れわれの基礎実験で確認している．

六君子湯は臨床薬理試験において胃排出能促進作用が確認されており，消化管のIMC発現を短縮させる[11]．この胃排出促進作用は六君子湯の成分であるL-アルギニンとヘスペリジンが関係しているとラットの実験から報告されている[12]．一酸化窒素（nitric oxide：NO）合成酵素阻害剤（L-NAMEなど）を用いて胃の受容性弛緩を傷害したモデルでは，六君子湯は胃排出を改善することから，六君子湯に含まれるL-アルギニンの作用が示唆されている．

近年の六君子湯における最も重要な研究は，Takedaら[13]による六君子湯がグレリン分泌を刺激する可能性に関する研究である．グレリンは28個のアミノ酸からなり胃から分泌され，成長ホルモン分泌刺激作用，食欲亢進作用，消化管の運動亢進作用などが報告されている消化管ホルモンである．Takedaらはラットのシスプラチン投与食欲不振モデルに対して六君子湯を投与し，血中の活性型グレリンの増加を示し，シスプラチン投与後の食欲不振を改善すると報告している．シスプラチンはEC細胞からのセロトニン分泌を刺激し，大量のセロトニンが血中，消化管内に分泌され，これが嘔吐中枢に作用し，嘔気，嘔吐，食欲低下を引き起こしている．これらの副作用抑制には一般に5-HT_3受容体拮抗薬が用いられているが，Takedaらは六君子湯が5-HT_2受容体に対して拮抗作用を有し，グレリンの低下を改善すると報告している．

一般臨床においては，1998年に原澤ら[14]が運動不全型のdyspepsia症例に対して六君子湯を用い，プラセボに対して上腹部愁訴を有意に改善したことを報告している．また幽門輪温存胃切除術後の胃排出遅延の患者に対して六君子湯を投与し，胃排出遅延が改善されることが報告されている[15]．六君子湯は食道逆流の症例に対しても使用されており，食道の食物酸クリアランスを改善して胃酸の食道逆流を有意に減少させる[16]．さらに，六君子湯はNOの基質であるL-アルギニンを多く含んでいるため，胃の受容性弛緩を増強し貯留機能を促進し，non-ulcer dyspepsia（NUD）に効果があると考えられている[12]．

おわりに

漢方は経験による治療法から科学的根拠にもとづいた治療法へと変わろうとしている．欧米においても漢方に注目が注がれており，DDWでも演題が取り上げられるようになった．また，米国人を対象とした漢方の臨床試験も進行中であり，西欧人があの苦い漢方薬を服用できる事実に驚いている．Japanese herbal medicineとしての漢方薬が世界中で使用されるためにも，科学的なmethodologyを用いてエビデンスの高い研究を発信することが重要と考える．

文　献

1) Mochiki E, Inui A, Satoh M *et al*：Motilin is a biosignal controlling cyclic release of pancreatic polypeptide via the vagus in fasted dogs. *Am J Physiol* **272**（2 Pt 1）：G224-G232, 1997
2) Mochiki E, Satoh M, Tamura T *et al*：Exogenous motilin stimulates endogenous release of motilin through cholinergic muscarinic pathways in the dog. *Gastroenterology* **111**：1456-1464, 1996
3) Kono T, Kanematsu T, Kitajima M：Exodus of Kampo, traditional Japanese medicine, from the complementary and alternative medicines：is it time yet? *Surgery* **146**：837-840, 2009
4) Shibata C, Sasaki I, Naito H *et al*：The herbal medicine Dai-Kenchu-Tou stimulates upper gut motility through cholinergic and 5-hydroxytryptamine 3 receptors in conscious dogs. *Surgery* **126**：918-924, 1999
5) Satoh K, Hashimoto K, Hayakawa T *et al*：Mechanism of atropine-resistant contraction induced by Dai-kenchu-to in guinea pig ileum. *Jpn J Pharmacol* **86**：32-37, 2001
6) Satoh K, Hayakawa T, Kase Y *et al*：Mechanisms for contractile effect of Dai-kenchu-to in isolated guinea pig ileum. *Dig Dis Sci* **46**：250-256, 2001

7) Kawasaki N, Nakada K, Nakayoshi T *et al* : Effect of Dai-kenchu-to on gastrointestinal motility based on differences in the site and timing of administration. *Dig Dis Sci* **52** : 2684-2694, 2007
8) Kono T, Koseki T, Chiba S *et al* : Colonic vascular conductance increased by Daikenchuto via calcitonin gene-related peptide and receptor-activity modifying protein 1. *J Surg Res* **150** : 78-84, 2008
9) Hayakawa T, Kase Y, Saito K *et al*: Effects of Dai-kenchu-to on intestinal obstruction following laparotomy. *J Smooth Muscle Res* **35** : 47-54, 1999
10) Endo S, Nishida T, Nishikawa K *et al* : Dai-kenchu-to, a Chinese herbal medicine, improves stasis of patients with total gastrectomy and jejunal pouch interposition. *Am J Surg* **192** : 9-13, 2006
11) Tatsuta M, Iishi H : Effect of treatment with liu-jun-zi-tang (TJ-43) on gastric emptying and gastrointestinal symptoms in dyspeptic patients. *Aliment Pharmacol Ther* **7** : 459-462, 1993
12) Kido T, Nakai Y, Kase Y *et al* : Effects of rikkunshi-to, a traditional Japanese medicine, on the delay of gastric emptying induced by N (G)-nitro-L-arginine. *J Pharmacol Sci* **98** : 161-167, 2005
13) Takeda H, Sadakane C, Hattori T *et al* : Rikkun-shito, an herbal medicine, suppresses cisplatin-induced anorexia in rats via $5\text{-}HT_2$ receptor antagonism. *Gastroenterology* **134** : 2004-2013, 2008
14) 原澤茂，三好秋馬，三輪剛ほか：運動不全型の上腹部愁訴（dysmotility-like dyspepsia）に対するTJ-43 六君子湯の多施設共同市販後臨床試験二重盲検群間比較法による検討．医のあゆみ **187** : 207-229, 1998
15) Takahashi T, Endo S, Nakajima K *et al* : Effect of rikkunshito, a chinese herbal medicine, on stasis in patients after pylorus-preserving gastrectomy. *World J Surg* **33** : 296-302, 2009
16) Kawahara H, Kubota A, Hasegawa T *et al* : Effects of rikkunshito on the clinical symptoms and esophageal acid exposure in children with symptomatic gastroesophageal reflux. *Pediatr Surg Int* **23** : 1001-1005, 2007

Original Article

Elmer Press

World J Oncol • 2010;1(6):232-235

Topical Application of Hangeshashinto (TJ-14) in the Treatment of Chemotherapy-Induced Oral Mucositis

Toru Kono[a, d], Machiko Satomi[b, c], Naoyuki Chisato[a], Yoshiaki Ebisawa[a], Manabu Suno[c], Toshiyuki Asama[a, b], Hidenori Karasaki[a], Kazuo Matsubara[c], Hiroyuki Furukawa[a]

Abstract

Background: The optimal treatment of chemotherapy-induced oral mucositis is not well established. A recent study showed that hangeshashinto (TJ-14) might be useful for periodontal disease via downregulating pro-inflammatory prostaglandins in the cyclooxygenase pathway in human. Our study aimed to determine whether TJ-14 is effective in the management of chemotherapy-induced oral mucositis.

Methods: Fourteen patients afflicted with chemotherapy-induced oral mucositis during mFOLFOX6 or FOLFIRI treatment for metastasis of advanced colorectal cancer were randomly assigned to topical TJ-14 treatment thrice daily for 7 days. Patients prepared a 50 ml solution with 2.5 g of TJ-14 dissolved in tap water and rinsed their oral mucosa for more than 5 seconds and then expectorated it. TJ-14 was also topically applied with a cotton pellet on the mucosal lesions. The severity of oral mucositis was evaluated using the Common Terminology Criteria for Adverse Events version 4 before and after one-week TJ-14 treatment.

Results: After the one-week topical treatment with TJ-14, thirteen of the fourteen patients (92.8 %) showed improvements in oral mucositis, with significantly decreased mean CTCAE grades ($P = 0.0012$). Compared to baseline, none of the patients' CTCAE grades worsened. The compliance of TJ-14-treatment was good and side effects from TJ-14 were not observed.

Conclusions: Topical application of TJ-14 may have therapeutic effects in patients with chemotherapy-induced oral mucositis via downregulation of pro-inflammatory prostaglandins. A prospective, randomized, controlled, double-blind studies are necessary to confirm the findings of this open-label, pilot study.

Keywords: Oral mucositis; Chemotherapy; Hangeshashinto; TJ-14; Topical treatment

Manuscript accepted for publication December 9, 2010

[a]Division of Gastroenterologic and General Surgery, Department of Surgery, Asahikawa Medical University, Asahikawa, Japan
[b]Division of Chemotherapy, Higashi-Asahikawa Hospital, Asahikawa, Japan
[c]Department of Hospital Pharmacy and Pharmacology, Asahikawa Medical University, Asahikawa 078-8510, Japan
[d]Corresponding author: Division of Gastroenterologic and General Surgery, Department of Surgery, Asahikawa Medical University, 2-1-1-1 Midorigaoka-Higashi, Asahikawa, Japan. Email: kono@asahikawa-med.ac.jp

doi:10.4021/wjon263w

Introduction

Oral mucositis is a common toxicity associated with cytotoxic chemotherapy used for cancer treatment and results in severe discomfort and impairs patients' ability to eat, swallow, and talk. Mucositis also has an indirect effect on tumor outcomes as its presence often necessitates an unfavorable modification of anti-cancer therapy such as breaks in chemotherapy or a dose reduction of chemotherapy [1-3].

Mucositis risk varies among patients with colorectal cancers who receive multicycle chemotherapy. Although the reported cycle 1 incidence varies, about 15% - 20% of patients being treated with commonly used chemotherapy regimens for cancers reportedly develop ulcerative oral mucositis [3, 4].

One of the factors associated with chemotherapy-induced oral mucositis (COM) exacerbation is the activation of cyclooxygenase pathway that mediates ulcer and pain through the upregulation of pro-inflammatory prostaglandins [5]. Chemotherapy-induced myelosuppression places patients at significant risk of bacteremia and sepsis from oral microorganisms resulting in increased COM [1-3, 6].

Hangeshashinto (TJ-14), a Japanese traditional medicine (kampo) [7], has been reported to downregulate the pro-inflammatory prostaglandins, such as prostaglandin E2 in colitis animal model [8, 9]. Moreover, one of the main ingredients of TJ-14, berberine, with broad-spectrum antibacterial activity has been shown to inhibit butyrate-induced colonic epithelial cell death [10, 11].

In light of the purported anti-inflammatory and antibac-

Table 1. Patient Characteristics

		No. of Patients (%)	
Gender	male	6	(43)
	female	8	(57)
Age	mean	62	
	range	34-80	
PS	0	12	(86)
	1	2	(14)
	2	0	
Concurrent Chemotherpy			
	FOLFOX	5	(36)
	FOLFIRI	9	(64)
Neutropenia			
	Yes	12	(86)
	No	2	(14)
Oral mucositis			
	G1	2	(14)
	G2	6	(43)
	G3	5	(36)
	G4	1	(7)

terial activities of TJ-14 in experimental models, we investigated whether TJ-14 had beneficial effects on COM in patients with advanced colorectal cancer.

Patients and Methods

We enrolled 14 patients with advanced colorectal cancer who underwent chemotherapy at Asahikawa Medical University Hospital and Higashi-Asahikawa Hospital from January 2009 through August 2010. Details of the study and testing procedures were explained, and a written informed consent was obtained from each participant. Fourteen patients who agreed to participate in the study by signing the written informed consent. All study participants were afebrile patients with lesions mostly on the movable mucosa of the buccal mucosa and lateral and ventral surfaces of the tongue that appeared 7 to 10 days after the chemotherapy. Table 1 summarizes the patient characteristics. The present study was conducted in accordance with the guidelines for the care for human study adopted by the ethics committee of Asahikawa Medical University and Higashi-Asahikawa Hospital.

Grading of oral mucositis

The severity of COM was graded by two blinded physicians using the Common Terminology Criteria for Adverse Events v4.0 (CTCAE). For the characterization of mucositis, CTCAE v4.0 grades were defined as follows: 1) grade 0: no mucositis; 2) grade 1: asymptomatic or mild symptoms; 3) grade 2: moderate pain, does not interfere with oral intake but modified diet is indicated; 4) grade 3: severe pain, interferes with oral intake; 5) grade 4: life-threatening consequence requiring urgent intervention.

TJ-14 application

The patients prepared a total 50 ml oral rinse solution with 2.5 g of TJ-14 (Tsumura & Co., Tokyo) and tap water and rinsed their oral mucosa three times daily after each meal. Patients were instructed to hold the solution in the mouth for 10 seconds and then expectorate it. Additionally, TJ-14 was topically applied with a cotton pellet on the oral mucositis at the time of ulcer lesion presentation. Food and drinks were prohibited within 30 minutes of each mouthwash. Treatment continued daily for 7 days. None of the patients received cryotherapy or other mucosal treatment concurrently with chemotherapy. No other mucosal treatment was used with over-the-counter drugs or other medications during the study.

Statistical analysis

All values were expressed as mean ± standard deviation of the mean (SD). Statistical calculations and analyses were performed with the use of Prism 5 (GraphPad Software, Inc., San Diego, CA) statistical software package for comparing the grades of mucositis before and after TJ-14 treatment. Mann-Whitney test was used. All statistical tests performed were two-sided. Differences were considered to be significant at $P < 0.05$.

Results

Fourteen patients with COM received TJ-14 and completed the study. The compliance was good and side effects from TJ-14 were not observed during the study period. Prior to TJ-14 treatment, one patient had grade 4, five patients had grade 3, six patients had grade 2, and two patients had grade 1. After the one-week TJ-14 topical treatment, the patient with grade 4 mucositis improved to grade 2. Of the five patients with grade 3 mucositis, three improved to grade 2 and two improved to grade 1. Among the six patients with grade 2 mucositis, three improved to grade 1, two improved to grade 0, and one had no change. Two patients with grade 1 mucositis improved to grade 0. None of the patients became worse compared to baseline. At the end of the study, thirteen of

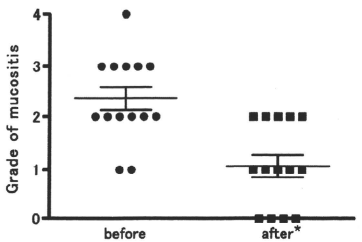

Figure 1. Improved CTCAE grades for oral mucositis following topical treatment of the oral mucosa with hangeshashinto (TJ-14). There was significant reduction in all the grades mucositis from 2.4 ± 0.8 to 1.1 ± 0.8 (* p = 0.0012). The severity of chemotherapy-induced oral mucositis was graded according to the Common Terminology Criteria for Adverse Events v4.0. Results are expressed as mean ± SD.

the fourteen patients (92.8 %) showed improvements in oral mucositis, with significantly decreased mean CTCAE grades (P = 0.0012) (Fig. 1).

Discussion

We found that repeated topical application of TJ-14, even on a short-term basis, improved the severity of COM symptoms in the majority of patients. We also noted that two-thirds of patients on FOLFIRI treatment developed COM.

The current standard of care for patients with advanced colorectal cancer includes varied schedules of chemotherapy like FOLFOX (leucovorin, 5-FU, and oxaliplatin) or FOLFIRI (leucovorin, 5FU, and irinotecan), with FOLFIRI posing a slightly higher risk of developing COM (35%) [4]. Mucosal lesions typically appear between 7 and 14 days after the initiation of chemotherapy [6, 12], mainly on the movable mucosa and rarely affecting the dorsum of the tongue, the hard palate, or the gingiva [3]. In this study, we noted similar observations in our patients, notably a higher incidence of COM among those on FOLFIRI regimen.

Previous reports implicate that some of the exacerbating factors of COM include pro-inflammatory cytokines, nitric oxide, ceramide, matrix metalloproteinases, and pro-inflammatory prostaglandins that lead to apoptosis and tissue injury [1, 3, 5]. Moreover, because prostaglandin E2, a pro-inflammatory prostaglandin, is known to act at pain receptors on neurons and to mediate tissue injury via release of matrix metalloproteinase [5, 13], targeted therapy to downregulate the cyclooxygenase pathway and inhibit prostaglandin synthesis appears important for pain control and mucosal healing in COM [5]. TJ-14 has been reported the anti-inflammatory effects, such as inhibition of pro-inflammatory prostaglandins, including prostaglandin E2, cytoplasmic phospholipase(cPLA), and COX-2 in both in vivo and vitro studies [8, 9]. In clinic, TJ-14 has been used for inflammatory diseases such as gastrointestinal catarrh and gastritis [14]. Although the precise mechanisms remain elusive, TJ-14 might show the anti-COM effect via down-regulation of pro-inflammatory prostaglandins, especially prostaglandin E2.

As most of the patients in the study had neutropenia, we also suspected the dynamic alteration of oral microbiota in promoting infective processes which occur more readily in the presence of neutropenia (as in chemotherapy patients) or other deterioration in host defense systems that causes reduced levels of salivary IgG, IgA, and IgM [15]. Myelosuppression is known to increase gram-negative organisms. Bacterial cell wall products such as lipopolysaccharide (LPS) amplify mechanisms that exaggerate and extend the injury by stimulating infiltrating macrophages to produce additional damaging cytokines [3, 6, 12].

TJ-14 is comprised of 7 extracted components: Pinelliae Tuber, Scutellariae Radix, Zingiberis Rhizoma, Ginseng Radix, Glycyrrhizae Radix, Zizyphi Fructus, and Coptidis Rhizoma. Berberine is the main ingredient of Coptidis Rhizoma with strong and wide-spectrum antimicrobial activity [10, 11]. We therefore speculated that compounds like berberine might be responsible for the antimicrobial and anti-inflammatory effects, suppression of prostaglandins, and alleviation of COM symptoms induced by TJ-14.

In conclusion, our findings support the possible benefits of TJ-14. This must be counter-balanced by cautions that further controlled, double blind studies are needed to confirm the findings.

References

1. Scardina GA, Pisano T, Messina P. Oral mucositis. Review of literature. N Y State Dent J 2010;76(1):34-38.
2. Napenas JJ, Shetty KV, Streckfus CF. Oral mucositis: review of pathogenesis, diagnosis, prevention, and management. Gen Dent 2007;55(4):335-344; quiz 345-336, 376.
3. Scully C, Sonis S, Diz PD. Oral mucositis. Oral Dis 2006;12(3):229-241.
4. Jones JA, Avritscher EB, Cooksley CD, Michelet M, Bekele BN, Elting LS. Epidemiology of treatment-associated mucosal injury after treatment with newer regimens for lymphoma, breast, lung, or colorectal cancer. Support Care Cancer 2006;14(6):505-515.
5. Lalla RV, Pilbeam CC, Walsh SJ, Sonis ST, Keefe DM, Peterson DE. Role of the cyclooxygenase pathway in chemotherapy-induced oral mucositis: a pilot study. Support Care Cancer 2009.

6. Sonis ST. The pathobiology of mucositis. Nat Rev Cancer 2004;4(4):277-284.
7. Kono T, Kanematsu T, Kitajima M. Exodus of Kampo, traditional Japanese medicine, from the complementary and alternative medicines: is it time yet? Surgery 2009;146(5):837-840.
8. Kase Y, Hayakawa T, Ishige A, Aburada M, Komatsu Y. The effects of Hange-shashin-to on the content of prostaglandin E2 and water absorption in the large intestine of rats. Biol Pharm Bull 1997;20(9):954-957.
9. Kase Y, Saitoh K, Ishige A, Komatsu Y. Mechanisms by which Hange-shashin-to reduces prostaglandin E2 levels. Biol Pharm Bull 1998;21(12):1277-1281.
10. Samosorn S, Tanwirat B, Muhamad N, Casadei G, Tomkiewicz D, Lewis K, Suksamrarn A, et al. Antibacterial activity of berberine-NorA pump inhibitor hybrids with a methylene ether linking group. Bioorg Med Chem 2009;17(11):3866-3872.
11. Grycova L, Dostal J, Marek R. Quaternary protoberberine alkaloids. Phytochemistry 2007;68(2):150-175.
12. Sonis ST. Pathobiology of mucositis. Semin Oncol Nurs 2004;20(1):11-15.
13. Shankavaram UT, Lai WC, Netzel-Arnett S, Mangan PR, Ardans JA, Caterina N, Stetler-Stevenson WG, et al. Monocyte membrane type 1-matrix metalloproteinase. Prostaglandin-dependent regulation and role in metalloproteinase-2 activation. J Biol Chem 2001;276(22):19027-19032.
14. Ohgimi Y. The rapid effect of kampo drugs for patienmts with acute symptoms. Jpn J Oriental Med 1995;46:301-308.
15. Lalla RV, Schubert MM, Bensadoun RJ, Keefe D. Anti-inflammatory agents in the management of alimentary mucositis. Support Care Cancer 2006;14(6):558-565.

Journal of Crohn's and Colitis (2010) 4, 161–170

available at www.sciencedirect.com

ScienceDirect

Anti-colitis and -adhesion effects of daikenchuto via endogenous adrenomedullin enhancement in Crohn's disease mouse model☆

Toru Kono [a,*], Atsushi Kaneko [a,b], Yoshiki Hira [c], Tatsuya Suzuki [a], Naoyuki Chisato [a], Nobuhiro Ohtake [b], Naoko Miura [b], Tsuyoshi Watanabe [c]

[a] *Division of Gastroenterologic and General Surgery, Department of Surgery, Asahikawa Medical College, Hokkaido 078-8510, Japan*
[b] *Tsumura Research Laboratories, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan*
[c] *Department of Anatomy, Asahikawa Medical College, Hokkaido 078-8510, Japan*

Received 10 July 2009; received in revised form 28 August 2009; accepted 19 September 2009

KEYWORDS
Adrenomedullin;
Crohn's disease;
Daikenchuto;
2,4,6-trinitrobenzenesulfonic acid;
Tumor necrosis factor-α;
Interferon-γ

Abstract

Background and aims: Adrenomedullin (ADM) is a member of the calcitonin family of regulatory peptides, and is reported to have anti-inflammatory effects in animal models of Crohn's disease (CD). We investigated the therapeutic effects of daikenchuto (DKT), an extracted Japanese herbal medicine, on the regulation of endogenous ADM in the gastrointestinal tract in a CD mouse model.

Methods: Colitis was induced in mice by intrarectal instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS); afterwards, DKT was given orally. Colonic damage was assessed on day 3 by macroscopic and microscopic observation, enzyme immunoassays of proinflammatory cytokines in the colonic mucosa, and serum amyloid A (SAA), a hepatic acute-phase protein. To determine the involvement of ADM, an ADM antagonist was instilled intrarectally before DKT administration. The effect of DKT on ADM production by intestinal epithelial cells was evaluated by enzyme immunoassay and real-time PCR.

Results: DKT significantly attenuated mucosal damage and colonic inflammatory adhesions, and inhibited elevations of SAA in plasma and the proinflammatory cytokines TNFα and IFN-γ in the colon. Small and large intestinal epithelial cells produced higher levels of ADM after DKT stimulation. A DKT-treated IEC-6 cell line also showed enhanced ADM production at protein and mRNA levels. Abolition of this effect by pretreatment with an ADM antagonist shows that DKT appears to exert its anti-colitis effect via up-regulation of endogenous ADM in the intestinal tract.

☆ Part of this work was presented at Digestive Disease Week 2009, American Gastroenterologic Association (AGA), Chicago, USA.

* Corresponding author. Division of Gastroenterologic and General Surgery, Department of Surgery, Asahikawa Medical College, 2-1 Midorigaoka-Higashi, Asahikawa, Hokkaido 078-8510, Japan. Tel.: +81 166 68 2503; fax: +81 166 68 2193.
E-mail address: kono@asahikawa-med.ac.jp (T. Kono).

doi:10.1016/j.crohns.2009.09.006

Conclusion: DKT exerts beneficial effects in a CD mouse model through endogenous release and production of ADM. Endogenous ADM may be a therapeutic target for CD.

1. Introduction

Adrenomedullin (ADM) is a peptide of the calcitonin family and a potent endogenous vasodilator.[1] ADM is ubiquitous in the gastrointestinal (GI) tract and plays important roles in microcirculation regulation; ADM also possesses anti-inflammatory actions via the inhibition of proinflammatory cytokines, most notably for its ability to inhibit tumor necrosis factor-α (TNFα).[2] The inhibitory effect of ADM on TNFα production has received considerable attention in the field of Crohn's disease (CD) research. Similar in many respects to therapeutic use of infliximab (which targets TNFα), ADM has advanced our understanding of possible treatment goals in CD.[3] Indeed, ADM has demonstrated anti-colitis effects in mouse[4] and rat[5] models of CD. Although the combined results of these studies suggest a novel approach to the treatment of CD with ADM, exogenous administration of ADM is clearly not practical because of the potential systemic effects of this agent and its metabolic clearance, which makes chronic delivery of small peptides impractical.[6–8]

Daikenchuto (DKT), an extracted Japanese herbal medicine, is manufactured as a recognized prescription drug with standardized quality and ingredient quantities.[9] The formulation is composed of extract granules of Japanese pepper, processed ginger, ginseng radix and maltose powder. DKT is prescribed in Japan to improve GI motility and prevent postoperative adhesion and paralytic ileus after abdominal surgery with defined clinical efficacy.[10–13] In experimental studies, DKT enhanced gastrointestinal motility in vivo[14–16] and in vitro,[17,18] and prevented formation of intestinal adhesions in a rat talc-induced adhesion model.[19,20] We have reported that intraduodenal[21] or intracolonic[22] administration of DKT in normal rats increases small and large intestinal blood flow in a dose-dependent manner, and that this activity is abolished completely by pretreatment with the calcitonin gene-related peptide (CGRP) antagonist $CGRP_{8-37}$. It is also known that $CGRP_{8-37}$ can block the signal pathway of ADM as well as that of CGRP, because these peptides bind to varying degrees to each other's receptors.[23,24] A heterodimer complex of calcitonin receptor-like receptor (CRLR) and receptor activity-modifying protein (RAMP) 1 is a known CGRP receptor, while a similar heterodimer complex of CRLR and RAMP2 or RAMP3 has been reported as the ADM receptor. ADM and CGRP, however, cross-react with one another's receptors, and thus these peptides share common biologic actions.[1,23] On the other hand, a decrease in endogenous CGRP is observed in animal models of CD and in clinical cases.[25] In animal models, this results in severe inflammation.[26–28]

Based on these observations, we hypothesize that DKT-induced up-regulation of endogenous ADM may compensate for the decrease of CGRP in CD and that DKT therapy may be beneficial in the management of CD. We investigated the beneficial effects of DKT in a mouse acute colitis model using 2,4,6-trinitrobenzenesulfonic acid (TNBS), which is widely used to test potential therapeutic agents of CD.[4,29]

2. Materials and methods

2.1. Test substances

DKT was obtained from Tsumura & Co. (Tokyo, Japan) as a water-soluble extract containing processed ginger (5.6%), ginseng radix (3.3%), Japanese pepper (2.2%), and maltose powder (88.9%). Prednisolone (PSL) was purchased from Shionogi & Co. (Osaka, Japan). The ADM antagonist ADM_{22-52} was purchased from Peptide Institute Inc. (Osaka, Japan).

DKT (300, 900, or 2700 mg/10 ml/kg) was suspended in distilled water and given orally to mice at 8, 24, 32, 48, and 56 h after colitis induction. To evaluate the participation of luminal endogenous ADM, ADM_{22-52} (3.57 μg/0.1 ml/mouse) was intrarectally instilled under ether anesthesia through a 3.5 F catheter inserted 3.5 cm from the anus 10 min before DKT administration in a subset of the mice. PSL (3 mg/10 ml/kg) was dissolved in distilled water, and given orally 2 and 18 h before and 8, 24, 32, 48, and 56 h after colitis induction.

2.2. Induction of acute colitis

Male BALB/c mice were purchased from Japan SLC (Shizuoka, Japan). Mice (6–8 weeks old) were anesthetized by intraperitoneal injection of sodium pentobarbital (55 mg/kg, Abbott Laboratories, North Chicago, IL) and atropine sulfate (3 mg/kg, Sigma-Aldrich, St. Louis, MO). To induce colitis, 1.5 mg TNBS (Tokyo Chemical Industry Co, Tokyo, Japan) dissolved in 0.1 ml of 50% ethanol was instilled transanally into the lumen of the colon 3.5 cm from the anus using a 3.5 F catheter. Control mice received 50% ethanol alone. Colonic damage was evaluated 3 days after the TNBS instillation. No mice died during the experiment. Ethical approval for the experimental procedures used in this study was obtained from the Asahikawa Medical College Animal Care and Use Committee. All experimental procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Assessment of colonic damage

Two observers blinded to treatment group scored the colon specimens for macroscopically visible damage on a 0–10 scale according to the previously published criteria[30] with slight modification. The large intestine was removed after evaluation of the adhesion score and opened longitudinally. After washing out the luminal contents, mucosal damage in the colon was assessed macroscopically. The criteria reflect both colonic inflammatory adhesions (0=no adhesions, 1=minor adhesions, 2=major adhesions) and mucosal damage described as the following: 0=no damage, 1=focal hyperemia with no ulcers, 2=ulceration without hyperemia or bowel wall thickening, 3=ulceration with inflammation at one site, 4=two or more sites of ulceration and inflammation, 5=two or

more major sites of ulceration and inflammation or one site of ulceration extending >1 cm along length of colon, 6–8 = damage covering >2 cm along length of colon, with score increased by 1 for each additional centimeter of involvement. A photograph was taken to evaluate necrotic areas by image analysis (ImageJ ver.1.37 software). For histological assessment, the colon was fixed in 4% buffered paraformaldehyde. Cross-sections were stained with hematoxylin and eosin. A microscopic score was assigned on a scale of 0–11 according to previously published criteria[30] by two pathologists blinded to the experimental groups.

2.4. Determination of cytokines in colon and serum amyloid A

For determination of cytokines in the colonic mucosa, protein extracts were obtained by homogenization of the colonic mucosa (0.5 mg tissue/ml) in 50 mM Tris HCl, pH 7.4, 0.5 mM dithiothreitol, and 10 μg/ml cocktail of proteinase inhibitors containing phenylmethylsulfonyl fluoride, pepstatin, and leupeptin (Sigma-Aldrich). Samples were centrifuged at 10,000*g* for 20 min at 4 °C, and the supernatants were stored at −80 °C until assay. Levels of the cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6), TNFα, and interferon-γ (IFNγ) were determined by a specific sandwich ELISA using capture/biotinylated detection. Antibodies from BD Biosciences (San Jose, CA) were used according to the manufacturer's recommendations. Serum amyloid A (SAA) in plasma samples was determined using a murine ELISA kit (Tridelta Development, Morris Plains, NJ).

2.5. Immunohistochemistry for ADM

Immunohistochemistry was performed as described below. Jejunal and distal colonic tissues obtained from normal mice 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 Laboratory, Inc., Belmont, CA) as the primary antibody, and peroxidase-conjugated goat anti-rabbit IgG (DAKO A/S, Glostrup, Denmark) were used as a secondary antibody. The reaction was developed by adding 3,3′ diaminobenzidine (Sigma-Aldrich) solution. All incubations were 20 min, with saturated antibody concentrations and followed by two washes.

2.6. Preparation of intestinal epithelium cells, mesenteric lymph node cells, and splenocytes

Intestinal epithelium (IE) cells were isolated according to the previously published protocol[31] with a slight modification. Briefly, the small or large bowel was removed, cut into 5 mm pieces, and washed 3 times with Hanks solution. Then, the fragments were incubated in Hanks solution (pH 7.4) containing 5 mM EDTA, 1 mM dithiothreitol, 15 mM HEPES, and 10% heat-inactivated fetal bovine serum (FBS) with continuous brisk stirring at 37 °C for 30 min. The supernatant was harvested and centrifuged at 300*g* for 10 min. The pellets were suspended in Ficoll-Hypaque (Pharmacia, Piscataway, NJ), and Hanks solution containing 10% FBS was overlaid. The layer of cells at the interface after centrifugation at 710*g* for 15 min was collected, washed, and applied to a 25–40% gradient of Percoll (Pharmacia). After centrifugation at 710*g* for 30 min, the interface containing IE cells was collected. Following these procedures, yields of >95% viable cells were routinely obtained. Further, purified IE cells were stained with rabbit anti-cytokeratin 12 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) following cytospin. The phenotypes of small and large intestinal IE cells were 80–90% and 95–98% cytokeratin 12$^+$, respectively. Twenty four hours after the culture, the cell viability was 35%, 28%, respectively.

Mesenteric lymph nodes (MLN) and spleen were removed aseptically from normal mice. Single-cell suspensions were prepared by teasing the tissues in Hanks solution containing 10% FBS. Splenocytes were prepared after being suspended in erythrocyte lysing buffer (0.155 M NH_4Cl, 0.1 mM EDTA, and 0.01 M $KHCO_3$).

2.7. Flow cytometry analysis

Single cells were suspended in Cytofix/Cytoperm solution (BD Biosciences) for 20 min at 4 °C, washed, and then pre-incubated for 5 min at 4 °C with goat polyclonal IgG antibody (Abcam, Cambridge, UK) to reduce non-specific binding of antibodies. Next, cells were incubated for 20 min at 4 °C with rabbit polyclonal IgG antibody (4 μg/ml) against rat ADM, cytokeratin 12, or isotype control IgG (Abcam). Cells were washed, incubated for 20 min with the Alexa Fluor 488-labeled goat polyclonal antibody against rabbit IgG (Invitrogen, Carlsbad, CA), and subjected to flow cytometry analysis using a FACScalibur analyzer and CellQuest Pro software (BD Biosciences).

2.8. ADM production test

IE cells of the small or large intestine were plated in 96-well round-bottom microtiter plates at 1×10^6 cells/ml in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, and 0.1% dimethyl sulfoxide (DMSO). DKT was suspended in DMSO, diluted in DMEM, passed through a 0.45 μm filter, and then added to the cultures at final concentrations of 90, 300, or 900 μg/ml. Cells were incubated for 24 h, and ADM in the culture fluids was quantified using enzyme immunoassay (EIA) kits specific for rat ADM according to the procedure provided by the manufacturer (Phoenix Pharmaceuticals, Burlingame, CA). The rat ADM assay has 100% cross-reactivity with murine ADM. The least level of detection for ADM was 10 pg/ml.

A rat small intestine epithelial cell line, IEC-6, was obtained from Dainippon Pharmaceuticals (Osaka, Japan) and grown in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10 mM HEPES. IEC-6 cells between the 30th and 37th passage were plated in 96-well flat-bottom microtiter plates at 1×10^4 cells/well in DMEM supplemented with the same additives as described above, allowed to settle overnight, and then culture fluids were replaced with fresh DMEM containing 3% FBS, 0.1% DMSO, and 90, 270, or 900 μg/ml DKT passed through a 0.45 μm filter. Cells were incubated for an additional 12, 24, 48, 72, and 96 h, and ADM in the supernatants was assessed using the EIA kit.

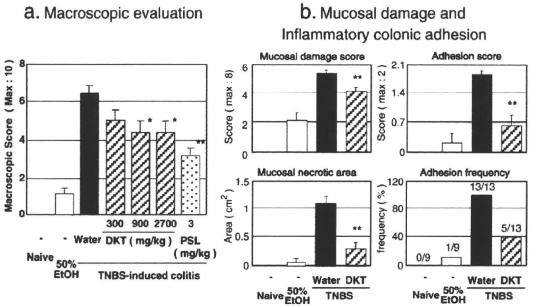

Figure 1 Protective effect of DKT on 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis model. Macroscopically visible damage was evaluated 3 days after TNBS instillation. (a) Colitis was scored on a 0–10 scale, which was a sum of the mucosal damage score (0–8) and the colonic adhesion score (0–2). DKT (300, 900, or 2700 mg/kg) was given orally 8, 24, 32, 48, and 56 h after TNBS instillation. $N=9$. (b) DKT was given orally at 900 mg/kg. Clinical severity was monitored by mucosal damage score, necrotic area of colonic mucosa, adhesion score, and adhesion frequency. $N=9$ (naive and 50% EtOH), 13 (colitis groups). *, **: $P<0.05$, 0.01 versus TNBS/water (colitis control), respectively.

2.9. Gene expression analysis

IEC-6 cell pellets were homogenized in QIAzol reagent (Qiagen, Valencia, CA) and total RNA was isolated using RNeasy kit (Qiagen) according to the manufacturer's recommendations. Expressions of ADM and ADM2 mRNAs were measured using a multiplex real-time quantitative RT-PCR method (TaqMan gene expression assays) and the ABI Prism 7900 sequence detection system (Applied Biosystems, Warrington, UK). Sample-to-sample variation in RNA loading was controlled by comparison with the housekeeping gene G3PDH.

2.10. Effect of ADM on proinflammatory cytokine production

MLN cells and splenocytes were cultured in 96-well flat-bottom microtiter plates pre-coated with 5 μg/ml of anti-

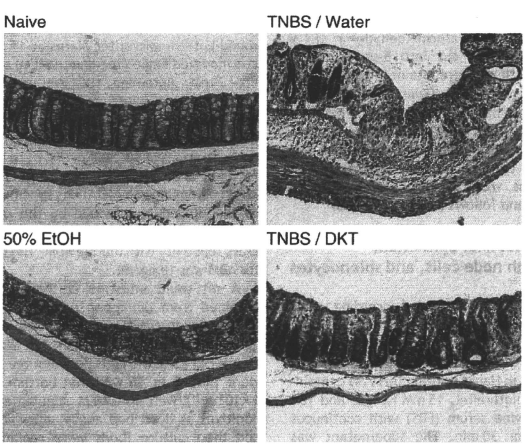

Figure 2 Effect of DKT on histological evaluation in colitis mice. DKT was given orally at 900 mg/kg after 2,4,6-trinitrobenzenesulfonic acid (TNBS) instillation. Three days after TNBS instillation, histopathological analysis (×40) of hematoxylin and eosin-stained sections of the colon was performed.

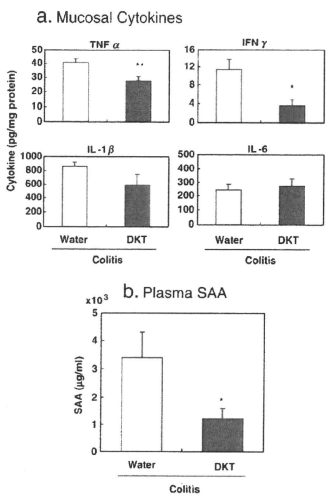

Figure 3 Effects of DKT on the mucosal and systemic inflammatory responses in colitis mice. Three days after colitis induction, colonic mucosa and plasma were collected. (a) Concentrations of cytokines in protein extracts of the colonic mucosa were determined by ELISA, $N=6$. (b) Serum amyloid A (SAA) concentration in plasma was determined by ELISA. $N=11$. SAA of naive and vehicle (50% EtOH)-treated control mice were less than 1 μg/ml, respectively. *, **: $P<0.05$, 0.01 versus colitis control, respectively.

CD3 antibody (clone 145-2C11, BD Biosciences) at 3×10^5 cells/well in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 50 μM 2-mercaptoethanol, and 10% FBS. Rat ADM (American Peptide, Sunnyvale, CA) was added at various concentrations (0.01, 0.1, or 1 μmol/L). After 24 h culture, TNFα and IFNγ concentrations in harvested culture fluids were determined as described above.

2.11. Statistics

All values are expressed as the mean±S.E.M. The statistical significance of differences between two groups was assessed using Student's *t*-test. For comparisons of multiple groups, Dunnett's test was used. A probability of less than 0.05 was considered significant.

3. Results

3.1. Effect of DKT on development of acute colitis

Macroscopic examination of the colons obtained 3 days after instillation of TNBS showed striking adhesion, hyperemia, inflammation, and necrosis compared with vehicle (50% EtOH)-instilled control mice. In contrast, the macroscopic evaluation scores of the colons of DKT-treated mice were lower than those of the colitis control mice in a dose-dependent manner (Fig. 1a). PSL was significantly effective. We performed another study to examine the effect of DKT (900 mg/kg) and to evaluate the individual results of mucosal damage and colonic inflammatory adhesion (Fig. 1b). DKT treatment provided significant protection against the respective parameters associated with colitis progression.

Histological examination of the colitis control specimens showed transmural inflammation with a marked increase in the thickness of the muscular layer, adherence to surrounding tissues, patchy ulceration, epithelial cell loss, pronounced depletion of mucin-producing goblet cells, reduction of the density of tubular glands, and focal loss of crypts (Fig. 2). A large number of inflammatory cells infiltrated the lamina propria. The microscopic score of colitis control mice was 6.4±0.1, whereas that of DKT-treated mice was 2.7±0.6.

We assessed the effect of DKT (900 mg/kg) on the induction of inflammatory mediators that are mechanistically linked to colitis development. As shown in Fig. 3a, DKT treatment significantly reduced TNFα and IFNγ levels in the colonic mucosa of colitis mice compared with colitis control mice. SAA, a hepatic acute-phase protein involved in tissue damage with inflammatory conditions, was prominently increased in the plasma of colitis control mice (3398±910 versus 0.5±0.1 μg/ml in vehicle control mice). As shown in Fig. 3b, DKT treatment significantly decreased SAA.

To examine the involvement of luminal endogenous ADM in the anti-colitis effect of DKT, ADM_{22-52} was instilled intrarectally before administration of 900 mg/kg DKT. DKT

Table 1 Abolition of anti-colitis effect of DKT by pretreatment with ADM antagonist.

Induction	Administration		*N*	Macroscopic score
	Oral	Intrarectal		(Max: 10)
Experiment 1				
–	–	–	6	0.00±0.00
50% EtOH	–	–	6	0.50±0.34
TNBS	Water	–	8	6.13±0.23
TNBS	DKT	–	8	4.25±0.59 [a]
TNBS	Water	Saline	8	5.38±0.46
TNBS	Water	ADM_{22-52}	8	6.00±0.42
TNBS	DKT	ADM_{22-52}	8	5.50±0.50 [NS]
Experiment 2				
–	–	–	6	0.00±0.00
50% EtOH	–	–	6	1.67±0.49
TNBS	Water	Saline	8	7.13±0.52
TNBS	DKT	Saline	8	5.25±0.45 [b]
TNBS	Water	ADM_{22-52}	8	6.88±0.35
TNBS	DKT	ADM_{22-52}	8	6.63±0.53 [NS]

DKT was given orally at doses of 900 mg/kg after 2,4,6-trinitrobenzene sulfonic acid (TNBS) instillation. An adrenomedullin (ADM) antagonist (ADM_{22-52}, 3.57 μg/0.1 ml/mouse) was instilled intrarectally 10 min before DKT administration. Macroscopic evaluation was performed 3 days after colitis induction. Significant analysis was performed by Student's *t*-test, Water versus DKT. [a,b]: $P<0.05$, NS: Not significant.

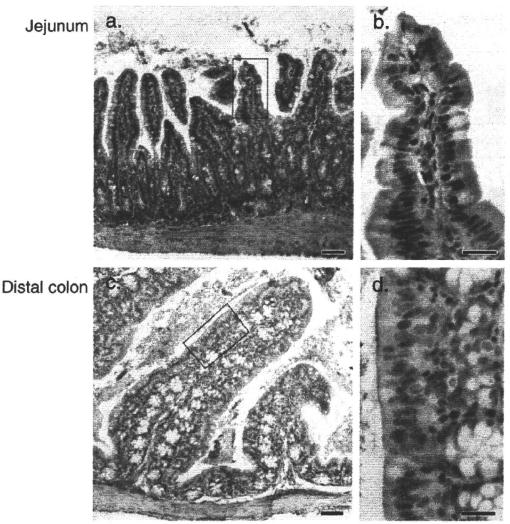

Figure 4 Expression of adrenomedullin (ADM) in mucosal epithelium of intestinal tract. Jejunum and distal colon were obtained from normal mice, and stained with rabbit anti-ADM antibody. Scale bar shown in panels a and c is 50 μm; scale bar shown in panels b and d is 20 μm.

alone showed an anti-colitis effect, whereas the effect of DKT was reduced by ADM_{22-52} pretreatment (Table 1).

3.2. ADM enhancement by DKT in cell culture systems

ADM immunoreactivity was mainly observed on the apical side of intestinal mucosa surface columnar epithelia (Fig. 4). In addition, ADM immunoreactivity was observed in the sub-epithelial sites. To investigate the possibility that DKT affects release of ADM from the IE, IE cells were isolated from the small and large intestines. First, phenotypic analysis was performed using flow cytometric techniques. As shown in Fig. 5, IE cells of both small and large intestines expressed cytokeratin and ADM. Next, an ADM production test was performed. As shown in Table 2, 900 μg/ml DKT enhanced ADM production in both small and large intestinal IE cells.

Because primary cultured IE cells do not proliferate during the ADM production test, a cell line of rat small intestinal epithelial cell IEC-6 was utilized to further examine the ADM-enhancing activity of DKT. As indicated by flow cytometric analysis, IEC-6 cells expressed intracellular ADM (Fig. 6a) and DKT enhanced ADM production in a concentration-dependent manner (Fig. 6b). Addition of 900 μg/ml DKT to culture fluids of IEC-6 cells resulted in an ADM concentration of 70±7 pg/ml, significantly higher than that of the control (39±4 pg/ml). Moreover, ADM production by IEC-6 cells treated with DKT (900 μg/ml) was higher than that of the control at any time point (Fig. 6c). Examination of the mRNA expression levels of ADM and ADM2 in IEC-6 cells treated with or without DKT (900 μg/ml) by real-time PCR revealed that DKT significantly up-regulated both ADM and ADM2 gene expression.

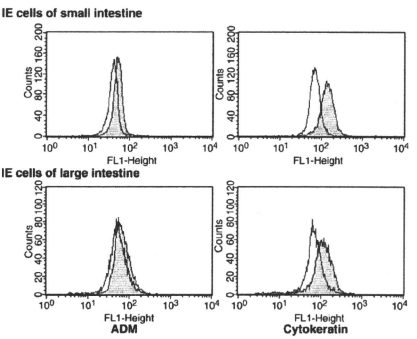

Figure 5 Phenotypic characterization of IE cells from small and large intestines. IE cells isolated from small and large intestines of normal mice were enriched using density gradient centrifugation and analyzed by flow cytometric techniques. Adrenomedullin (ADM) and cytokeratin were stained with the respective specific antibody (grey histograms) after cells were suspended in Cytofix/Cytoperm solution. Isotype controls are shown as open histograms.

Table 2 Increase of ADM production in murine intestinal epithelial cells by DKT.

Cells	DKT concentration (μg/ml)			
	0	90	270	900
IE cells of small intestine	31±4	38±7	46±5	54±2 [a]
IE cells of large intestine	3±1	13±1 [b]	11±3 [b]	11±1 [b]

Intestinal epithelial (IE) cells were isolated from the small or large intestine of normal mice and stimulated with DKT (90, 270, or 900 μg/ml) at 1×10^6 cells/ml in 96-well round-bottom plates for 24 h. Concentrations (pg/ml) of adrenomedullin (ADM) in culture fluids were determined using the EIA method. N=4–6, [a, b]: $P<0.05$, 0.01 versus no DKT control, respectively.

3.3. Inhibitory effect of ADM on cytokine production by MLN cells and splenocytes

To test the anti-inflammatory effect of ADM, MLN cells and splenocytes were cultured in plates coated with anti-CD3 in the presence of various concentrations of ADM. TNFα and IFNγ were determined in this study as representative proinflammatory cytokines related to enteritis. As shown in Fig. 7, both cytokines were significantly decreased in ADM-treated cells compared with untreated control.

4. Discussion

In this study, ADM immunoreactivity was abundant in intestinal epithelial mucosa. Several investigators have suggested that ADM plays an important role in mucosal defense as an antimicrobial peptide.[32,33] Invasion of microbes through the mucosal barrier stimulates the host immune system and intimately correlates with the development of morbidity in experimental and human inflammatory bowel disease.[34] Besides epithelial cells, ADM is known to be synthesized and secreted from vascular smooth muscle cells, endothelial cells, fibroblasts, neuronal cells, and immune cells,[35] however, we did not identify the ADM-staining cells in subepithelial sites.

It has already been verified that ADM diminishes proinflammatory cytokine production.[36] Thus, ADM may play a regulatory role in inflammatory gut diseases such as CD. Actually, the anti-colitis effect of ADM was previously demonstrated in mouse[4] and rat[5] models of CD. Combined results of these studies support the potential treatment of CD with ADM, as exogenous ADM administration has proven efficacy in animal models.

However, clinical application of exogenous ADM as a therapeutic agent is clearly impossible because of its effects on the entire systemic circulation, and rapid metabolic clearance makes delivery impractical.[6–8] Thus, it is more desirable to develop an agent that causes release and production of endogenous ADM in the bowel rather than supplying exogenous ADM. As shown in Table 2 and Fig. 6, DKT stimulates and enhances ADM production by IE cells and the IEC-6 cell line. Messenger RNA expressions of ADM and ADM2 were prominently increased by DKT stimulation. ADM2 is a peptide with 33% sequence homology to ADM, binding to ADM and CGRP receptors.[37] Indeed, ADM2 has many biological effects similar to those of ADM and CGRP, and could be important for regulation of diverse physiological processes that have been attributed to CGRP and ADM.

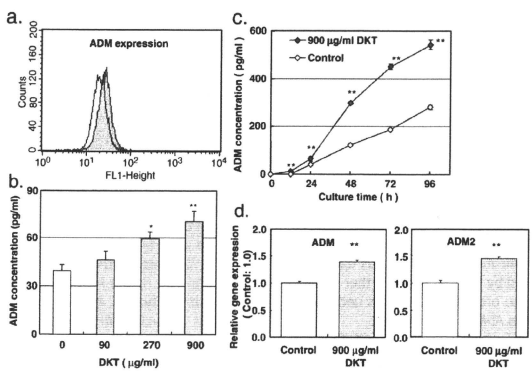

Figure 6 DKT-enhanced ADM production by IEC-6 cell line. (a) A line of rat small intestine epithelial cells, IEC-6, was stained with anti-adrenomedullin (ADM) polyclonal antibody, and determined by flow cytometry. (b) IEC-6 cells were plated in 96-well flat-bottom plates at 1×10^4 cells/well. The next day, culture fluids were replaced with fresh medium containing DKT (90, 270, or 900 μg/ml). Cells were allowed to incubate an additional 24 h, and the supernatants were harvested. Concentrations (pg/ml) of adrenomedullin (ADM) in culture fluids were determined using the EIA method. N=3. (c) A time-course study was performed. IEC-6 cell was cultured with or without 900 μg/ml of DKT for 12, 24, 48, 72, or 96 h. N=3. (d) Total RNA was isolated from IEC-6 cell cultured with or without 900 μg/ml of DKT for 24 h. Expressions of ADM and ADM2 mRNA were measured by real-time PCR and normalized by GAPDH expression. N=5. *, **: $P<0.05$, 0.01 versus no DKT control, respectively.

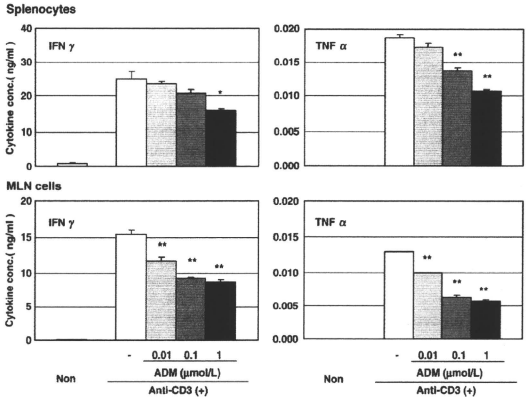

Figure 7 Inhibitory effect of ADM on TNFα and IFNγ production by immune cells. Mesenteric lymph node (MLN) cells and splenocytes were isolated from normal mice and cultured at 3×10^5 cells/well for 24 h in 96-well flat-bottom microtiter plates pre-coated with anti-CD3 antibody. Adrenomedullin (ADM) was added at final concentrations of 0.01, 0.1, or 1 μmol/l. Concentrations (pg/ml) of TNFα and IFNγ in culture fluids were determined by ELISA. $N=3$. *, **: $P<0.05$, 0.01 versus no ADM control, respectively.

Several investigators demonstrated that various cells produce ADM and ADM2 and that their production profiles are modified by inflammation-related substances including cytokines and steroid hormones.[35,38]

The main ingredients of DKT have been identified by three-dimensional high-performance liquid chromatography.[14] A study of the active ingredients that enhance ADM production is now being performed. According to our preliminary tests, 6-shogaol and hydroxy α-sanshool, the primary ingredients of DKT, have the ability to enhance ADM synthesis in an ADM production test using IEC-6 cells. It is of interest that these ingredients elevate intestinal blood flow when they are administered to the intestinal tract.[21,22] In the current study, we investigated the relationship between the anti-colitis effect of DKT and endogenous ADM in the bowel using ADM_{22-52}. Pretreatment with ADM_{22-52} reduced the anti-colitis effect of DKT. Moreover, we demonstrated that DKT induces the release of ADM from IE cells in a dose-dependent manner. These lines of evidence indicate that endogenous epithelial ADM is directly up-regulated by luminal DKT. Further studies are needed to elucidate the precise mechanisms underlying up-regulation of endogenous ADM in the intestine.

We confirmed that DKT increased the blood flow at ischemia sites found in chronically inflamed colons in a TNBS-induced rat colitis model (data not shown), as well as in a normal rat model.[21,22] Recurrent strictures frequently emerge around anastomosis sites in CD. This morbidity is related to local ischemia at the anastomosis site, although its precise mechanism is not clear. It is speculated that recurrent lesions at an anastomosis site in clinical practice may be reduced by postoperative DKT administration.

The inhibitory effect of ADM on TNFα and IFNγ production has been reported previously.[5,36] As shown in Fig. 3a, administration of DKT, an enhancer of endogenous ADM, resulted in reduction of TNFα and IFNγ levels in the colonic mucosa of colitis mice. DKT-induced inhibition of TNFα and IFNγ production has received considerable attention in the field of CD, similar in many respects to the advances in CD treatment with infliximab, which targets TNFα.[3] Recent study revealed that there is a potential advantage of targeting IFNγ for the treatment of CD. Neutralization of IFNγ may interrupt the cytokine cascade that leads to TNFα up-regulation, resulting in decreased TNFα and IFNγ levels.[39] In fact, some of the clinical effects of anti-IFNγ therapy have been reported.[40,41] Based on these facts, targeting endogenous ADM may be a potential advantage for the CD treatment. Additionally, DKT may be a unique therapeutic agent for CD as an intestinal endogenous ADM enhancer.

Finally, as shown in Fig. 1b, DKT dramatically inhibited formation of inflammatory adhesions between the inflamed colon and adjacent tissue. Bowel adhesions are frequently found in CD, associated with morbidity in the form of obstruction and fistula formation, and may require surgical removal of the bowel. Recent studies have confirmed that IFNγ plays a crucial role in the regulation of fibrous tissue formation by disrupting the balance between plasminogen activator inhibitor type 1 and tissue-type plasminogen activator, which reciprocally regulate fibrin deposition.[42] It is plausible that the anti-adhesion effect of DKT is due to the reduction in IFNγ, which can be down-regulated by ADM; however, a precise mechanism of the anti-adhesion effect of ADM is still unclear. Interestingly, for nearly a decade in Japan, DKT has been employed to speed the recovery from postoperative ileus after abdominal surgery and its efficacy has been reported in clinical[11] and animal study.[19] The Japanese government has covered DKT as a prescription drug

under their national health insurance since 1986. In addition, DKT has been on the market in Japan for several decades and is associated with very few side effects.[43]

In summary, our findings indicate that DKT attenuates mucosal damage, colonic inflammatory adhesions, systemic inflammation, and inhibited mucosal proinflammatory cytokines, including TNFα and IFNγ, in a CD mouse model via up-regulation of endogenous ADM in the IE. Endogenous ADM in epithelial cells may be a unique therapeutic target for CD morbidity.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research provided by the Ministry of Education, Culture, Sports, Science and Technology, Japan. We thank Drs. Yuji Omiya, Masahiro Yamamoto, Shinichi Kasai, and Yoshio Kase for the helpful discussions and Akihito Mase for the technical assistance.

References

1. Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, et al. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem Biophys Res Commun* 1993;**192**:553–60.
2. Wu R, Zhou M, Wang P. Adrenomedullin and adrenomedullin binding protein-1 downregulate TNF-alpha in macrophage cell line and rat Kupffer cells. *Regul Pept* 2003;**112**:19–26.
3. Sandborn WJ. Clinical perspectives in Crohn's disease. Moving forward with anti-TNF-alpha therapy: current needs and future treatments. *Rev Gastroenterol Disord* 2007;**7**(Suppl 2):S23–35.
4. Gonzalez-Rey E, Fernandez-Martin A, Chorny A, Delgado M. Therapeutic effect of urocortin and adrenomedullin in a murine model of Crohn's disease. *Gut* 2006;**55**:824–32.
5. Talero E, Sanchez-Fidalgo S, de la Lastra CA, Illanes M, Calvo JR, Motilva V. Acute and chronic responses associated with adrenomedullin administration in experimental colitis. *Peptides* 2008;**29**:2001–12.
6. Eto T, Kitamura K, Kato J. Biological and clinical roles of adrenomedullin in circulation control and cardiovascular diseases. *Clin Exp Pharmacol Physiol* 1999;**26**:371–80.
7. Meeran K, O'Shea D, Upton PD, Small CJ, Ghatei MA, Byfield PH, et al. Circulating adrenomedullin does not regulate systemic blood pressure but increases plasma prolactin after intravenous infusion in humans: a pharmacokinetic study. *J Clin Endocrinol Metab* 1997;**82**:95–100.
8. Westphal M, Booke M, Dinh-Xuan AT. Adrenomedullin: a smart road from pheochromocytoma to treatment of pulmonary hypertension. *Eur Respir J* 2004;**24**:518–20.
9. Kono T, Kanematsu T, Kitajima M. Exodus of Kampo, traditional Japanese medicine, from the complementary and alternative medicines: is it time yet? *Surgery* July 30 2009;**146**:837–40.
10. Endo S, Nishida T, Nishikawa K, Nakajima K, Hasegawa J, Kitagawa T, et al. Dai-kenchu-to, a Chinese herbal medicine, improves stasis of patients with total gastrectomy and jejunal pouch interposition. *Am J Surg* 2006;**192**:9–13.
11. Itoh T, Yamakawa J, Mai M, Yamaguchi N, Kanda T. The effect of the herbal medicine dai-kenchu-to on post-operative ileus. *J Int Med Res* 2002;**30**:428–32.
12. Iwai N, Kume Y, Kimura O, Ono S, Aoi S, Tsuda T. Effects of herbal medicine Dai-Kenchu-to on anorectal function in children with severe constipation. *Eur J Pediatr Surg* 2007;**17**:115–8.
13. Suehiro T, Matsumata T, Shikada Y, Sugimachi K. The effect of the herbal medicines dai-kenchu-to and keishi-bukuryo-gan on bowel movement after colorectal surgery. *Hepatogastroenterology* 2005;**52**:97–100.
14. Tokita Y, Yuzurihara M, Sakaguchi M, Satoh K, Kase Y. The pharmacological effects of Daikenchuto, a traditional herbal medicine, on delayed gastrointestinal transit in rat postoperative ileus. *J Pharmacol Sci* 2007;**104**:303–10.
15. Fukuda H, Chen C, Mantyh C, Ludwig K, Pappas TN, Takahashi T. The herbal medicine, Dai-Kenchu-to, accelerates delayed gastrointestinal transit after the operation in rats. *J Surg Res* 2006;**131**:290–5.
16. Satoh K, Kase Y, Yuzurihara M, Mizoguchi K, Kurauchi K, Ishige A. Effect of Dai-kenchu-to (Da-Jian-Zhong-Tang) on the delayed intestinal propulsion induced by chlorpromazine in mice. *J Ethnopharmacol* 2003;**86**:37–44.
17. Satoh K, Hashimoto K, Hayakawa T, Ishige A, Kaneko M, Ogihara S, et al. Mechanism of atropine-resistant contraction induced by Dai-kenchu-to in guinea pig ileum. *Jpn J Pharmacol* 2001;**86**:32–7.
18. Satoh K, Hayakawa T, Kase Y, Ishige A, Sasaki H, Nishikawa S, et al. Mechanisms for contractile effect of Dai-kenchu-to in isolated guinea pig ileum. *Dig Dis Sci* 2001;**46**:250–6.
19. Tokita Y, Satoh K, Sakaguchi M, Endoh Y, Mori I, Yuzurihara M, et al. The preventive effect of Daikenchuto on postoperative adhesion-induced intestinal obstruction in rats. *Inflammopharmacology* 2007;**15**:65–6.
20. Hayakawa T, Kase Y, Saito K, Hashimoto K, Ishige A, Komatsu Y, et al. Effects of Dai-kenchu-to on intestinal obstruction following laparotomy. *J Smooth Muscle Res* 1999;**35**:47–54.
21. Murata P, Kase Y, Ishige A, Sasaki H, Kurosawa S, Nakamura T. The herbal medicine Dai-kenchu-to and one of its active components [6]-shogaol increase intestinal blood flow in rats. *Life Sci* 2002;**70**:2061–70.
22. Kono T, Koseki T, Chiba S, Ebisawa Y, Chisato N, Iwamoto J, et al. Colonic vascular conductance increased by Daikenchuto via calcitonin gene-related peptide and receptor-activity modifying protein 1. *J Surg Res* 2008;**150**:78–84.
23. Hay DL, Howitt SG, Conner AC, Schindler M, Smith DM, Poyner DR. CL/RAMP2 and CL/RAMP3 produce pharmacologically distinct adrenomedullin receptors: a comparison of effects of adrenomedullin22–52, CGRP8-37 and BIBN4096BS. *Br J Pharmacol* 2003;**140**:477–86.
24. Xia CF, Yin H, Borlongan CV, Chao J, Chao L. Postischemic infusion of adrenomedullin protects against ischemic stroke by inhibiting apoptosis and promoting angiogenesis. *Exp Neurol* 2006;**197**:521–30.
25. Eysselein VE, Reinshagen M, Patel A, Davis W, Nast C, Sternini C. Calcitonin gene-related peptide in inflammatory bowel disease and experimentally induced colitis. *Ann N Y Acad Sci* 1992;**657**:319–27.
26. Mazelin L, Theodorou V, Fioramonti J, Bueno L. Vagally dependent protective action of calcitonin gene-related peptide on colitis. *Peptides* 1999;**20**:1367–74.
27. Reinshagen M, Flamig G, Ernst S, Geerling I, Wong H, Walsh JH, et al. Calcitonin gene-related peptide mediates the protective effect of sensory nerves in a model of colonic injury. *J Pharmacol Exp Ther* 1998;**286**:657–61.
28. Reinshagen M, Patel A, Sottili M, French S, Sternini C, Eysselein VE. Action of sensory neurons in an experimental at colitis model of injury and repair. *Am J Physiol* 1996;**270**:G79–86.
29. Kawada M, Arihiro A, Mizoguchi E. Insights from advances in research of chemically induced experimental models of human inflammatory bowel disease. *World J Gastroenterol* 2007;**13**:5581–93.
30. Appleyard CB, Wallace JL. Reactivation of hapten-induced colitis and its prevention by anti-inflammatory drugs. *Am J Physiol* 1995;**269**:G119–125.
31. Zhu Q, Thomson CW, Rosenthal KL, McDermott MR, Collins SM, Gauldie J. Immunization with adenovirus at the large intestinal

mucosa as an effective vaccination strategy against sexually transmitted viral infection. *Mucosal Immunology* 2008;1:78–88.
32. Allaker RP, Kapas S. Adrenomedullin and mucosal defence: interaction between host and microorganism. *Regul Pept* 2003;**112**:147–52.
33. Marutsuka K, Nawa Y, Asada Y, Hara S, Kitamura K, Eto T, et al. Adrenomedullin and proadrenomedullin N-terminal 20 peptide (PAMP) are present in human colonic epithelia and exert an antimicrobial effect. *Exp Physiol* 2001;**86**:543–5.
34. Clavel T, Haller D. Bacteria- and host-derived mechanisms to control intestinal epithelial cell homeostasis: implications for chronic inflammation. *Inflamm Bowel Dis* 2007;**13**:1153–64.
35. Minamino N, Kikumoto K, Isumi Y. Regulation of adrenomedullin expression and release. *Microsc Res Tech* 2002;**57**:28–39.
36. Miksa M, Wu R, Cui X, Dong W, Das P, Simms HH, et al. Vasoactive hormone adrenomedullin and its binding protein: anti-inflammatory effects by up-regulating peroxisome proliferator-activated receptor-gamma. *J Immunol* 2007;**179**:6263–72.
37. Takei Y, Inoue K, Ogoshi M, Kawahara T, Bannai H, Miyano S. Identification of novel adrenomedullin in mammals: a potent cardiovascular and renal regulator. *FEBS Lett* 2004;**556**:53–8.
38. Lin Chang C, Roh J, Park JI, Klein C, Cushman N, Haberberger RV, et al. Intermedin functions as a pituitary paracrine factor regulating prolactin release. *Mol Endocrinol* 2005;**19**:2824–38.
39. Ghosh S, Chaudhary R, Carpani M, Playford R. Interfering with interferons in inflammatory bowel disease. *Gut* 2006;**55**:1071–3.
40. Reinisch W, Hommes DW, Van Assche G, Colombel JF, Gendre JP, Oldenburg B, et al. A dose escalating, placebo controlled, double blind, single dose and multidose, safety and tolerability study of fontolizumab, a humanised anti-interferon gamma antibody, in patients with moderate to severe Crohn's disease. *Gut* 2006;**55**:1138–44.
41. Hommes DW, Mikhajlova TL, Stoinov S, Stimac D, Vucelic B, Lonovics J, et al. Fontolizumab, a humanised anti-interferon gamma antibody, demonstrates safety and clinical activity in patients with moderate to severe Crohn's disease. *Gut* 2006;**55**:1131–7.
42. Kosaka H, Yoshimoto T, Yoshimoto T, Fujimoto J, Nakanishi K. Interferon-gamma is a therapeutic target molecule for prevention of postoperative adhesion formation. *Nat Med* 2008;**14**:437–41.
43. Ohya T, Usui Y, Arii S, Iwai T, Susumu T. Effect of dai-kenchu-to on obstructive bowel disease in children. *Am J Chin Med* 2003;**31**:129–35.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2011, Article ID 418481, 8 pages
doi:0.1093/ecam/nep200

Original Article

Efficacy of Goshajinkigan for Peripheral Neurotoxicity of Oxaliplatin in Patients with Advanced or Recurrent Colorectal Cancer

Toru Kono,[1] Noriaki Mamiya,[1,2] Naoyuki Chisato,[1] Yosiaki Ebisawa,[1] Hirotaka Yamazaki,[3] Jiro Watari,[4] Yasuhiro Yamamoto,[5] Shigetaka Suzuki,[5] Toshiyuki Asama,[1] and Kazunori Kamiya[6]

[1] *Division of Gastroenterologic and General Surgery, Department of Surgery, Asahikawa Medical University, 2-1 Midorigaoka-higashi, Asahikawa, Hokkaido, 078-8510, Japan*
[2] *Division of Cancer Chemotherapy Center, Higashiasahikawa Hospital, Asahikawa, Japan*
[3] *Department of Surgery, Kushiro City Medical Association Hospital, Kushiro, Japan*
[4] *Deptartment of Gastroenterology, Kushiro City Medical Association Hospital, Kushiro, Japan*
[5] *Department of Surgery, Kobayashi Hospital, Kitami, Japan*
[6] *Department of Surgery, Karasawa Hospital, Asahikawa, Japan*

Correspondence should be addressed to Toru Kono, kono@asahikawa-med.ac.jp

Received 9 July 2009; Accepted 26 October 2009

Peripheral neurotoxicity is the major limiting factor for oxaliplatin therapy. Goshajinkigan (GJG), a traditional Japanese herbal medicine, was recently shown to be effective in protecting against the neurotoxicity of taxanes in Japan. We retrospectively investigated the effect of GJG on peripheral neurotoxicity associated with oxaliplatin therapy. Ninety patients with metastatic colorectal cancer that received FOLFOX4 or modified FOLFOX6 therapy were assigned to receive one of the following adjuncts: oral GJG at 7.5 g day^{-1} (Group A, $n = 11$), intravenous supplementation of calcium gluconate and magnesium sulfate (1 g each before and after FOLFOX) (Group B, $n = 14$), combined GJG and calcium gluconate and magnesium sulfate therapies (Group C, $n = 21$), or no concomitant therapy (Group D, $n = 44$). The incidence of peripheral neurotoxicity was investigated when the cumulative dose of oxaliplatin exceeded 500 mg m^{-2}. When the cumulative dose of oxaliplatin exceeded 500 mg m^{-2}, the incidence of neuropathy (all grades) in Groups A–D was 50.0%, 100%, 78.9%, and 91.7%, respectively. It was lowest in the group that received GJG alone. Concomitant administration of GJG reduced the neurotoxicity of oxaliplatin in patients that received chemotherapy for colorectal cancer.

1. Introduction

In recent years, the standard chemotherapy for advanced/recurrent colorectal cancer is a continuous intravenous infusion of 5-fluorouracil (5-FU) combined with either oxaliplatin (FOLFOX, FOLFOX4 or modified FOLFOX6) or irinotecan (FOLFIRI) [1–3]. Acute and persistent peripheral neuropathy is the characteristic of oxaliplatin therapy [4], and the oxaliplatin dose must be limited to avoid toxicity. The prevalence of peripheral neurotoxicity increases with the total accumulated dose of oxaliplatin, and often interferes with the continuation of FOLFOX therapy [5]. Gamelin et al. [6, 7] reported that administration of calcium gluconate and magnesium sulfate (Ca/Mg) before and after oxaliplatin therapy could alleviate peripheral neurotoxicity. Other similar treatments have been described, including carbamazepine [8–10] or glutathione [11], but an effective remedy for peripheral neurotoxicity related to oxaliplatin therapy has not yet been established.

Goshajinkigan (GJG) is an extracted traditional Japanese herbal medicine (Kampo) that is mainly used for the improvement of symptoms like numbness, cold sensation and limb pain associated with diabetic neuropathy [12–15]. Moreover, Mamiya et al. [16] and Shindo et al. [17] recently reported that peripheral neurotoxicity due to oxaliplatin was relieved by administration of GJG in patients with advanced colorectal cancer that were receiving FOLFOX therapy.

We conducted the present retrospective study to compare the efficacy of GJG with that of Ca/Mg for alleviation of peripheral neurotoxicity in patients with advanced or recurrent colorectal cancer that received either FOLFOX4 therapy or modified FOLFOX6 (mFOLFOX6) therapy at our hospital and affiliated institutions in Japan.

2. Patients and Methods

2.1. Patients. This retrospective analysis included 90 patients with advanced or recurrent colorectal cancer that had received either FOLFOX4 or mFOLFOX6 therapy from August 2005 to January 2008 at our hospital and five affiliated institutions. Patients were classified into the following four groups: chemotherapy + GJG, chemotherapy + Ca/Mg, chemotherapy + GJG + Ca/Mg and chemotherapy alone. Full ethical approval for this study has been obtained from all of each responsible Ethics Committees in each hospital according to Japanese Ministry of Health, Labour and Welfare guidelines. All patients provided written informed consent. All records will be kept confidential and the patient's; name will not be released at any time.

2.2. Chemotherapy. On Day 1, patients treated with FOLFOX4 received a 2-h intravenous infusion of oxaliplatin ($85\,mg\,m^{-2}$) combined with levofolinate (1-LV, $100\,mg\,m^{-2}$), followed by a bolus injection of 5-FU ($400\,mg\,m^{-2}$), and then continuous infusion of 5-FU ($600\,mg\,m^{-2}$) for 22 h. On Day 2, 1-LV ($100\,mg\,m^{-2}$) was administered in a 2-h intravenous infusion, and then a bolus of 5-FU was administered ($400\,mg\,m^{-2}$), followed by a 22-h continuous 5-FU infusion ($600\,mg\,m^{-2}$). This regimen comprised one course of therapy and was repeated once every 2 weeks.

On Day 1, patients treated with mFOLFOX6 therapy received a 2-h intravenous infusion of oxaliplatin ($85\,mg\,m^{-2}$) combined with 1-LV ($100\,mg\,m^{-2}$), followed by a rapid intravenous infusion of 5-FU ($400\,mg\,m^{-2}$), and then a 46-h continuous infusion of 5-FU ($2400\,mg\,m^{-2}$). This regimen comprised one course of therapy and was repeated once every 2 weeks.

GJG ($7.5\,mg\,day^{-1}$ divided into 2-3 doses) was administered during FOLFOX therapy, given orally before meals or between meals on a daily basis. Ca and Mg (1 g each) were administered before and after FOLFOX therapy by intravenous infusion.

2.3. Endpoints and Evaluation. Each group was evaluated to determine the total dose of oxaliplatin, the median and mean numbers of courses, the incidence of each grade of peripheral neuropathy, the incidence of peripheral neuropathy when the total dose of oxaliplatin exceeded $500\,mg\,m^{-2}$, the total dose of oxaliplatin at which 50% of patients showed peripheral neuropathy and the time to treatment failure (TTF). Peripheral neuropathy evaluations were based on the Neurotoxicity Criteria of DEBIOPHARM (DEBNTC) [18]. The assessment of the occurrence of peripheral neuropathy in relation to the total dose of oxaliplatin, and the TTF comparisons were based on Kaplan-Meier analyses. The attending physicians assessed the anti-tumor effect of chemotherapy with the new Guidelines for Evaluation of the Response to Treatment in Solid Tumors (RECIST) [19]. In this retrospective study, Groups A–C were compared with Group D (no adjunct treatment) with the log-rank test to identify differences in the incidence of peripheral neuropathy and TTF. Differences observed among groups in the incidence of peripheral neuropathy at a total oxaliplatin dose $>500\,mg\,m^{-2}$ and differences in the anti-tumor activity were assessed with the chi-square test.

3. Results

3.1. Patient Characteristics. There were an unequal number of patients in each group (Table 1). Group D (no concomitant therapy) was the largest group and Group A (GJG alone) was the smallest group. There were no between-group differences of sex (P-values are for Groups A ($P = .890$), B ($P = .223$) and C ($P = .745$) versus Group D by the χ^2-test) and age (P-values are for Groups A ($P = .954$), B ($P = .470$), and C ($P = .790$) versus Group D by the t-test). The performance status (PS) was evaluated with The Eastern Cooperative Oncology Group criteria. A higher percentage of Group A patients had a PS = 1 compared with the other groups. None of the patients in Groups A or B had a PS = 2, but there were no significant differences in PS between the groups (P-values are for Groups A ($P = .373$), B ($P = .316$) and C ($P = .702$) versus Group D by the χ^2-test). There were no differences in the locations of the primary and metastatic tumors (P-values are for Groups A ($P = .498$), B ($P = .431$) and C ($P = .993$) versus Group D by the χ^2-test).

3.2. FOLFOX Therapy. The chemotherapy regimen most commonly administered in Group A was mFOLFOX6, and all of the patients in Groups B and C received mFOLFOX6 therapy. Only Group D had a relatively large number of patients that received FOLFOX4 therapy. Table 2 shows the median and mean total doses of oxaliplatin for each group. Both the median and mean doses were highest in Group A, followed by Groups C, D and B in descending order. Compared with Group D, Group B had a smaller percentage of patients ($P = .004$ by the χ^2-test.) that received a total dose of oxaliplatin exceeding $500\,mg\,m^{-2}$, but there was no difference between the other two groups ($P = .466$ and .366 by the χ^2-test.). Groups A, C and D had similar medians and mean numbers of courses, but Group B had the lowest number of courses (Table 2).

3.3. Peripheral Neuropathy. Kaplan-Meier analyses showed that peripheral neuropathy of Grade 1 or worse (Figure 1) and Grade 2 or worse (Figure 2) occurred less frequently in Group A compared with the other groups; the difference was most marked for neuropathy of Grade 1 or worse. Peripheral neuropathy of Grade 3 (Figure 3) did not occur in either Groups A or C, the two groups that received GJG therapy.

The incidence of peripheral neuropathy at a total oxaliplatin dose $>500\,mg\,m^{-2}$ was lower in the two groups given GJG (Group A and Group C; Table 3). In Group A, there were no cases of Grades 2 or 3 peripheral neuropathy. In Group

Table 1: Patient characteristics for the four groups.

	Group A ($n = 11$)	Group B ($n = 14$)	Group C ($n = 21$)	Group D ($n = 44$)	Total ($n = 90$)
Sex					
Male	7	6	12	27	52
Female	4	8	9	17	38
Age, median (range)	62 (47–78)	61.5 (54–75)	63 (36–82)	64 (43–87)	63 (36–87)
Body weight, median (range)	59 (41–76)	60 (40–75)	58 (38–77)	59 (39–76)	59 (38–77)
PS					
0	7	13	15	38	73
1	4	1	4	3	12
2	0	0	2	3	5
Primary tumor					
Colon	4	5	10	21	40
Rectum	7	9	11	23	50
Metastasis					
Liver	9	9	12	28	58
Lung	3	5	4	18	30
Lymph nodes	0	2	1	1	4
Other	2	4	7	8	21

Group A, GJG; Group B, Ca/Mg; Group C, GJG + Ca/Mg; Group D, no therapy.
GJG, goshajinkigan; Ca, calcium gluconate; Mg, magnesium sulfate; PS, performance status.

Table 2: Details of FOLFOX therapy.

	Group A ($n = 11$)	Group B ($n = 14$)	Group C ($n = 21$)	Group D ($n = 44$)	Total ($n = 90$)
FOLFOX					
FOLFOX4	4	0	0	33	37
mFOLFOX6	7	14	21	11	53
Cumulative oxaliplatin Dose ($mg\,m^{-2}$)					
Median	807.5	500.0	750.0	680.0	680.0
Mean	726.3	534.3	686.7	625.0	632.3
Range	300–850	170–850	180–850	235–850	170–850
Total oxaliplatin dose $\geq 500\,mg\,m^{-2}$	90.9%	42.9%	90.5%	81.8%	78.9%
Percentage of patients in group	($n = 10$)	($n = 6$)	($n = 19$)	($n = 36$)	($n = 71$)
No. of courses					
Median	10.0	6.0	10.0	8.0	8.5
Mean	8.9	6.8	8.8	7.9	8.0
Range	4–10	2–10	3–10	3–10	2–10

Group A, GJG; Group B, Ca/Mg; Group C, GJG + Ca/Mg; Group D, no therapy.
GJG, goshajinkigan; Ca, calcium gluconate; Mg, magnesium sulfate.

B (Ca/Mg alone), the overall incidence of neuropathy was comparable to that in Group D (no concomitant therapy), but Group B had a higher rate of Grade 3 peripheral neuropathy (Table 3).

The total dose of oxaliplatin at which 50% of the patients developed peripheral neuropathy was $765\,mg\,m^{-2}$ in Group A for Grade 1 or worse neuropathy (Table 4). In Group A, 50% level was not reached for Grade 2 or worse neuropathy; this suggested that a higher oxaliplatin dose could be administered to Group A when compared to the other groups before peripheral neurotoxicity occurred.

Table 3: Frequency of peripheral neuropathy at a total oxaliplatin dose of 500 mg m^{-2}.

	Group A, (n = 10)		Group B, (n = 6)		Group C, (n = 19)		Group D (n = 36)
	Percentage	P-value	Percentage	P-value	Percentage	P-value	
All grades	50.0	.002	100	.463	78.9	.178	91.7
Grade 2	0	.130	16.7	.873	5.3	.156	19.4
Grade 3	0	.345	33.3	.080	0	.196	8.3

Group A, GJG; Group B, Ca/Mg; Group C, GJG + Ca/Mg; Group D, no therapy.
P-values are for Groups A, B and C versus. Group D by the χ^2-test.
GJG, goshajinkigan; Ca, calcium gluconate; Mg, magnesium sulfate.
n: numbers are patients received over 500 mg m^{-2} dose of total oxaliplatin.

Table 4: Total dose of oxaliplatin at which 50% of patients developed neuropathy.

	Group A (n = 11)	Group B (n = 14)	Group C (n = 21)	Group D (n = 44)
Grade ≥1	765	255	340	255
Grade ≥2	Not reached	510	765	670
Grade 3	—	850	—	Not reached

Total oxaliplatin doses are shown in mg/m^2.
Grade ≥ 1, Grade 1 or worse neuropathy; Grade ≥ 2, Grade 2 or worse neuropathy;
Group A, GJG; Group B, Ca/Mg; Group C, GJG + Ca/Mg; Group D, no therapy.
GJG, goshajinkigan; Ca, calcium gluconate; Mg, magnesium sulfate.

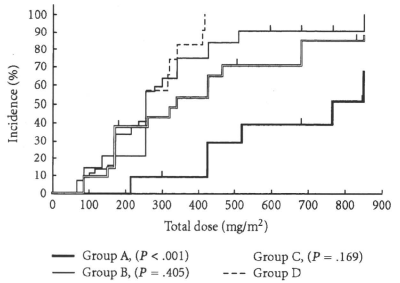

Figure 1: Kaplan-Meier analysis of Grade 1 or worse peripheral neuropathy in relation to the total dose of oxaliplatin. Group A, GJG; Group B, calcium gluconate (Ca) and magnesium sulfate (Mg); Group C, GJG + Ca/Mg; Group D, no therapy. P-values are for comparison to Group D with the log-rank test.

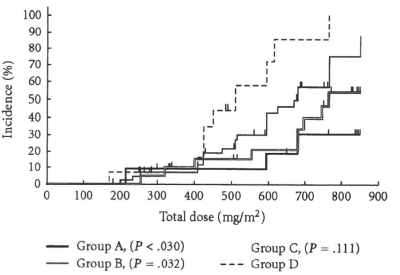

Figure 2: Kaplan-Meier analysis of Grade 2 or worse peripheral neuropathy in relation to the total dose of oxaliplatin. Group A, GJG; Group B, calcium gluconate (Ca) and magnesium sulfate (Mg); Group C, GJG + Ca/Mg; Group D, no therapy. P-values are for comparison to Group D with the log-rank test.

3.4. Treatment Failure and Discontinuation. The TTF was ~2 months longer in the groups given GJG than in the groups without GJG (Figure 4). The rate of discontinuation of treatment due to tumor progression was 18.2% and 20.5% in patients that did not receive Ca/Mg (Groups A and D, resp.), but only 7.1% and 4.8% in those that received Ca/Mg (Groups B and C, resp.) (Table 5). In Group A, no patients discontinued therapy due to peripheral neurotoxicity; in the other groups, the rates ranged from 19.0% to 42.9%. However, Group A showed a higher discontinuation due to hematological toxicity than the other groups. There were no discontinuations due to patient refusal or change of therapy in the groups administered GJG. Also, in Group A, nearly half the patients continued the treatment throughout the study (Table 5).

3.5. Tumor Response. The response rate was 54.5% (6/11) in Group A, 35.7% (5/14) in Group B, 42.9% (9/21) in Group C and 45.5% (20/44) in Group D. The disease control rate (stable disease or better) was 90.9% (10/11) in Group A, 71.4% (10/14) in Group B, 90.5% (19/21) in Group C and 88.6% (39/44) in Group D (Table 6).