, the level of αCGRP mRNA was not significantly decreased after treatment with TNBS. The level of ADM2 mRNA in the colitis rats was greater than that in control rats (4.72 $\pm$ 1.36 vs.

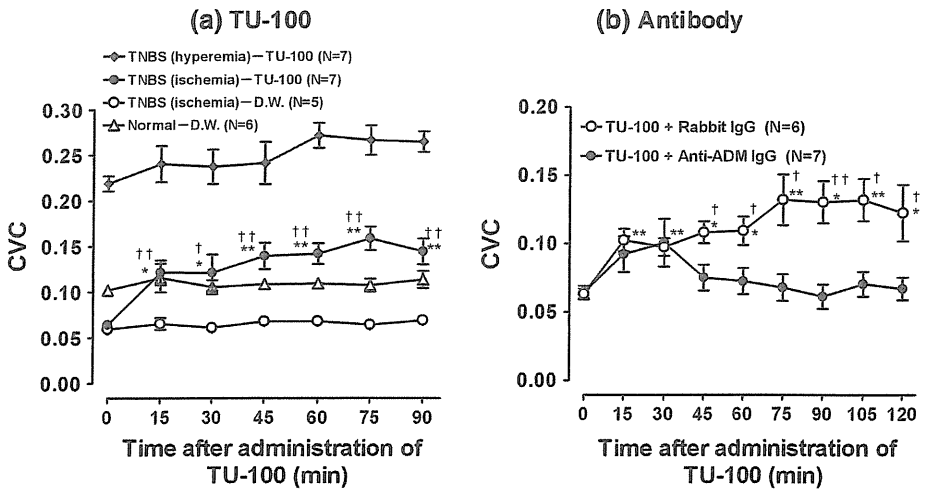

Fig. 3 Involvement of endogenous adrenomedullin (*ADM*) in vasodilatory effect of TU-100 in colitis rats. **a** TU-100 and vehicle (distilled water [*D.W.*]) were evaluated. $N = 5$–7. **b** Rabbit polyclonal IgG specific to ADM was infused intravenously at 50 μg/mL/kg 15 min before TU-100 administration. Rabbit non-specific IgG was injected as the matched control ($N = 6$–7 rats). Factorial two-way analysis of variance (ANOVA) revealed significant effects of group [$F(2, 125) = 23.70$ $p < 0.0001$] and time [$F(5, 125) = 6.08$, $p < 0.0001$]. $^{*}p < 0.05$, $^{**}p < 0.01$ versus pre-administration (0 min) (Dunnett's test). $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ versus no-antibody control, respectively (Student's *t*-test). *CVC* Colonic vascular conductance

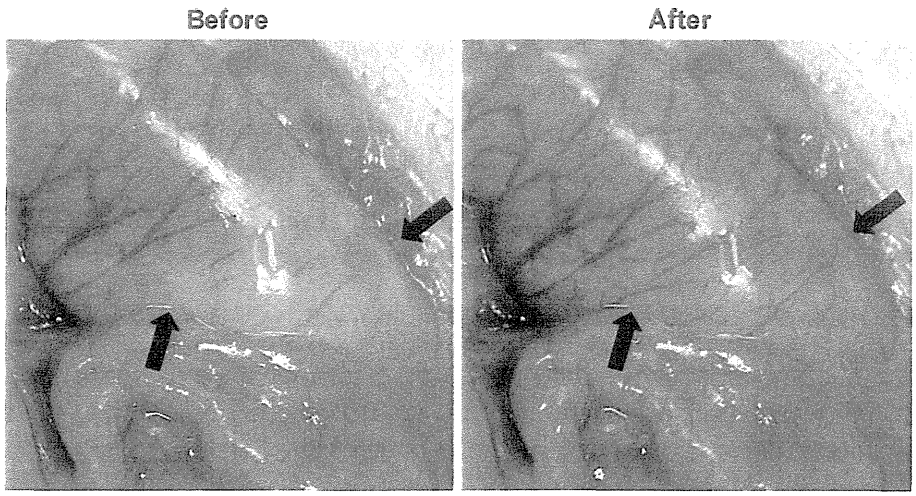

Fig. 4 Vasodilatation by TU-100 in TNBS-treated colon. Photographs show the colon exposed by a midline laparotomy before and 15 min after administration of TU-100. Vasodilated microvessels are visible in the ischemic site of a TNBS-treated rat (*arrows*)

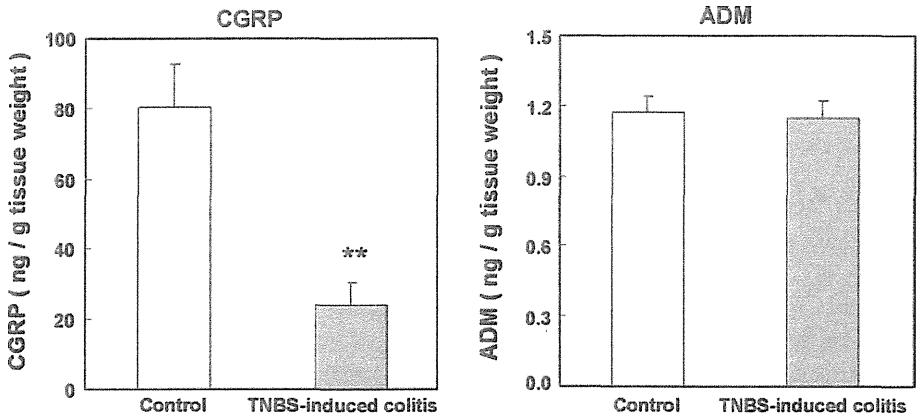

Fig. 5 Down-regulation of calcitonin gene-related peptide (*CGRP*), but not ADM, concentration in segments of ischemic colon. Ten days after colonic instillation of TNBS or 0.9% NaCl (control), colon segments were isolated. CGRP and ADM in each sample were measured using an enzyme immunoassay (EIA) kit. $N = 6$. $^{**}p < 0.01$ versus control (Student's *t*-test)

Table 1 mRNA expressions in segments of ischemic colon

	ΔΔ CT		Relative mRNA expression	
	Control	TNBS-induced colitis	Control	TNBS-induced colitis
αCGRP	14.34 ± 0.25	13.12 ± 0.59	1.00 ± 0.16	3.05 ± 1.09
βCGRP	9.54 ± 0.14	10.40 ± 0.39*	1.00 ± 0.09	0.62 ± 0.12*
ADM	6.00 ± 0.12	5.60 ± 0.27	1.00 ± 0.09	1.39 ± 0.28
ADM2	12.66 ± 0.33	10.75 ± 0.41*	1.00 ± 0.19	4.72 ± 1.36*
CRLR	7.12 ± 0.13	6.67 ± 0.14*	1.00 ± 0.08	1.35 ± 0.12*
RAMP1	3.82 ± 0.09	3.69 ± 0.23	1.00 ± 0.06	1.14 ± 0.14
RAMP2	6.71 ± 0.06	5.91 ± 0.11**	1.00 ± 0.04	1.70 ± 0.11**
RAMP3	8.22 ± 0.11	6.63 ± 0.33**	1.00 ± 0.04	3.18 ± 0.65**

Quantitative gene expression was analyzed 10 days after administration of TNBS or saline (control) by real-time reverse transcription polymerase chain reaction (RT-PCR) using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference gene. The mRNA expressions of αCGRP, βCGRP, ADM, ADM2, CRLR, RAMP1, RAMP2, and RAMP3 were calculated by the change in the cycle threshold (ΔΔ CT) method. Each value is expressed as the mean ± SEM. $N = 6$

CGRP calcitonin gene-related peptide, *TNBS* 2,4,6-trinitrobenzenesulfonic acid, *ADM* adrenomedullin, *CRLR* calcitonin receptor-like receptor, *RAMP* receptor activity-modifying protein

* $p < 0.05$, ** $p < 0.01$ versus control, respectively (Student's *t*-test)

1.00 ± 0.19, $p < 0.05$), while the mRNA expression of ADM was unchanged. Moreover, the mRNA expressions of CRLR, RAMP2, and RAMP3 were significantly increased in the colitis rats. TU-100 treatment had no effect on the mRNA levels of these CGRP receptors.

Immunohistochemistry of ADM in intestinal epithelial cells

To clarify the source of ADM, immunohistochemistry of the small and large intestines isolated from normal rats was investigated. ADM immunoreactivity was observed in the mucosal epithelium and crypts of the intestinal tract (Fig. 6).

ADM enhancement by TU-100 and its components in vitro

TU-100 significantly enhanced ADM release from IEC-6 cells dose-dependently (Fig. 7a). The concentration of ADM in the culture fluid from IEC-6 cells stimulated with 900 μg/mL of TU-100 was 167 ± 6 pg/mL, which was 2.3 times that of the control. Moreover, as shown in Fig. 7b, extracts of processed ginger and Japanese pepper, but not of ginseng radix and maltose syrup, enhanced ADM release. In particular, the ADM concentration after the administration of 100 μg/mL of Japanese pepper extract was 2.4 times that of the control. As indicated in Fig. 7c, HAS significantly enhanced ADM release, dose-dependently, to 2.7 times that of the control at 30 μmol/L.

Effects of constituents of TU-100 on CVC

To identify the active constituents of TU-100, tests were performed using the main components, 6-shogaol (processed ginger), ginsenoside Rb1 (ginseng radix), HAS (Japanese pepper), and maltose (maltose syrup). The CVC was not significantly changed in colitis rats after the intracolonic administration of ginsenoside Rb1, 6-shogaol, maltose, or the vehicle (Fig. 8). In contrast, HAS (0.3 mg/5 mL/kg) increased the CVC at 60, 75, and 90 min compared with the basal value.

Discussion

Blood flow is an important factor in the pathogenesis of CD. Ileal ulcers tend to occur along the mesenteric margin of the bowel wall in CD and in experimental models of inflammatory bowel disease [30–32]. Wakefield et al. [33] proposed the hypothesis that the primary pathological abnormality in CD is in the mesenteric blood supply. They also demonstrated that granulomatous vasculitis caused ischemia in areas of small bowel mucosa supplied by small feeding arteries along the mesenteric margin [34]. Although the overall blood flow was measured, rather than that at only the mesenteric margin, these lines of evidence suggest that the administration of TU-100 may have beneficial effects on an anastomotic site as well as on ulcer healing by improving intestinal blood flow.

The present study demonstrated that the endogenous CGRP level in the colon was significantly lower in

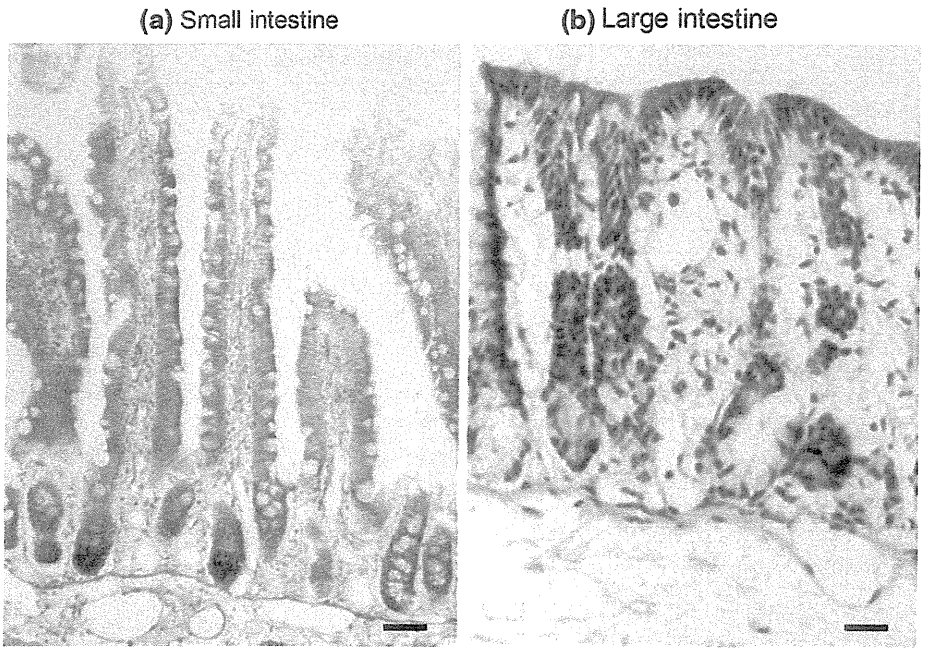

Fig. 6 Immunohistochemistry of ADM in rat intestines. Small and large intestines were obtained from normal rats, and stained with rabbit anti-ADM antibody. *Scale bar* shown in **a** is 50 μm; *scale bar* shown in **b** is 20 μm

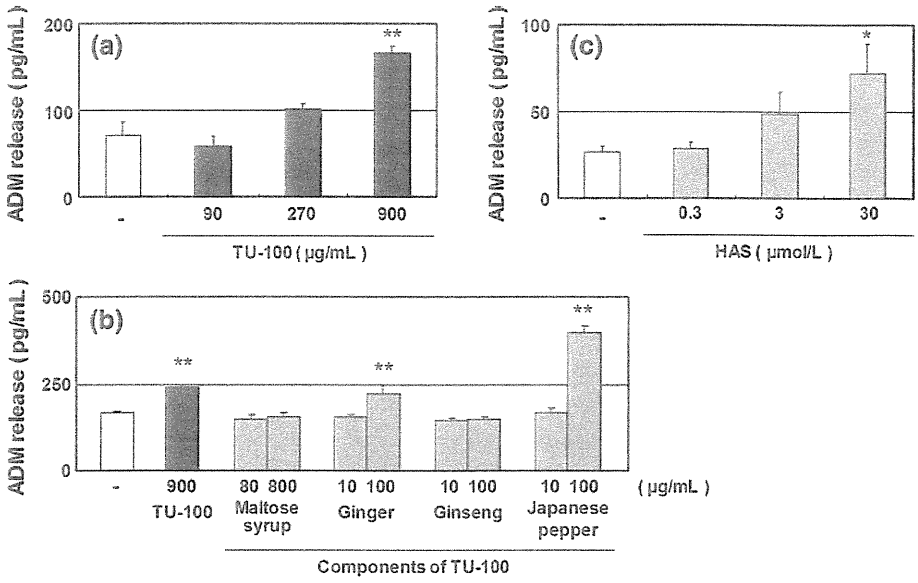

Fig. 7 Effects of TU-100 and its components on ADM release by rat epithelial cell line. a TU-100 was evaluated at various concentrations. b Maltose syrup powder, processed ginger, ginseng radix, and Japanese pepper were evaluated at the concentrations indicated in the figure. c Hydroxy α-sanshool (*HAS*) was evaluated at various concentrations. The concentration of ADM in each sample was measured using an EIA kit. $N = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$ versus control, respectively (Dunnett's test)

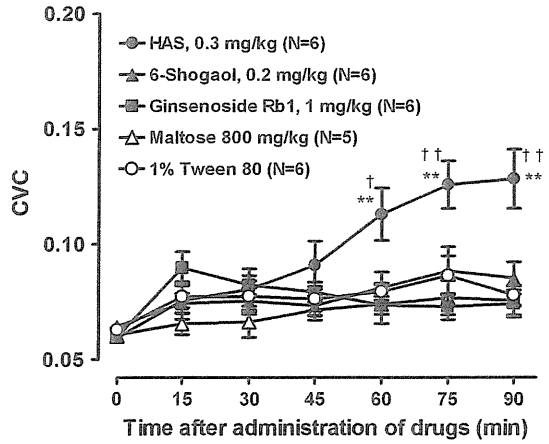

Fig. 8 Effects of specific constituents of TU-100 on colonic blood flow in colitis rats. HAS, ginsenoside Rb1, 6-shogaol, and maltose were evaluated at the respective doses contained in 900 mg/kg of TU-100. The vehicle (1% Tween 80) was also evaluated. $N = 5$–6 each. Factorial two-way ANOVA revealed significant effects of group [$F(4, 144) = 21.17$ $p < 0.0001$] and time [$F(5, 144) = 3.95$, $p = 0.0022$]. $^{**}p < 0.01$ versus pre-administration (0 min) (Dunnett's test). $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ versus the vehicle control, respectively (Student's *t*-test)

TNBS-induced colitis rats than in control rats, while ADM was unchanged at the protein and mRNA levels (Fig. 5). It has been reported that the CGRP concentrations in the colons of patients with CD were decreased compared with that of the normal colon [7]. We demonstrated that the βCGRP mRNA expression was 23.4 times that of αCGRP mRNA in the colon of control rats, and that the gene expression of βCGRP, but not αCGRP, was decreased in ischemic segments of the TNBS-treated colon. Therefore, the decrease of the CGRP concentration in the CD intestine may be related to the decrease of βCGRP, which is expressed in intrinsic primary afferent neurons [24, 26]. Previous observations of several structural and functional abnormalities of the enteric nervous system in patients with CD support our findings [35, 36].

As we previously reported, TU-100 increased colonic blood flow in normal rats via up-regulation of the CGRP system [19]. The present study using the colons of TNBS rats, however, suggests that the increase of blood flow induced by TU-100 may be mediated predominantly via the ADM system (Fig. 3). Further, in the present TNBS model, TU-100 did not increase the expression of CGRP receptors, which was reported to be upregulated in normal rat intestines [19]. Thus, it seems that the CGRP system is heavily damaged in CD [7, 27] and in TNBS-instilled intestines. Activation of the ADM system, which appears to be intact even in TNBS-instilled intestines (Fig. 5), may therefore be a better therapeutic option than the restoration/reinforcement of a damaged CGRP system.

In the present study, ADM immunoreactivity was observed in the mucosal epithelium and in the crypts of small and large intestines (Fig. 6). We and other researchers have observed similar results in various species, including humans [17, 37–40]. TU-100 was administered directly into the colon in the present study, and therefore it is reasonable to consider that TU-100 may induce the release and/or production of ADM by directly interacting with intestinal epithelial cells. Based on the above assumption, we screened several intestinal epithelial cell lines for ADM production and found that IEC-6, a non-transformed intestinal epithelial cell line derived from the rat intestinal crypt, produced ADM. IEC-6 is derived from the small intestine; however, we have observed that the intraduodenal administration of TU-100 increased blood flow in the jejunum and that an antibody to ADM abolished the vasodilatory effect of TU-100 (unpublished observation). Further, we have

previously shown that TU-100 increases the amount of ADM released from intestinal epithelial cells isolated from both small and large intestines [17]. To investigate the active components of TU-100's effect, therefore, we assayed the in vitro ADM release from IEC-6 cells.

Previous and present data on TU-100's effect on ADM release from IEC-6 cells have focused on the induction of ADM expression/synthesis, which was evaluated 24 h after the addition of TU-100. The induction of ADM mRNA expression, detailed time course of ADM release, and flowcytometric detection of intracellular ADM protein have been reported previously [17]. However, we confirmed that 6-h incubation with TU-100 (or TU-100 components) was sufficient to observe an elevation of ADM levels in the culture supernatant of IEC-6 cells (unpublished observation). These data suggest that TU-100 may enhance not only the expression and synthesis of ADM but also its transport and release from the cells. However, extensive future studies are necessary to clarify this point.

Although most TU-100 activities, at least 45 min after TU-100 administration, were abrogated by anti-ADM antibody treatment, the initial elevation at 15–30 min was not affected by the antibody treatment (Fig. 3b). Therefore, the effect of TU-100 may be exerted partially by a mechanism other than activation of the ADM system. This mechanism needs to be elucidated in the future, but the possible involvement of the CGRP system is plausible and needs to be investigated first.

In contrast to its effects in ischemic segments, TU-100 did not alter blood flow in hyperemic segments of the TNBS-treated colon. We speculate that this is because the ADM/CGRP system was already activated and had increased the blood flow due to acute inflammation. Acute inflammation has been reported to increase CGRP and ADM release from intestinal nerves and epithelium, respectively [7, 41]. If TU-100 utilizes the endogenous ADM/CGRP system for its vasodilatory effect, an additional increase of the blood flow, which is already augmented via the same or similar host defense systems, could be difficult. Thus, it seems that TU-100 is unlikely to be a direct vasodilator.

TU-100 is one of the traditional Japanese medicines (Kampo) whose production is well controlled compared with conventionally known herbal medicinal products [10]. Contamination studies have certified that Tsumura Kampo medicines are free of toxins, pesticides, microbes, and heavy metals. They are primarily extract granules, and their pharmacological actions have been studied and elucidated at the molecular level. TU-100 is composed of maltose syrup powder, processed ginger, ginseng radix, and Japanese pepper, and the main constituents of these components are maltose, 6-shogaol, ginsenoside Rb1, and HAS, respectively (shown in Fig. 1). By examination on a galenical level, we previously confirmed the vasodilatory effect of TU-100 and its galenical component, Japanese pepper, in normal rats [19]. HAS is the main active constituent of Japanese pepper. The present study showed that HAS improved the blood flow of the ischemic colon in TNBS-induced colitis and enhanced ADM release from IEC-6 cells. Therefore, it seems that the TU-100-induced vasodilatory effect may be due to HAS via ADM.

In conclusion, our findings indicate that the ischemia complicating TNBS-induced colitis was associated with down-regulation of the CGRP system, particularly a decrease of βCGRP in intrinsic primary afferent neurons, while the ADM system was maintained at a normal level. Endogenous ADM significantly improved the decreased blood flow, demonstrating its potential as a new therapeutic target for CD by promoting mucosal healing of the intestinal microvasculature. TU-100 increased blood flow in the ischemic colon, but not in the hyperemic colon, by up-regulating endogenous ADM. HAS, the main active constituent in TU-100, enhanced ADM release from intestinal epithelial cells in a concentration-dependent manner. Thus, it appears that TU-100 may have therapeutic and preventive effects on the CD intestine, especially in reducing recurrence at the anastomotic site.

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Conflict of interest The authors declare that they have no conflict of interest.

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今日のがん治療を支える漢方薬のエビデンス

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Key Points

- 六君子湯の成分ヘプタメソキシフラボンがセロトニンと拮抗的に働いて，胃や十二指腸にあるグレリン分泌細胞のグレリン分泌を増強させ，食欲亢進させる．
- 牛車腎気丸は抗がん薬による末梢神経障害を抑制する可能性がプラセボ対照二重盲検試験で証明された．
- 半夏瀉心湯の成分バイカリンがβ-グルクロニダーゼを阻害することで，イリノテカンの下痢を予防，含嗽するとベルベリンの抗菌作用，プロスタグランジンの抑制で口内炎治療に有効である．
- 補剤の高いエビデンスの臨床研究はないが，未踏の分野の薬剤として注目される．

はじめに

近年，がん領域で女性の死亡原因のトップ，男性でも第2位となった大腸がんは毎年，日本で5万人以上，新規に診断されている．しかも75%は進行がんで発見されている．その半分は再発，転移を起こし，化学療法が必要であるといわれている．一方，大腸がんは固形がんのなかで化学療法がもっとも成功した1つで，20年前には余命半年だった進行がんでも現在では化学療法の進歩によってQOLを良好に保ちながら3年程度まで延命が可能となってきた．

その原動力となったのがオキサリプラチンとイリノテカンの登場である．イリノテカンは植物から成分が抽出されたものであることはあまり知られていない．イリノテカンは街路樹として植えられていた早蓮木（かんれんぼく）の根のアルカロイド成分である．植物をベースにした医療が古代から世界中で盛んに行われてきたが，いまだに代替補完医療の枠組みのなかにあり，エビデンス重視の現代医療のなかに組み込まれていないのが現状である．

米国では医療費削減と合成薬剤の限界から代替補完医療に対する期待感が巻き起こり，年間1億ドル以上の巨額の研究費が毎年投資され，エビデンス構築が行われてきている．しかし，これまで行われてきたすべての大規模臨床試験結果はネガティブデータであった．そこでFDAは，日本の伝統的薬剤である漢方薬のほかに類をみない品質の高さと，日本の高度に発達した医療のなかで薬として標準化され西洋薬と併用されている点に注目した[1]．

FDAが最初にターゲットにしたのが，安全

性と品質が均一であるツムラの大建中湯で，合剤であるにもかかわらず臨床治験薬として認可したことは知られていない．その契機となったのは薬効機序に関する基礎研究であり，これまで西洋医学的な立場から理解ができなかった作用機序に関して，成分レベルで多くの新知見を得ることができたためである[1～4]．また，漢方薬として初めて大建中湯の有効成分が吸収され，血中レベルが上昇することが明らかとなった[5]．これらの研究をベースに，臨床的エビデンスとしては最高レベルであるプラセボを使用した二重盲検試験がメイヨー・クリニックで数ヵ月間というハイスピードで行われ，大建中湯の腸管運動に対する有効性が2010年に証明された[6]．これを皮切りに，炎症性腸疾患患者が100万人以上いる米国で，診断治療のメッカであるシカゴ大学が中心となって全米20ヵ所でクローン病に対する大建中湯の有効性を多施設共同二重盲検プラセボ対照比較試験で検証することが決定され，2011年9月から始まろうとしている．一方，日本では最近まで漢方薬に対する偏見から医師，薬剤師も大きな関心を示すことは少なかった．

しかし，大建中湯の基礎研究を契機に全国大学病院の80%が参加する大建中湯の多施設共同二重盲検プラセボ対照比較試験グループ(北島代表，DKTフォーラム)が組織され，高いエビデンスレベルを獲得するため2009年から症例集積中である．その成果が大いに渇望されるが，黒船来襲前に日本の医師や薬剤師が漢方薬を理解し，積極的にがん治療に取り入れるきっかけとなってほしいと考え，とくに西洋薬では十分対処することができていない抗がん薬の有害事象に使って有効性を実感できやすく，しかも機序に関してもエビデンスレベルで理解が進んでいる漢方薬を概説する(表1)．

六君子湯 (Rikkunshito，TJ-43)

TJ-43と食思不振

"君子"は最高のもの，"湯"は水溶性を意味

表1 化学療法の副作用対策に役立つ漢方薬

漢方薬	適応症状	有効成分と作用機序	臨床試験	副作用
六君子湯 (TJ-43)	食思不振	ヘプタメソキシフラボン：セロトニン拮抗作用によるグレリン分泌増強	プラセボなし多施設二重盲検前向き試験	電解質異常 偽アルドステロン症
牛車腎気丸 (TJ-107)	末梢神経障害	成分レベル未確定：一酸化窒素誘導による血流改善／ダイノルフィン，オピオイド受容体を介した鎮痛作用	プラセボ対照多施設二重盲検前向き試験 第Ⅱ相終了 第Ⅲ相試験中	とくになし
半夏瀉心湯 (TJ-14)	下痢	バイカリン：β-グルクロニダーゼ阻害による下痢発生予防	後ろ向き試験	とくになし
	口内炎	ベルベリン：抗菌作用 成分未定：プロスタグランジン抑制による鎮痛作用	プラセボ対照多施設二重盲検前向き試験 第Ⅱ相試験中	とくになし
補中益気湯 (TJ-41) 十全大補湯 (TJ-48)	Biological response modifier		後ろ向き試験	電解質異常 偽アルドステロン症

する．名前の意味するところは胃腸に効果のある最高の6種類の生薬を組み合わせたものである．しかし，TJ-43（7.5g/日）は8種類の生薬，蒼朮，人参，半夏，茯苓，大棗，陳皮，甘草，生姜の合剤である．漢方発祥時に中国の中医から名前だけを拝借し，その後，日本で独自に発達したためと考えられている．日中間の新幹線問題と似ている状況である．

余談はさておき，TJ-43は漢方薬のなかでもっとも機序解明が進んでいるものの1つである．とくに食欲増進作用に関する機序は驚くべきスピードで解明が進んでいる．日本の寒川先生らが発見した，生体がもつ唯一の食欲増進ペプチドであるグレリン分泌細胞の産生抑制スイッチとなるセロトニン受容体に対して，TJ-43の構成生薬の1つである陳皮（温州ミカンの皮）の主要成分であるヘプタメソキシフラボンがセロトニンと拮抗的に働いて，胃や十二指腸にあるグレリン分泌細胞のグレリン分泌を間接的に増強させることが判明した[7]．抗がん薬のなかでもとくに食思不振を招きやすいシスプラチンは，消化管エンテロクロマフィン細胞を刺激してセロトニン産生を促し，グレリン分泌細胞を抑制して食思不振を起こさせることが知られており，シスプラチンを用いた食思不振モデルにおいてもTJ-43の効果が確認された[7]．これまでもプラセボ試験ではないが常用量の1/30という低用量と常用量との多施設二重盲検前向き試験で効果が確認されているが，プラセボを対照とした二重盲検試験は行われていないので臨床的エビデンスレベルとしては十分ではないが，抗がん薬による食思不振にTJ-43を用いることは強く推奨される．

2 TJ-43のその他のエビデンス

TJ-43は胃部不快感など消化管運動不全に起因すると思われる症状に用いられる．プラセボを対照薬とした多施設二重盲検群間比較試験にて運動不全型神経性胃炎，機能性ディスペプシアに対する臨床的有用性が証明されている．薬効機序として，一酸化窒素依存性の胃排出能である胃適応性弛緩を促進させる作用や，胃粘膜血流改善作用などが考えられている．

3 TJ-43の安全性

グリチルリチンを主成分とする甘草が多く含まれているため，長期連用する際には，偽アルドステロン症・低カリウム血症に注意が必要である．とくに，担がん患者や抗がん薬を使用する場合に体力低下などの理由で後述する補中益気湯，十全大補湯などの補剤を併用する場合も多いが，これらの漢方薬には甘草が含まれており過剰投与となることが懸念され，カリウムなど電解質異常に注意を払うことが重要である．

牛車腎気丸（Goshajinkigan，TJ-107）

1 TJ-107と末梢神経障害

TJ-107は，八味地黄丸をベースに牛膝（ヒナタイノコズチの根）と車前子（オオバコの種子）を加えた10種類の生薬から構成されている．“腎”は排尿経路全体を意味する．TJ-107（7.5g/日）は腰痛，下肢痛，しびれ，排尿困難，糖尿病性末梢神経障害に用いられている．TJ-107の作用機序については，一酸化窒素（NO）誘導による血流改善や，ダイノルフィン，オピオイド受容体を介した鎮痛作用が推測されている．

タキサン系，白金製剤，とくにオキサリプラチンなど，末梢神経障害を呈する抗がん薬は多い[8]．しかも，末梢神経障害によって抗がん薬の使用制限や中止など担がん患者の予後

を左右する副作用となっているにもかかわらず，有効な手段がいまだにみつかっていない[9]．そのため，抗がん薬による末梢神経障害に対して多くの治療薬や予防薬の臨床試験がくり返されてきた．残念ながら，その多くは小規模の後ろ向き試験が行われたにすぎない．その後のプラセボを使用した第Ⅲ相試験で有効性が明らかとなったものはきわめて少なく，コンセンサスを得たものはない[10,11]．

オキサリプラチンは大腸がんのキードラッグの1つであるが，末梢神経障害が90%以上発生することが報告されていて，オキサリプラチンの末梢神経障害(手・足指のしびれ感など)は，治療継続の大きな障壁となっている．神経障害の発現機序について，オキサリプラチンが感覚神経細胞である脊髄後根神経節に蓄積し，その代謝産物であるシュウ酸がナトリウムチャンネルに作用し，神経障害が発現すると考えられている．そのため神経障害の抑制に，シュウ酸をキレートするカルシウムやマグネシウムの投与が試みられ，有効性が後ろ向き試験で報告され，引き続きプラセボ二重盲検試験が計画され，症例集積が開始されたが，オキサリプラチンの抗腫瘍効果を減弱させる可能性が指摘され，中止となってしまった．その後，一部解析が行われたが，その有効性は極めて限局的であり，神経毒性抑制効果は証明されていない[10,12]．

日本では，以前よりタキサン系の抗がん薬による神経毒性に対してTJ-107が用いられ，有効性が報告されていた．そこで，筆者らはオキサリプラチンを使用した化学療法を6クール以上完遂した進行・再発大腸がん90症例を対象に後ろ向き試験を行った結果，TJ-107が末梢神経障害発生を抑制する可能性を報告した[13]．次に，小規模前向き試験を行った結果，TJ-107の有効性が再び示唆された[14]．そこで，多施設プラセボ対照前向き二重盲検第Ⅱ相試験(GONE試験)を計画し，症例集積を行った[15]．予定期間より大幅に短い11ヵ月で目標症例数以上の94例が集積され，解析を行った結果，神経毒性のグレード2以上の発生率は25%，治療継続が困難となるグレード3の発生率は50%低下させることが明らかとなった．現在，310例の大規模プラセボ対照前向き二重盲検第Ⅲ相試験(GENIUS試験)が厚生労働省研究費で行われ，症例集積中である．これらの結果が明らかになれば，世界中でTJ-107を併用した大腸がん化学療法が行われることが期待される．

2 TJ-107の安全性

副作用は重篤なものは報告されていない．

半夏瀉心湯 (Hangeshashinto, TJ-14)

1 TJ-14と下痢

“瀉”とは余計なものを取り除くという意味で，“心”は心下部のみぞおち付近をさし，半夏を主要生薬として胃のつかえなどを取り除くという意味の漢方薬である．TJ-14の構成生薬は，7種類，半夏，黄芩，黄連，人参，乾姜，大棗，甘草である．

がん領域ではイリノテカンによる遅発性下痢発症予防で使用されている．イリノテカンによる下痢の特徴は，投与開始24時間以内に発現する早期性下痢と，24時間以降とくに投与数日後に発現することが多い遅発性下痢の2種類に分かれる．早期性下痢の原因は，イリノテカンのアセチルコリンエステラーゼ阻害作用により副交感神経が刺激され，腸管運動の亢進，水分吸収阻害が起こり，下痢を起こす．遅発性下痢は，イリノテカンの活性代謝物SN-38のグルクロン酸抱合体が，腸内細菌のβ-グルクロニダーゼによりSN-38に

脱抱合され，濃度依存的にClイオンの分泌を増加させ，これが腸管粘膜の細胞傷害をきたし下痢を起こす．TJ-14の黄芩の成分フラボノイド配糖体のバイカリンには，β-グルクロニダーゼを阻害する活性があるため，活性型の腸管での再生成を抑え，イリノテカンの下痢を抑制すると考えられている[16]．したがって，イリノテカンの投与数日前から使用することが肝要である．イリノテカンの抗腫瘍効果にTJ-14は影響しないことが確認されている．

2 TJ-14と口内炎

がん化学療法中の口内炎の発症率は領域によって差がある．頭頸部がん領域ではほぼ100%であるが，大腸がん領域では20%程度である．発症原因としては，抗がん薬によって発生する活性酸素による口腔粘膜細胞のDNA障害，各種サイトカインなどによるアポトーシス誘導，各種炎症性プロスタグランジンの増強による疼痛出現，宿主の免疫能低下による細菌増殖などがあげられている．化学療法時の口内炎はQOLを著しく低下させるにもかかわらず，有効な治療手段はほとんどなく，予防的な手法として口腔内清潔や，抗がん薬の口腔内に到達する薬剤濃度を低下させる目的で氷などを利用したクライオテラピーが報告されている．治療に関して最近，遺伝子操作で合成したケラチノサイト増殖因子が血液がんの化学療法に起因する口内炎に対し治療的効果が確認され米国FDAで承認されたが，日本では未承認である．そのケラチノサイト増殖因子に関して安全性の面，つまりがん細胞に対する増殖因子となる可能性について十分な検討はなされていないことが危惧されている．

口内炎による痛みは摂食障害の原因となり，大きな問題点となる．したがって，口内炎による痛みをコントロールすることが治療における大きな目標となる．口内炎の痛みは感覚神経へのプロスタグランジンE2の作用で誘発されると考えられており，TJ-14は炎症部位の痛み発生物質であるプロスタグランジン誘導型E2の濃度依存性の産生抑制効果が報告されており[17]，痛みを早期に減弱させる効果が期待できる．抗がん薬による免疫力低下に伴い，口腔内環境，とくに口腔内細菌叢による二次感染も口内炎増悪への関与が示唆されているが，TJ-14の構成生薬である黄連の主要成分であるベルベリンは広い抗菌作用を有しており，細菌性細胞障害に対する抑制効果が報告されていることから，口腔内の細菌増殖抑制効果が期待される．そこで，われわれはこれらの局所作用を最大にするためにTJ-14をコップ半分程度に1包(2.5g)を撹拌し，数回に分けて口腔に含んでもらい1回5秒以上ゆすいでもらい，痛みが強い部位には直接TJ-14をつけることを考案し，大腸がん化学療法中に発生した口内炎に対してTJ-14を使用した後ろ向きの臨床試験を行ったところ，期待以上に有効であった[18]．現在，多施設プラセボ対照前向き二重盲検第Ⅱ相試験(HANGESHA試験)で，胃がん，大腸がん化学療法中に発生する口内炎を予防できるか検証中である．

本来，服用するのが漢方薬の原則だが，口内炎に関して局所濃度を高める目的で行ったが，抗がん薬による吐き気がある場合でも可能な方法であることは患者に推奨しやすい．

補　剤

1 補剤と体力低下，免疫力低下

補中益気湯，十全大補湯はいずれも"補剤"に分類され，BRM(Biological response modifier)として，免疫機能改善作用や栄養状態改善作用，生体防御機能作用をもち，術後のQOL

改善，がん化学療法・放射線療法の副作用軽減，手術侵襲軽減，がん転移・再発予防などに使用されている．作用機序に関して成分レベルでのエビデンスは不十分であるが，西洋薬の未踏の分野の薬剤として今後の成分レベルの研究成果，エビデンスレベルの高い臨床研究の成果が待たれる．

2 補中益気湯(Hochuekkito, TJ-41)

TJ-41の"中"とは消化管を意味し，病後や術後の全身倦怠感に対して胃腸の働きを高め，体力を補い元気をつけるという意味である．最大の狙いは感染症であり，抗がん薬による副作用軽減などに使用される．作用機序として，これまでに抗ウイルス効果(インフルエンザなど)，NK細胞活性化，CTL免疫誘導などが考えられている．最近の報告では，外科的ストレスによる免疫能低下の防止作用，さらにはがん増殖抑制効果も報告されている．

3 十全大補湯(Juzentaihoto, TJ-48)

TJ-48の"十全"とは完全無欠を意味し，幅広く大いに補うという意味である．とくにがんの転移・再発の予防効果が期待されている．最近ではクッパー細胞の酸化ストレス抑制による肝がん発生を抑制する効果が報告された．抗腫瘍効果は免疫能向上だけでなく，血管新生を抑制する効果も報告されている．また，抗がん薬による骨髄造血能低下，とくに血小板減少に効果があるとされる[19]．

4 補剤の使用選択順と副作用

補剤は終末期や抗がん薬による副作用，免疫力低下を改善することを期待して使用するが，日本のがん治療の指導的立場にあるがん研有明病院の星野内科部長は，最初にTJ-41，次にTJ-48の順番で使用することを推奨している(NHK教育「健康応援フェスタ」2010年8月放映「漢方と西洋医学の新たな融合～がん治療に漢方医学が果たす役割～」)．また，長期連用による副作用として，甘草による低カリウム血症，偽アルドステロン症やミオパシーのほか，間質性肺炎による発熱，咳嗽，呼吸困難に注意する．

おわりに

漢方薬が西洋薬と肩をならべて使用される時代の到来には漢方薬のエビデンスが必要であり，日本においてがん治療にたずさわるすべての医師，薬剤師，看護師ががん治療における漢方薬の効果を実感できる可能性が高い漢方薬をそのエビデンスとともに紹介した．

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漢方のCAMからの脱出：大建中湯を中心に

河野　透

要約：植物由来物を利用した医薬は代替補完医療complementary and alternative medicine（CAM）の枠組みの中にあり，エビデンス重視の現代医療では異端的扱いであった．世界中から日本の伝統的医薬である漢方薬が高品質および標準化されている点に注目され始めた．その契機となったのが大建中湯の薬効機序に関する分子レベルの研究である．大建中湯は3つの生薬（山椒，乾姜，人参）が含まれ，術後の腸管運動麻痺改善，および腸管血流改善作用が，大建中湯の主要成分であるhydroxy-α-sanshool，6-shogaolを中心にカルシトニン・ファミリー・ペプチドを介して発現していることが明らかとなった．この研究を契機に全国の大学病院で二重盲検プラセボ比較試験が開始された．同時に米国でも臨床試験が行われ大建中湯の有効性がいち早く証明され漢方薬のCAMからの脱出が始まった．

はじめに

世界中で古くから植物由来の抽出物を利用した医療，例をあげればアロパシー医学などが盛んに行われてきたが，欧米諸国では代替補完医療complementary and alternative medicine（CAM）の枠組みの中にあり，エビデンス重視の現代医療の中に組み込まれることを阻んできた(1)．しかしながら，高騰する医療費削減と合成薬剤の開発，適応の限界から代替補完医療に対する期待感が沸き起こり，年間1億2千万ドル以上の巨額の研究費が投資され，代替補完医療の研究が行われてきた(2)．アメリカの食品医薬品局（FDA）で認可されるにいたったのは代替補完医療としてではなく，そのような状況下で日本の伝統的医薬である漢方薬の例外的な高品質および標準化されている点である．その契機となったのが大建中湯（TJ-100，TU-100，Daikenchuto，DKT）の薬効機序に関する分子レベルの研究である(3-5)．最近まで日本でも，漢方に対する偏見から多くの医師が関心を示すことはなかった．しかし，大建中湯の研究を契機に全国大学病院の80%が参加する大建中湯の二重盲検プラセボ比較試験が2009年から開始された．同時に米国のメイヨークリニックで臨床試験が行われ大建中湯の有効性がいち早く証明された(6)．本稿では，漢方薬がCAMからの脱出を試みる契機となった研究を紹介する．

1．なぜ欧米から漢方が注目されるのか

2002年，WHO世界保健機構はグローバル戦略として伝統医療を世界中の医療に組み込むように提言していた．日本ではすでに漢方薬が保険医療として組み込まれている．漢方医学は日本独自に発展してきた伝統医療で，起源は中国であるが，中国では中医学として発展してきた．アメリカで代替補完医療が注目されるきっかけは，ある上院議員が長年悩んでいた喘息がherbal medicineで治ったことである．彼が中心となって議会に働きかけ，1992年200万ドルという少額予算でNIHにOffice of Alternative Medicineが初めて誕生した．その後，組織は改組され国立補完代替医療センターNCCAMとなり，予算は年々増加し，現在では1億2千万ドル以上の巨額の予算で運営されている．風邪治療にエキナセア（Echinacea）が有効であると考えられていたが，大規模臨床試験で無効であると判定され，エビデンス獲得のための臨床試験がことごとく失敗に終わった(2,7)．そこで困ったFDAは安全性，信頼性が極めて高い漢方薬に注目した．従来，アメリカには中国から中医薬が多く輸入されていたが，製品に含まれる重金属，抗生物質，農薬などによるアレルギーなど副作用が多く，医師たちも患者に説明や推奨

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することをためらっていた．FDAは欧米の医師が納得できるエビデンスレベルの高い薬理作用機序，さらには臨床試験の結果を(株)ツムラと日本の医師に求めてきた．

2. 大建中湯とは

これから述べていく大建中湯（TJ-100, TU-100）は(株)ツムラで抽出されたものである．大建中湯は日本で最も多く使用されている漢方薬である．構成生薬は3種類で山椒（2.2%），乾姜（5.6%），人参（3.3%）で，残りはマルトース（膠飴）でできている(4)．中医薬にも大建中湯があるが，山椒は入っていない．日本では全て抽出されたエキス製剤である．3D-HPLCで成分をみても多数の化学物質が含まれていることがわかる（図1）．適応症は腹部膨満感，腹部の冷えの改善であるが，実際臨床では術後の腸管運動麻痺改善目的で使用されることが多い(8-10)．

3. 腸管血流増加作用

最初に注目されたのは腸管運動亢進作用で，神経ペプチドでカルシトニン・ファミリー・ペプチドの1つであるCGRP（calcitonin gene related peptide）が大建中湯によって刺激されることであった．さらにアセチルコリン，セロトニンなど神経因子やモチリン，バニロイド受容体が関与することも報告された(11-16)．CGRPは末梢血管拡張作用が最も強いペプチド(17)で，われわれは機序解明が遅れていた腹部の冷えの改善に関与しているという仮説の元に研究を進めた(4, 5)．その結果，CGRPだけでなくCGRP受容体関連因子も大建中湯によって刺激を受けることが明らかとなった．CGRP受容体は恒常的に存在せず，未成熟な受容体であるCRLR（calcitonin receptor-like receptor）が成熟化プロセスに必要で，その成熟化にはRAMP（receptor activity-modifying membrane protein）が必須である．RAMPには3種類のタイプがあり，RAMP1が関与するとCGRP受容体になるが，RAMP2，RAMP3が関与するとCGRPと同じカルシトニン遺伝子関連ペプチドであるADM（adrenomedullin）の受容体に変化することが解明された（図2)(17)．

われわれの実験結果から，大建中湯によってRAMP1, 2, 3のいずれも増加することが明らかとなり，カルシトニン・ファミリー・ペプチドの2つのペプチド，CGRPとADMおよび，その受容体関連因子が大建中湯の血流改善機序に関与していることが示唆された．両ペプチドの生理学的作用で共通しているのは強い末梢血管拡張作用である(17-19)．さらにCGRPは腸管運動亢進作用，分泌作用(20)があり，ADMには抗炎症性サイトカイン作用(21)がある．従って，大建中湯

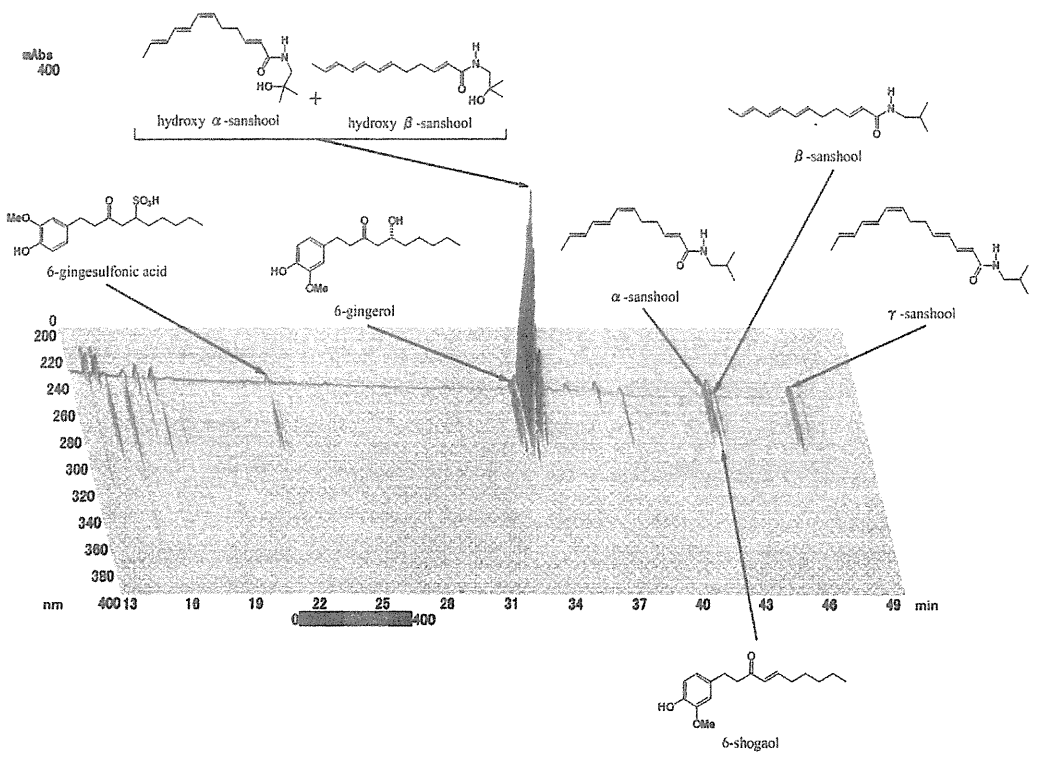

図1 大建中湯の3D-HPLC
3-D HPLC（three-dimensional high-performance liquid chromatography）によって大建中湯（TJ-100, TU-100）の構成生薬に含まれる主要成分以外の農薬，重金属，抗生物質などが含まれていないことが明らかとなった．