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β-Caryophyllene attenuates dextran sulfate sodium-induced colitis in mice via modulation of gene expression associated mainly with colon inflammation

Jae Young Cho[b], Hwa Yeon Kim[a], Sung-Kyu Kim[c], Jung Han Yoon Park[d], Hong Jin Lee[a], Hyang Sook Chun[a,*]

[a] Department of Food Science and Technology, Chung-Ang University, Naeri 72-1, Ansung, Kyonggi 456-756, Republic of Korea
[b] CKD Research Institute, Dongbaekjukjeon-daero 315-20, Yungin, Kyonggi 446-916, Republic of Korea
[c] Nutra R&BT Inc., 371-47 Gasan, Geumcheon-gu, Seoul 153-788, Republic of Korea
[d] Department of Food Science and Nutrition, Hallym University, Hallymdaehak-gil 39, Chuncheon, Kangwon 200-702, Republic of Korea

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ABSTRACT

We examined the modulatory activity of β-caryophyllene (CA) and gene expression in colitic colon tissues in a dextran sulfate sodium (DSS)-induced colitis model. Experimental colitis was induced by exposing male BALB/c mice to 5% DSS in drinking water for 7 days. CA (30 or 300 mg/kg) was administered orally once a day together with DSS. CA administration attenuated the increases in the disease activity index, colon weight/length ratio, inflammation score, and myeloperoxidase activity in DSS-treated mice. Microarray analysis showed that CA administration regulated the expression in colon tissue of inflammation-related genes including those for cytokines and chemokines (Ccl2, Ccl7, Ccl11, Ifitm3, IL-1β, IL-28, Tnfrsf1b, Tnfrsf12a); acute-phase proteins (S100a8, Saa3, Hp); adhesion molecules (Cd14, Cd55, Cd68, Mmp3, Mmp10, Sema6b, Sema7a, Anax13); and signal regulatory proteins induced by DSS. CA significantly suppressed NF-κB activity, which mediates the expression of a different set of genes. These results suggest that CA attenuates DSS-induced colitis, possibly by modulating the expression of genes associated mainly with colon inflammation through inhibition of DSS-induced NF-κB activity.

1. Introduction

β-Caryophyllene (trans-(1R,9S)-4,11-11-trimethyl-8-methylene bicyclo[7.2.0] undec-4-ene; CA) is a major constituent of many essential oils obtained from a number of plant species such as *Betula* (~30%), *Syzygium* (~13%), and *Strobilanthes* (~7%) species [2,12,24]. CA shows potential antimicrobial, antioxidant, and anti-carcinogenic properties [16,19,32] as well as anti-inflammatory activity against gastric mucosal injuries and carrageenan- or prostaglandin E1-induced edema [5,35]. In our previous study, we demonstrated that CA ameliorated dextran sulfate sodium (DSS)-induced colonic inflammation by regulating the proinflammatory cytokine interleukin 6 (IL-6) in BALB/c mice [9]. Following our study, Bento et al. reported that the anti-inflammatory property of CA is mediated through the activation of the cannabinoid receptor 2 and peroxisome proliferator-activated receptor-γ, leading to the inhibition of proinflammatory cytokines in DSS-induced ulcerative colitis model [6].

Inflammatory bowel disease (IBD) such as Crohn disease (CD) and ulcerative colitis (UC) have an unpredictable clinical course characterized by the involvement of many pleiotropic molecules that orchestrate both the innate and adaptive immune responses [8,7]. To understand the genomic changes involved in the pathogenesis of IBDs, many studies have tried to identify a set of genes that is expressed distinctively in inflamed colonic tissues compared with normal colonic tissues [11,17,42]. Using a genome-wide cDNA microarray, [11]. found differences in the gene expression profiles between CD and UC [11]. This finding suggests that is important to study the roles of naturally occurring dietary components in regulating the genomic expression in addition to the well-known genes related to inflammation.

Abbreviations: CA, β-caryophyllene; CD, crohn disease; Cebpb, CCAAT/enhancer-binding protein β DSS, dextran sulfate sodium; Hp, haptoglobin; IBD, inflammatory bowel disease; IκB, inhibitor κB; IL, interleukin; MPO, myeloperoxidase; NF-κB, nuclear factor-kappa B; S100a8, S100 calcium binding protein a8; Saa3, serum amyloid A3; SAL, sulfasalazine; TNF-α, tumor necrosis factor-α; UC, ulcerative colitis.

* Corresponding author. Fax: +82 31 675 4853.
E-mail address: hschun@cau.ac.kr (H.S. Chun).

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Because of its ability to damage the epithelium and because the DSS-induced colitis animal model mimics the morphology and clinical symptoms of human UC, DSS is the chemical used most often to induce colitis [13,26]. cDNA microarray analysis has shown that DSS affected the expression of different sets of genes, in particular those involved in the acute inflammatory response, leukocyte activation, and cell adhesion, proliferation, and differentiation and these genes were regulated by the treatment of lactic acid bacteria and xanthorrhizol [10,18]. However, the profile of the genes regulated by CA treatment has not been investigated using transcriptome analysis in UC colon tissues.

In the present study, we examined the effects of CA in a mouse DSS-induced colitis model. To investigate the molecular targets for the anti-colitis actions of CA, we used cDNA microarray analysis to assess gene expression in colon tissues from these mice. We also evaluated the possible roles of CA in modulating the upstream signaling mediators such as nuclear factor-κB (NF-κB) in the RAW 264.7 murine macrophage cell line.

2. Materials and methods

2.1. Materials and reagents

CA (purity 98%) was obtained from Bordas (Sevilla, Spain), and DSS was obtained from MP Biomedicals (Solon, OH, USA). Dimethyl sulfoxide, hydrogen peroxide, lipopolysaccharide (LPS), olive oil, sodium dodecyl sulfate (SDS), and sulfasalazine (SAL) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals used were of reagent grade. All primary antibodies including anti-S100 calcium-binding protein a8 (S100a8), anti-serum amyloid A3 (Saa3), anti-haptoglobin (Hp), anti-IL-1β, anti-tumor necrosis factor-α (TNF-α), anti-IL-6, anti-CCAAT/enhancer-binding protein β (Cebpb), anti-phospho-p65-NF-κB, anti-phospho-inhibitor of κB (IκB), anti-actin, and secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Mice and experimental protocol

Six-week-old male BALB/c mice were purchased from Daehan Biolink (Choongchung, Korea) and maintained in a room controlled at 23 ± 2 °C with a relative humidity of 50–55% and a 12/12 h light/dark cycle. To induce colitis, the DSS-treated mice were fed 5% DSS dissolved in sterile distilled water for 7 days. The control mice received plain drinking water only. CA (30 or 300 mg/kg body weight) and a positive drug control, SAL (100 mg/kg body weight) dissolved in olive oil, were administered concomitantly by gavage once a day for 7 days. The mice in the control group and colitis group were given only the vehicle (olive oil, 0.2 mL/day). Water consumption did not differ between groups in the experiment. This study was approved by the Institutional Animal Care and Use Committee of Korea Food Research Institute (#KFRI-M-110006), and animal care was in accordance with institutional guidelines.

2.3. Assessment of colitis

In all mice, a disease activity (DAI) score was determined by macroscopic examination using the following criteria of [36] with slight modification: weight loss score (0, none; 1, 1–5%; 2, 5–10%; 3, 10–15%; 4, >15%); diarrhea score (0, normal stool; 1, mildly soft stool; 2, very soft stool; 3, very soft stool (no regular shape); 4, watery stool); and bloody feces score (0, normal-colored stool; 1, brown stool; 2, brown/red stool; 3, reddish stool; 4, bloody stool). The colon length and weight were measured, and excised segments of the colon were then fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. The samples were evaluated histologically by a pathologist. Neutrophil infiltration into the colon was assessed indirectly by measuring myeloperoxidase (MPO) activity, as described previously [30]. MPO activity was defined as the quantity of enzyme that degraded 1 μmol/mL of peroxide at 37 °C and is expressed as units/g protein [20].

2.4. RNA extraction and cDNA microarray

RNA from homogenized colon tissue (n = 3/group) was extracted and purified using Qiagen RNeasy mini kits (Qiagen, Valencia, CA, USA). Purified total RNA was analyzed using an 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 colon tissue RNA samples. The sequences used for microarray probe design were from the Celera Genomics Database (http://www3.appliedbiosystems.com). Digoxigenin-UTP-labeled cDNA was generated and linearly amplified from 1 μg of total RNA using an 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 an Applied Biosystems Chemiluminescence Detection Kit and an Applied Biosystems 1700 Chemiluminescent Microarray Analyzer. Scanned images were submitted to an Auto-Grid, and the chemiluminescent signals were quantified, corrected for background, and spot and spatially normalized.

2.5. Microarray data analysis and validation

Applied biosystems expression system software was used to extract the assay signals and determine the signal-to-noise values from the microarray images. The exported data file was 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 10 equal sets. Genes having at least 2.0-fold differential expression levels dysregulated by DSS were classified into functional categories based on the Gene Ontology database (http://david.abcc.ncifcrf.gov/) and Mouse Genome Informatics (http://www.informatics.jax.org/). From these lists, genes regulated by CA were selected using a 1.5-fold deviation compared with the DSS-treated group.

To validate the microarray data, 500 ng of total RNA were reverse transcribed to cDNA using the Superscript™ II RT-PCR System (Invitrogen, Karlsruhe, Germany). The reaction mixture consist of cDNA and 12.5 μL of TaqMan Universal PCR Master Mix (Applied Biosystems). PCR condition were as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min in the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). All RT-PCR were performed in triplicate and data were analyzed using the Sequence Detector software (Applied Biosystems). Relative mRNA expression levels were calculated for each sample normalized by reference gene of Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) using the delta–delta Ct method.

2.6. Western blot assay

Colon tissues were homogenized, and the lysates were centrifuged for 20 min at 12,000 × g at 4 °C. Thirty micrograms of protein was separated by 10% SDS-PAGE and transferred to a polyvinylidene fluoride membrane. The membrane was incubated with a specific primary antibody followed by horseradish

Table 1
Effects of oral administration of CA on symptomatic and histological changes during acute colitis induced by DSS in mice.

Experimental group	DAI score (0–4)	Colon weight/length (mg/cm)	Inflammation score (0-4)	MPO (U/g protein)
CON	0.1 ± 0.0*	43.9 ± 3.4*	0*	1.4 ± 0.2*
DSS	3.1 ± 0.6*	52.1 ± 4.1*	2.9 ± 0.4*	3.0 ± 0.9*
CA30	2.5 ± 0.7*	46.7 ± 3.7*	2.2 ± 0.4*	1.8 ± 0.8*
CA300	2.0 ± 0.8*	44.1 ± 2.8*	2.2 ± 0.5*	1.7 ± 0.5*
SAL100	2.1 ± 0.9*	51.4 ± 4.1*	2.6 ± 0.9*	2.3 ± 0.6*

Data are expressed as the mean ± SD.
* $p < 0.05$ vs. DSS colitis group.

peroxidase-conjugated secondary antibodies and was then visualized using an ECL system (Amersham, Buckinghamshire, UK).

2.7. Cell culture and electrophoretic mobility shift assay

The murine macrophage cell line, RAW 264.7, was obtained from American Type Culture Collection (Manassas, VA, USA). Raw 264.7 cells were cultured in DMEM supplemented with 10% heat-inactivated bovine calf serum (Gibco BRL, Grand Island, NY, USA), 100 units/ml penicillin, and 100 μg/mL streptomycin at 37 °C in 5% CO_2 in an incubator. The cells (1.5×10^5 cells/mL) were pretreated with 1–100 μM CA for 8 h and then incubated with 500 ng/mL of LPS for 2 h. For electrophoretic mobility shift assay, nuclear extracts were prepared using nuclear extraction kits from Panomics Inc. (Fremont, CA, USA). Briefly, cells were lysed with buffer A, the nuclei were pelleted by centrifugation at 14,000 × *g* for 3 min, and the nuclei were lysed with buffer B. Following lysis, the samples were centrifuged at 14,000 × *g* for 5 min, and the supernatant was retained for use in the DNA binding assay and western blot assay. Oligonucleotides containing an NF-κB binding site were used as the probe supplied with the Panomics gel shift kits. Nuclear extracts (10 μg protein) were incubated with 1 μg of poly[d(I-C)] and DNA probe in the binding buffer for 5 min at room temperature. A further incubation step was performed at 15 °C. Protein–DNA binding complexes were separated from the free probe using 6% nondenaturing PAGE. After electrophoresis, the gel was dried and subjected to autoradiography.

2.8. Statistical analysis

The data are presented as the mean ± SD. The data were analyzed using Student's *t* test (SPSS Inc., Chicago, IL, USA), and $p < 0.05$ was accepted as significant.

3. Results

3.1. Effects of CA on DSS-induced colitis in mice

We first confirmed the symptomatic and histological changes in the DAI, colon weight/length ratio, and MPO activity (Table 1). Following 5% DSS treatment for 7 days, mice exhibited reproducible colitis characterized by decreased body weight, loose stools, and gross bleeding. As shown in Table 1, the DAI, which represents the combined score for weight loss, stool consistence, and rectal bleeding, was significantly higher in the DSS-treated mice than in the control mice. Animals treated with 30 or 300 mg/kg of CA exhibited lower DAI scores compared with the mice treated with DSS alone, but the difference was significant only at the higher dose ($p < 0.05$). The colon weight/length ratio, a marker of tissue edema, was significantly lower in CA-treated mice than in mice treated with DSS alone ($p < 0.05$, Table 1). The inflammation score, which reflects the infiltration of inflammatory cells into both the mucosa and submucosa, was markedly elevated in DSS-treated mice, but was significantly reduced by CA treatment ($p < 0.05$, Table 1). Representative photomicrographs of hematoxylin and eosin-stained paraffin sections of colons are shown in Fig. 1. Consistent with the inflammation scores, colonic sections in the DSS colitis group showed typical inflammatory changes in colonic architecture such as crypt and surface epithelial loss as well as infilteration of inflammatory cells (Fig. 1B). These alterations caused by DSS were ameliorated by the treatment with 30 and 300 mg/kg of CA (Fig. 1C and 1D). The treatment with DSS alone also increased MPO activity, an inflammatory marker, in colon tissue. The oral administration of CA significantly inhibited colonic MPO activity in DSS-treated mice ($p < 0.05$, Table 1). Mice treated with the lower concentration of CA showed significant changes in colon weight/length ratio, inflammation score, and MPO activity (Table 1). Oral treatment with SAL, as a positive drug control, caused significant improvement in DAI score and MPO activity ($p < 0.05$), but not in colon weight/length ratio and inflammation score compared with the mice treated with DSS alone (Table 1).

3.2. Inflammation-related genes affected by CA in colon tissue

To assess the profile of molecular markers of inflammation in relation to the attenuation of colitis by CA, mRNA expression and the protein levels in the colon were assessed on day 7 after the induction of colitis. In the microarray analysis, genes upregulated or downregulated by DSS (≥2-fold difference and $p < 0.05$ between DSS-treated and control mice) were first selected. Twenty-six genes were identified and these responsive genes were classified into four categories by gene ontology analysis (Table 2). All genes except *Anxa13* were upregulated after DSS exposure for 7 days. Notably, the mRNA expression of acute-phase proteins such as S100a8, Saa3, and Hp was increased markedly by >30-fold by DSS. Analysis of the proinflammatory genes showed that those for several C–C motif chemokine ligands (Ccl2, Ccl7, and Ccl11), cytokines (IL-1β and IL-28), and TNF family members were also affected by DSS. Increased expression of CD antigens (CD14, CD55, and CD68), matrix metalloproteinases (Mmp3 and Mmp10), and transcription factors CCAAT/enhancer-binding proteins (Cebpb and Cebpd) were found in colitic colon tissues. Twenty-six representative inflammation-related genes were effectively modulated by the CA treatment. The microarray results for the selected genes (S100a8, Saa3, Hp, IL-1β, Mmp3, and Cebpb; marked with an asterisk in Table 2) were validated using real-time RT–PCR. Although the change ratio of each transcript was different, both results of array analysis and real time RT-PCR showed same alteration tendency for seven genes (Fig. S1). The expression of IL-6, which has been detected previously in a DSS-induced colitis model [9], was confirmed by western blot analysis (Fig. 3). CA administration blocked the expression of six proteins related to inflammation (S100a8, Saa3, Hp, IL-1β, TNF-α, and Cebpb) whose expression was induced by DSS.

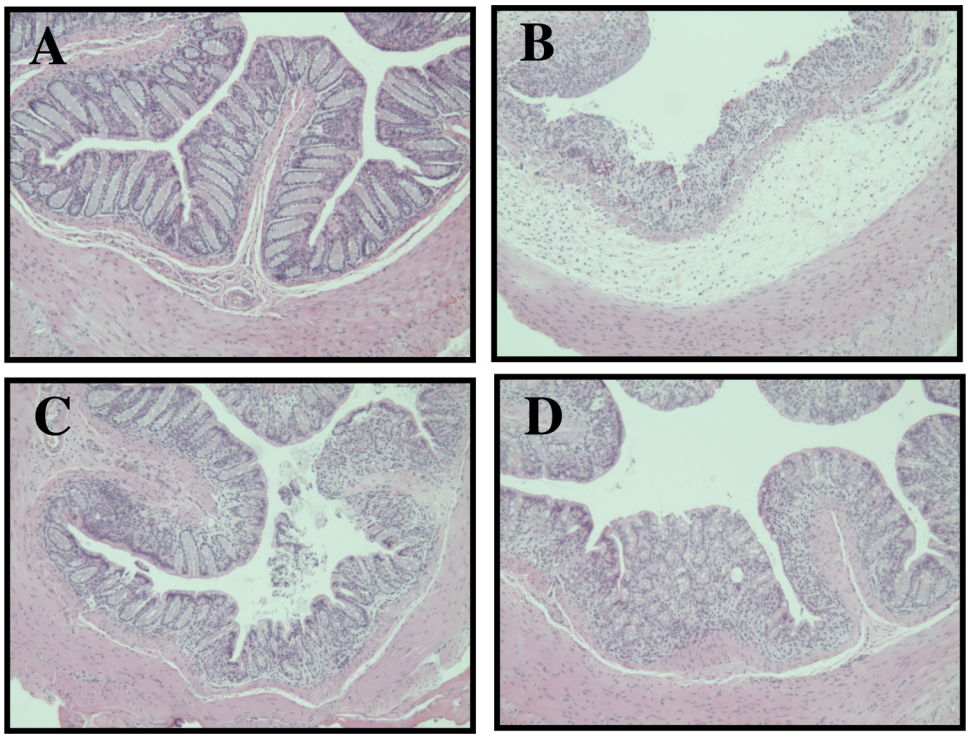

Fig. 1. Representative photomicrographs of hematoxylin and eosin-stained paraffin sections of colons (Magnifications, ×100). (A) Control group, (B) DSS colitis group, (C) CA-treated (30 mg/kg), (D) CA-treated (300 mg/kg).

Table 2
Inflammatory and immune response-related gene variations after CA treatment during acute colitis induced by DSS in mice.

Gene	Gene	DSS		CA30		CA300		SAL100	
function	symbol	Fold change	*p*-value	Fold change	*p*-value	Fold change	*p*-value	Fold change	*p*-value
Chemokine/ cytokine	Ccl2	15.2 ± 1.2	0.000	21.1 ± 0.8	0.000	8.8 ± 0.8	0.000	10.2 ± 1.5	0.003
	Ccl7	10.2 ± 1.6	0.001	5.7 ± 0.8	0.000	2.9 ± 0.9	0.012	6.2 ± 1.2	0.002
	Ccl11	3.9 ± 1.2	0.003	3.6 ± 1.1	0.070	2.0 ± 0.8	0.008	3.2 ± 1.2	0.012
	Ifitm3	4.3 ± 0.9	0.000	1.9 ± 0.5	0.002	1.9 ± 0.7	0.190	4.1 ± 0.9	0.002
	IL-1β*	9.5 ± 1.2	0.000	3.7 ± 0.6	0.000	5.9 ± 0.8	0.002	5.5 ± 1.4	0.007
	IL28	2.5 ± 0.8	0.004	1.7 ± 0.8	0.520	3.3 ± 0.4	0.000	1.7 ± 0.8	0.043
	Tnfrsf1b	3.4 ± 0.7	0.000	-1.7 ± 0.6	0.350	1.3 ± 0.5	0.046	2.2 ± 0.7	0.008
	Tnfrsf12a	3.2 ± 0.9	0.002	-1.1 ± 0.6	1.000	1.4 ± 0.5	0.025	2. ± 0.7	0.004
Acute phase protein	S100a8*	147.6 ± 1.7	0.000	112.9 ± 2.3	0.000	71.6 ± 1.2	0.000	33.7 ± 1.9	0.001
	Saa3*	148.1 ± 2.6	0.000	27.3 ± 2.5	0.001	60.2 ± 2.5	0.000	67.8 ± 2.9	0.001
	Hp*	31.5 ± 2.2	0.000	2.7 ± 1.6	0.029	3.6 ± 1.7	0.029	23.5 ± 2.3	0.002
Adhesion molecule	Cd14	3.7 ± 1.0	0.001	2.6 ± 0.8	0.002	2.7 ± 0.8	0.001	2.0 ± 0.9	0.020
	Cd55	3.1 ± 0.8	0.002	2.5 ± 0.6	0.030	1.9 ± 0.4	0.023	2.0 ± 0.7	0.012
	Cd68	2.7 ± 1.4	0.022	-5.4 ± 1.1	0.030	−2.6 ± 0.8	0.029	2.6 ± 1.3	0.030
	Mmp3*	18.0 ± 2.0	0.001	4.4 ± 1.2	0.220	4.7 ± 1.1	0.003	12.8 ± 1.5	0.001
	Mmp10	13.3 ± 1.8	0.001	8.8 ± 0.9	0.007	8.3 ± 0.9	0.000	8.7 ± 1.7	0.000
	Sema6b	2.5 ± 0.5	0.000	1.4 ± 0.5	0.800	1.6 ± 0.4	0.002	1.7 ± 0.9	0.048
	Sema7a	4.0 ± 0.8	0.000	1.5 ± 0.6	0.045	2.0 ± 0.6	0.003	2.1 ± 0.9	0.017
	Anxa13	-4.1 ± 1.2	0.004	-1.5 ± 0.4	0.920	−1.3 ± 0.4	0.024	-3.9 ± 1.3	0.014
Signal regulatory Protein	Bcl3	3.4 ± 0.8	0.000	1.4 ± 0.6	0.032	2.1 ± 0.7	0.032	2.1 ± 0.7	0.007
	Cebpb*	4.1 ± 0.9	0.001	2.7 ± 0.6	0.060	2.0 ± 0.5	0.009	3.2 ± 0.7	0.006
	Cebpd	4.7 ± 1.3	0.001	2.4 ± 1.2	0.060	2.2 ± 1.1	0.009	3.4 ± 1.5	0.006
	G1p2	7.9 ± 2.3	0.009	1.1 ± 1.4	1.000	3.4 ± 1.2	0.013	6.2 ± 2.4	0.031
	Ier3	5.3 ± 1.0	0.000	2.8 ± 0.7	0.080	2.6 ± 0.6	0.001	4.4 ± 1.0	0.003
	Inhbb	7.2 ± 1.5	0.002	5.2 ± 0.9	0.003	5.0 ± 1.0	0.004	4.6 ± 1.2	0.004
	Ly86	2.5 ± 0.7	0.002	1.7 ± 0.9	0.450	2.5 ± 0.7	0.006	1.9 ± 1.0	0.034

Fold change are shown by the comparison with the mRNA expression level of normal control mice. Data are represented as the means ± SD.
* These six genes were validated by a quantitative real-time RT-PCR method.

3.3. Inhibition of NF-κB activation by CA in vivo and in vitro

Because NF-κB is one of the most well-known transcription factors that can mediate the regulatory effects of CA on gene expression in DSS-induced colitis, we used western blot analysis to measure the protein expression of phospho-NF-κB (p65) and phospho-IκB. As shown in Fig. 2A, phospho-NF-κB and IκB proteins were almost undetectable in the colon tissue of normal control mice. By contrast, the expression of these proteins was augmented markedly in the DSS-induced inflamed colon, and treat-

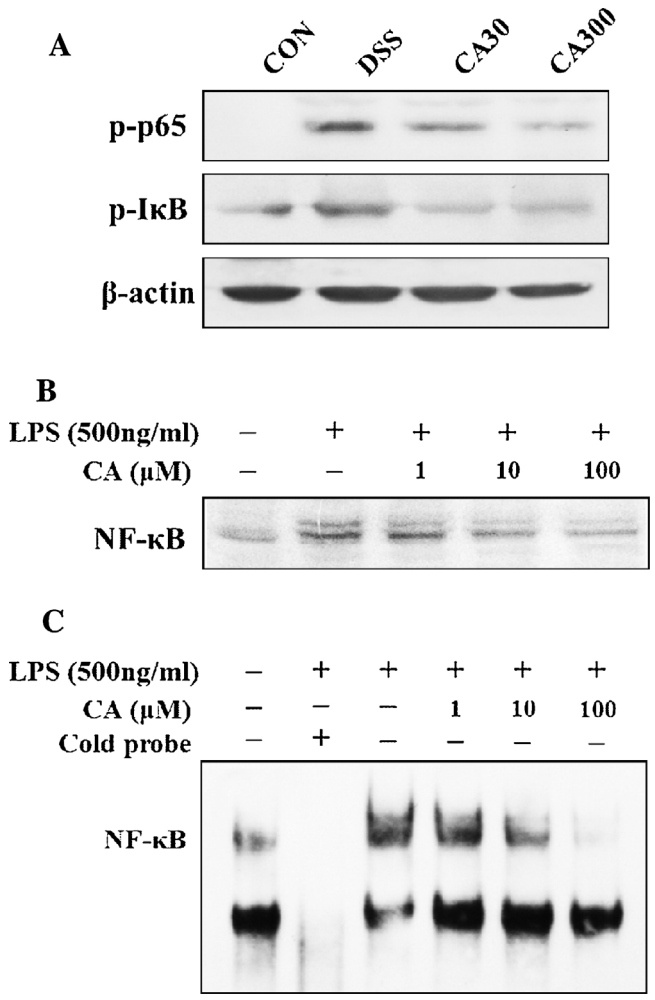

Fig. 2. Effects of oral administration of CA on protein expression during acute colitis induced by DSS in mice. Western blot analysis was performed on colon protein extracts after 7 days of DSS treatment with or without CA (30 or 300 mg/kg): (A) acute phase proteins; (B) cytokines (C), transcription factor. The results are representative of at least three independent experiments.

ment with CA inhibited the phosphorylation of NF-κB and IκB. We next examined the in vitro NF-κB translocation and activation following treatment with LPS (500 ng/mL) and/or CA in RAW 264.7 cells. The expression of NF-κB in the nuclear extract fraction increased following LPS treatment (Fig. 2B, lane 2). However, in cells treated with CA in the presence of LPS, NF-κB expression decreased in a dose-dependent manner (Fig. 2B, lanes 3–5). After treatment with LPS, the DNA binding activity of NF-κB was greater in the nuclear extract fraction than in the control and cold probe-treated negative control (Fig. 2C, lanes 1 and 2). In cells treated with CA in the presence of LPS, the DNA-binding activity of NF-κB decreased in a dose-dependent manner.

4. Discussion

Plant compounds, especially essential oils, are important constituents of fragrances and are used increasingly as essential oils in the pharmaceutical and food industries [33], and the application of natural products provides an attractive and relatively nontoxic alternative for controlling inflammatory disorders [31]. A natural bicyclic sesquiterpene, CA is known to exhibit various biological activities and has been granted "generally recognized as safe" status by the Flavor and Extract Manufacturers Association and is approved for food use by the US Food and Drug Administration because of its low toxicity [30]. Therefore, it has recently attracted interest as an effective and safe natural compound for nutraceuticals. Moreover, CA is commonly ingested with vegetable food, and an estimated daily intake of 10–200 mg of CA could be a dietary factor with potential to modulate the inflammatory signs associated with colitis [6].

We studied the anti-inflammatory activity of CA in a DSS-induced colitis animal model and identified the molecular mediators responsible for its anti-inflammatory actions. Oral administration of CA reduced the severity of acute colonic damage, as indicated by the DAI score, colon weight/length ratio, and inflammation score after 7 days of DSS treatment in mice (Table 1), which is consistent with those of previous reports [30,23,9]. In the present study, the main focus was on the colonic damage induced by DSS. But several lines of evidences indicate that DSS- induced damage in mice is not confined to the colon, but also induces morphological and biochemical changes in the small intestine [43]. In addition, DSS-treated mice exhibited increased MPO activity, a marker of neutrophil infiltration and perturbation of the inflammatory system, which can trigger the pathological responses and symptoms of colitis [21,29]. CA suppressed MPO activity, which may have helped ameliorate neutrophil infiltration, as evidenced by the histological signs of less-severe tissue inflammation in colon tissue.

In this study, SAL was used as a positive control and significantly improved DAI score and MPO activity. The mechanism of action of SAL in colitis has not been fully elucidated, but the 5-aminosalicylic acid moiety is considered to have the major therapeutic action via blocking the production of prostaglandins and leukotrienes, inhibiting bacterial peptide-induced neutrophil chemotaxis and adenosine-induced secretion, and scavenging reactive oxygen metabolites [4]. Regarding the intestinal absorption, SAL is known to be poorly absorbed from the intestine. Compared to SAL, CA showed superior effects in terms of colon weight/length ratio and inflammation score. Possible explanations for this phenomenon are the differences in the intestinal absorption and mechanism of action of orally administered SAL and CA. But a further research is needed to elucidate what the most likely explanation is.

Transcriptome analysis using cDNA microarray has shown that cytokines, chemokines, adhesion molecules, and acute-phase proteins such as S100a8, Saa3, and Hp play an important role in the regulation of inflammation [10,18,21,37,1,39]. In the present study, CA treatment suppressed the expression of DSS-induced inflammatory genes including acute-phase proteins such as S100a8 and Saa3, proinflammatory cytokines such as IL-1β and TNF-α, and adhesion molecules such as Mmp3 and Mmp10 (Table 2). The acute-phase proteins are involved in triggering an immune reaction in the colon mucosa and in initiating an inflammatory response [10,25]. In particular, S100 calcium-binding proteins S100a6 and S100a8 have been shown to stimulate the adhesion and migration of neutrophils [27]. The expression of S100a9 is higher in inflamed human colon tissue than in normal colon tissue [17], which suggests that S100a6 and/or S100a8 may be molecular markers of IBDs. Interestingly, CA caused significant inhibition of S100a8 in the microarray analysis, which was confirmed by real-time reverse transcription-polymerase chain reaction (RT-PCR) and western blot analysis (Fig. 1).

Transcriptome expression is mediated by transcriptional regulators and their upstream signaling modulators. NF-κB has been identified as a key transcription factor involved in the regulation of genes related to inflammation, especially those for cytokines including IL-1β, TNF-α, and IL-6, during inflammatory processes including IBD. NF-κB is thus considered an ideal target molecule for the treatment of colitis [22,28]. To provide the possible link between NF-κB activity and the expression of the regulated genes in the microarray, we investigated the effect of CA on NF-κB

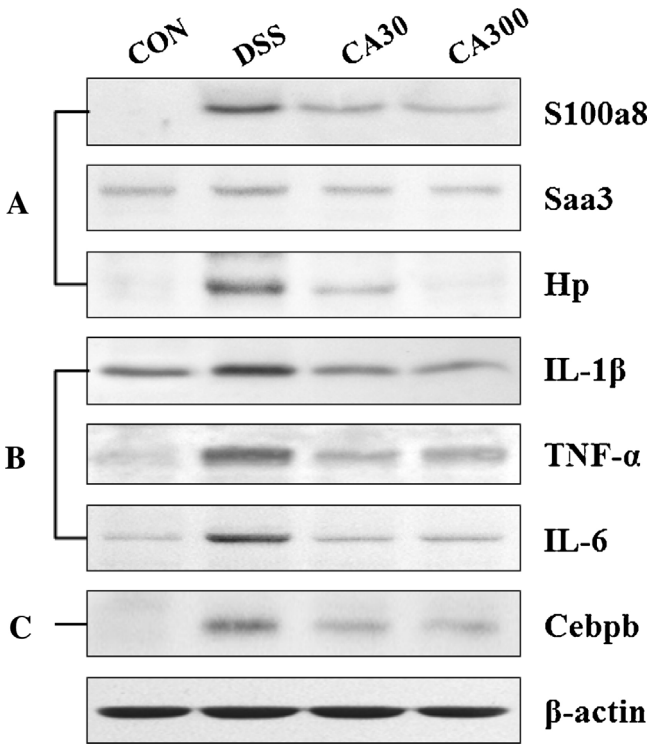

Fig. 3. Effects of CA on NF-κB activation during acute colitis induced by DSS in mice and LPS-induced RAW 264.7 cells. (A) Western blot analysis was performed on colon protein extracts after 7 days of DSS treatment with or without CA (30 or 300 mg/kg). (B) RAW 264.7 cells were pretreated with CA (1–100 μM) for 8 h and then stimulated with LPS (500–ng/mL) for 2 h. NF-κB protein was detected in the nuclear faction from RAW 264.7 cells. (C) DNA binding activity of NF-κB was measured by electrophoretic mobility shift assay using the NF-κB oligonucleotide. An unlabeled probe was used as a control in the competition assay and is indicated as "Cold".

activation in colitic colon tissues induced by DSS and confirmed in LPS-stimulated murine macrophage RAW 264.7 cells. In DSS-treated colon tissue, the phosphorylation of IκB and NF-κB p65 was increased by DSS, indicating the activation of the NF-κB signaling pathway, and phosphorylation was blocked by CA treatment (Fig. 2A). The efficacy of CA in regulating NF-κB translocation and its promoter-binding activity was also validated in an in vitro system (Fig. 2B). These results are in agreement with another study using an experimental colitis model, which showed that the beneficial effect of CA was dependent on activation of NF-κB [6].

In addition to the common roles of NF-κB in inflammatory cytokines, recent studies have reported S100a8/a9-induced NF-κB activation and NF-κB-dependent transcription in rheumatoid arthritis and in colon cancer cells [34,40]. The present study suggests that oral administration of CA effectively suppresses colon inflammation and that the beneficial effect of CA is linked, in part, to the regulation of NF-κB activity and the downstream inflammation-related genes. In addition to the above findings, the involvement of intestinal epithelial cells [38,3], toll like receptors [41] and transient receptor potential channels [14] in the action of CA on the DSS-induced colitis cannot be ruled out. Furthermore, [15] showed that CA was a selective agonist of cannabinoid receptor type-2 (CB2) and exerted cannabimimetic anti-inflammatory effects in mice. These suggest that further studies are needed to elucidate the molecular mechanism underlying the action of CA on the DSS-induced colitis (Fig. 3).

The present study confirmed that CA, given orally, decreased the DAI score, colon weight/length ratio, histological tissue inflammation, and colon MPO activity in a mouse model of DSS-induced colitis. In the colitic colon tissue, 26 inflammatory genes regulated by CA were identified and the increase in NF-κB activity by DSS was significantly suppressed by CA. These results suggest that CA attenuates DSS-induced colitis in mice and might be a potent natural treatment for ameliorating IBDs.

Conflict of interest

There is no conflict of interest in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.toxrep.2015.07.018

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