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CLINICAL TRIAL

Adrenomedullin for biologic-resistant Crohn's disease: A randomized, double-blind, placebo-controlled phase 2a clinical trial

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Key words
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Abstract

Background and Aim: Adrenomedullin is a bioactive peptide with many pleiotropic effects, including mucosal healing and immunomodulation. Adrenomedullin has shown beneficial effects in rodent models of inflammatory bowel disease and, more importantly, in clinical trials including patients with ulcerative colitis. We performed a successive clinical trial to investigate the efficacy and safety of adrenomedullin in patients with Crohn's disease (CD).
Methods: This was a multicenter, double-blind, placebo-controlled phase 2a trial that evaluated 24 patients with biologic-resistant CD in Japan. Patients were randomly assigned to three groups and were given an infusion of 10 or 15 ng/kg/min of adrenomedullin or placebo for 8 h per day for 7 days. The primary endpoint was the change in the CD activity index (CDAI) at 8 weeks. The main secondary endpoints included changes in CDAI from week 4 to week 24.
Results: No differences in the primary or secondary endpoints were observed between the three groups by the 8th week. Changes in CDAI in the placebo group gradually decreased and disappeared at 24 weeks, but those in the adrenomedullin-treated groups (10 or 15 ng/kg/min group) remained at steady levels for 24 weeks. Therefore, a significant difference was observed between the placebo and adrenomedullin-treated groups at 24 weeks ($P = 0.043$) in the mixed-effects model. We noted mild adverse events caused by the vasodilatory effect of adrenomedullin.
Conclusion: In this trial, we observed a long-lasting (24 weeks) decrease in CDAI in the adrenomedullin-treated groups. Adrenomedullin might be beneficial for biologic-resistant CD, but further research is needed.

Declaration of conflict of interest: Takayuki Matsumoto has received lecture fees from Takeda Pharmaceutical, AbbVie GK, EA pharma, Jansen Pharmaceutical, Mitsubishi Tanabe Pharma, Kyorin Pharmaceutical, Sekisui Medical, and Nippon Kayaku. Hiroshi Nakase has received lecture fees from AbbVie GK, Kissei Pharmaceutical, Kyorin Pharmaceutical, Mitsubishi Tanabe Pharma, Jansen Pharmaceutical, Takeda Pharmaceutical, Pfizer, Celgene, and EA pharma; and research funding from HOYA Group Pentax Medical, Mitsubishi Tanabe Pharma, Pfizer, AbbVie GK, Mochida Pharmaceutical, Takeda Pharmaceutical, Nippon Kayaku, Otsuka Pharmaceutical, and EA Pharma. Satoshi Motoya has received lecture fees from Mitsubishi Tanabe Pharma and Takeda Pharmaceutical, and research funding from EA pharma, AbbVie GK, Takeda Pharmaceutical, and Pfizer. Keiichi Mitsuyama has received research funding from Kirin, Pfizer, and Nippon Kayaku. Tadakazu Hisamatsu has received lecture fees from EA pharma, AbbVie GK, Celgene, Jansen Pharmaceutical, Pfizer, Nichi-iko Pharmaceutical, Mitsubishi Tanabe Pharma, Kyorin Pharmaceutical, and JIMRO; and research funding from Alfresa Pharma, EA Pharma, Mitsubishi Tanabe Pharma, AbbVie GK, JIMRO, Zeria Pharmaceutical, Nippon Kayaku, Takeda Pharmaceutical, Pfizer, and Mochida Pharmaceutical. Masayuki Saruta has received lecture fees from AbbVie GK, Jansen Pharmaceutical, Mitsubishi Tanabe Pharma, Mochida Pharmaceutical, Zeria Pharmaceutical, Takeda Pharmaceutical, Kissei Pharmaceutical, Phizer, and EA Pharma; and research funding from EA Pharma, Kissei Pharmaceutical, Zeria Pharmaceutical, and Mochida Pharmaceutical.

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Introduction

Crohn's disease (CD) is a chronic, intractable inflammatory disease of the gastrointestinal tract, including the small intestine and colon. The number of CD patients is steadily increasing, even in Japan.[1] Intestinal complications and the risk of surgery are gradually increasing in CD patients, and the resulting digestive damage is progressive.[2,3] Recently, there have been made changes in the therapeutic strategies for CD, and the early introduction of biologics is recommended.[3–5] The introduction of biologics such as anti-tumor necrosis factor (TNF) agents has achieved incredible therapeutic effects and may alter the natural history of CD patients.[6] With the rapid growth of biologic therapy, there has been a rapid increase in the number of biologic-resistant cases of CD. The annual risk of loss of infliximab response was reported to be 13% per patient-year.[7] New biologics for different molecular targets are being developed,[8–10] but the loss of response remains challenging. More importantly, therapy for patients with multi-biologic failure is quite challenging.[11] New and effective drugs for biologic-resistant CD are required.

Adrenomedullin (AM) is a biologically active peptide with vasodilating and anti-inflammatory actions. AM also stimulates mucosal regeneration.[12] Our study group and other researchers have confirmed the beneficial effects of AM in experimental rodent models of inflammatory bowel disease (IBD).[13–19] We confirmed the effectiveness of AM in a phase 2a trial involving patients with steroid-resistant UC.[20] Recently, an infliximab-resistant patient with CD received AM administration for 7 days and had recovery of infliximab response.[21] AM might be a potent medication for biologic-resistant CD because it acts via a novel mechanism that is not dependent on excessive immunosuppression and it promotes mucosal regeneration. Additionally, since AM is an endogenous peptide, it is considered safe for patients and could be added to existing biologics. We planned another investigator-initiated trial to obtain proof of concept (POC) that AM may be an alternative medication for biologic-resistant patients with CD.

Methods

Study design. This phase 2a, randomized, double-blind, multi-center, placebo-controlled study was conducted at 16 medical centers in Japan. After approval by the Pharmacological and Medical Device Agency, ethical approval for this study was obtained from the institutional review board of the University of Miyazaki and those of the other centers. This clinical trial was conducted in compliance with the ethical principles of the Declaration of Helsinki, good clinical practice (GCP) of the Japanese Ministerial Ordinance, and other related regulatory requirements. The trial is registered in the JAPIC clinical trials information (JAPIC CTI-183947 [200612912663]).

Subjects. Eligible subjects were patients aged 18–75 years, who had a definitive diagnosis of CD and were receiving maintenance therapy with the same biologics for at least 3 months, without adequate response to the therapy (200 ≤ Crohn's disease activity index [CDAI] < 450). The regimens were limited to the maximum dose for each allowed biologic, such as infliximab 5 mg/kg with a 4-week interval, infliximab 10 mg/kg with an 8-week interval, adalimumab 80 mg/kg with a 2-week interval, and ustekinumab 90 mg/kg with an 8-week interval. The main exclusion criteria were as follows: non-responders to biologics (CDAI ≥ 450), patients with severe CD, patients with a small intestinal lesion that has fear for ileus, patients requiring an emergency operation, patients with malignancy or a history of malignancy, and patients with an active infection. We excluded patients with a systolic blood pressure of < 90 mmHg and a pulse rate of less than 45 bpm for safety reasons, due to the vasodilatory effect of AM. All patients provided written informed consent for all study-related procedures and required hospitalization for 1 week.

Randomization and masking. Eligible patients were enrolled by the principal investigator or a designee based on the above inclusion and exclusion criteria. Patients were randomized to one of three groups at a rate of 1:1:1 to receive a placebo or 10 or 15 ng/kg/min of AM. Randomization was performed by an independent contract research organization, CAC Croit (Sapporo, Japan), using a block size of three, to attain a 1:1:1 ratio of randomization to receive a placebo or one of the two doses of AM. The institute adjusted the allocations.

Screening and evaluation. Patients whose condition did not improve, defined as 200 ≤ CDAI < 450, with a regular period of biologic treatment, were enrolled. Screening tests, including the measurement of vital signs, blood and urine examinations, and 12-lead electrocardiography (ECG), were conducted within 2 weeks prior to the therapy. Demographic data and disease characteristics, including medical history of biologics use, were also collected before randomization. If possible, endoscopic assessment using a simple endoscopic score for Crohn's disease (SESCD) was conducted by on-site investigators and the assessments were not subjected to central review. Clinical disease activity was assessed using a questionnaire administered by expert doctors, according to the patients' CDAI. Response was defined as an improvement of 50 points or more in CDAI, and remission was defined as CDAI ≤ 150. Stool samples were collected before the administration of the investigational drug and after 8 days of treatment initiation. Fecal calprotectin (fluorescence enzyme immunoassay) and immunochemical blood tests (FITs) were performed by a commercially available laboratory testing service (BML, Tokyo, Japan) in compliance with GCP.

Interventions. The AM formulation we used in the present trial is of the same lot as that we used in our phase 2 clinical trial for steroid-resistant UC.[20] After hospitalization, the first administration of the investigational drugs (AM or placebo) was on the day of the administration of the biologic. The patients received continuous infusion of the assigned drug, 8 h per day for 7 days. After the final administration of the investigational drug, the patients were subjected to safety examinations and then discharged. Patients were instructed to visit the hospital 4, 6, and 8 weeks subsequently and undergo testing to obtain their CDAI. After the 8-week follow-up period, patients were asked to permit additional follow-up at 16 and 24 weeks, at which time their CDAI was

assessed. This additional follow-up was discontinued if there were changes in biologic treatment, such as an increase in the dose of biologics or switch to other biologics, or additional drugs, such as steroids or immunosuppressants, were used, or a major accident occurred. Changes in the doses of other drugs, such as aminosalicylic acid, were prohibited throughout the trial.

Data assessment. All data were collected at each institute from May 2018 to March 2020. The primary endpoint was the change in CDAI at 8 weeks. Secondary endpoints included the change in CDAI at 4 and 6 weeks and the rate of clinical response assessed by CDAI at 8 weeks, the rate of clinical response assessed by SESCD at 8 weeks, defined as an improvement in SESCD of at least 50% compared to baseline; changes in fecal calprotectin and FIT at 1 week; and finally, changes in c-reactive protein (CRP) levels at 2, 4, and 8 weeks. Additionally, changes in the plasma concentrations of infliximab or adalimumab were assessed at 8 weeks. In an extended observation of up to 24 weeks, changes in CDAI at 16 and 24 weeks were evaluated.

Safety evaluations, including the evaluation of adverse events (AEs) and serious adverse events (SAEs), were conducted throughout the study. The evaluation of vital signs, blood and urine tests, and 12-lead ECG were performed at specified times after drug administration.

The serum concentration of AM was assessed using an automated enzyme-linked immunosorbent assay (ELISA) system (AIA-1800, Toso, Tokyo, Japan). Samples were collected before and at 4, 8, and 10 h after drug administration on the first and seventh day of administration. Samples were collected at 4 and 8 weeks after the administration. The blood sampling method and the procedure for the measurement of AM concentration have been described.[22] Serum concentrations of infliximab or adalimumab were measured using SHIKARI Q-INFLIXI and SHIKARI Q-ADA ELISA kits (Matriks Biotek, Ankara, Turkey), respectively. Serum concentrations of antibodies for infliximab or adalimumab were measured using Infliximab (Remicade) ADA and Adalimumab (Humira) ADA ELISA kits (Somru BioScience, Charlottetown, Canada), respectively. Samples for the assessment of plasma concentrations of infliximab and adalimumab and their antibodies were collected immediately before the administration of the biologic. All measurements and data processing were performed by the Bozo Research Centre (Tsukuba, Japan). Evaluated pharmacokinetic parameters for AM include the maximum measured plasma concentration (C_{max}), the time to the maximum measured plasma concentration (T_{max}), and the cumulative area under

Table 1 Basal characteristics of the patients

Characteristics	Placebo	Adrenomedullin	
		10 ng/kg/min	15 ng/kg/min
Number of cases	8	8	8
Age (years)	32.6 ± 9.1	39.5 ± 11.1	35.4 ± 8.6
Male/female	5/3	4/4	5/3
Body weight (kg)	49.5 ± 6.4	55.7 ± 10.6	57.3 ± 20.3
Current smoking	2 (25%)	2(25%)	1 (13%)
Disease duration (years)*	12.3 (2.7–30.0)	18.8 (2.0–38.3)	7.7 (1.3–24.3)
Involved intestinal area			
Ileum	1 (13%)	2 (25%)	1 (13%)
Ileum and colon	7 (88%)	5 (63%)	7 (88%)
Colon	0 (0%)	1 (13%)	0 (0%)
Previous segmental resection(s)	2 (25%)	4 (50%)	4 (50%)
Current biologics			
Infliximab 5 mg/kg	3 (38%)	2 (25%)	4 (50%)
Infliximab 10 mg/kg	0 (0%)	1 (13%)	0 (0%)
Adalimumab	2 (25%)	2 (25%)	1 (13%)
Ustekinumab	3 (38%)	3 (38%)	3 (38%)
Other biologics used history			
None	3 (38%)	1 (13%)	2 (25%)
One drug	2 (25%)	4 (50%)	4 (50%)
Two drugs	3 (38%)	3 (38%)	2 (25%)
Immunomodulators			
Azathioprine	3 (38%)	4 (50%)	2 (25%)
Mercaptopurine	1 (13%)	0 (0%)	2 (25%)
Any steroid use	3 (38%)	3 (38%)	3 (38%)
CDAI	295 ± 86	269 ± 65	268 ± 29
SESCD (number of cases)	13.0 ± 4.1 (6)	7.8 ± 4.3 (4)	13.2 ± 6.1 (5)
CRP (mg/dL)	1.74 ± 2.62	1.53 ± 2.76	1.29 ± 1.71
Adrenomedullin (pg/mL)	10.5 ± 1.9	10.6 ± 3.4	9.9 ± 2.8

CDAI, Crohn's disease activity index; CRP, C-reactive protein; SESCD, simple endoscopic score for Crohn's disease.
†Median (range).

the plasma concentration-time curve (AUC) from time 0 to time 10 h.

Data collection and statistical analysis. All data, except for data that were processed at the Bozo Research Centre, were collected using an electronic data collection system (cubeCDMS) and analyzed by Intellim (Tokyo, Japan), an independent contract research organization. All 24 patients completed the 7-day administration of the test drug; thus, all patients were included in the primary full analysis set (FAS) and the safety analysis. Changes in CDAI, SESCD, fecal calprotectin, FIT, and CRP were analyzed using an unpaired *t*-test and analysis of variance (ANOVA), followed by Fisher's multiple comparison test. Clinical response rates were analyzed using chi-square analysis and Fisher's exact test. The changes in CDAI from 0 to 24 weeks were analyzed using a mixed-effects model. The significance level for each test was set at 5%. All analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA). The C_{max} and T_{max} values were obtained directly from the data, while the AUC was calculated using the Phoenix WinNonlin software 6.1 (Pharsight, CA, USA). All data are shown as mean ± standard deviation (SD).

Results

Randomization and clinical characteristics of patients at baseline. All 24 patients completed the 7 days of administration of the test drug. Of the 24 patients, 22 patients completed the 8-week follow-up period. Only one patient (high-dose AM [15 ng/kg/min] group) quit the study before 8 weeks due to low improvement. Another patient (high-dose AM group) quit the study at 8 weeks due to SAE associated with the endoscopic examination, but CDAI was obtained before SAE. The clinical characteristics of the 24 patients who completed the 7-day drug administration, adapted for FAS, are shown in Table 1. No significant differences were observed between the three groups. Notably, 18 of 24 patients (75%) had previously used other biologic(s), and 10 patients (42%) had a history of operation(s) for CD.

Clinical efficacy. We did not observe any differences in the primary endpoint and the changes in CDAI at 8 weeks between the three groups (Table 2). In addition, there were no differences in the changes in CDAI at 4, 6, 16, and 24 weeks (Table 2). The CDAI of one patient in the middle-dose (10 ng/kg/min) AM group

Table 2 Changes in CDAI

	Placebo		Adrenomedullin				
			10 ng/kg/min	15 ng/kg/min		10 + 15 ng/kg/min	
	n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD
Baseline CDAI	8	295 ± 86	8	269 ± 65	8	268 ± 29	16	269 ± 49
Changes in CDAI								
4 weeks	8	−50 ± 103	8	−77 ± 90	8	−64 ± 81	16	−71 ± 83
6 weeks	8	−81 ± 86	7	−79 ± 62	7	−65 ± 76	14	−72 ± 67
8 weeks	8	−69 ± 96	8	−74 ± 77	7	−88 ± 74	15	−81 ± 73
16 weeks	7	−37 ± 127	6	−45 ± 42	4	−84 ± 62	10	−61 ± 51
24 weeks	7	−13 ± 131	6	−82 ± 56	3	−78 ± 26	9	−81 ± 46
P-value (*vs* placebo)*								
4 weeks	8	—	8	0.42	8	0.54	16	0.45
6 weeks	8	—	7	0.43	7	1.00	14	0.74
8 weeks	8	—	8	0.64	7	0.38	15	0.49
16 weeks	7	—	6	0.77	4	0.27	10	0.43
24 weeks	7	—	6	0.21	3	0.41	9	0.13
Response (response/non-response, %)								
4 weeks	8	4/4 (50%)	8	5/3 (63%)	8	5/3 (63%)	16	10/6 (63%)
6 weeks	8	5/3 (63%)	7	4/3 (57%)	7	4/3 (57%)	14	8/6 (57%)
8 weeks	8	6/2 (75%)	8	4/4 (50%)	7	5/2 (71%)	15	9/6 (60%)
P-value (*vs* placebo)**								
4 weeks	8	—	8	1.00	8	1.00	16	0.67
6 weeks	8	—	7	1.00	7	1.00	14	1.00
8 weeks	8	—	8	0.61	7	1.00	15	0.66
Remission (CDAI ≤ 150/others, %)								
6 weeks	8	2/6 (25%)	7	1/6 (14%)	7	2/5 (29%)	14	3/11 (21%)
8 weeks	8	1/7 (13%)	8	2/6 (25%)	7	3/4 (43%)	15	5/10 (33%)
P-value (*vs* placebo)**								
6 weeks	8	—		1.00	7	1.00	14	1.00
8 weeks	8	—		1.00	7	0.28	15	0.37

CDAI, Crohn's disease activity index.
†Analysis of covariance (ANOVA).
‡Fisher's exact test.

at 6 weeks was excluded because the CDAI was calculated using the hemoglobin value at 4 weeks. The response rates of the three groups were similar (Table 2). The number of patients who experienced remission at 8 weeks seemed higher in the AM-treated groups, although there was no statistical difference. The changes in CDAI in AM-treated (10 ng/kg/min or 15 ng/kg/min) groups mostly remained improved for 24 weeks, but the changes in CDAI in most placebo groups diminished gradually and disappeared at 24 weeks (Fig. 1). The backgrounds of the patients were heterogeneous, as shown in Table 1; thus, changes in CDAI were evaluated using a mixed-effects model. The differences between the placebo group and the AM-treated group reached statistical significance at 24 weeks in the mixed-effect model (1.0 ± 33.3 *vs* -95.6 ± 43.3, $P = 0.043$) (Fig. 1, Table S1). Changes in SESCD at 8 weeks were similar in the placebo, middle-dose AM, and high-dose AM groups (−1.3 ± 3.4, 0.3 ± 0.5, −2.0 ± 6.3, respectively). In

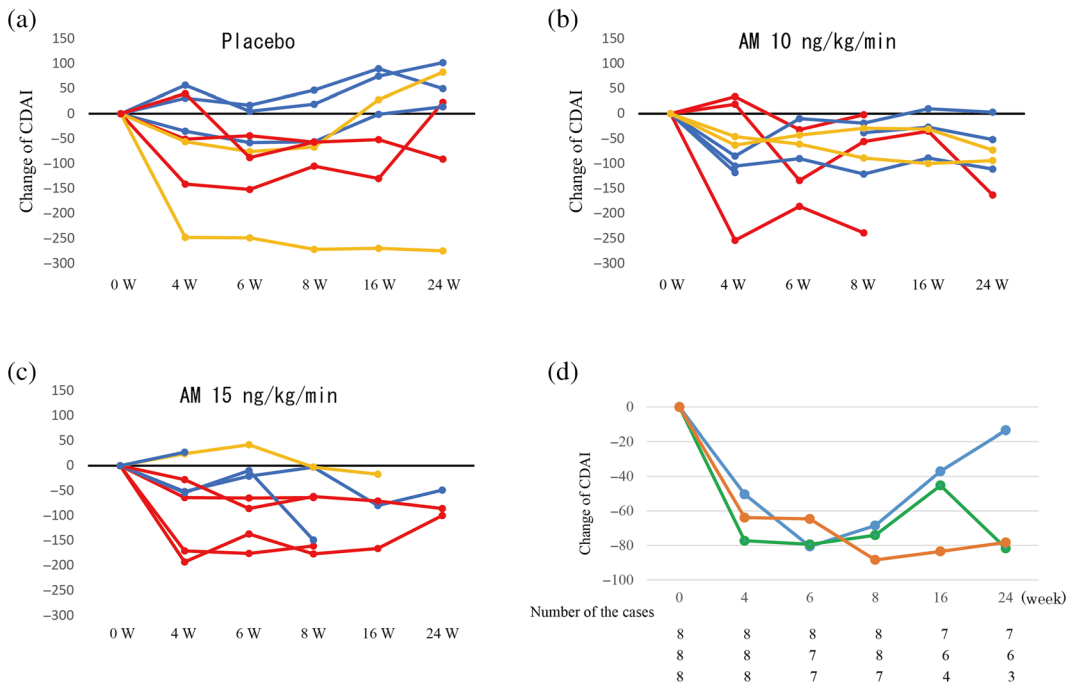

Figure 1 Time course of each change in CDAI of every patients in three groups (a–c). Time course of each change in CDAI in three groups in mixed-effect model (d). (a–c) —●—, Infliximab; —●—, Adalimumab; —●—, Ustekinumab. (d) ●, Placebo; ●, 10 ng/kg/min;●, 15 ng/kg/min. [Color figure can be viewed at wileyonlinelibrary.com]

Table 3 Trough levels of infliximab and anti-infliximab antibody

Infliximab dose	Group	Case	Day 1			4 weeks			8 weeks		
			Infliximab (μg/mL)	Anti-IFX antibody		Infliximab (μg/mL)	Anti-IFX antibody		Infliximab (μg/mL)	Anti-IFX antibody	
				1st Ex	2nd Ex		1st Ex	2nd Ex		1st Ex	2nd Ex
5 mg/kg 4-week interval	Placebo	01–02	11.40	0.0124 (−)	0.0146 (+)	12.06	0.0106 (−)	0.0108 (−)	12.55	0.0107 (−)	0.0101 (+)
		09–01	3.98	0.0378 (−)	0.0120 (−)	4.93	0.0416 (−)	0.0115 (−)	4.13	0.0401 (−)	0.0112 (−)
		16–01	10.37	0.0423 (−)	0.0202 (+)	9.09	0.0563 (−)	0.0114 (−)	11.21	0.0471 (−)	0.0099 (−)
	10 ng/kg/min	04–01	4.90	0.0147 (−)	0.0116 (−)	5.45	0.0165 (−)	0.0141 (+)	7.46	0.0193 (−)	0.0164 (+)
		04–04	21.88	0.0115 (−)	0.0116 (−)	21.36	0.0123 (−)	0.0104 (−)	22.14	0.0123 (−)	0.0121 (−)
	15 ng/kg/min	01–03	5.86	1.1299 (+)	0.5822 (+)	5.18	1.0962 (+)	0.6243 (+)	8.24	0.7770 (+)	0.2582 (+)
		01–04	14.05	0.0415 (+)	0.0212 (+)	16.27	0.0465 (+)	0.0231 (+)	16.25	0.0308 (+)	0.0180 (+)
		08–01	9.95	0.0874 (+)	0.0442 (+)	9.04	0.0699 (+)	0.0350 (+)	8.95	0.0474 (+)	0.0253 (+)
		10–01	21.03	0.0159 (−)	0.0121 (−)	19.17	0.0181 (+)	0.0165 (+)	20.54	0.0227 (+)	0.0176 (+)
10 mg/kg 8-week interval	10 ng/kg/min	01–01	BLQ	> 4.00 (+)	3.1297 (+)	—	—	—	BLQ	> 4.00 (+)	2.9083 (+)

The values of antibody are presented as absorbance and (+) indicates that a value exceeds the cutoff point.
BLQ, below the lower limit of quantification.

addition, the response rates measured by SESCD did not differ. There were no differences in the changes in CRP levels and the changes in fecal calprotectin and FIT (data not shown).

Trough levels of biologics and their antibodies. We measured trough levels of infliximab and adalimumab and their antibodies using the available ELISA kits, but we could not obtain the ELISA kit for ustekinumab. The trough levels of infliximab and adalimumab and their antibodies in each patient are shown in Tables 3 and 4. Contrary to our expectations, all patients maintained relatively high trough levels of biologics, except for one patient (case 01-01). The antibody concentrations in the patients, except for the above patient, were marginal; thus, we repeatedly confirmed the values of anti-biologic antibodies. The trough levels of biologics did not change after AM administration (Tables 3 and 4).

Plasma concentration of adrenomedullin. Plasma concentrations of AM were similarly increased after AM administration, as previously reported in patients with UC.[20] The increase in plasma concentration of AM was dose-dependent and showed good reproducibility (Fig. 2). The increased plasma concentration of AM mostly returned to basal levels within 2 h after the termination of AM infusion. The C_{max} at 7 days in the middle-dose and high-dose AM groups were 34.8 ± 9.8 and 65.3 ± 43.0 pg/mL, respectively. The AUC_{0-10h} after 7 days in the middle-dose and high-dose AM groups was shown to be 247.3 ± 60.0 and 440.9 ± 244.9 h*pg/mL, respectively. The AUC_{0-10h} after 7 days

Table 4 Trough levels of adalimumab and anti-adalimumab antibody

Group	Case	Day 1			4 weeks			8 weeks		
		Adalimumab (μg/mL)	Anti-ADA antibody		Adalimumab (μg/mL)	Anti-ADA antibody		Adalimumab (μg/mL)	Anti-ADA antibody	
			1st Ex	2nd Ex		1st Ex	2nd Ex		1st Ex	2nd Ex
Placebo	10–02	17.43	0.0093 (–)	0.0104 (–)	18.17	0.0092 (–)	0.0098 (–)	20.17	0.0103 (–)	0.0090 (–)
	14–03	4.97	0.0106 (–)	0.0113 (–)	6.03	0.0114 (–)	0.0169 (+)	4.67	0.0191 (+)	0.0096 (–)
10 ng/kg/min	14–02	7.88	0.0260 (–)	0.0090 (–)	7.64	0.0209 (–)	0.0086 (–)	7.52	0.0288 (+)	0.0099 (–)
	15–01	18.32	0.0284 (+)	0.0086 (–)	22.77	0.0191 (–)	0.0098 (–)	22.56	0.0185 (–)	0.0131 (+)
15 ng/kg/min	14–01	8.36	0.0127 (–)	0.0099 (–)	8.12	0.0137 (–)	0.0087 (–)	7.72	0.0172 (–)	0.0093 (–)

The values of antibody are presented as absorbance and (+) indicates that a value exceeds the cutoff point.

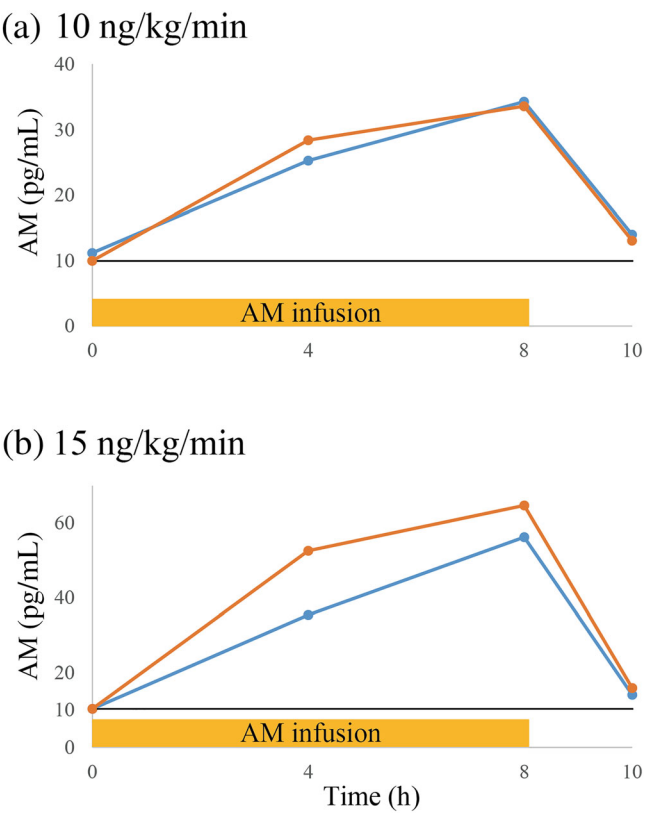

Figure 2 Mean plasma concentration-time profiles of adrenomedullin (AM) at 1 and 7 days of repeated infusion. (a, b)—●—, day 1; —●—, day 7. [Color figure can be viewed at wileyonlinelibrary.com]

in the high-dose AM group was significantly higher than that in the middle-dose AM group ($P < 0.05$).

Safety assessments. All 24 patients completed the 7-day administration of the test drug and were thus eligible for safety assessments. The reported AEs are summarized in Table 5. Symptoms related to the vasodilatory effect of AM, such as headaches, palpitations, and flushes, were more frequently observed in the AM-treated groups than in the placebo group. SAE, namely bacteremia, occurred after an endoscopic examination which was conducted after the 7-day AM administration in the high-dose AM group. The patient was treated in the hospital; he recovered and remained stable with regular biologic treatment. In the past, the patient had similar episodes of bacteremia after endoscopic examination; therefore, we considered that this SAE was not related to AM administration. All other symptoms listed in Table 5 were mild and tolerable. We did not observe any statistically significant changes in blood pressure and pulse rate in all groups throughout the drug administration period (data not shown). No deaths occurred during the trial.

Discussion

This is the first randomized, placebo-controlled, phase 2a trial of AM in Japanese patients with biologic-resistant CD. In this trial, the test drug and biologics were simultaneously administered, and similar decreases in CDAI were observed in both the AM-treated group and the placebo group within 8 weeks. Therefore, no difference in the primary and secondary endpoints was found between the AM-treated group and the placebo group. The decrease in CDAI in the placebo group disappeared at 24 weeks, but the AM-treated group maintained a decrease in CDAI for 24 weeks; thus, the differences were statistically significant at 24 weeks in the mixed-effects model. Except for one patient in the high-dose AM group, all patients exhibited high tolerance for AM, with no AM administration-related SAE.

This trial challenged the unmet need to assess very difficult cases of CD in which there is a history of biologic failure (75%) and/or a history of surgery (42%), and long disease duration. Additionally, six of 24 patients (25%) were treated with these biologics first and currently, the effects of these biologics have not reached a sufficient level (Table 1). These refractory patients progressively showed resistance to every treatment. As a result, AM treatments were not statistically different compared to those of placebo in CDAI, CRP, and fecal markers within 8 weeks. The situation changed after 16 weeks, where differences in changes in CDAI between the placebo and AM-treated groups gradually increased. Notably, almost all patients including early-dropped patients who were treated by AM remained in an improved state, as shown by minus changes in CDAI (Fig. 1). This progress seems to be an unpopular phenomenon that cannot be easily explained. However, the favorable effect of AM for highly refractory patients with CD seems attractive; thus, a future large-scale trial will be expected.

Measurement of serum trough level and antibodies to infliximab (ATI) is crucial for good management.[23] High trough levels of infliximab were related to favorable clinical outcomes in patients with CD receiving regular maintenance therapy.[24] The threshold of the clinical responses in major trials for rheuma-

Table 5 Summary of reported adverse events

	Placebo[8]	Adrenomedullin	
		10 ng/kg/min[8]	15 ng/kg/min[8]
Any adverse events	4 (50%)	7 (88%)	8 (100%)
Moderate to severe adverse events	1 (13%)	1 (13%)	3 (38%)
Serious adverse events	0	0	1 (13%)
Deaths	0	0	0
Major adverse events			
Nervous system disorders			
Headache	0	3 (38%)	7 (88%)
Head discomfort	0	0	1 (13%)
Dizziness	0	1 (13%)	0
Cardiovascular disorders			
Palpitation	0	1 (13%)	2 (25%)
Flushing	1 (13%)	1 (13%)	1 (13%)
Right bundle branch block	0	0	1 (13%)
Others			
Vomiting	0	0	1 (13%)
Dehydration	0	0	1 (13%)
Increase in transaminase level	0	0	1 (13%)
Fever up	1 (13%)	0	3 (38%)
Nasopharyngitis	0	1 (13%)	2 (25%)
Lumbago	1 (13%)	0	0
Rash	0	1 (13%)	1 (13%)
Decrease in body weight	1 (13%)	0	0

toid arthritis was 1 μg/mL,[25,26] and the same value was also observed in CD.[27] The presence of ATI does not relate to a lower response rate in CD,[28] but ATI may reduce the trough level of infliximab and the effective period of regular administrations of infliximab.[29] We expected low trough levels of infliximab and relatively high levels of ATI in the patients who participated in this trial. However, all patients except for case 01-01 showed sufficient trough levels of infliximab and marginal levels of ATI. Originally sufficient levels of infliximab were not changed by AM treatment. A similar phenomenon was observed in patients treated with adalimumab.

The elevation of plasma AM levels has been suggested as an endogenous counter-factor for CD. Similarly, as previously reported in UC,[20] plasma concentration of AM was higher in patients with CD than in healthy volunteers in the phase 1 single-dose study (10.4 ± 2.6 pg/mL (n = 24) *vs* 7.2 ± 1.4 pg/mL (n = 23); $P < 0.0001$).[22] Significant changes in the plasma concentration of AM were not observed at 4 and 8 weeks in all groups (data not shown). The time duration of increasing AM concentrations after the administration of middle or high doses of AM in CD patients were also similar to those in UC patients (Fig. 2).[20]

This study had several limitations. First, the backgrounds of the patients were heterogeneous. In particular, concomitant biologics were varied, and ustekinumab, which suppresses different targets from TNF, was administered to 38% of the patients. Unmatched patient characteristics may decrease the detection power for the effect of AM. Second, five of eight patients in the high-dose AM group dropped out of the trial by 24 weeks. Although three of the five drop-out patients maintained good condition at 24 weeks, this loss also decreased the detection power. Therefore, statistical differences were detected only in the comparison between the active and placebo groups, using the mixed-effects model at 24 weeks. Finally, the endoscopic evaluations were not subjected to central review; therefore, inter-observer variations might have been unavoidable.

In conclusion, despite the limited number of patients, we observed sustained improvement at 24 weeks after 1-week administration of AM with biologics in biologic-resistant patients with CD. AM might be beneficial for biologic-resistant CD; however, our data are insufficient, and thus, further reconfirmation of our findings in a future large-scale trial is necessary.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1: Changes of CDAI in the mixed-effects model.