Safety of Emulsifying Lipid Formulation Containing Omega-3 Polyunsaturated Fatty Acids for Patients with Crohn's Disease

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Abstract. *Background/Aim: The efficacy of omega-3 supplementation by oral capsule for patients with Crohn's disease (CD) remains controversial. We investigated the safety and efficacy of an omega-3 emulsified formulation. Patients and Methods: Six patients with CD in remission participated in this open-label clinical trial. Patients ingested one bottle (100 ml) of the test formulation (IMARK S®) daily for 28 days. After a 1-month washout period, patients ingested two bottles of the formulation daily for 28 days. Anthropometric and blood tests were performed before and after each intervention. Results: The omega-3 emulsifying formulation was safe with minimal side-effects. Body weight and body-mass index were not altered; however, CD activity index scores tended to decrease after ingested one bottle of formulation. Blood tests revealed no severe adverse effects. Conclusion: Supplementation with an omega-3 emulsifying formulation can be safe and useful for maintaining remission in patients with CD and warrants further studies.*

Crohn's disease (CD) and ulcerative colitis, generally known as inflammatory bowel disease (IBD), are chronic, relapsing and refractory disorders of the intestine (1, 2). Some patients exhibit severe intestinal and extraintestinal complications, such as fistula, stenosis and skin lesions, which can impair quality of life. Chronic inflammation is associated with increased risk of some cancers in patients with IBD (3-8). Laharie *et al.* (9) reported that patients with CD who have not achieved clinical remission have a significantly higher clinical relapse rate. To increase the quality of life and decrease the risk of cancer for IBD patients, various therapeutic approaches to induce and maintain clinical remission have been investigated (2, 10, 11).

Patients with severe IBD, especially CD, have low body weight and low body fat (12), which may be due to nutritional therapies containing low levels of fat and fiber, decreased consumption and malabsorption due to intestinal inflammation and/or self-dietary management considering symptoms, such as diarrhea and abdominal pain. Patients are recommended to ingest low fiber and fat to maintain remission, since intake of excess fiber and animal fat can exacerbate inflammation. Our group recently reported that nutritional status affects the therapeutic effects of infliximab in patients with CD (13), indicating that appropriate nutritional support can help ameliorate intestinal inflammation.

N-3 polyunsaturated fatty acids (n-3 PUFA, also called omega-3 fatty acids) have a double bond (C=C) at the third carbon atom from the end of the carbon chain (14), which includes α-linoleic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Omega-3 fatty acids have various physiological effects, including reduction of serum triglyceride levels, blood pressure and heart rate; improvement of endothelial function; and anti-arrhythmic and anti-inflammatory effects (14-20). These functions derive from eicosanoids converted from EPA and DHA. Wiktorowska-Owczarek *et al.* (21) described the functional mechanism of PUFAs as follows. Eicosanoids (prostaglandins and leukotrienes) are products of ALA and EPA acid metabolism. Eicosanoids derived from ALA biosynthesize prostaglandin E_2 (PGE_2), which is an inflammatory mediator; prostacyclin I_2 (PGI_2), responsible for blood vessel dilation; and thromboxane A_2 (TXA_2), which activates blood platelet aggregation and vasospasm. The 4-series leukotrienes are formed by the actions of lipoxygenase and play crucial roles in the development and maintenance of the inflammatory response. On the other

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hand, EPA is metabolized in a similar way with participation of the same enzymes; however, their metabolic products are different: 3-series prostanoids and 5-series leukotrienes are formed and they have different functions, such as anti-inflammatory (PGE_3, LTA_5, LTB_5, LTC_5, LTD_5), anti-aggregatory (TXA3) and vasodilative (PGI3) effects (21). PUFA derived from omega-6 comprises most of the ingested fatty acids; the intake ratio of omega-6 to that of omega-3 is almost 4:1 in Japanese (22). The elemental diet (ED) is an essential nutritional therapy with no side-effects that is widely used for patients with CD (23, 24), especially those of younger age (25, 26). ED contains almost no or very low amounts of omega-3 fatty acid. Even patients with CD in remission, using these enteral nutrients, may not take in adequate amounts of omega-3.

Various types of dietary supplements containing omega-3 fatty acids have recently come on the market. While some studies of omega-3 supplementation by capsule for patients with CD have demonstrated efficacy, the results are controversial (27). On the other hand, very few studies have examined the efficacy of an emulsifying lipid formulation for patients with CD. Emulsifying lipids are more efficiently absorbed by the intestine than non-emulsifying lipids (28). Here, we planned a preliminary study to clarify the safety and efficacy of an omega-3 emulsified formulation in a small number of patients prior to performing a large intervention. As the primary end point, we investigated the safety and optimum dosage of the emulsifying lipid formulation containing omega-3 for patients with CD in remission. Other effects of the emulsifying omega-3 formulation, such as anti-inflammatory effects and body weight, as well as body fat reduction, were also investigated in the present study.

Patients and Methods

The present study was approved by the ethics committee of Osaka University Hospital in 2014 (UMIN #000012585). Patients with CD in remission, defined as a Crohn's disease clinical activity index (CDAI) of less than 150 (29), were recruited. One subject (Subject 6) with CDAI of 324.52 was also included because the high value of CDAI of the subject was due to habitual frequent defecation, not by active disease, and the patient was clinically and endoscopically regarded as remission. All patients experienced intestinal resection before intervention and stayed in remission state. Written informed consent was obtained from the six patients. Patients treated with enteral nutrients except for the elemental diet (Elental®, AJINOMOTO PHARMACEUTICALS CO., INC., Tokyo, Japan), steroids and anti-tumor necrosis factor-α therapy at the time of recruitment were excluded.

Protocol therapy. This study was an open-label study to evaluate the safety of the testing formulation per dose. The testing formulation (IMARK S®), which is commercially available and contains high amounts of omega-3, was provided by Nippon Suisan Kaisha, Ltd. (Tokyo, Japan). The nutritional composition and raw materials of the formulation are indicated in Table I. The subjects ingested one bottle (100 ml) of the testing formulation daily for 28 days. After a 1-month washout period, subjects ingested two bottles daily for 28 days. The protocol of the present study is shown in Figure 1.

Table I. *Nutritional composition and raw material of testing formulation per bottle (100 ml).*

Nutritional	Composition	Content
Energy (kcal)	26	
Protein (g)	0	
Fat (g)	2.7	
	EPA(mg)	600
	DHA(mg)	260
	Oleic acid (mg)	180
	Linoleic acid (mg)	30
Carbohydrate (g)	0.5	
Sodium (mg)	65	

Raw Material:
purified fish oil, emulsifier, acidulant, flavoring, antioxidant, sweetener

EPA, Eicosapentaenoic acid ; DHA, docosahexaenoic acid.

Assessment. The CDAI score is the most prevalent index for evaluating CD disease activity (29). We recorded the CDAI score and performed anthropometric and blood tests before and after each intervention (4 evaluation points). Blood tests included white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Ht), platelet (Plt), lymphocyte count (Lympho), C-reactive protein (CRP), albumin (Alb), total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-bil), cholinesterase (ChE), estimated glomerular filtration rate (e-GFR), creatinine (Cre), blood urea nitrogen (BUN), total cholesterol (T-cho), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). Abnormalities of these parameters were evaluated and recorded. To investigate the subjective symptoms, a nutritional inquiry to evaluate adverse events was performed before the first and after the last intervention (points 1 and 4).

Statistical analysis. Numerical data are presented as median (range). Statistical analysis was performed using the JMP Pro10 (SAS Institute Inc., Cary, NC, USA). A paired *t*-test was used to compare values before and after the intervention. A p-value of <0.05 was considered statistically significant. Data for one case in which the subject withdrew from the study were not included in the statistical analysis.

Results

Six subjects were enrolled into the study. One subject (Subject 4) withdrew from the study after the evaluation at point 1 due to discomfort associated with colostomy. None of the other subjects had stomas. The flow chart of procedures is shown in Figure 1. The characteristics of each subject at baseline (point 1) are shown in Table II.

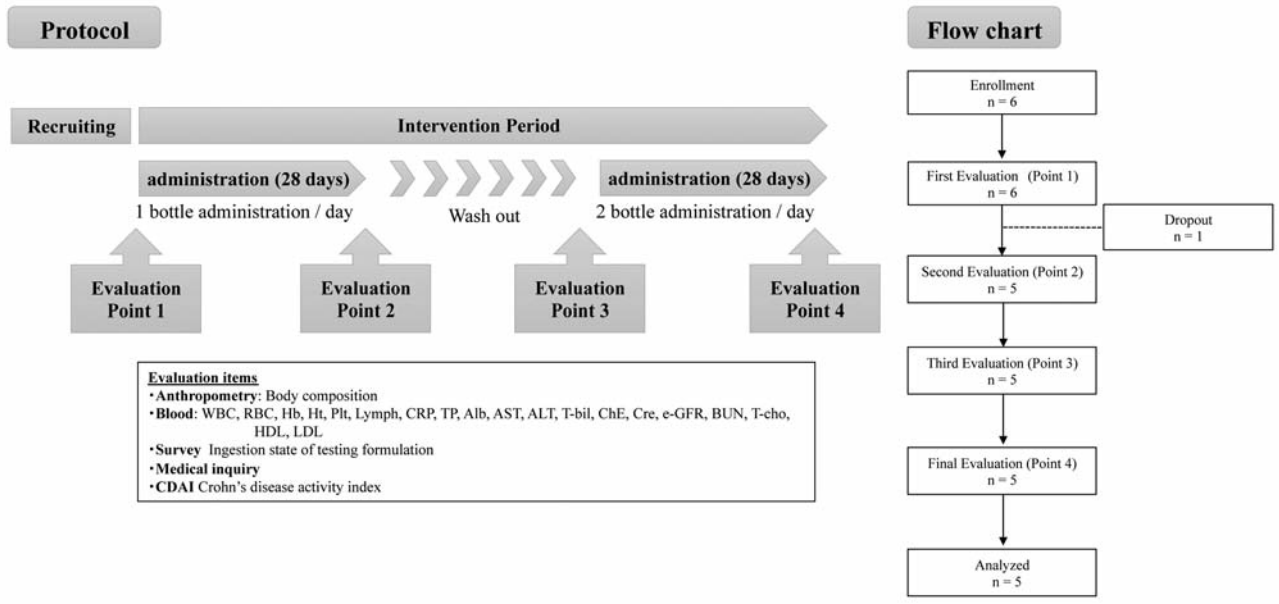

Figure 1. *Protocol and flow chart of the study. Protocol: Evaluations before therapy were performed (point 1). After a 28-day intervention (ingestion of one bottle of testing formulation), evaluations were performed again (point 2). After a 28-day washout period, a third evaluation was performed (point 3). Then, a second intervention (ingestion of 2 bottles of testing formulation) was performed for 28 days. After intervention, final evaluation was performed (point 4). Flow chart: After point 1 evaluation, one subject withdrew due to discomfort associated with colostomy. Five other patients completed the schedule set. BMI, Body mass index; CDAI, Crohn's disease clinical activity index; WBC, white blood cell count; RBC, red blood cell count; Hb, hemoglobin; Ht, hematocrit; Plt, platelet; Lympho, lymphocyte count; CRP, C-reactive protein; Alb, albumin; TP, total protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-bil, total bilirubin; ChE, cholinesterase; e-GFR, estimated glomerular filtration rate; Cre, creatinine; BUN, blood urea nitrogen; T-cho, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.*

To evaluate the possibility of hepatotoxicity, nephrotoxicity and other organ toxicities, anthropometric measurements and blood tests were performed. The results of the anthropometric measurements and blood tests are shown in Table II. In the anthropometric category, however, there were no changes in body weight or body mass index. The blood test results indicated no severe changes that threatened safety. Significant changes were recorded in e-GFR, Cre and Alb from point 3 to point 4 but these changes were within the standard range and not considered clinically significant. In the subjective data, there were no significant changes except for an increase in stool frequency (+2 [-1-+3], compared to point 1) in some subjects (Table III). As shown in Figure 2, CDAI scores tended to decrease from point 1 (105.7 [27.16-324.52]) to point 2 (61.51 [35.94-201.92]); however, the difference was not significant. On the other hand, no remarkable changes in CDAI scores were observed from point 3 (61.06 [28.16-181]) to point 4 (66 [34.81-213]).

Discussion

To establish a protocol, the present study was undertaken as a preliminary trial prior to a large study to investigate the safety of an emulsified formulation containing omega-3 for patients with CD in remission. We, therefore, performed the present study to clarify the safety and investigate the dose setting. Ingesting two bottles of the testing formulation per day led to a slightly increased CDAI score that was not statistically significant. Some subjects complained of an increase in the number of episodes of diarrhea and difficulty ingesting two bottles of the emulsifying formulation (containing 1,200 mg of EPA and 520 mg of DHA) per day. Even one daily bottle of supplementation (containing 600 mg of EPA and 260 mg of DHA) tended to decrease the CDAI score. Taken together, these data suggest that one daily bottle of supplementation is enough to maintain remission and avoid adverse effects of PUFA supplementation.

There are some reports demonstrating no benefit of omega-3 fatty acids. Lorenz-Meyer *et al*. (30) performed a randomized controlled trial on 204 patients with CD and demonstrated no effect of omega-3 fatty acids for extending remission. Similarly, Feagan *et al*. (31) performed two randomized, double-blind, placebo-controlled trials with 363 and 375 patients with CD, respectively. They also demonstrated that treatment with omega-3-free fatty acids was not effective for the prevention of relapse in patients with

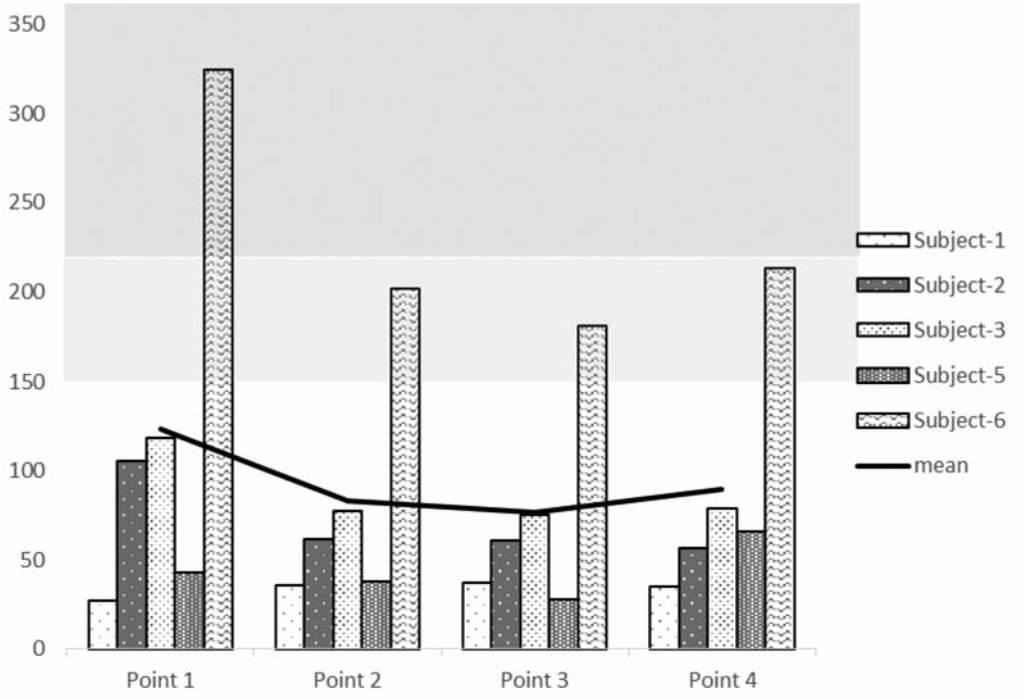

Figure 2. *Disease activity was defined by Crohn's disease clinical activity index (CDAI) score: mild (150-220), moderate (220-450), severe (>450). CDAI score of each subject is indicated in bar graph. Mean data of all subjects is indicated by the line graph.*

Table II. *Patients' characteristics are indicated for each subject at baseline. Case No.4 withdrew from this study due to difficulty in ingesting the test formulation after point 1 evaluation.*

Case No.	Age (recruited point)	Gender	Disease duration (years)	Disease location	Disease behavior	Medical before inclusion	Postoperative (years)	Surgery before inclusion	CDAI	CRP
1	67	F	27	Ileitis	Nonperforating	ED, 5-ASA	4.87	Ileosecal resection	27.16	0.02
2	44	M	0	Ileitis	Perforating	ED	0.74	Partial resection of S-shaped colon Appendectomy	105.7	0.42
3	54	M	25	Ileitis	Perforating	ED	1	Partial resection of small intestine Ileosecal resection Fistula resection	118.45	0.1
5	29	M	13	Ileitis	Perforating	ED, 5-ASA	2.18	Ileosecal resection	42.89	0.05
6	34	M	2	Ileitis	Perforating	ED, 5-ASA	1.37	Partial resection of ileosecal and ileum	324.52	0.02

ED, Elemental diet; 5-ASA, 5-aminosalicylic acid; CDAI, Crohn's disease clinical activity index; CRP, C-reactive protein.

CD. On the other hand, Belluzzi *et al*. (32) performed a 1-year, double-blind, placebo-controlled trial for 78 patients with CD in remission. They reported that omega-3 supplementation had significant effects to reduce the rate of relapse (32). As described in the review by Lev-Tzion *et al*. (27), although some large trials have demonstrated efficacy, the effects of a capsular form of omega-3 supplementation remain controversial. In the present study, we used a testing drug that was an emulsifying liquid. Raatz *et al*. (28) reported that a single dose of emulsified fish oil enhanced the absorption of total EPA and DHA, as evidenced by changes in the phospholipid fatty acid composition compared to a capsular triglyceride fish oil. Considering that the capsule form of the testing drugs was used in their study, a trial using an emulsifying formulation with different drug delivery system from the conventional capsular form is warranted.

The present investigation had some limitations. Insufficient collection of objective evaluation data, such as

Table III. *Change in anthropometric and blood data from point 1 to point 4. Statistical significance of changes between each evaluation point was evaluated.*

Parameters				Point 1 (n=5) Median (range)	Point 2 (n=5) Median (range)	Point 3 (n=5) Median (range)	Point 4 (n=5) Median (range)
Subjective	Stool frequency		Times/day	baseline 0	0 (-1-2)	0 (-1-1)	2 (-1-3)
	Stool state			1 (0-2)	1 (0-2)	1 (0-2)	1 (0.5-2)
	Skin condition			1 (1-2)	1 (0-1)	1 (0-1)	0 (0-2)
Objective	Anthropometric	Body Weight	(kg)	56.8 (48.7-64.9)	56.7 (49.5-63.7)	54.5 (49.5-63.1)	54.7 (50.7-63.6)
		Body Fat	(%)	14.2 (9.9-30.6)	14.3 (10.9-28)	13.3 (10.3-28.3)[d]	13.8 (12.2-29.3)[d]
		BMI	(m^2/kg)	20.79 (17.9-22.6)	20.5 (18.2-22.5)	19.9 (18.2-21.7)	20.4 (18.6-21.7)
	Blood	CDAI		105.7 (27.16-324.52)	61.51 (35.94-201.92)	61.06 (28.16-181)	66 (34.81-213)
		WBC	($\times10^9$/l)	5.64 (4.44-8.28)	4.39 (3.17-5.71)	4.91 (3.64-6.97)	5.22 (3.74-7.42)
		RBC	($\times10^{12}$/l)	5.2 (4.56-5.27)	4.9 (4.65-4.93)	4.97 (4.73-5.34)	4.935 (4.6-5.43)
		Hb	(g/dl)	13.8 (13-14.4)	13.2 (12.7-15.2)	13.9 (12.8-14.8)	13.8 (12.7-14.4)
		Ht	(%)	43.2 (41.7-44.1)	42.6 (39.6-44.3)	43.1 (40.4-45.9)	42.1 (41-44.5)
		Plt	($\times10^9$/l)	241 (174-374)	248 (168-382)	246 (156-400)	238.5 (225-361)
		Lympho	(%)	20.1 (15.6-38.4)	27.6 (20.3-38.9)	24.9 (17.1-37.3)	24.6 (20.9-37.4)
		CRP	(mg/dl)	0.05 (0.02-0.42)	0.02 (0.02-0.79)	0.04 (0.02-0.52)	0.02 (0.02-0.65)
		Alb	(g/dl)	4.2 (4.1-4.3)	4 (3.8-4.3)	4.3 (4.2-4.5)d	4.1 (3.9-4.4)d
		TP	(g/dl)	6.8 (6.6-7.2)	6.6 (6.1-6.9)	7 (6.7-7.1)	6.7 (6.5-6.8)
		AST	(U/l)	27 (20-32)	28 (14-30)	25 (17-32)	23 (17-32)
		ALT	(U/l)	22 (17-54)	25 (11-43)	16 (11-52)	19 (15-54)
		T-bil	(mg/dl)	0.6 (0.5-0.9)	0.6 (0.5-0.8)	0.8 (0.6-0.9)	0.5 (0.4-0.7)
		ChE	(U/l)	306 (282-385)	297 (271-376)	295 (288-295)	291 (280-385)
		e-GFR	(ml/min/1.73 m^2)	77.8 (63.1-82.8)	79.3 (57.3-87.6)	75.8 (63.1-83.8)[d]	79.8 (63.8-91.7)[d]
		Cre	(mg/dl)	0.89 (0.58-0.98)[a]	0.86 (0.57-1.07)[a]	0.89 (0.64-0.98)[d]	0.82 (0.61-0.97)[d]
		BUN	(mg/dl)	14 (9-15)	12 (9-20)	12 (9-18)	13 (8-15)
		T-cho	(mg/dl)	147 (95-163)	141 (94-161)cc	145 (98-183)	145 (101-167)[cc]
		HDL	(mg/dl)	41 (36-75)	44 (33-72)	40.5 (37-86)	40 (34-76)
		LDL	(mg/dl)	73.5 (44-92)	77 (46-87)	75.5 (48-86)	73 (46-88)

Stool frequency: change from baseline (0; point 1), "+", increase, "-", decrease.
Stool state: 0: normal stool, 1: loose stool, 2: diarrhea.
Skin condition, 0: normal skin, 1: dry skin, 2: severely dry skin
[a]$p<0.05$ between points 1 and 2.
[b]$p<0.05$ between points 1 and 4.
[c]$p<0.05$ between points 2 and 4. (cc: $p<0.005$ between points 2 and 4).
[d]$p<0.05$ between points 3 and 4.
BMI, Body mass index; CDAI, Crohn's disease clinical activity index; WBC, white blood cell count; RBC, red blood cell count; Hb, hemoglobin; Ht, hematocrit; Plt, platelet; Lympho, lymphocyte count; CRP, C-reactive protein; Alb, albumin; TP. total protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-bil, total bilirubin; ChE, cholinesterase; e-GFR, estimated glomerular filtration rate; Cre, creatinine; BUN, blood urea nitrogen; T-cho, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

by endoscopy and computed tomography, were obtained. Furthermore, the timing of the intake was not completely consistent. In addition, the exact dosage was recorded using a confirmation form, but the number of empty bottles was not counted. Further, the present investigation was performed with only a small sample size, which was insufficient to completely elucidate the effects of the formulation containing omega-3 for maintaining the remission state.

In conclusion, our results suggested that an emulsifying formulation containing omega-3 could be safely applied with minimal side-effects for patients with CD in remission. Furthermore, these results suggest that this therapy could be useful for maintaining remission in patients with CD. Although further studies with a longer duration and a larger number of subjects are required to investigate whether the formulation is effective for maintaining remission, an emulsifying omega-3 formulation is one option for adjuvant therapy to maintain remission in patients with CD.

Conflicts of Interest

Nippon Suisan Kaisha, Ltd. provided the IMARK S® testing formulation. The Department of Integrative Medicine, Osaka University Graduate School of Medicine and the Department of

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