Arvelexin Inhibits Colonic Inflammation by Suppression of NF-κB Activation in Dextran Sulfate Sodium-Induced Mice and TNF-α-Induced Colonic Epithelial Cells

Eu-Jin Cho,[†,∥] Ji-Sun Shin,[†,∥,§,∇] Kyung-Sook Chung,[†,⊥] Yong Sup Lee,[⊥] Young-Wuk Cho,[§,∇] Nam-In Baek,[#] Hae-Gon Chung,[⊗] and Kyung-Tae Lee*,[†,⊥]

[†]Department of Pharmaceutical Biochemistry, College of Pharmacy, and [⊥]Department of Life and Nanopharmaceutical Science, College of Pharmacy, Kyung Hee University, Seoul, Korea

[§]Reactive Oxygen Species Medical Research Center, and [∇]Department of Physiology, School of Medicine, Kyung Hee University, Seoul, Korea

[#]Graduate School of Biotechnology and Plant Metabolism Research Center, Kyung Hee University, Suwon, Korea

[⊗]GangHwa Agricultural R&D Center, Incheon, Korea

ⓢ Supporting Information

ABSTRACT: Recently, we reported the anti-inflammatory effects of arvelexin isolated from *Brassica rapa* in macrophages. In the present study, the effects of arvelexin were investigated in a dextran sulfate sodium (DSS)-induced colitis mouse model and in a cellular model. In the DSS-induced colitis model, arvelexin significantly reduced the severity of colitis, as assessed by disease activity, colonic damage, neutrophil infiltration, and levels of colonic iNOS. Moreover, arvelexin inhibited the expressions of IL-8, IP-10, ICAM-1, and VCAM-1 in HT-29 colonic epithelial cells. Arvelexin also inhibited the TNF-α-induced adhesion of U937 monocytic cells to HT-29 cells. Furthermore, arvelexin reduced p65 NF-κB subunit translocation to the nucleus and IκBα degradation in the colonic tissues and in TNF-α-induced HT-29 cells. These results demonstrate that the ameliorative effects of arvelexin on colonic injury are mainly related to its ability to inhibit the inflammatory responses via NF-κB inactivation, and support its possible therapeutic role in colitis.

KEYWORDS: adhesion molecule, Arvelexin, Brassica rapa, chemokine, NF-κB, ulcerative colitis

■ INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory bowel diseases (IBD) characterized by aberrant responses to luminal bacteria in genetically susceptible subjects and by a chronic inflammatory disorder of the digestive tract.[1,2] Although the etiologies of IBDs have not been established, evidence indicates that the involvement of damaged epithelia and activated immune cells in inflamed mucosa play an important role.[3] In addition, patients with long-standing IBD are at great risk of developing colitis-associated cancer.[4] At present, the medical treatment of IBD relies mainly on traditional drugs, such as 5-aminosalicylates (5-ASA), corticosteroids, and immunosuppressants like azathioprine and 6-mercaptopurine. Furthermore, antibiotics are becoming increasingly important in the settings of steroid resistant disease and steroid-dependent patients.[5] However, their side effects and systemic actions are so severe that they disturb quality of life, particularly when administered long-term.[6] Thus, it is important to develop an optimal therapy for IBD.

The intestinal epithelium serves as a barrier between luminal triggers and the host, and an impaired barrier function could lead to increased uptake of luminal antigens that promote mucosal inflammation. In addition, the activation of the proinflammatory gene transcription program in colonic epithelial cells challenged by bacterial products, such as lipopolysaccharide (LPS) or inflammatory cytokines like

tumor necrosis factor-α (TNF-α) or interleukin-1β (IL-1β), is associated with acute and chronic intestinal inflammation.[7] The infiltration and migration of inflammatory cells, such as neutrophils and monocytes, into tissue lesion sites are dependent on cytokine and chemokine levels. Chemokines, such as interleukin-8 (IL-8) and interferon-γ inducible protein-10 (IP-10), play critical roles by activating and attracting leukocytes to sites of inflammation and by upregulating adhesion molecules. Furthermore, IL-8 and IP-10 have been reported to be markedly expressed in the mucosa of colitis, and IL-8 expression was even found to be correlated with the degree of colon inflammation.[8,9] In addition to chemokines, intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), both members of the immunoglobulin superfamily of proteins, are crucially involved in the trafficking of inflammatory cells through endothelial and epithelial barriers in the inflamed colon.[10,11]

Nuclear factor-kappa B (NF-κB) activation appears to be strongly associated with IBD.[12] NF-κB is composed mainly of two proteins; p65 and p50. Under normal conditions in intestinal epithelial cells, NF-κB dimers are present in
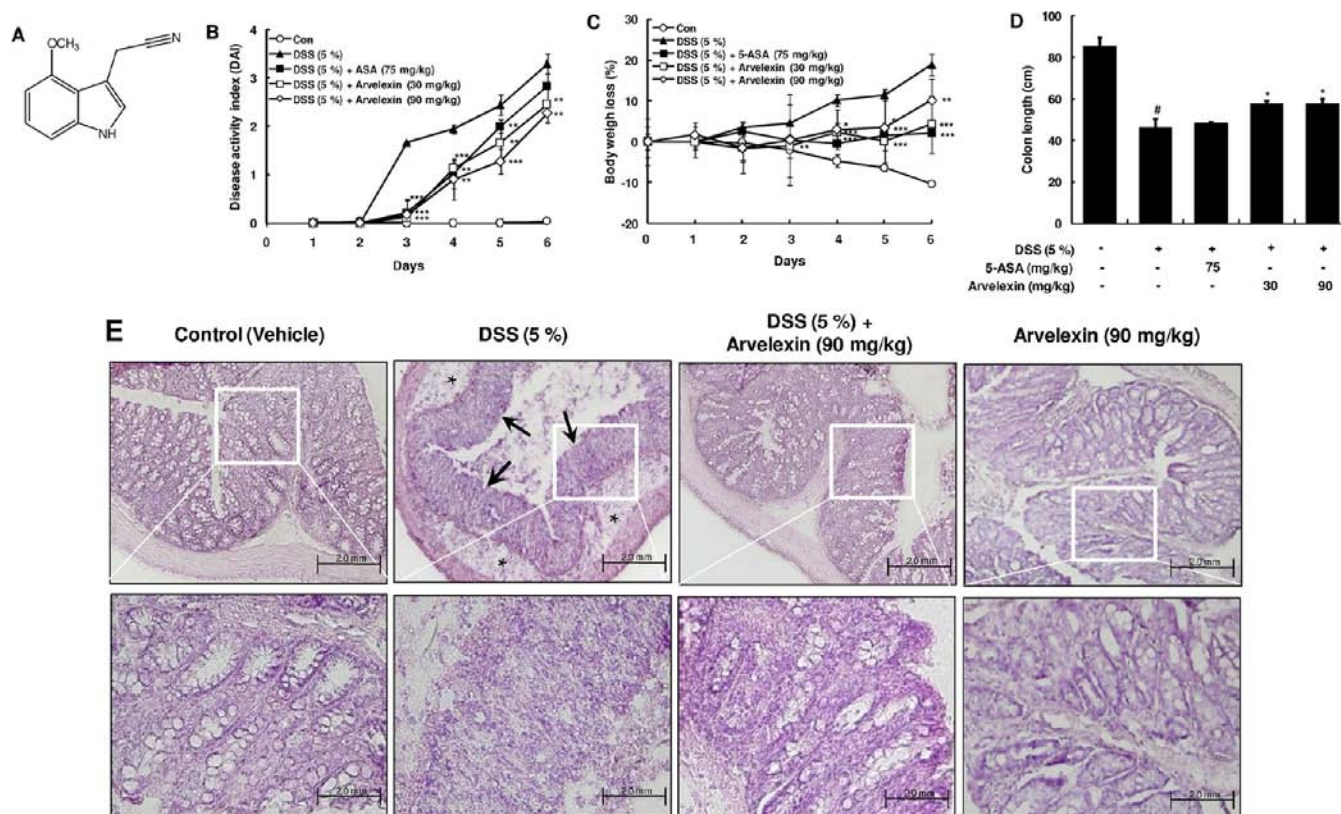
Figure 1. Arvelexin attenuated the progression of DSS-induced colitis in mice. A: Chemical structure of arvelexin. B: Mice were administered 5% DSS in drinking water (ad libitum) for 6 days with or without arvelexin (30 or 90 mg/kg/day po). 5-ASA (75 mg/kg/day po) was used as a positive control. Changes in DAI levels were evaluated daily throughout the 6 day administration period. C: Changes in body weights were monitored daily. Body weight losses are expressed as percentages of body weights on immediately prior to treatment. D: Colons were obtained after 6 days of DSS administration, and their lengths were measured. Values are the mean $\pm$ SD ($n = 8$); [#]$P < 0.05$ vs the vehicle-treated control group; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs the DSS-induced group; significances between treated groups were determined using ANOVA and Dunnett's post hoc test. E: Histological analysis of DSS induced damage. Representative H&E sections of colonic tissues were obtained from control mice (vehicle), DSS-induced mice (5% DSS, ad libitum), and DSS-induced mice treated with arvelexin (90 mg/kg/day po) for 6 days. Asterisks indicate distension of the submucosa caused by edema. Black arrows mark lesions with complete loss of crypt morphology.

cytoplasm as NF-κB-Inhibitor of kappa B (IκB) complex, and activation of this complex by proinflammatory cytokines leads to the phosphorylation of IκB proteins and their subsequent recognition by ubiquitin ligases. The resulting proteasomal degradation of IκB proteins leads to the translocation of NF-κB to the nucleus, where it binds to its consensus DNA binding sites to regulate the transcriptions of a large number of genes that include proinflammatory cytokines, adhesion molecules, chemokines, and inducible enzymes.[13,14] Furthermore, increases in intestinal epithelial tight-junction permeability require NF-κB activation, which suggests that NF-κB signaling is a potential therapeutic target for the treatment of IBD.[15−17]

Arvelexin (4-methoxyindole-3-acetonitrile; Figure 1A) is a biologically active compound found in *Brassica rapa* L. (Brassicaceae), a species that includes many important crops, such as the Chinese cabbage and the turnip, the latter of which are cultivated in quantity commercially in South Korea to make kimchi.[18] In traditional medicine, *B. rapa* has been used to treat a variety of diseases and conditions, such as hepatitis, jaundice, furuncle, and sore throat. Furthermore, several studies have suggested that extracts of *B. rapa* prevent high-fat diet-induced obesity,[19] modulate the deleterious effects of diabetes,[18] protect against cisplatin-induced nephrotoxicity,[20] and exert anti-inflammatory effects on LPS-induced macrophages and carrageenan-induced paw edema.[21] Although *B. rapa* contains

numerous biologically active compounds, such as flavonoids (isorhamnetin, kaempferol, and quercetin glycosides),[22] phenylpropanoid derivatives,[22] indole alkaloids,[23] and sterol glucosides,[23] the molecular targets and the mechanisms underlying the biological activities of these constituents are poorly defined. Among them, it is known that arvelexin, one of the phytoalexins secreted in Brassicaceae, exerts antifungal activity.[24] Several phytoalexins harbor various biological activities; especially resveratrol found in many plants including grapes and berries has potent anti-inflammatory, anticancer, and cardioprotective activities in vitro and in vivo.[25] To explore the beneficial effects of arvelexin on IBD, we investigated its anti-inflammatory properties and the underlying molecular mechanisms responsible in a DSS-induced colitis murine model and in TNF-α-induced HT-29 intestinal epithelial cells.

■ MATERIALS AND METHODS

Isolation of Indole Compounds from the Root of *B. rapa*. *Brassica rapa* L. emend. Metzg was obtained from the GangHwa Agricultural R&D Center (Incheon, Republic of Korea), and its identity was confirmed by one of the authors (H.-G.C.). A voucher specimen (number 05157) has been deposited at the Laboratory of Natural Product Chemistry, Kyung Hee University (Suwon, Republic of Korea). Five kilograms of the fresh root skin of *B. rapa* were immersed in 80% MeOH solution (10 L) and left for 24 h at room temperature with occasional stirring. The extract was filtered and the

remainder was extracted twice more using the same method. The resulting filtrates were combined and concentrated under reduced pressure at 40 °C to obtain the MeOH extract (102 g). The MeOH extract (100 g) was then suspended in water (1 L) and extracted with ethyl acetate (EtOAc, 1 L × 2). The EtOAc and aqueous layers were then concentrated under reduced pressure at 40 °C to afford an EtOAc fraction (BRE, 7 g) and a H_2O fraction (BRW, 89 g). The BRE (7 g) was applied to the silica gel column chromatography, eluted with n-hexane−EtOAc (10:1 → 5:1 → 3:1→ 1:1) and $CHCl_3$−MeOH (10:1 → 7:1 → 5:1 → 3:1 → 2:1 → 1:1) to afford 23 fractions (BRE1−BRE23). The fraction BRE9 (268 mg) was subject to octadecyl silica gel column chromatography, and MeOH−H_2O was used as an eluting solution to give a purified indole compound, arvelexin [BRE9-3, 21 mg, V_e/V_t 0.50−0.63, TLC (SiO_2 F_{254}) R_f = 0.42 in n-hexane−EtOAc (1:1), (RP-18 F_{254}) R_f = 0.62 in MeOH−H_2O (2:1)]. The chemical structures of arvelexin were determined by comparing their physicochemical and spectroscopic data reported values.[26] The compound used for this study were examined by HPLC and were >97% pure. The Shimadzu LC-20A was used for HPLC analysis, equipped with a UV detector (268 nm) (Shimadzu, Tokyo, Japan). Chromatography was performed on a Waters C_{18} column (5 μm, 250 × 4.6 mm) set at 30 °C. Aliquots of 20 μL of each sample solution were injected and eluted according to the following program at a flow rate of 0.7 mL/min: 45% MeOH → 70% MeOH from 0 to 10 min, 75% MeOH → 90% MeOH from 20 to 25 min, 90% MeOH to 40 min. The peak of arvelexin was appeared at 16.62 min.

Experimental Animals. All animal care and experimental procedures complied with the Guidelines of the Committee for Animal Care and Use of laboratory animals, College of Pharmacy, Kyung Hee University, according to an animal protocol (Approval No. # KHP-2009-12-2). Male ICR mice weighing 28−30 g were purchased from Daehan Biolink (Eumsung, Korea). All mice were housed 4/cage and fed standard laboratory chow in the animal room with 12 h dark/light cycles and constant temperature (temperature, 20 ± 5 °C; humidity, 40−60%; light/dark cycle, 12 h) for 2 weeks or more.

Induction of Colitis. Experimental colitis was induced by giving mice drinking water ad libitum containing 5% (w/v) DSS for 6 days. Mice of each group were monitored carefully every day to confirm that they consumed an approximately equal volume of DSS-containing water. 5-ASA was dissolved in 0.9% saline, and arvelexin was dissolved in vehicle (0.9% saline containing 0.01% Tween 20 and 0.5% carboxymethyl cellulose sodium). Control groups were given vehicle daily for 6 days. 5-ASA (75 mg/kg/day po) and arvelexin (30 or 90 mg/kg/day po) were administrated daily for 6 days, commencing at the same time DSS exposure began. The experiments were conducted two times with eight mice in each group.

Evaluation of Disease Activity Index (DAI). Body weight, stool consistency, and gross bleeding were recorded daily. Disease activity index (DAI) was determined by combining scores of (i) body weight loss, (ii) stool consistency, and (iii) gross bleeding, divided by 3. Each score was determined as follows: change in body weight loss (0, none; 1, 1−5%; 2, 5−10%; 3, 10−20%; 4, >20%), stool blood (0, negative; 1, +; 2, ++; 3, +++; 4, ++++) and stool consistency (0, normal; 1 and 2, loose stool; 3 and 4, diarrhea). Body weight loss was calculated as the percent difference between the original body weight (day 0) and the body weight on any particular day (Table 1.). At the end of the experiment, mice were killed and the colons were separated from the proximal rectum, close to its passage under the pelvisternum. The

colon length was measured between the ileo−cecal junction and the proximal rectum.

Histopathology. The resected large intestine was grossly examined for the mucosal defect, hemorrhage, or ulcerative lesions, and then fixed immediately in 4% neutral formalin. For histopathological analysis, tissue sections were made from the representative region of large intestine by the conventional tissue preparation methods, and viewed under the light microscope (× 10−1000) after hematoxylin and eosin (H&E) staining. For immunohistochemistry, frozen midcolons were sectioned on a cryostat at 5 μm, and stored in storage solution at −70 °C until required. After air-drying, sections were treated with 3% H_2O_2 for 5 min in the dark, and sections were incubated with polyclonal anti-inducible nitric oxide synthase (iNOS) antibody and anti-IκB antibody (Santa Cruz, CA, USA) diluted 1:100 in PBS containing 3% Triton X-100, 0.5 mg/mL of bovine serum albumin and 1.5% normal horse serum, overnight at 4 °C in a humidified chamber. After incubation with primary antibody, the sections were then incubated with biotinylated secondary antibody (1:200 dilution; Vector Laboratories Inc., Burlingame, CA, USA) for 90 min, avidin−biotin−peroxidase complex (1:100 dilution; Vector Laboratories Inc., CA, USA) at room temperature for 1 h. Peroxidase activity was visualized with the use of diaminobenzidine.

Myeloperoxidase (MPO) Assay. MPO accumulation was measured in soft tissue from colon, as a marker of neutrophil influx into the tissue. The tissue was thawed and homogenized in lysis beffer (10 mM Tris pH 7.4, 200 mM NaCl, 5 mM EDTA, 10% glycerol, 1 mM phenylmethylsulfonylfluoride (PMSF), 1 μg/mL leupeptide and 28 μg/mL aprotonine). The homogenate was then centrifuged at 1500g for 15 min, and the resulting supernatant was assayed for MPO assay using EIA kits (Hycult Biotech, Netherlands).

Western Blot Analysis. Segments of colon or cell were homogenized and resuspended in PRO-PREP protein extraction solution (Intron Biotechnology, Seoul, Korea) and incubated for 20 min at 4 °C. Tissue or cell debris was removed by microcentrifugation, followed by quick freezing of the supernatants. Nuclear proteins were extracted with NE-PER (Thermo Fisher Scientific, Waltham, MA, USA), followed by the Thermo manufacturer's instructions. The protein concentration was determined using the Bio-Rad protein assay reagent according to the manufacturer's instruction. Cellular protein was electroblotted onto a PVDF membrane following separation on 10% SDS−polyacrylamide gel electrophoresis. The immunoblot was incubated for 1 h with blocking solution (5% skim milk) at room temperature, followed by incubation overnight with a primary antibody. Blots were washed four times with Tween 20/Tris-buffered saline and incubated with a 1:1000 dilution of horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature. Blots were again washed three times with Tween 20/Tris-buffered saline, and then developed by enhanced chemiluminescence (GE Healthcare, WI, USA).

Cell Culture and Sample Treatment. The HT-29 human colonic epithelial cell line and U937 human monocyte cell line were obtained from the Korea Cell Line Bank (Seoul, Korea). These cells were grown at 37 °C in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin (100 units/mL) and streptomycin sulfate (100 μg/mL) in a humidified atmosphere of 5% CO_2. Cells were incubated with arvelexin at concentrations of 25, 50, 100, or 200 μM, and then stimulated with TNF-α (10 ng/mL) for the indicated time. Various concentrations of tested compounds dissolved in DMSO were added to the medium. The final concentration of DMSO did not exceed 0.05%.

RNA Preparation and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). Total cellular RNA was isolated using Easy Blue kits (Intron Biotechnology, Seoul, Korea). From each sample, 1 μg of RNA was reverse-transcribed (RT) using MuLV reverse transcriptase, 1 mM deoxyribonucleotide triphosphate (dNTP), and oligo (dT$_{12−18}$) 0.5 μg/μL. PCR analyses were performed on aliquots of the cDNA preparations to detect IL-8, IP-10, ICAM-1, VCAM-1, and GAPDH (as an internal standard) gene expression using a thermal cycler (Perkin-Elmer Cetus, CA, USA). Reactions were carried out in a volume of 25 μL containing 1 unit of

Table 1. Evaluation of Disease Activity Index (DAI)

DAI score	wt loss (%)	stool consistency	occult/gross bleeding
0	none	normal	normal
1	1−5		
2	5−10	loose stools	hemoccult positive
3	10−20		
4	>20	diarrhea	gross bleeding

Taq DNA polymerase, 0.2 mM dNTP, ×10 reaction buffer, and 100 pmol of 5′ and 3′ primers. After an initial denaturation for 2 min at 95 °C, 26 or 30 amplification cycles were performed for IL-8 (1 min of 94 °C, 1 min of 56 °C, and 1 min 72 °C), IP-10 (1 min of 94 °C, 1 min of 55 °C, and 1 min 72 °C), ICAM-1 (1 min of 94 °C, 1 min of 50 °C and 1 min 72 °C), and VCAM-1 (1 min of 94 °C, 1 min of 50 °C, and 1 min 72 °C). The PCR primers used in this study are listed in Table 2

Table 2. PCR Primer Sequence

gene		sequence
IL-8	sense	5′-CAAACCTTTCCACCCCAAAT-3′
	antisense	5′-ACCCTCTGCACCCAGTTTTC-3′
IL-10	sense	5′-CTGCCATTCTGATTTGCTGC-3′
	antisense	5′-ACGTGGACAAAATTGGCTTG-3′
ICAM-1	sense	5′-TTATACACAAGAACCAGACC-3′
	antisense	5′-TGTATCTGACTGAGGACAAT-3′
VCAM-1	sense	5′-GAGAAACCATTTACTGTTGA-3′
	antisense	5′-ACATAAAGTGTTTGCGTACT-3′
GAPDH	sense	5′-ATGGTTTACATGTTCCAATA-3′
	antisense	5′-GTCTCTCTCTTCCTCTTGTG-3′

and were purchased from Bioneer (Seoul, Korea). After amplification, the PCR reactions were electrophoresed on 2% agarose gel and visualized by ethidium bromide staining and UV irradiation.

RNA Interference. Cells were transiently transfected with 100 nM of NF-κB p65 siRNA or control siRNA (Bioneer, Seoul, Korea) using Lipofectamine RNAi MAX reagent (Invitrogen, CA, USA) for 24 h by following the manufacturer's instructions.

Monocyte Adhesion Assay. Monocyte-epithelial cell adhesion was evaluated using human leukemia premonocytic U937 cells. U937 cells were labeled with 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acethoxymethyl ester (BCECFAM, 10 μg/mL; Sigma, MO, USA) for 1 h at 37 °C. HT-29 cells cultured in a 48-well plate were pretreated with arvelexin for 1 h and then incubated with TNF-α for an additional 8 h. Then, HT-29 cells were coincubated with BCECFAM-prelabeled U937 cells (4×10^5 cells/well) for 1 h at 37 °C. Nonadhering U937 cells were removed by washing gently with PBS. For quantitative analysis, the cells were lysed in 0.1% Triton X-100 in 0.1 M Tris at room temperature for 30 min in the dark. Fluorescence intensity was measured using a FluostarOptima microplate reader (Perkin-Elmer, CA, USA) with excitation at 485 nm and emission at 520 nm.

Statistical Analyses. Results are expressed as the mean ± SD. Statistically significant values were compared using ANOVA and Dunnett's post hoc test, and P-values of less than 0.05 were considered statistically significant.

■ RESULTS

Arvelexin Attenuated Severity, Weight Loss, and Colon Length Shortening in the DSS-Induced Colitis Model. To determine the effects of arvelexin on colitis, mice were administered 5% DSS in drinking water for 6 days with or without arvelexin (30 or 90 mg/kg/day, po) once a day. Progressive body weight loss, hematochezia, and diarrhea were noted from 3 days after administration of 5% DSS. Following induction of colitis, a combinatorial DAI, which incorporate body weight loss, stool consistency, and gross bleeding, was significantly increased in DSS-treated mice, but the administration of arvelexin (30 or 90 mg/kg/day, po) significantly reduced DAI values versus the DSS-treated group from 3 days post-treatment (Figure 1B). In particular, arvelexin had better

suppressive effects than 5-ASA (75 mg/kg/day, po), the reference drug. As shown Figure 1C, DSS-exposed mice body weights significantly decreased for experiment periods. However, treatment with arvelexin or ASA reduced this weight loss. In addition, colon lengths in DSS-treated mice were markedly shorter than in vehicle-treated control mice, and arvelexin significantly reduced the colon shortening caused by DSS (Figure 1D). To investigate the protective effects of arvelexin on DSS-induced colitis, pathological examinations of colon and rectum sections were carried out after H&E staining. As shown in Figure 1E, tissue sections from vehicle-treated control mice exhibited a histologically normal colon structure, whereas histologic examination of colon sections of mice with DSS-induced colitis showed mucosal inflammation in the rectum with reduced proximal severity, as previously described.[27] Colon structures in the DSS group showed severe disruption of the tissue architecture, edema, and mixed inflammatory cell infiltration with ulcerations and large areas of epithelial denudation. Interestingly, the evaluation of colons from arvelexin-administered mice (90 mg/kg) revealed an obvious reduction in inflammatory response, which resulted in a prominent decrease in microscopic tissue damage as compared with colons from DSS-treated mice. In order to identify possible side effects of arvelexin, we administered arvelexin at 90 mg/kg for 7 days without DSS exposure. A microscopic examination of colon sections revealed no evident changes (Figure 1E).

Arvelexin Administration Reduced MPO Activity and iNOS Expression in DSS-Induced Colon Tissues. DSS-induced colon damage is associated with an influx of inflammatory cells, such as neutrophils and lymphocytes, into the intestinal mucosa that promote extensive oxidant and protease-dependent injury.[28,29] Therefore, we assessed whether the protective effects of arvelexin on DSS-induced colitis are associated with alterations in the infiltrations of inflammatory cells by determination of MPO levels. This enzyme is produced mainly by polymorphonuclear leukocytes, and its levels are correlated with the degree of neutrophil infiltration in tissues.[30] After 6 days of DSS treatment, MPO activity was markedly increased to a level about 11 times higher than that in untreated control group (Figure 2A), and this increase in MPO activity was significantly reduced by arvelexin administration (30 and 90 mg/kg) or 5-ASA (75 mg/kg).

The expression of iNOS in intestinal mucosa appears to be enhanced in active ulcerative colitis, and when present in excess, it plays a proinflammatory role and increases oxidative stress by producing excessive amounts of NO.[31] Therefore, we evaluated the effects of arvelexin on DSS-induced iNOS expression in colitis tissues by Western blot analysis. As shown in Figure 2B, arvelexin significantly inhibited DSS-induced iNOS expression in mice at day 6 post-treatment. In addition, these results were confirmed by H&E stained colonic sections (Figure 2C).

Arvelexin Suppressed NF-κB Activation in DSS-Induced Colitis. NF-κB is not only a key regulator in the immunological setting but also strongly influences mucosal inflammation.[32] Thus, we examined the effects of arvelexin on the nuclear translocation of the p65 NF-κB subunit in colonic tissues by Western blotting. As shown in Figure 3A, treatment with DSS for 6 days markedly increased the translocation of p65 to the nucleus, whereas arvelexin significantly suppressed the DSS-induced nuclear translocation of p65 in colonic tissues. Since NF-κB remains inactive in the cytosol because it is bound
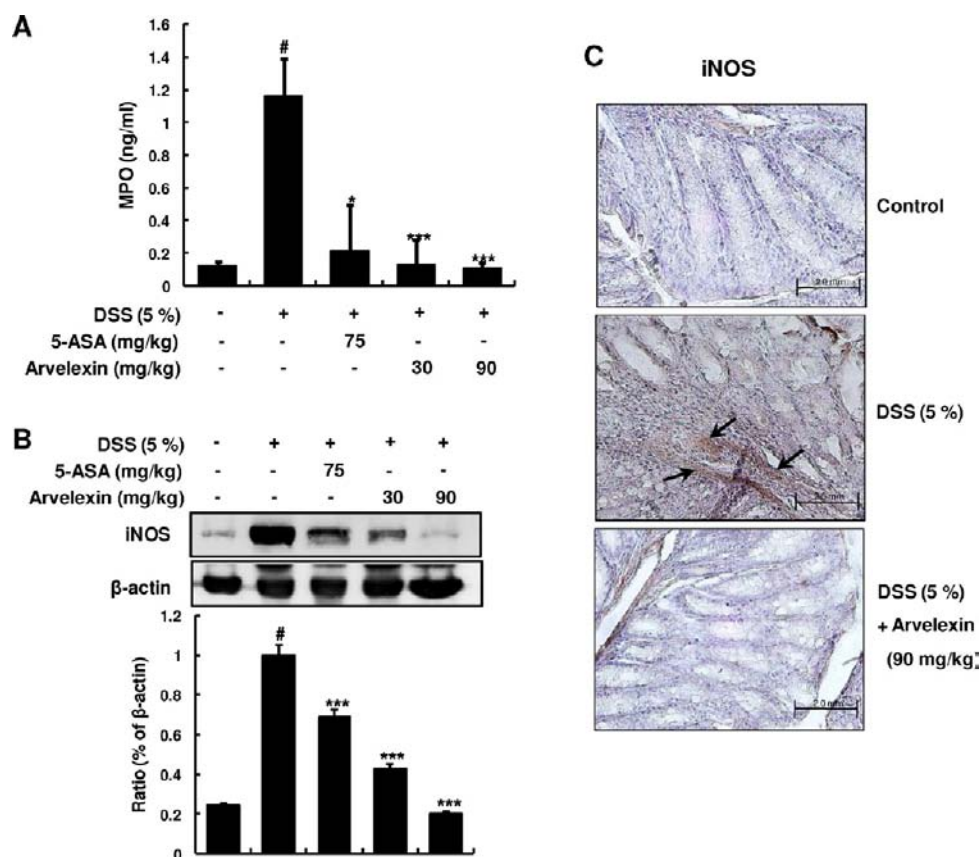
Figure 2. Arvelexin reduced the influx of inflammatory cells and iNOS expression in inflamed colons. After 6 days of DSS treatment with or without arvelexin, colon tissues were processed to determine MPO activity or iNOS expression levels. A, B: Colon segments from control mice (saline or vehicle), DSS-induced mice (5% DSS, ad libitum), and DSS-induced mice treated with arvelexin (30 or 90 mg/kg/day po) or 5-ASA (75 mg/kg/day po) were subjected to EIA to determine MPO levels. Values are the means $\pm$ SDs ($n = 8$); $^{\#}P < 0.05$ vs the vehicle-treated control group; $^{*}P < 0.05$, $^{***}P < 0.001$ vs DSS-induced group; significances between treated groups were determined using ANOVA and Dunnett's post hoc test. B: Whole proteins were prepared from mice for the Western blotting of iNOS using specific anti-iNOS monoclonal antibodies. β-Actin was used as internal control. Representative data is shown. C: Representative colonic sections from control mice (saline or vehicle), DSS-induced mice (5% DSS, ad libitum), and DSS-induced mice treated with arvelexin (90 mg/kg/day po) for 6 days were immunostained for iNOS as described in Materials and Methods. Arrows indicate iNOS-positive cells in mucosa.

by IκB, and activated and translocated to the nucleus by inflammatory signals,[33] we examined whether arvelexin inhibits the degradation of IκB in colonic tissue. Arvelexin was found to significantly prevent DSS-induced IκBα degradation in mice, and immunohistochemistry analysis showed that IκBα protein was more strongly stained in the colon tissues of control mice than in those of DSS-treated mice, and that this staining was recovered to the normal level by arvelexin administration (90 mg/kg) (Figure 3B).

Arvelexin Reduced TNF-α-Induced Monocyte-Epithelial Adhesion by Suppression of ICAM-1, VCAM-1, IL-8, and IP-10 Expressions in HT-29 Colonic Epithelial Cells. In IBD, it has been shown that the expressions of chemokines and adhesion molecules play important roles in inflammatory cell recruitment to inflamed intestines and developing colitis.[10,34,35] To investigate the cellular mechanisms responsible for DSS-induced colitis attenuation by arvelexin, we quantified the expressions of IL-8, IP-10, ICAM-1, and VCAM-1 in HT-29 intestinal epithelial cells by RT-PCR. TNF-α significantly upregulated the expressions of the IL-8, IP-10, and ICAM-1 genes and pretreatment of arvelexin significantly and concentration-dependently reduced these mRNA expressions. Interestingly, arvelexin so strongly inhibited TNF-α-induced VCAM-1 gene expression even at low concentration (Figure

4A). We next examined the inhibitory effects of arvelexin on the TNF-α-induced adhesion of U937 premonocytic cells to HT-29 human colonic epithelial cells to mimic the initial step of colon inflammation. HT-29 colonic epithelial cells were pretreated with arvelexin (25, 50, 100, or 200 μM) for 1 h and then treated with TNF-α (10 ng/mL) for 8 h. As shown in Figure 4B, TNF-α significantly increased the adhesion of U937 monocytes to HT-29 colonic epithelial cells, and arvelexin significantly and concentration-dependently reduced this TNF-α-induced monocyte-epithelial adhesion. In addition, MTT assays indicated that the suppressive effects of arvelexin on TNF-α-induced monocyte adhesion and chemokines and adhesion molecule expressions were not due to nonspecific cytotoxicity (Sup. Figure 1 in the Supporting Information).

Arvelexin Suppressed TNF-α-Induced NF-κB Activation in HT-29 Colonic Epithelial Cells. In IBD, the line of defense provided by intestinal epithelial cell is breached, and this results in an uncontrolled immune response.[36-38] Since it is generally known that NF-κB activation in epithelial cells induces the expressions of cytokines, chemokines, and adhesion molecules,[32] we evaluated the suppressive effects of arvelexin on TNF-α-induced NF-κB activation in HT-29 colonic epithelial cells. As demonstrated in Figure 5A, it was found that TNF-α markedly induced the translocation of p65 to the
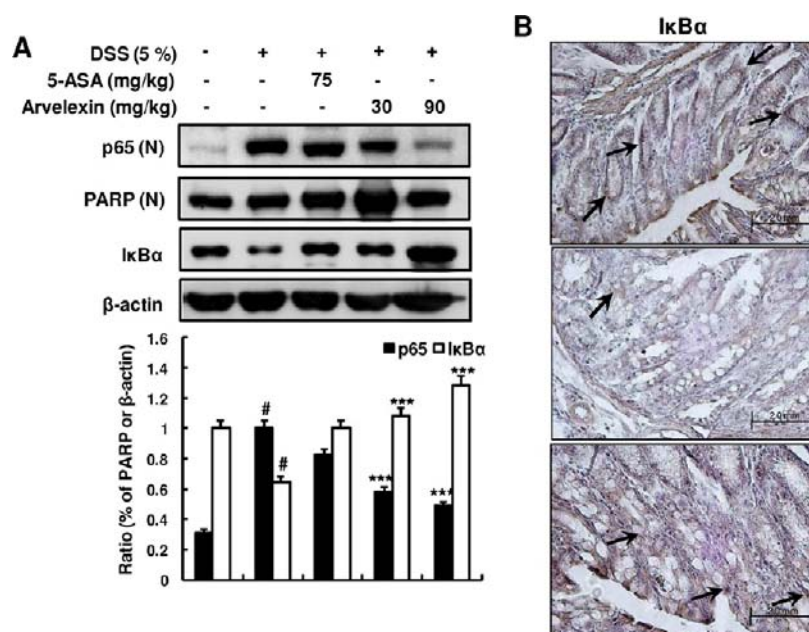
Figure 3. Arvelexin inhibited the translocation of NF-κB and degradation of IκBα in DSS-induced colitis mice. A: Colons were obtained after 6 days of DSS treatment (5% DSS, ad libitum) with or without arvelexin (30 or 90 mg/kg/day po) or 5-ASA (75 mg/kg/day, po), and colon tissues were investigated with respect to the translocation of NF-κB p65 and IκBα expression. Nuclear or whole proteins were prepared for the Western blotting of p65 and IκBα using specific anti-p65 and anti-IκBα monoclonal antibodies. PARP and β-actin were used as internal controls. Representative data is shown. B: Representative colonic sections from control mice (saline or vehicle), DSS-induced mice (5% DSS, ad libitum), and DSS-induced mice treated with arvelexin (90 mg/kg/day po) for 6 days were immunostained for IκBα as described in Materials and Methods. Arrows indicate IκBα-positive cells in mucosa.

nucleus and that arvelexin pretreatment significantly suppressed this translocation. Furthermore, TNF-α-induced IκBα degradation was prevented by arvelexin in HT-29 colonic epithelial cells. Furthermore, we confirmed our observation using NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC) and RNA interference technique to knockdown p65, which is an important subunit of NF-κB. Results showed that the NF-κB inhibition by inhibitor and p65 siRNA led to the inhibition of TNF-α-induced ICAM-1, VCAM-1, IL-8, and IP-10 expressions (Figure 5B). Taken together, these data showed that NF-κB is a potential target of arvelexin for inhibition of TNF-α-induced ICAM-1, VCAM-1, IL-8, and IP-10 expressions in HT-29 cells.

DISCUSSION

A DSS-induced murine colitis model exhibits symptoms comparable to those of human ulcerative colitis, that is, body weight loss, diarrhea, bloody feces, mucosal ulceration, and colonic shortening.[40−42] Although the mechanisms of DSS in experimental colitis remain poorly understand, there are several possible mechanisms of DSS-induced colitis: direct cytotoxic effects on epithelial cells[43] and indirect damage due to changes in resident bacteria,[42] upregulation of lymphocyte adhesion molecules on intestinal epithelial cells[44] and activation of gut macrophages and T cells.[42,45,46] These facts suggest that the initial insult in the DSS model is most likely at the epithelial cell level; and similar to human IBD, DSS induced intestinal inflammation in mice results in higher concentrations of proinflammatory mediators and excessive infiltration of immune cells within the colonic mucosa.[47,48] Previously, we found that arvelexin isolated from *B. rapa* inhibits proinflammatory mediators, and that these inhibitions resulted in the inhibition of IKK-dependent NF-κB activation in macrophages.

More importantly, arvelexin was found to decrease the serum levels of inflammatory mediators in vivo and to protect mice from LPS-induced lethality.[39] In the present study, we extended our study to ameliorative effects of arvelexin on experimental colitis. Our findings demonstrate for the first time that arvelexin suppresses DSS-induced colitis in mice and TNF-α-induced inflammatory responses in colon epithelial cells by blocking NF-κB activation and, thus, reducing the expressions of inflammatory mediators.

Our findings show that arvelexin suppresses DSS-induced colitis in mice through increasing the body weight and stool consistency, decreasing bloody feces, and reducing colon length shortening. Furthermore, histopathologic findings confirmed that arvelexin treatment protected DSS-treated mice from intestinal epithelial damage, that is, from mucosal erosion, the distortion and loss of crypts and villi, ulceration, and epithelial layer erosion. Importantly, these events, in combination with weakening of epithelial tight junctions, can allow increased contact between antigens from the intestinal lumen and inflammatory and immune cells,[49] and as the present study shows arvelexin reduced all of these inflammatory changes. Especially, arvelexin effectively inhibited MPO activity, which is used as a marker of neutrophil infiltration.[50] This finding suggests that the inhibitory effects of arvelexin observed in DSS-induced colitis mice may, in part, be the results of reduced cell infiltration. It has been well established that inflammatory cell infiltration is responsible for extensive colon damage and the release of toxic components and free radicals.[40]

In IBD, oxidative stress is believed to be a major factor of tissue destruction,[51] and several authors have suggested that reactive oxygen and nitrogen metabolites are involved in the initiation and progression of IBD, prompted by the knowledge that nitrogen reacts with superoxide anions (O_2^-), produced
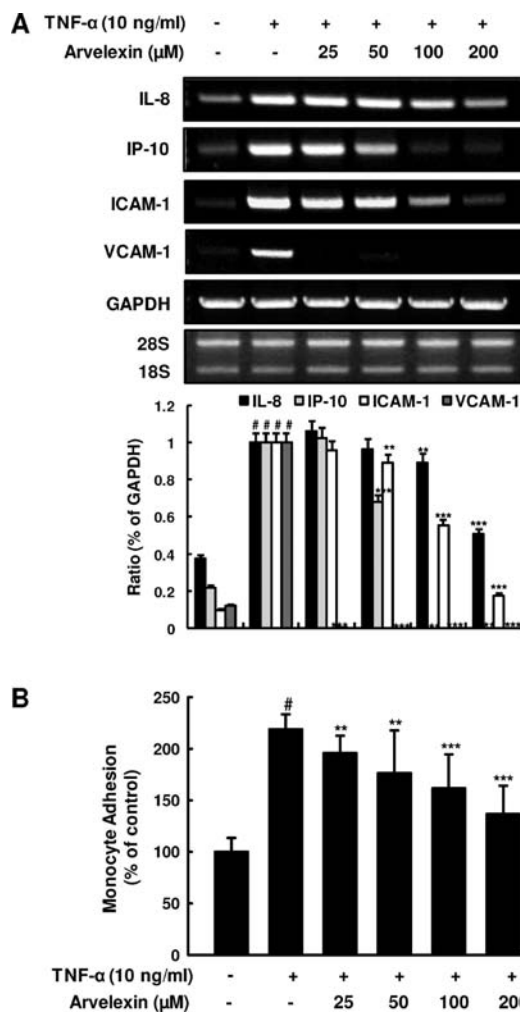
Figure 4. Arvelexin inhibited the TNF-α-induced expressions of chemokines and adhesion molecules in HT-29 cells and inhibited TNF-α-induced monocyte-epithelial cell adhesion. A: Total RNA was prepared from cells pretreated with arvelexin (25, 50, 100, or 200 μM) for 1 h and then stimulated with TNF-α (10 ng/mL) for 4 h (for IL-8, IP-10, and ICAM-1) or 12 h (for VCAM-1). mRNA levels of IL-8, IP-10, ICAM-1, and VCAM-1 were determined by RT-PCR, as described in Materials and Methods. The experiment was repeated three times, and similar results were obtained. B: HT-29 cells were pretreated with arvelexin (25, 50, 100, or 200 μM) for 1 h and, then, stimulated with TNF-α (10 ng/mL) for 8 h. HT-29 cells were then cocultured with U937 cells previously labeled with 10 μg/mL BCECF-AM. Cell adhesion was determined by measuring fluorescence intensities (excitation 485 nm and emission 520 nm). The values shown are means ± SDs of three independent experiments; $^#P < 0.05$ vs control group; $^{**}P < 0.01$, $^{***}P < 0.001$ vs TNF-α-induced group.

Figure 5. Arvelexin inhibited the activation of NF-κB in TNF-α-induced HT-29 cells. A: Cells were pretreated with arvelexin (25, 50, 100, or 200 μM) for 1 h and then stimulated with TNF-α (10 ng/mL) for 10 min or 1 h. Nuclear and whole proteins were prepared for the Western blotting of p65 and IκBα using specific anti-p65 and anti-IκBα monoclonal antibodies. PARP and β-actin were used as internal controls. B: Total RNA was prepared from cells pretreated with PDTC (10, 20, or 40 μM) for 1 h and then stimulated with TNF-α (10 ng/mL) for 4 h (for IL-8, IP-10, and ICAM-1) or 12 h (for VCAM-1). For p65 knockdown, cells were transfected with indicated concentration of p65 siRNA or control siRNA using Lipofectamine RNAi MAX reagent for 24 h. mRNA levels of IL-8, IP-10, ICAM-1, and VCAM-1 were determined by RT-PCR, as described in Materials and Methods. The experiment was repeated three times, and similar results were obtained.

during the course of inflammation, to form peroxynitrite (ONOO⁻), a highly cytotoxic oxidant.[52,53] In one study, the severity of DSS-induced colitis was found to be significantly attenuated by iNOS knockout and by treating mice with a specific iNOS inhibitor.[54] Recently, we suggested that arvelexin potently reduces the LPS-induced NO production and iNOS expression by inhibiting NF-κB and by activating the NF-E2-related factor 2 (Nrf2)/HO-1 signaling,[39] suggesting a possible protective action of arvelexin on oxidative stress. Similarly, in the present study, arvelexin reduced iNOS expression in the colonic tissues of mice treated with DSS.
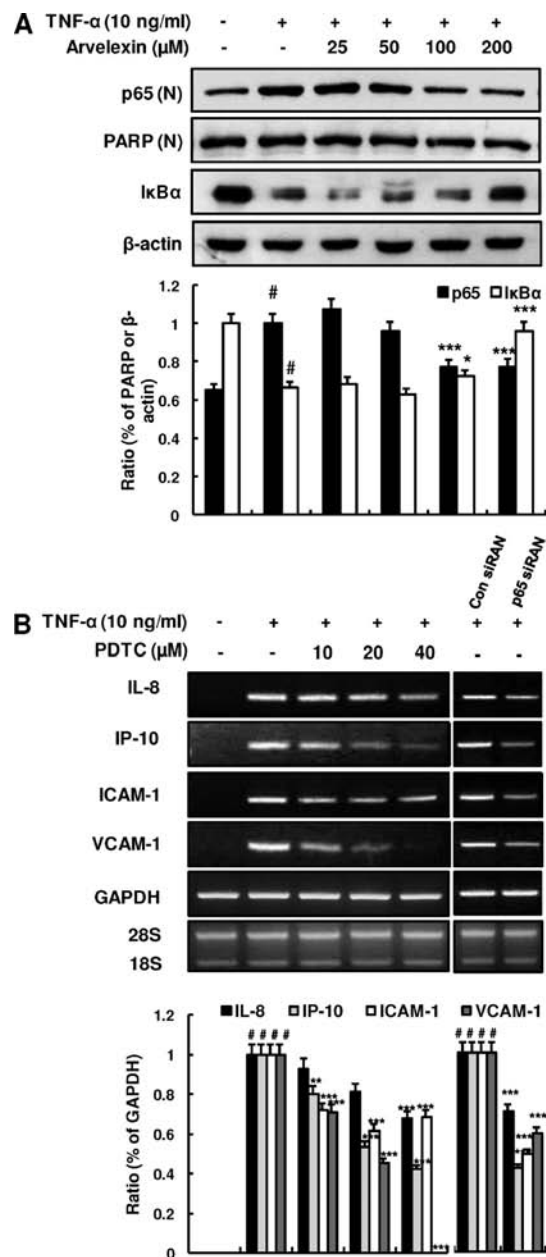
As mentioned above, colonic epithelial cells are one of the important factors during the progress of IBD. In particular, their hyperactivation of these cells facilitates the excessive infiltration of immune cells to tissue lesions which initiate and promote inflammation.[49] Furthermore, elevated levels of

chemokines and adhesion molecules, which activate and recruit leukocytes to an injured site and result in colonic tissue damage, in intestinal epithelial cells have been implicated in the pathogenesis of inflammatory diseases.[9,55] It has also been reported that expressions of IP-10, IL-8, and MCP-1 are significantly enhanced in UC patients,[8] and that ICAM-1 and VCAM-1, which are normally present in intestinal epithelial cells, are upregulated in the inflamed mucosa and serum of IBD patients.[56,57] Accordingly, these findings suggest that forced reductions in the expressions of chemokines and adhesion molecules offer an effective approach of preventing and treating IBD.[55,58] In the present study, it was observed that pretreatment of TNF-α-induced HT-29 colonic epithelial cells with arvelexin potently reduced the expressions of chemokines (IL-8 and IP-10) and adhesion molecules (ICAM-1 and VCAM-1), and consequently inhibited TNF-α-induced monocyte-epithelial cell adhesion. Furthermore, these results are in line with the observed marked inhibition of MPO activity (a surrogate of neutrophil infiltration) by arvelexin in DSS-induced colitis.

Since NF-κB regulates the expressions of proinflammatory enzymes, cytokines, and chemokines, it has been described as "a central mediator of immune response", and thus, the NF-κB signaling cascade is an attractive therapeutic target of inflammatory and autoimmune disorders.[59] NF-κB is activated in the gut of IBD patients, and the levels of activated NF-κB are correlated with the severity of intestinal inflammation.[60,61] Therefore, NF-κB activation appears to be strongly associated with IBD, which suggests that modulation of NF-κB signaling might be a potential target for the treatment of IBD.[62] Many of the already established immunosuppressive drugs in IBD like corticosteroids, sulfasalazine, methotrexate, and anti-TNF-α antibodies are known to mediate their anti-inflammatory effects at least partly via inhibition of NF-κB activity.[32] In agreement with these data, arvelexin induce an increased expression of IκBa, which in turn retains NF-κB in the cytoplasm and interact physically with p65, thereby preventing the transactivation of NF-κB. These results suggest that arvelexin ameliorates DSS-induced acute colitis by inhibiting NF-κB signaling. Furthermore, to concur with these fundamental data in vivo, we found that the inhibitory effects of arvelexin on NF-κB activation correlated with its ability to suppress the expressions of chemokines and adhesion molecules in TNF-α-induced HT-29 cells.

In summary, our results demonstrate that arvelexin prevents DSS-induced colitis development (based on DAI values and observations of histological severity), and that it significantly reduces MPO activity and iNOS expression by inhibiting the NF-κB pathway in DSS-induced colitis mice. In addition, arvelexin inhibits the expression of chemokines and adhesion molecules and, thus, suppresses the adhesion of inflammatory cells to colonic epithelial cells. Taken together, these results suggest that arvelexin had a protective effect on intestinal inflammation by inactivating NF-κB and downregulating the NF-κB-regulated transcription activation of proinflammatory mediator genes; and the modulation of pathophysiological activity during colonic inflammation by arvelexin offers a promising therapeutic means for treating colitis. However, based on present study, further extended research on the bioavailability of arvelexin is needed to properly assess their usefulness for treatment of disease.

ASSOCIATED CONTENT

Ⓢ Supporting Information
Figure depicting the effect of arvelexin on cell viability in HT-29 cells and U937 cells. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*Department of Pharmaceutical Biochemistry, College of Pharmacy, Kyung Hee University, Dongdaemun-Ku, Hoegi-Dong, Seoul, 130-701, Republic of Korea. Phone: +82 2 961 0860. Fax: +82 2 966 3885. E-mail: ktlee@khu.ac.kr.

Author Contributions
‖These authors contributed equally to this work.

Notes
The authors declare no competing financial interest.

REFERENCES

(1) Owczarek, D.; Cibor, D.; Szczepanek, M.; Mach, T. Biological therapy of inflammatory bowel disease. *Pol. Arch. Med. Wewn.* **2009**, *119*, 84−8.
(2) Peyrin-Biroulet, L.; Desreumaux, P.; Sandborn, W. J.; Colombel, J. F. Crohn's disease: beyond antagonists of tumour necrosis factor. *Lancet* **2008**, *372*, 67−81.
(3) Onizawa, M.; Nagaishi, T.; Kanai, T.; Nagano, K.; Oshima, S.; Nemoto, Y.; Yoshioka, A.; Totsuka, T.; Okamoto, R.; Nakamura, T.; Sakamoto, N.; Tsuchiya, K.; Aoki, K.; Ohya, K.; Yagita, H.; Watanabe, M. Signaling pathway via TNF-alpha/NF-kappaB in intestinal epithelial cells may be directly involved in colitis-associated carcinogenesis. *Am. J. Physiol.* **2009**, *296*, G850−9.
(4) Potack, J.; Itzkowitz, S. H. Colorectal cancer in inflammatory bowel disease. *Gut Liver* **2008**, *2*, 61−73.
(5) Hanauer, S. B. Inflammatory bowel disease. *N. Engl. J. Med.* **1996**, *334*, 841−8.
(6) Xu, C. T.; Meng, S. Y.; Pan, B. R. Drug therapy for ulcerative colitis. *World J Gastroenterol.* **2004**, *10*, 2311−7.
(7) Jobin, C.; Sartor, R. B. The I kappa B/NF-kappa B system: a key determinant of mucosalinflammation and protection. *Am. J. Physiol.* **2000**, *278*, C451−62.
(8) Uguccioni, M.; Gionchetti, P.; Robbiani, D. F.; Rizzello, F.; Peruzzo, S.; Campieri, M.; Baggiolini, M. Increased expression of IP-10, IL-8, MCP-1, and MCP-3 in ulcerative colitis. *Am. J. Pathol.* **1999**, *155*, 331−6.
(9) Helwig, U.; Gionchetti, P.; Rizzello, F.; Lammers, K.; Kuhbacher, T.; Schreiber, S.; Baggiolini, M.; Uguccioni, M.; Campieri, M. CXC and CC chemokine expression in inflamed and noninflamed pelvic ileal pouch tissue. *Int. J. Colorectal Dis.* **2004**, *19*, 165−70.
(10) Sans, M.; Panes, J.; Ardite, E.; Elizalde, J. I.; Arce, Y.; Elena, M.; Palacin, A.; Fernandez-Checa, J. C.; Anderson, D. C.; Lobb, R.; Pique, J. M. VCAM-1 and ICAM-1 mediate leukocyte-endothelial cell adhesion in rat experimental colitis. *Gastroenterology* **1999**, *116*, 874−83.
(11) Hopkins, A. M.; Baird, A. W.; Nusrat, A. ICAM-1: targeted docking for exogenous as well as endogenous ligands. *Adv. Drug Delivery Rev.* **2004**, *56*, 763−78.
(12) Li, Q.; Verma, I. M. NF-kappaB regulation in the immune system. *Nat. Rev. Immunol.* **2002**, *2*, 725−34.

(13) Karin, M.; Ben-Neriah, Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu. Rev. Immunol.* **2000**, *18*, 621−63.

(14) Takeda, K.; Kaisho, T.; Akira, S. Toll-like receptors. *Annu. Rev. Immunol.* **2003**, *21*, 335−76.

(15) Auphan, N.; DiDonato, J. A.; Rosette, C.; Helmberg, A.; Karin, M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science* **1995**, *270*, 286−90.

(16) De Bosscher, K.; Vanden Berghe, W.; Vermeulen, L.; Plaisance, S.; Boone, E.; Haegeman, G. Glucocorticoids repress NF-kappaB-driven genes by disturbing the interaction of p65 with the basal transcription machinery, irrespective of coactivator levels in the cell. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3919−24.

(17) Yamamoto, Y.; Gaynor, R. B. Therapeutic potential of inhibition of the NF-kappaB pathway in the treatment of inflammation and cancer. *J. Clin. Invest.* **2001**, *107*, 135−42.

(18) Jung, U. J.; Baek, N. I.; Chung, H. G.; Bang, M. H.; Jeong, T. S.; Lee, K. T.; Kang, Y. J.; Lee, M. K.; Kim, H. J.; Yeo, J.; Choi, M. S. Effects of the ethanol extract of the roots of Brassica rapa on glucose and lipid metabolism in C57BL/KsJ-db/db mice. *Clin. Nutr.* **2008**, *27*, 158−67.

(19) An, S.; Han, J. I.; Kim, M. J.; Park, J. S.; Han, J. M.; Baek, N. I.; Chung, H. G.; Choi, M. S.; Lee, K. T.; Jeong, T. S. Ethanolic extracts of Brassica campestris spp. rapa roots prevent high-fat diet-induced obesity via beta(3)-adrenergic regulation of white adipocyte lipolytic activity. *J. Med. Food* **2010**, *13*, 406−14.

(20) Kim, Y. H.; Kim, Y. W.; Oh, Y. J.; Back, N. I.; Chung, S. A.; Chung, H. G.; Jeong, T. S.; Choi, M. S.; Lee, K. T. Protective effect of the ethanol extract of the roots of Brassica rapa on cisplatin-induced nephrotoxicity in LLC-PK1 cells and rats. *Biol. Pharm. Bull.* **2006**, *29*, 2436−41.

(21) Shin, J. S.; Yun, C. H.; Cho, Y. W.; Baek, N. I.; Choi, M. S.; Jeong, T. S.; Chung, H. G.; Lee, K. T. Indole-Containing Fractions of Brassica rapa Inhibit Inducible Nitric Oxide Synthase and Pro-Inflammatory Cytokine Expression by Inactivating Nuclear Factor-kappaB. *J. Med. Food* **2011**, *14* (12), 1527−37.

(22) Romani, A.; Vignolini, P.; Isolani, L.; Ieri, F.; Heimler, D. HPLC-DAD/MS characterization of flavonoids and hydroxycinnamic derivatives in turnip tops (Brassica rapa L. Subsp. sylvestris L.). *J. Agric. Food Chem.* **2006**, *54*, 1342−6.

(23) Schonhof, I.; Krumbein, A.; Bruckner, B. Genotypic effects on glucosinolates and sensory properties of broccoli and cauliflower. *Nahrung* **2004**, *48*, 25−33.

(24) Pedras, M. S.; Zheng, Q. A.; Gadagi, R. S.; Rimmer, S. R. Phytoalexins and polar metabolites from the oilseeds canola and rapeseed: differential metabolic responses to the biotroph Albugo candida and to abiotic stress. *Phytochemistry* **2008**, *69*, 894−910.

(25) Sadruddin, S.; Arora, R. Resveratrol: biologic and therapeutic implications. *J. CardioMetab. Syndr.* **2009**, *4*, 102−6.

(26) Pedras, M. S.; Chumala, P. B.; Suchy, M. Phytoalexins from Thlaspi arvense, a wild crucifer resistant to virulent Leptosphaeria maculans: structures, syntheses and antifungal activity. *Phytochemistry* **2003**, *64*, 949−56.

(27) Tanaka, F.; Tominaga, K.; Ochi, M.; Tanigawa, T.; Watanabe, T.; Fujiwara, Y.; Ohta, K.; Oshitani, N.; Higuchi, K.; Arakawa, T. Exogenous administration of mesenchymal stem cells ameliorates dextran sulfate sodium-induced colitis via anti-inflammatory action in damaged tissue in rats. *Life Sci.* **2008**, *83*, 771−9.

(28) Baumgart, D. C.; Carding, S. R. Inflammatory bowel disease: cause and immunobiology. *Lancet* **2007**, *369*, 1627−40.

(29) Sasaki, M.; Bharwani, S.; Jordan, P.; Joh, T.; Manas, K.; Warren, A.; Harada, H.; Carter, P.; Elrod, J. W.; Wolcott, M.; Grisham, M. B.; Alexander, J. S. The 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor pravastatin reduces disease activity and inflammation in dextran-sulfate induced colitis. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 78−85.

(30) Islam, M. S.; Murata, T.; Fujisawa, M.; Nagasaka, R.; Ushio, H.; Bari, A. M.; Hori, M.; Ozaki, H. Anti-inflammatory effects of phytosteryl ferulates in colitis induced by dextran sulphate sodium in mice. *Br. J. Pharmacol.* **2008**, *154*, 812−24.

(31) Guslandi, M. Nitric oxide and inflammatory bowel diseases. *Eur. J. Clin. Invest.* **1998**, *28*, 904−7.

(32) Atreya, I.; Atreya, R.; Neurath, M. F. NF-kappaB in inflammatory bowel disease. *J. Intern. Med.* **2008**, *263*, 591−6.

(33) Ghosh, S.; Karin, M. Missing pieces in the NF-kappaB puzzle. *Cell* **2002**, *109* (Suppl.), S81−96.

(34) Vainer, B.; Nielsen, O. H.; Horn, T. Comparative studies of the colonic in situ expression of intercellular adhesion molecules (ICAM-1, −2, and −3), beta2 integrins (LFA-1, Mac-1, and p150,95), and PECAM-1 in ulcerative colitis and Crohn's disease. *Am. J. Surg. Pathol.* **2000**, *24*, 1115−24.

(35) Gerard, C.; Rollins, B. J. Chemokines and disease. *Nat. Immunol.* **2001**, *2*, 108−15.

(36) Danese, S.; Gasbarrini, A. Chemokines in inflammatory bowel disease. *J. Clin. Pathol.* **2005**, *58*, 1025−7.

(37) Keates, S.; Keates, A. C.; Mizoguchi, E.; Bhan, A.; Kelly, C. P. Enterocytes are the primary source of the chemokine ENA-78 in normal colon and ulcerative colitis. *Am. J. Physiol.* **1997**, *273*, G75−82.

(38) Lichtenstein, G. R. Chemokines and cytokines in inflammatory bowel disease and their application to disease treatment. *Curr. Opin. Gastroenterol.* **2000**, *16*, 83−8.

(39) Shin, J. S.; Noh, Y. S.; Lee, Y. S.; Cho, Y. W.; Baek, N. I.; Choi, M. S.; Jeong, T. S.; Kang, E.; Chung, H. G.; Lee, K. T. Arvelexin from Brassica rapa suppresses NF-kappaB-regulated pro-inflammatory gene expression by inhibiting activation of IkappaB kinase. *Br. J. Pharmacol.* **2011**, *164*, 145−58.

(40) Wirtz, S.; Neurath, M. F. Mouse models of inflammatory bowel disease. *Adv. Drug Delivery Rev.* **2007**, *59*, 1073−83.

(41) Lee, S. J.; Lim, K. T. Glycoprotein isolated from Ulmus davidiana Nakai regulates expression of iNOS and COX-2 in vivo and in vitro. *Food Chem. Toxicol.* **2007**, *45*, 990−1000.

(42) Okayasu, I.; Hatakeyama, S.; Yamada, M.; Ohkusa, T.; Inagaki, Y.; Nakaya, R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* **1990**, *98*, 694−702.

(43) Cooper, H. S.; Murthy, S. N.; Shah, R. S.; Sedergran, D. J. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab. Invest.* **1993**, *69*, 238−49.

(44) Ni, J.; Chen, S. F.; Hollander, D. Effects of dextran sulphate sodium on intestinal epithelial cells and intestinal lymphocytes. *Gut* **1996**, *39*, 234−41.

(45) Takizawa, H.; Asakura, H.; Sasakawa, T.; Bannai, H.; Nomoto, M. Immunological studies of the mucosa in colitis induced by sodium dextran sulfate in rats. *Adv. Exp. Med. Biol.* **1995**, *371B*, 1383−7.

(46) Takizawa, H.; Shintani, N.; Natsui, M.; Sasakawa, T.; Nakakubo, H.; Nakajima, T.; Asakura, H. Activated immunocompetent cells in rat colitis mucosa induced by dextran sulfate sodium and not complete but partial suppression of colitis by FK506. *Digestion* **1995**, *56*, 259−64.

(47) Beck, P. L.; Xavier, R.; Wong, J.; Ezedi, I.; Mashimo, H.; Mizoguchi, A.; Mizoguchi, E.; Bhan, A. K.; Podolsky, D. K. Paradoxical roles of different nitric oxide synthase isoforms in colonic injury. *Am. J. Physiol.* **2004**, *286*, G137−47.

(48) Beck, P. L.; Li, Y.; Wong, J.; Chen, C. W.; Keenan, C. M.; Sharkey, K. A.; McCafferty, D. M. Inducible nitric oxide synthase from bone marrow-derived cells plays a critical role in regulating colonic inflammation. *Gastroenterology* **2007**, *132*, 1778−90.

(49) Elson, C. O.; Cong, Y.; McCracken, V. J.; Dimmitt, R. A.; Lorenz, R. G.; Weaver, C. T. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. *Immunol. Rev.* **2005**, *206*, 260−76.

(50) Malle, E.; Furtmuller, P. G.; Sattler, W.; Obinger, C. Myeloperoxidase: a target for new drug development? *Br. J. Pharmacol.* **2007**, *152*, 838−54.

(51) Kruidenier, L.; Verspaget, H. W. Review article: oxidative stress as a pathogenic factor in inflammatory bowel disease–radicals or ridiculous? *Aliment. Pharmacol. Ther.* **2002**, *16*, 1997−2015.

(52) Boughton-Smith, N. K. Pathological and therapeutic implications for nitric oxide in inflammatory bowel disease. *J. R. Soc. Med.* **1994**, *87*, 312−4.

(53) Cross, R. K.; Wilson, K. T. Nitric oxide in inflammatory bowel disease. *Inflammatory Bowel Dis.* **2003**, *9*, 179−89.

(54) Krieglstein, C. F.; Cerwinka, W. H.; Laroux, F. S.; Salter, J. W.; Russell, J. M.; Schuermann, G.; Grisham, M. B.; Ross, C. R.; Granger, D. N. Regulation of murine intestinal inflammation by reactive metabolites of oxygen and nitrogen: divergent roles of superoxide and nitric oxide. *J. Exp. Med.* **2001**, *194*, 1207−18.

(55) Yusuf-Makagiansar, H.; Anderson, M. E.; Yakovleva, T. V.; Murray, J. S.; Siahaan, T. J. Inhibition of LFA-1/ICAM-1 and VLA-4/VCAM-1 as a therapeutic approach to inflammation and autoimmune diseases. *Med. Res. Rev.* **2002**, *22*, 146−67.

(56) Goke, M.; Hoffmann, J. C.; Evers, J.; Kruger, H.; Manns, M. P. Elevated serum concentrations of soluble selectin and immunoglobulin type adhesion molecules in patients with inflammatory bowel disease. *J. Gastroenterol.* **1997**, *32*, 480−6.

(57) Jones, S. C.; Banks, R. E.; Haidar, A.; Gearing, A. J.; Hemingway, I. K.; Ibbotson, S. H.; Dixon, M. F.; Axon, A. T. Adhesion molecules in inflammatory bowel disease. *Gut* **1995**, *36*, 724−30.

(58) Zhong, W.; Kolls, J. K.; Chen, H.; McAllister, F.; Oliver, P. D.; Zhang, Z. Chemokines orchestrate leukocyte trafficking in inflammatory bowel disease. *Front. Biosci.* **2008**, *13*, 1654−64.

(59) Uwe, S. Anti-inflammatory interventions of NF-kappaB signaling: potential applications and risks. *Biochem. Pharmacol.* **2008**, *75*, 1567−79.

(60) Jobin, C.; Sartor, R. B. NF-kappaB signaling proteins as therapeutic targets for inflammatory bowel diseases. *Inflammatory Bowel Dis.* **2000**, *6*, 206−13.

(61) Schottelius, A. J.; Baldwin, A. S., Jr. A role for transcription factor NF-kappa B in intestinal inflammation. *Int. J. Colorectal Dis.* **1999**, *14*, 18−28.

(62) Kim, J. M.; Kang, H. W.; Cha, M. Y.; Yoo, D.; Kim, N.; Kim, I. K.; Ku, J.; Kim, S.; Ma, S. H.; Jung, H. C.; Song, I. S.; Kim, J. S. Novel guggulsterone derivative GG-52 inhibits NF-kappaB signaling in intestinal epithelial cells and attenuates acute murine colitis. *Lab. Invest.* **2010**, *90*, 1004−15.