RESEARCH ARTICLE

Oral administration of docosahexaenoic acid attenuates colitis induced by dextran sulfate sodium in mice

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Scope: Dietary supplementation of n-3 PUFAs, containing docosahexaenoic acid (DHA), modulates the symptoms of colitis. Hence, we investigated the effects of oral administration of pure DHA and the therapeutic agent sulfasalazine (SAL) on chemically induced colitis in mice, and analyzed the expression levels of DHA-responsive genes in colonic tissue using cDNA arrays.

Methods and results: Colitis in BALB/c mice was induced by feeding 5% dextran sulfate sodium (DSS) in drinking water for 7 days. DHA (30 mg/kg/day, DHA) or SAL (100 mg/kg/day, SAL) was administered orally throughout the treatment along with DSS. The DHA-treated group showed significant reduction of the weight loss and colon shortening compared to the DSS-treated colitis group. In contrast, SAL treatment was effective in reducing colon shortening, stool consistency and bleeding scores. DHA and SAL treatments also significantly reduced the changes in inflammation of the colon, and reversed the increase in myeloperoxidase activity induced by DSS. Among DSS-responsive genes, those for inflammatory cytokines (IL-1β, CD14 antigen and tumor necrosis factor receptor superfamily, member 1b), membrane remodeling genes (matrix metalloproteinase-3, -10 and -13) and acute phase proteins (S100 calcium-binding protein A8), which were increased by DSS, were down-regulated by DHA or SAL treatment.

Conclusions: DHA was effective in alleviating DSS-induced colitis in mice, partly by modulating the expression levels of genes involved in colitis.

Keywords:
Colitis / Dextran sulfate sodium / Docosahexaenoic acid / Gene expression / Microarray

1 Introduction

Long-chain PUFAs synthesized from dietary precursors such as α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6) are major components of membrane phospholipids in neurons, microglia and immune cells [1]. Consumption of fish oil containing high levels of n-3 PUFAs such as docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) has been reported to improve the prognosis of several inflammatory diseases, including atherosclerosis, systemic lupus erythematosus and rheumatoid arthritis [2–5].

Because of their anti-inflammatory effects, dietary fats can ameliorate inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease, characterized by a chronic relapsing inflammation with unknown etiology [6, 7]. In a rat colitis model, dietary administration of n-3 PUFAs (in fish oil; 30% of total fat in the diet) led to the recovery of histological scores, lower colonic alkaline phosphatase and γ-glutamyl transpeptidase activity as well as lower mucosal levels of prostaglandin E_2 and leukotriene B_4 [8]. Dietary oil supplement enriched with n-3 PUFAs attenuated major colitis parameters (length of the colon, histological scores and myeloperoxidase (MPO) activity) when colitis was induced by incorporating dextran sulfate sodium (DSS) in drinking water [9]. Therefore, based

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Abbreviations: DHA, docosahexaenoic acid; **DSS,** dextran sulfate sodium; **EPA,** eicosapentaenoic acid; **H & E,** hematoxylin and eosin; **IBD,** inflammatory bowel disease; **IU,** international unit; **MPO,** myeloperoxidase; **SAL,** sulfasalazine; **UC,** ulcerative colitis

on the clinical and experimental results, dietary supplementation with n-3 PUFAs could be an effective strategy for treating patients with UC [10].

To date, most studies investigating the anti-inflammatory effects of n-3 PUFAs have employed fish oils, which contain a heterogeneous mixture of EPA and DHA. The use of such mixtures of EPA and DHA in fish oil has prevented a comparison and understanding of the individual effect of these fatty acids. Although a few studies have shown that pure DHA and/or EPA can reduce inflammation *in vitro* and *in vivo* [11, 12], there is little information on the possible modulatory effect of pure DHA on colitis.

The aim of this study was to examine whether oral administration of pure DHA could modulate the symptoms of acute colitis in a murine model. Further, a cDNA microarray system was applied for better understanding of the effects of DHA on the expression levels of inflammatory genes. The results strongly suggested that DHA, as an n-3 PUFA, was effective in alleviating DSS-induced colitis in mice, partly by modulating the expression levels of genes such as inflammatory cytokines, membrane remodeling genes and acute phase proteins.

2 Materials and methods

2.1 Reagents

DSS was obtained from MP Biomedicals (Solon, OH, USA). Hexadecyl-trimethyl-ammonium bromide was obtained from TCI (Kyoto, Japan). All other reagents, including *o*-dianisidine and sulfasalazine (SAL), were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Animals

Male BALB/c mice weighing about 20 g were purchased from Daehan Biolink (Choongchung, Korea). They were maintained in a room controlled at $23 \pm 2^{\circ}C$ with a relative humidity of 50–55% and a 12/12 h light/dark cycle. Standard laboratory rodent chow (E-joeun, Jeong-eup, Korea) and sterile water were provided *ad libitum*. After 7 days of acclimatization, weight-matched animals were randomized into four groups ($n = 10$ *per* group). The experimental protocol was approved by the Animal Experiment Ethics Committee of the Korea Food Research Institute (KFRI-IACUC-5).

2.3 Induction and general assessment of colitis

The untreated control group (Control) received only drinking water without DSS for 7 days. The DSS-induced colitis group (DSS) was fed 5% DSS dissolved in sterile distilled water for 7 days. DHA was dissolved in olive oil and administered orally at 30 mg/kg once a day concurrently with DSS for 7 days. As a positive drug control, 100 mg/kg SAL dissolved in olive oil was administered orally once a day, concurrently with DSS for 7 days. The dose of DHA selected in this study was equivalent to 1.8–2.1 g/day in a 60–70 kg human, based on the previously published studies [13, 14]. A symptomatic score was generated based on the ratings of 0–4 each for stool consistency and intestinal rectal bleeding [15]. Animals were weighed at the beginning and end of the study to obtain a weight change ratio. All mice were sacrificed by cervical dislocation 7 days after the start of the experiment. After measuring the colon length, excised segments of the proximal colon were fixed in 10% buffered formalin, processed for histology and stained with hematoxylin and eosin (H & E). Histological scoring for inflammation and crypt destruction (0–4 each) was performed in a blinded fashion by a pathologist [15]. Colonic tissue was weighed and assessed for myeloperoxidase activity using a spectrophotometer at 460 nm as described [16]. One international unit (IU) of MPO activity is defined as the quantity of enzyme able to convert 1 μmol of hydrogen peroxide to water in 1 min at room temperature. MPO activity was expressed in IU *per* gram of protein.

2.4 RNA extraction and cDNA microarray analysis

RNA from homogenized colonic tissue ($n = 3$ *per* group) was extracted and purified using Qiagen RNeasy mini kits (Qiagen, Valencia, CA, USA). Purified total RNAs were analyzed using Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Applied Biosystems Mouse Genome Survey Arrays (Applied Biosystems, Foster City, CA, USA) were used to analyze the transcriptional profiles of colonic tissue RNA samples. Sequences used for microarray probe design were from the Celera Genomics Database (http://www3.appliedbiosystems.com). Digoxigenin-UTP labeled cDNA was generated and amplified linearly from 1 μg of total RNA using Applied Biosystems Chemiluminescent RT-IVT Labeling Kit in accordance with the manufacturer's protocol. Array hybridization, chemiluminescence detection, image acquisition and analysis were performed using Applied Biosystems Chemiluminescence Detection Kit and Applied Biosystems 1700 Chemiluminescent Microarray Analyzer. Scanned images were auto-gridded and the chemiluminescent signals were quantified, corrected for background and spots, and normalized spatially.

2.5 Microarray data analysis

Applied Biosystems Expression System software was used to extract assay signals and assay signal-to-noise ratio values from the microarray images. Bad spots flagged by the software were removed from the analysis. Raw data were exported to Excel spreadsheet software (Microsoft 2003) for further manipulations. Exported data files were analyzed using

Avadis prophetic software (Version. 4.3, Strand Life Sciences, Carlsbad, CA, USA). Genes with a minimum *p*-value of 0.05 were identified as candidates for significantly differentially expressed genes. To minimize the number of falsely significant genes, the identified set of genes was filtered by signal-dependent fold change thresholds. To calculate the thresholds, the same sample assay signal ratios were ordered by assay signal intensity and binned in ten equal sets. Genes having at least 2.0-fold differential expression levels dysregulated by DSS treatment were classified into functional categories based on the Gene Ontology database (http://pantherdb.org/). From these lists, genes dysregulated by DHA, with 1.5-fold deviations when compared with the DSS-treated group, were selected, and Gene Ontology analysis was performed using the PANTHER classification system.

2.6 DNA synthesis and quantitative TaqMan RT – PCR amplification

cDNA was produced using the Superscript™ II RT-PCR System (Invitrogen, Karlsruhe, Germany) according to the manufacturer's recommendations for oligo (dT) primed-cDNA synthesis. cDNA synthesis was performed on 500 ng of RNA, at 42°C. PCR was performed in an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) in 384-well microtiter plates. Optimum reaction conditions were obtained with 12.5 μL of Universal Master Mix (Applied Biosystems) containing dNUTPs, $MgCl_2$, reaction buffer and Ampli Taq Gold, 200 μM of primer(s) and fluorescence labeled TaqMan probe. Finally, template cDNA was added to the reaction mixture. Amplifications were performed starting with a 10 min template denaturation step at 95°C, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. All samples were amplified in triplicate and data were analyzed using Sequence detector software (Applied Biosystems).

2.7 Statistical analysis

All data are shown as the mean ± SD. For comparison of symptomatic and histological data among treatments, one-way analysis of variance and Duncan's test were used, and a probability value (*p*-value) less than 0.05 was regarded as significant (SPSS software, version 12.0, SPSS, Chicago, IL, USA). For comparison of microarray data between treatments, *t*-test and false discovery rate control with *p*-value less than 0.01 were used. The correlation coefficients between treatments were calculated utilizing a statistical software R (R.2.8.1, www.r-project.org).

3 Results

3.1 Effects of DHA on DSS-induced colitis

Mice exposed to 5% DSS for 7 days showed symptoms of acute colitis characterized by decreased body weight and colon length, and exhibited severe diarrhea accompanied by rectal bleeding after 4 or 5 days. In the DSS-treated group, body weight and colon length were reduced significantly compared with those of the untreated control mice (Table 1, $p < 0.05$, $n = 10$). Treatment with DHA significantly reduced the weight loss and colon shortening induced by DSS. Stool consistency and rectal bleeding scores induced by DSS were slightly, but not significantly, improved by DHA treatment. As a positive drug control, the SAL-treated group showed significant improvements of colon shortening, stool consistency and rectal bleeding scores compared with the DSS-treated group ($p < 0.05$, $n = 10$). However, SAL was mildly effective in inhibiting weight loss.

Colon samples obtained from control mice showed no signs of inflammation or changes in the cytoarchitecture of the colon mucosa (Fig. 1A). In the DSS-treated group, there were extensive distortion of crypt structure and infiltration of neutrophils (Fig. 1B). In contrast, daily administration of DHA or SAL significantly reduced the changes in morphological features induced by DSS (Figs. 1C and D). Taken together, the inflammation and crypt destruction scores were significantly ($p < 0.05$, $n = 10$) better in the DHA- or SAL-treated group than in the DSS-treated mice (Table 2). The MPO activity in control mice was 1.1 ± 0.1 IU/g protein, whereas it increased to 8.9 ± 0.1 IU/g protein in DSS-treated animals. Treatment of DSS concomitantly with DHA or SAL significantly reduced the DSS-induced increase of colonic MPO by over 30% ($p < 0.05$, $n = 10$). These data were

Table 1. Effects of treatments with 5% DSS and concomitant 30 mg/kg DHA or 100 mg/kg SAL on symptomatic markers in the murine DSS-induced colitis

Group	Body weight (g)		Colon length (cm)	Stool consistency score (0–4)	Bleeding score (0–4)
	0 day	7 day (ratio, %)			
Control	21.2 ± 0.9	21.4 ± 0.6 (101.0%)	8.8 ± 0.7	0	0
DSS	21.1 ± 1.0	18.6 ± 0.8 (88.4%)	5.6 ± 0.4	3.6 ± 0.8	3.6 ± 0.8
DHA	21.2 ± 0.8	19.4 ± 0.7 (91.8%)*	6.4 ± 0.3*	3.4 ± 1.0	3.4 ± 1.0
SAL	21.1 ± 1.3	19.1 ± 1.3 (90.4%)	6.1 ± 0.2*	2.4 ± 0.8*	2.4 ± 0.8*

Data are represented as the means ± SD. *$p < 0.05$ *versus* DSS-treated colitis group ($n = 10$).

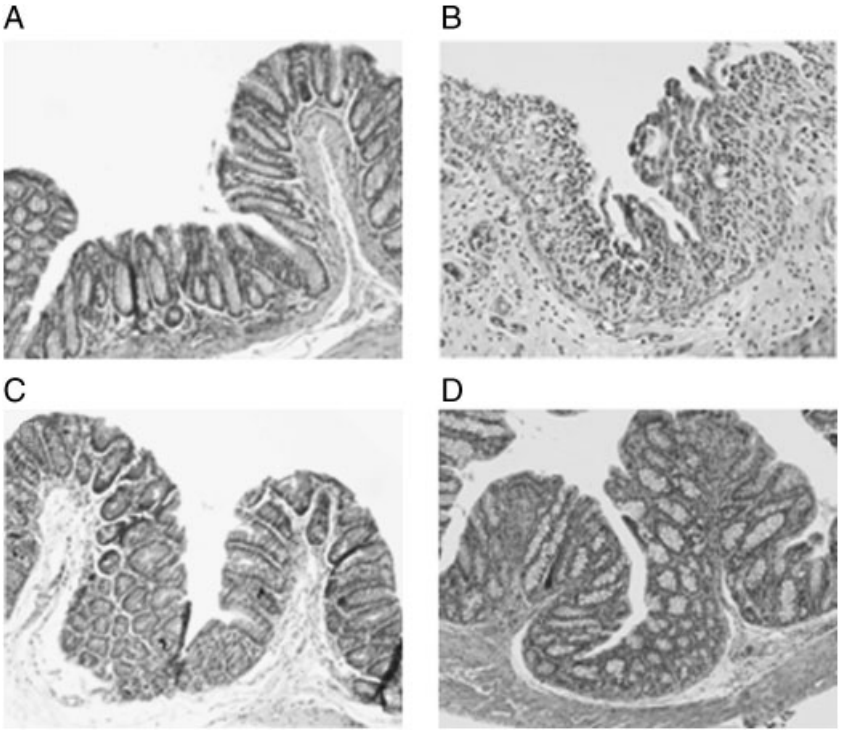

Figure 1. Histology of colonic tissue in mice with DSS-induced colitis. Colon sections were stained with H & E and representative micrographs are shown. (A) Normal control; (B) DSS (5% *per* day for 7 days); (C) DHA (30 mg/kg) and (D) SAL (100 mg/kg) with 5% DSS for 7 days. Magnification × 40.

Table 2. The severity of histopathology in mice with DSS-induced colitis

Group	Inflammation (0–4)	Crypt destruction (0–4)	MPO activity (IU/g protein)
Control	0.1±0.1	0.1±0.2	1.1±0.4
DSS	2.3±0.3	2.9±0.3	8.9±3.1
DHA	1.7±0.4*	2.6±0.5	6.1±2.9*
SAL	1.6±0.3*	2.4±0.5*	4.9±1.6**

Groups are defined as in Table 1. Data are represented as the means±SD. Inflammation and crypt destruction were each scored from 0 to 4 in a double-blind test. *$p<0.05$, **$p<0.01$ *versus* DSS-treated colitis group ($n=10$).

consistent with the histology of the colon section shown by H & E staining.

3.2 Regulation of gene expression by DHA in mice with DSS-induced colitis

On microarray analysis, 933 probes were regulated differentially between the control- and DSS-treated groups. From these, 521 were overexpressed and 412 were underexpressed in the DSS group compared with untreated controls (data not shown). As shown in Table 3, seven genes for immunity and defense, five genes for protein metabolism and modification and one gene for signal transduction were detected and grouped as being DHA-responsive in this model of DSS-induced colitis. The correlation analysis revealed that correlation coefficient of gene expression between DHA- and SAL-treated groups was 0.934 ($p<0.05$). To validate the microarray data, mRNA expressions of S100 calcium-binding protein A8 (S100a8), IL-1β and matrix metalloproteinase-3, previously found in patients with UC [17, 18], were confirmed by real-time RT-PCR quantification (Fig. 2).

4 Discussion

In this study, we observed that oral administration of DHA modulated the symptoms of acute colitis induced by DSS in mice. In addition, DHA treatment affected the expression levels of genes encoding inflammatory cytokines [IL-1β, CD14 antigen (CD14) and tumor necrosis factor receptor superfamily member 1b (Tnfrsf1b)], membrane remodeling (Mmp-3, -10 and -13) and acute phase protein (S100a8), which are all associated with inflammatory response and/or colitis. Our results suggest that oral administration of DHA might be beneficial for ameliorating DSS-induced colitis in mice, partly by modulating the expression levels of inflammation- and/or colitis-related genes.

Briefly, n-3 PUFAs have been suggested as a treatment for IBD, because of their anti-inflammatory effects [6, 19, 20]. In the clinical study, symptoms of colitis in ten UC patients who were given a conventional treatment (steroids and salicylates) with PUFAs for 8 weeks were improved significantly in seven out of ten patients [21]. In a similar double-blind study, patients with active UC, who

Table 3. DHA-responsive genes in mice with DSS-induced colitis

Gene group	GeneBank accession number	Gene symbol	DSS		DHA		SAL	
			Fold change[a)]	*p*-Value	Fold change[a)]	*p*-Value	Fold change[a)]	*p*-Value
Immunity and defense								
Acute-phase protein	AK157837	S100a8	134.5±17	2.2E–9	108.7±1.9	1.9E–6	32.2±1.9	2.6E–6
Inflammatory cytokine	AK156396	IL-1β	8.7±1.2	3.9E–5	6.2±1.1	1.0E–2	5.0±1.3	2.2E–3
	AK149744	CD14	3.5±0.9	2.9E–4	2.8±0.8	4.4E–2	2.0±0.9	9.0E–2
	AK004752	Tnfrsf1b	3.2±0.7	2.0E–6	2.4±0.5	5.7E–3	2.1±0.7	1.7E–3
Receptor protein	BC049354	Clec4a1	4.5±0.9	1.7E–6	3.5±0.7	1.7E–3	3.2±0.8	1.7E–4
	AK019479	Slit3	3.6±0.9	8.8E–6	2.8±0.6	7.3E–3	3.0±0.7	1.6E–4
Regulation of transcription	AK154830	Mafb	3.0±0.6	1.5E–5	2.4±0.5	1.0E–2	1.9±0.7	1.0E–2
Protein metabolism and modification								
Membrane remodeling protein	AK148467	Mmp3	16.1±2.0	1.0E–5	9.5±1.4	7.2E–3	11.6±1.4	1.2E–4
	AK020292	Mmp10	13.6±1.7	3.2E–6	3.9±1.0	9.9E–2	9.0±1.1	7.3E–5
	AK150728	Mmp13	10.0±1.3	3.6E–7	5.3±0.9	3.0E–3	6.4±1.0	2.4E–5
Protein binding	M33960	Serpine1	7.8±1.5	1.1E–4	6.1±1.4	1.4E–2	4.9±1.2	3.5E–3
Peptidase	U43525	Prtn3	5.5±1.0	1.8E–7	3.6±0.6	4.4E–3	3.1±0.8	2.5E–4
Signal transduction								
	AK078630	Scube1	3.3±0.7	1.4E–7	2.4±0.5	1.5E–3	3.1±0.5	8.7E–7

Treatment groups are as defined in Table 1.

a) Fold changes are shown on a $\log_2$ scale compared with the mRNA expression level of normal control mice. Data were represented as the means±SD.

received the PUFAs, showed gain of body weight, improvement of the histological score and reduction of LTB_4 production [22]. Several studies had reported that dietary supplementation with n-3 PUFAs has an anti-inflammatory activity in the experimental model of UC [9, 13, 14]. However, it is still unclear whether pure DHA or EPA as a single compound has an anti-inflammatory effect against colitis models.

A few studies have examined the effects of the individual effects of EPA and DHA *in vitro* studies [23–25]. Some evidences suggested that DHA and EPA may differentially influence the metabolic pathway [23, 26]. For example, EPA competes with arachidonic acid and it is also able to act as a substrate for both cyclooxygenase and lipoxygenase enzymes, but DHA does not [24]. In contrast, DHA suppressed T-cell activation when assessed by cytokine expression, whereas EPA did not [27]. In endothelial cells stimulated by TNF-α, DHA counteracted the TNF-α-induced deactivation of extracellular signal-related kinases, more effectively than EPA, and only DHA reduced the mRNA expression of Jun amino-terminal kinases [25]. In LPS-stimulated macrophages, 25 μM of DHA had an inhibitory effect greater than EPA on the production of cytokines such as IL-1β and IL-6. DHA significantly decreased nuclear factor-kappa B as inflammation-related transcription factor [24]. Therefore, we investigated DHA for its possible influence on inflammation in DSS-induced colitis, and compared the result with that of SAL which is an amino-salicylic acid-based drug.

The DSS-induced colitis model used in this study is one of the commonly used experimental models, because of its ease of administration and reproducibility. This model, resembling human UC, represents well-characterized mucosal injury with cellular infiltration and abnormal intestinal immune system [28–30]. In the present study, oral administration of DHA significantly attenuated the loss of body weight caused by DSS, whereas it did not significantly attenuate stool variation and rectal bleeding. The possible reasons why DHA was less effective on stool variation and rectal bleeding might be due to low dosage (30 mg/kg), high concentration of DSS (5%) and short-term study design (7 days) used in this study. From the published studies using a heterogeneous mixture of DHA and EPA [14, 15], the calculated dosages of DHA were ranged from 65 to 110 mg/kg in the animal model of experimental colitis. It is also reported that dietary PUFAs (DHA+EPA) was significantly effective on stool variation and rectal bleeding, when dietary PUFAs were administered for 2 weeks before DSS exposure and until the end of the experiment, and DSS concentration used was changed from 5% for 5 days to 2% for the following 10 days [10]. The shortening of colon induced by DSS was effectively inhibited by DHA or SAL. Administration of DSS resulted in the destruction of colon tissue as assessed macroscopically and histopathologically. The inflammation score of the DHA-treated group was nearly equivalent to that of the SAL-treated groups. In addition, when treated with DHA or SAL, the reduction of MPO activity which is an index of neutrophil infiltration and inflammation [31] was observed in the colonic mucosa. Therefore, the present findings

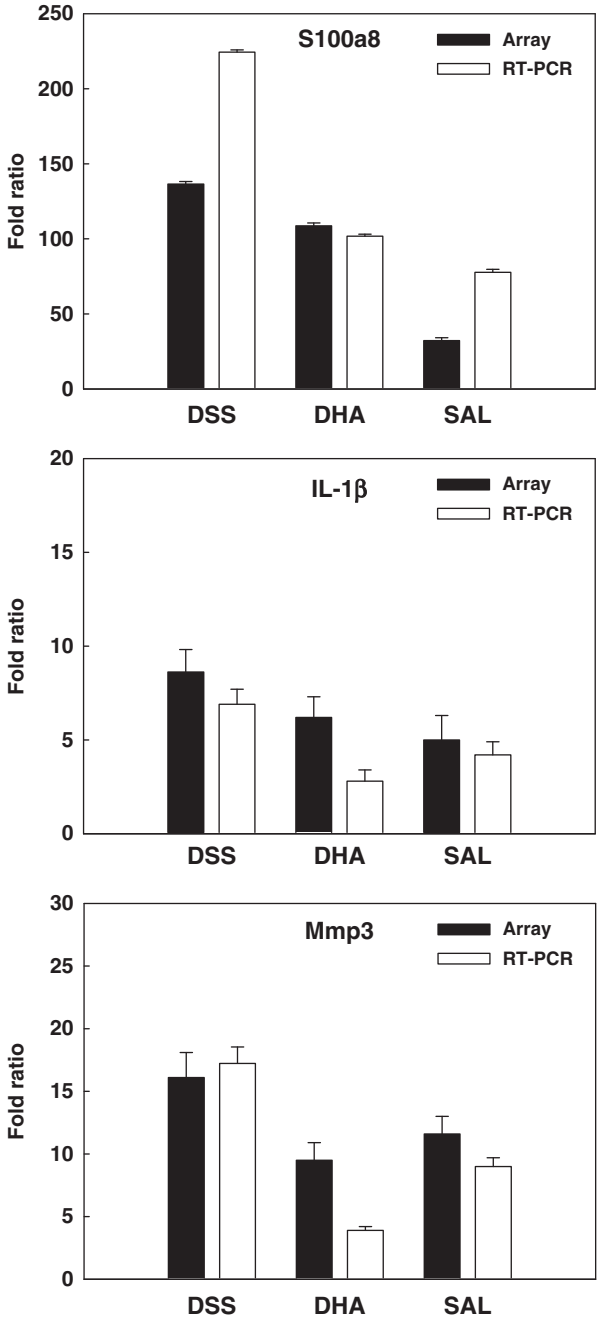

Figure 2. Real-time RT-PCR analysis of selected genes that were regulated by DHA in mice colonic tissue. Data are represented as the means ± SD. Fold change was compared with the mRNA expression level of normal control mice.

demonstrate the ameliorating effects of DHA on symptoms of colitis including inflammation.

Various studies have analyzed altered gene expressions in patients with IBD and in animal models using cDNA microarray techniques, and a number of differentially expressed genes were verified to evaluate the anti-inflammatory effects by food factors at the molecular level [32–34]. To better understand the beneficial effects of DHA on the DSS-induced colitis, colonic gene expression was examined using cDNA microarray analysis in this study. Among differentially expressed genes induced by DSS exposure, seven genes for immunity and defense [S100a8, IL-1β, CD14, Tnfrsf1b, C-type lectin domain family 4, member a1 (Clec4a1), slit homolog 3 (Slit3) and v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (Mafb)], five genes for protein metabolism and modification [Mmp-3, Mmp-10, Mmp-13, serine peptidase inhibitor, clade E, member 1 (Serpine1) and proteinase 3 (Prtn3)] and one gene for signal transduction [signal peptide, CUB domain, EGF-like 1 (Scube1)] were detected and grouped as being DHA-responsive in this model of DSS-induced colitis. In addition, the correlation coefficient of gene expression between DHA- and SAL-treated groups was 0.934 ($p < 0.05$), suggesting that the response of DHA treatment is similar to that of SAL, a drug control used in this study.

In the immunity and defense group, the highly expressed gene of S100a8 following DSS exposure for 7 days, an acute phase protein, was suppressed to about 20 and 70% of the levels after treatment of DHA and SAL, respectively. S100a8 exists during various inflammatory conditions. It is associated with neutrophil recruitment and detected in human patient with IBD, in animal model [35, 36] and microarray results [34]. IL-1β and TNF-α are involved in inflammatory leukocyte recruitment and retention, and upregulated in the inflamed colon [37]. Our microarray results are consistent with DHA effects against inflammatory cytokines in LPS-stimulated macrophage [25]. Increased colonic expression of CD14 was demonstrated during the course of experimental colitis in animals [38, 39]. In our results, CD14 was 2-fold overexpressed by DSS treatment. This was consistent with other microarray results in experimental mice model [40]. Backdahl *et al.* [41], recently reported that Clec4a1 was a candidate gene in inflammatory disease such as rheumatoid arthritis. Mafb is one of transcription factors involved during macrophage differentiation and/or inflammatory response [42]. The present results appear to be the first to show that Clec4a1 and Mafb might be also potential markers of UC, although more researches are needed.

The expression of MMPs as the membrane remodeling proteins, in the mucosa of patients with IBD, are a marker of disease activity and potential targets for therapy [43, 44]. Previously, DHA effects against Mmp-3 and -13 were reported in a model of joint inflammation [45]. In the present study, Mmp-3, -10 and -13 induced by DSS were significantly downregulated by DHA or SAL. Prtn3 is a granule serine protease present in neutrophils and monocytes, and inactivates p65 nuclear factor-kappa B as inflammation-related transcription factor [46]. In our results, Prtn3 increased by DSS exposure was downregulated by DHA treatment.

The accumulation of DSS resulted in the epithelial injury and infiltration of large numbers of leukocytes into mucosa [15]. Accordingly, increased expression level of leukocyte-

related gene, Slit [47], and one inflammatory gene, Serpine1 [48] were attenuated by DHA and SAL. In signal transduction group in our array analysis, Scube1 is downregulated in vascular endothelium after IL-1β and TNF-α treatment *in vitro* and after LPS injection *in vivo*, thus suggesting a possible role of the Scube gene family in the inflammatory response [49]. Therefore, DHA might exert its ameliorating effects on DSS-induced colitis in mice, partly through regulation of these inflammatory genes.

In conclusion, we have shown that oral administration of DHA had a potent effect to ameliorate DSS-induced colitis, at least in a murine model. Microarray analysis indicated that DHA-responsive genes were mostly associated with inflammatory response and/or colitis, suggesting that the observed effect of DHA might be associated with the modulation of the expression levels of these genes at the molecular level.

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The authors have declared no conflict of interest.

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