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Soyasaponin I Attenuates TNBS-Induced Colitis in Mice by Inhibiting NF-κB Pathway

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Soybean, which contains soyasaponins and isoflavones as representative constituents, exhibits anti-inflammatory and antioxidant effects. To understand the anti-inflammatory effects of soyasaponins, we isolated soyasaponin I, a major constituent of soybean, and investigated the inhibitory effects on inflammatory markers in LPS-stimulated mouse peritoneal macrophages and 3,4,5-trinitrobenzenosulfonic acid (TNBS)-induced colitic mice. Soyasaponin I, which exhibited lipid peroxidation-inhibitory effects *in vitro*, inhibited the production of proinflammatory cytokines (TNF-α and IL-1β), inflammatory mediators (NO and PGE2), and inflammatory enzymes (COX-2 and iNOS) in LPS-stimulated peritoneal macrophages. Soyasaponin I also suppressed the phosphorylation of IκB-α and the nuclear translocation of NF-κB. However, these soyasaponins barely inhibited mitogen-activated protein kinases. Oral administration of soyasaponin I (10 and 20 mg/kg) to TNBS-treated colitic mice significantly reduced inflammatory markers, colon length, myeloperoxidase, lipid peroxide (malondialdehyde and 4-hydroxy-2-nonenal), proinflammatory cytokines and NF-κB activation in the colon, as well as increased glutathione content, superoxide dismutase, and catalase activity. Based on these findings, soyasaponin I may attenuate colitis by inhibiting the NF-κB pathway.

KEYWORDS: Soyasaponin I; inflammation; colitis; lipid peroxidation; peritoneal macrophage; NF-κB

INTRODUCTION

Inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease, are chronically relapsing disorders of the intestine (*1*, *2*). Their pathogenic mechanism is assumed to be dysregulation of the intestinal immune response to intestinal environmental antigens, such as intestinal microflora, and is characterized by several factors, such as T cell-rich infiltrates, IL-6 increment and reactive oxygen species (ROS) overproduction in the inflamed mucosa of the terminal ileum and colon, where many intestinal microbes reside (*3*, *4*). These findings suggest that intestinal microflora and ROS may play an important role in initiating and perpetuating colonic inflammation. Intestinal bacterial endotoxins, such as lipopolysaccharides (LPS), penetrate the epithelial barrier, either due to damage or via paracellular pathways, and directly stimulate the mucosal immune system (*3*, *5*). This results in the production of proinflammatory cytokines and other inflammatory mediators, causing inflammatory reactions in the mucosal immune system via signaling pathways through Toll-like receptors (TLRs) and/or cytokine receptors (*6*−*8*). ROS, such as the peroxide anion, hydrogen peroxide, hypochlorous acid, and 4-hydroxy-2-nonenal (4-HNE), are not merely byproducts of the inflammatory process but are actually involved in the pathogenesis of IBD (*9*−*11*). Superoxide dismutase ameliorates TNBS-induced colitis (*12*).

Soybeans and soy foods contain phytochemicals including isoflavines, saponins, phytic acids, phytosterols and trypsin inhibitors (*13*). Many clinical studies have shown that daily intake of soy foods may reduce the risk of osteoporosis (*14*), heart attack, hyperlipidemia, coronary heart disease (*15*), cardiovascular and chronic renal diseases (*16*), and cancers including prostate, colon and breast cancers (*17*). Soybean also exhibits strong antioxidant activity (*18*). Of the soy constituents, soyasaponins may play an important role in the aging-prevention effects of soybeans via their antioxidant effects (*19*). However, most researchers have reported that isoflavones possess strong antioxidant potency in both *in vitro* and *in vivo* experiments (*18*, *20*).

In our preliminary study, soyasaponin I, a major constituent in soybeans, inhibited lipid peroxidation in liposomes prepared from L-α-phosphatidylcholine. Therefore, to determine whether the antioxidant constituent soyasaponin I could improve colitis, we investigated the anti-inflammatory effects of soyasaponin I in LPS-induced peritoneal macrophages and TNBS-induced colitic mice.

MATERIALS AND METHODS

Materials. RPMI 1640, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), penicillin−streptomycin, 2,4,6-trinitrobenzene sulfonic acid (TNBS), and LPS purified from *Escherichia coli* O111:B4 were purchased from Sigma Co. (St. Louis, MO). Antibodies for cyclooxygenase (COX)-2, inducible NO synthase (iNOS), and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies for IκB-α, p65, p-p65, p38, p-p38, JNK, p-JNK, ERK, and p-ERK were purchased from Cell Signaling

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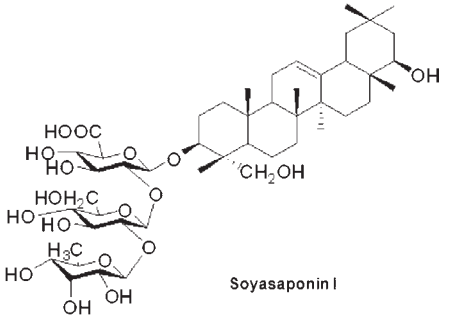

Figure 1. Chemical structure of soyasaponin I.

Technology (Beverly, MA). Enzyme-linked immunosorbent assay (ELISA) kits for cytokines and PGE2 were purchased from R&D Systems (Minneapolis, MN). Other chemicals used were of the highest grade available. Soyasoponin I (**Figure 1**) was isolated from soybeans as previously reported by Chang et al. (*21*).

Antioxidant Activity Assay. DPPH radical scavenging activity was assayed according to the method of Choo et al. (*22*). The reaction mixture containing 2.0×10^{-4} M DPPH in the presence or absence of soyasaponin I was incubated for 30 min at 37 °C, and then the absorbance was measured at 517 nm.

Lipid peroxidation-inhibitory activity was assayed according to the method of Lee et al. (*23*). The reaction mixture containing liposomal suspension (100 μL), 1.5 mL of 50 mM sodium phosphate buffer (pH 7.4), 100 μL of 2 mM ferrous chloride, and 100 μL of 4 mM ascorbic acid was incubated for 1 h at 37 °C in the presence or absence of soyasaponin B. Lipid peroxide in the reaction mixture was quantified as thiobarbituric acid-reactive substances (TBARS).

For liposome preparation, L-α-phosphatidylcholine (0.1 g, type XV-E from egg yolk) was dissolved in diethyl ether (10 mL) and mixed with distilled water (0.6 mL). The mixture was sonicated with an ultrasonic disrupter (Eyeler Co., Tokyo, Japan) and evaporated under vacuum on ice. The resulting extract was suspended in 30 mL of 0.1 M *N*-(2-acetamido)-imidinodiacetic acid (ADA) sodium buffer (pH 6.7), sonicated for 15 min on ice, and centrifuged at 1500*g* for 10 min at 4 °C. The supernatant was used as the liposome suspension.

Animals. Male ICR mice (20−22 g, 4 weeks) and male C57BL/6 (18−22 g, 6 weeks) were supplied from the Orient Animal Breeding Center (Sungnam, Korea). All animals were housed in wire cages at 20−22 °C and 50 ± 10% humidity, fed standard laboratory chow (Samyang Co., Seoul, Korea), and allowed water ad libitum. All experiments were performed in accordance with the NIH and Kyung Hee University guidelines for Laboratory Animals Care and Use and approved by the Committee for the Care and Use of Laboratory Animals in the College of Pharmacy, Kyung Hee University.

Isolation and Culture of Peritoneal Macrophages. Male C57BL/6 mice were intraperitoneally injected with 2 mL of 4% thioglycolate solution (*24*). Mice were sacrificed 4 days after injection, and the peritoneal cavities were flushed with 10 mL of RPMI 1640. The peritoneal lavage fluids were centrifuged at 200*g* for 10 min, and the cells were resuspended with RPMI 1640 and plated. After incubation for 1 h at 37 °C, the cells were washed three times and nonadherent cells were removed by aspiration. Cells were cultured in 24-well plates (0.5×10^6 cells/well) at 37 °C in RPMI 1640 plus 10% FBS. The attached cells were used as peritoneal macrophages. To examine the anti-inflammatory effects of soyasaponin I, peritoneal macrophages were incubated in the absence or presence of soyasaponin I with 50 ng/mL LPS.

Preparation of Experimental Colitic Mice. The ICR mice were randomly divided into 5 groups: normal and TNBS-induced colitic groups treated with or without soyasaponin I or sulfasalazine. Each group consisted of 10 mice. TNBS-induced colitis was induced by the administration of 2.5% (w/v) TNBS solution (100 μL) in 50% ethanol into the colon of anesthetized mice via a thin round-tip needle equipped with a 1 mL syringe (*25*). The normal group was treated with just the vehicle. The needle was inserted so that the tip was 3.5−4 cm proximal to the anal verge. To distribute the agents within the entire colon and cecum, mice were held in a vertical position for 30 s after the injection. Using this procedure, >95% of the mice retained the TNBS enema. If an animal quickly excreted the TNBS−ethanol solution, it was excluded from the remainder of the study. Soyasaponin I (10 and 20 mg/kg) or sulfasalazine (50 mg/kg) dissolved in 2% Tween 80 was orally administered once a day for 5 days beginning 3 days before TNBS administration. The mice were sacrificed on the third day after TNBS administration. The colon was quickly removed, opened longitudinally, and gently cleared of stool by PBS. Macroscopic assessment of the disease grade was scored according to a previously reported scoring system (0, no ulcer and no inflammation; 1, ulceration and local hyperemia; 2, ulceration without hyperemia; 3, ulceration and inflammation at one site only; 4, two or more sites of ulceration and inflammation; 5, ulceration extending more than 2 cm (*26*). Then, the entire colon tissue was used for immunoblot and enzyme-linked immunosorbent assay (ELISA) analysis. For the histological exam, the middle part of the colon was fixed in 10%-buffered formalin solution, embedded in paraffin using standard methods, cut into 7 μm sections, stained with hematoxylin−eosin, and assessed under light microscopy.

Colon Tissue Preparation. Colon tissues were excised, perfused with ice-cold perfusion solution containing 0.15 M KCl and 2 mM EDTA (pH 7.4), and homogenized in 50 mM Tris-HCl buffer (pH 7.4). The homogenates were centrifuged at 10000*g* at 4 °C for 30 min. The supernatant was used for the estimation of the antioxidant defense system.

Assay of Myeloperoxidase Activity in Colonic Mucosa. Colons isolated from the mice were homogenized in a solution containing 0.5% hexadecyl trimethyl ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7.0), and then centrifuged for 30 min at 20000*g* at 4 °C. A 50 μL aliquot of the supernatant was added to the reaction mixture consisting of 1.6 mM tetramethyl benzidine and 0.1 mM H_2O_2, and incubated at 37 °C. Following that, the absorbance was measured at 650 nm spectophotometrically time-scanned. The myeloperoxidase activity was defined as the quantity of enzyme degrading 1 μmol/mL of peroxide at 37 °C, and expressed in unit/mg protein (*27*).The protein content was assayed by Bradford's method (*28*).

Assay of Lipid Peroxide (Malondialdehyde, MDA). Lipid peroxidation was estimated in colon homogenates as described by Ohkawa et al (*23*). Briefly, a reaction mixture containing 50 mM Tris-HCl buffer (pH 7.4), 500 μM *tert*-butyl hydroperoxide (BHP) (in ethanol) and 1 mM ferrous chloride was incubated with the samples (dissolved in 0.1% dimethyl sulfoxide) at 37 °C for 90 min. The reaction was terminated by adding 0.2 mL of 8% sodium dodecyl sulfate followed by 1.5 mL of 20% acetic acid (pH 3.5). The amount of malondialdehyde formed during the incubation was assessed by adding 1.5% thiobarbituric acid and then heating at 95 °C for 45 min. After cooling, the reaction mixtures were centrifuged, and the absorbance of TBARS in the supernatant was measured at 532 nm. The levels of lipid peroxidation are expressed in terms of nmol TBARS/90 min/mg protein.

Assay of Glutathione Level and Superoxide Dismutase and Catalase Activities. The amount of glutathione in the tissue homogenate was measured according to the method of Paglia and Valentine (*23*). Catalase and superoxide dismutase activities were measured according to the method of Prakash et al. (*23*).

Analysis of 4-Hydroxy-2-nonenal (4-HNE) by High Performance Lipid Chromatography (HPLC). The colon (1 g) was homogenized in 1 mL of lysis buffer, and centrifuged twice at 10000*g* for 20 min. The supernatant was analyzed for 4-HNE using HPLC (Younglin high performance lipid chromatography system): column, Develosil ODS-UG-5 (4.6 mm i.d. × 150 mm, 5.8 μm particle diameter); mobile phase, linear-gradient mixture of 10% acetonitrile and 90% acetonitrile for 0−20 min and 100% acetonitrile for 20−30 min; flow rate, 1 mL/min; and detection, UV at 230/233 nm.

Determination of Nitric Oxide and Cytokines. Nitrite was measured in culture media using Griess reagent (*24*). The culture medium (100 μL) was combined with 100 μL of Griess reagent [mixture of equal volume of 1% sulfanilamide in 5% H_3PO_4 and 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride in H_2O] in a 96-well plate, and then the absorbance was measured spectrophotometrically at 550 nm. Nitrite concentration was determined by using sodium nitrite as a standard.

PGE2 and proinflammatory cytokines, TNF-α, IL-1β and IL-6, were assessed by ELISA according to the manufacturer's instructions.

Immunoblot Analysis. The supernatant extracts prepared from colon and peritoneal macrophages were separated by 9% SDS−PAGE and

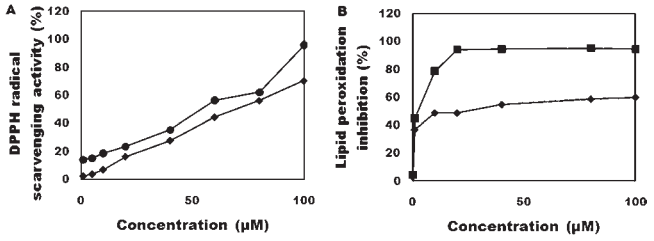

Figure 2. Antioxidant effects of soyasaponin. (**A**) 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging activities of soyasaponin I (◆) and α-tocopherol (●). (**B**) Inhibitory effect of soyasaponin I (◆) on butylated hydroxylanisole (■) lipid peroxidation of liposome. Lipid peroxide in liposome was estimated by thiobarbituric acid-reactive substance assay. All values are the mean ± SD (n = 3).

transferred onto polyvinylidene difluoride membranes (PVDF). The membranes were blocked with 5% nonfat dried-milk proteins in PBST, and probed with COX-2, iNOS, IκB-α, p65, p-p65 or β-actin antibody. After washing with PBST, proteins were detected with HRP-conjugated secondary antibodies for 1 h. Bands were visualized with enhanced chemiluminescence (ECL) reagent (*23*).

Immunofluorescent Confocal Microscopy. For the p65 assay, peritoneal macrophages were stimulated with LPS (50 ng/mL) in the presence or absence of soysaponin I for 60 min. The cells were then fixed with 4% formaldehyde and permeabilized with 0.2% Triton X-100. The cells were stained with goat polyclonal anti-p65 antibody for 2 h at 4 °C and then incubated with secondary antibodies conjugated with Alexa 488 and propidium iodide (10 μg/mL, Calbiochem Co., San Diego, CA) for 1 h. Images were observed by confocal microscopy.

Statistical Analysis. Data are presented as the means ± standard deviation of at least three replicates. ANOVA was used for comparisons between multiple groups (SPSS for win 8.0, SPSS Inc., Chicagio, IL). The Student t test was used for the statistical analysis of the difference noted. P values of 0.05 or less were considered statistically significant.

RESULTS

Antioxidant Effect of Soyasaponin I. To investigate the relationship between the antioxidant activity of soyasaponin and its anti-inflammatory effects, we first isolated soyasaponin I, a major constituent of soybean, and measured its antioxidant effects (**Figure 2**). Soyasaponin I showed concentration-dependent DPPH radical-scavenging effects with a 50% inhibitory concentration of 70.2 μM. Soyasaponin I showed DPPH radical scavenging activity comparable to that of α-tocopherol (IC_{50} = 52.1 μM). Soyasaponin I also inhibited lipid peroxidation concentration-dependently in liposomes prepared from L-α-phosphatidylcholine (IC_{50} = 19.5 μM).

Effect of Soyasaponin I on Inflammatory Markers in LPS-Stimulated Peritoneal Macrophages. To investigate the anti-inflammatory effects of soyasaponin I, peritoneal macrophages were stimulated with LPS in the presence or absence of soyasaponin I. Treatment with LPS alone significantly increased the expression of proinflammatory cytokines, IL-1β and TNF-α, in peritoneal macrophages according to ELISA and immunoblot analysis (**Figure 3**). Treatment with LPS in the presence of soyasaponin I significantly reduced IL-1β and TNF-α expression. Soyasaponin I also inhibited PGE2 and NO production as well as COX-2 and iNOS expression in LPS-treated peritoneal macrophages. To determine whether soyasaponin I has cytotoxic effects, we used the crystal violet method (*24*) to assess cell viability. No cytotoxic effect of soyasaponin I was observed under the conditions of these experiments.

To investigate the effect of soyasaponin I on the degradation and phosphorylation of IκB-α in LPS-induced peritoneal macrophages, the cells were cultured with LPS in the presence or

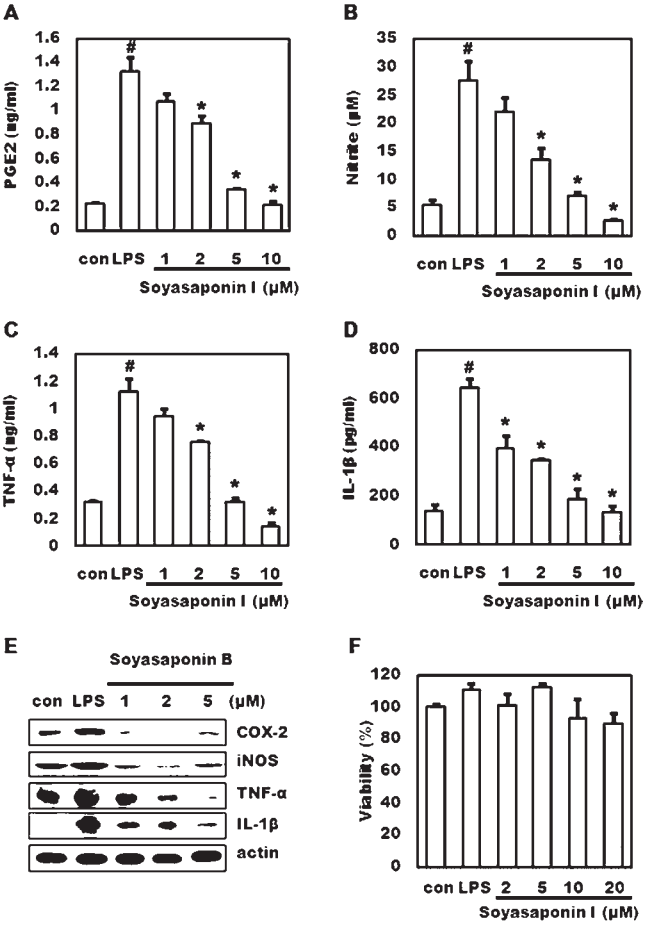

Figure 3. Effects of soyasaponin I on inflammatory mediators in LPS-stimulated mouse peritoneal macrophages. After 16 h incubation with LPS in the absence or presence of soyasaponin I (1, 2, 5, and 10 μM), PGE2 (**A**), nitrite (**B**), TNF-α (**C**) and IL-1β (**D**) in the culture medium were measured using ELISA kits (for PGE2, TNF-α and IL-1β) and Griess reagents (for nitrite). Protein expression levels of COX-2, iNOS, TNF-α and IL-1β levels were measured by Western blotting following incubation with LPS in the absence or presence of soyasaponin I (1, 2, and 5 μM). β-Actin was used as an internal control (**E**). Cytotoxicity (**F**) of soyasaponin I (2, 5, 10, and 20 μM) in the presence of LPS (50 ng/mL); cells were incubated for 20 h, and then cell viability was measured by crystal violet staining. All data are expressed as mean ± SD (n = 3 in a single experiment). *P < 0.5; **P < 0.01; P < 0.001 vs LPS control.

absence of soyasaponin I (**Figure 4**). Stimulation with LPS resulted in the phosphorylation and degradation of IκBα. Co-treatment with soyasaponin I and LPS inhibited this degradation and phosphorylation of IκBα. Next, we used confocal microscopy to examine whether soyasaponin I could inhibit the nuclear translocation of NF-κB in LPS-stimulated peritoneal macrophages. LPS decreased NF-κB (p65) levels in the cytosolic fraction, but increased NF-κB in the nuclear fraction. Co-treatment with soyasaponin I and LPS significantly prevented accumulation of the p65 subunit in the cytosolic fraction and decreased the p-p65 level in the nucleus based on immunoblot analysis.

To investigate whether inhibition of the inflammatory response by soyasaponin I is mediated via a MAPK pathway, we examined their inhibitory effects on the LPS-stimulated phosphorylation of ERK1/2, JNK1/2, and p38 MAPK in peritoneal macrophages (**Figure 5**). Soyasaponin I exhibited low inhibition toward the activation of ERK and p38, although the activation of JNK was

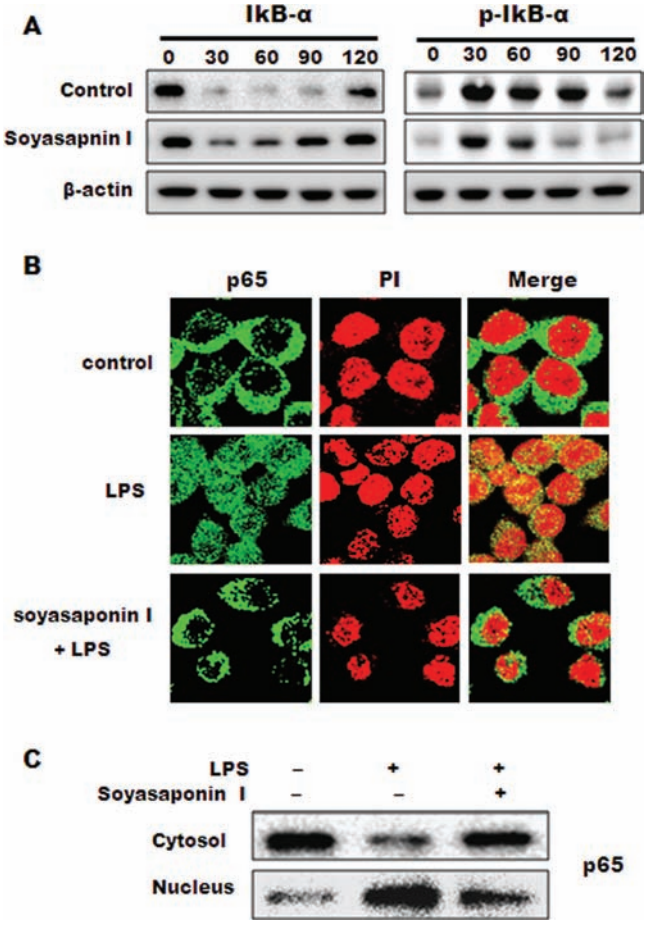

Figure 4. Effects of soyasaponin I on IκB-α degradation and NF-κB activation. Peritoneal macrophages were treated with 50 ng/mL LPS in the absence or presence of soyasaponin I (5 μM). (**A**) Degradation and phosphorylation of IκB-α were assessed at 0, 30, 60, and 120 min after addition of LPS to the cultures. β-Actin was used as a control. (**B**) NF-κB nuclear translocation was detected by confocal analysis using an antibody for the p65 subunit. Soyasaponin I prevented NF-κB nuclear translocation in LPS-stimulated macrophages. (**C**) NF-κB activation was determined by immunoblot analysis, with cytosolic and nuclear fractions prepared from peritoneal macrophages.

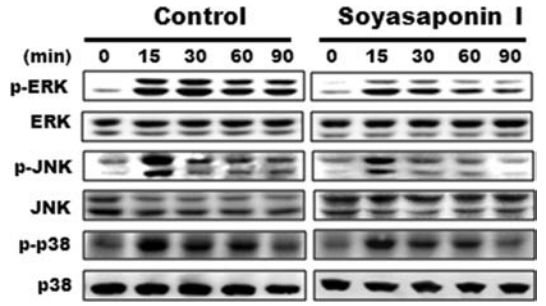

Figure 5. Effects of soyasaponin I on MAP kinase activation in LPS-stimulated peritoneal macrophages. The cells were incubated with 50 ng/mL LPS in absence or presence of soyasaponin I (5 μM) for the indicated times.

weakly inhibited. No cytotoxic effect of soyasaponin I was observed under the conditions of these experiments.

Inhibitory Effect of Soyasaponin I in TNBS-Induced Colitis. Intrarectal injection of TNBS caused inflammation, manifested by shortened, thickened and erythematous colons in mice.

Table 1. Effect of Soyasaponin I (10 and 20 mg/kg) and Sulfasalazine (50 mg/kg) on Macroscopic Score, Changes in Colonic Length and MOP Activity in TNBS Chronic Colitis with Relapse[a]

group	dose (mg/kg)	colon length	macroscopic score	MPO activity
normal		7.9 ± 0.4	0.0 ± 0.1	5.7 ± 6.2
control (TNBS)		6.4 ± 0.6[#]	2.0 ± 0.3[#]	33.0 ± 10.7[#]
soyasaponin I	10	7.1 ± 0.6	1.1 ± 0.4*	8.7 ± 2.6
soyasaponin I	20	7.3 ± 0.4*	0.9 ± 0.2*	6.9 ± 1.1*
sulfasalazine	50	7.3 ± 0.2*	1.1 ± 0.2*	8.8 ± 6.1*

[a] The test agents were orally administered once a day for 3 days prior to TNBS treatment. The mice were anesthetized with ether and killed 3 days after TNBS treatment. All values are the mean ± SD (n=10). [#]Significantly different vs normal group in each column of mice ($P < 0.05$). *Significantly different vs TNBS-treated control group in each column of mice ($P < 0.05$).

Treatment with soyasaponin I in TNBS-treated mice ameliorated body weight reduction, colon shortening, macroscopic score, and myeloperoxidase activity (**Table 1**). TNBS also increased malondialdehyde and 4-HNE levels in colons, but lowered glutathione content as well as superoxide dismutase and catalase activity. Treatment with soyasaponin I in TNBS-treated mice potently inhibited lipid peroxidation, malondialdehyde and 4-HNE levels, but increased the glutathione content as well as superoxide dismutase and catalase activity (**Table 2**). We also measured the inhibitory effect of soyasaponin I on the expression of proinflammatory cytokines, TNF-α, IL-1β and IL-6, in the colons of TNBS-induced colitic mice (**Figure 6**). TNBS increased protein expression of the proinflammatory cytokines, IL-1β, IL-6 and TNF-α, and the proinflammatory enzymes, iNOS and COX-2. TNBS also significantly activated the transcription factor NF-κB. Soyasaponin I prevented the TNBS-induced expression of TNF-α, IL-1β, IL-6, iNOS and COX-2. Soyasaponin I also attenuated activation of the transcription factor NF-κB. The inhibitory effects of soyasaponin I were more potent than those of sulfasalazine.

DISCUSSION

The inflammatory process not only induces NF-κB activation but also produces ROS, such as peroxide anion, hydrogen peroxide, 4-HNE, and hypochlorous acid. These ROS may be involved in the pathogenesis of IBD (*9*, *10*). ROS are involved in TNF- α, IL-8 and IL-1β production in LPS stimulated macrophages. TNBS, which causes colitis by intrarectal injection, increases ROS in the colon. Treatment with superoxide dismutase significantly ameliorates TNBS-induced colitis (*29*). We observed in the present study that TNBS significantly increased lipid peroxides, malondialdehyde and 4-HNE, as well as NF-κB activation in the colon of mice intrarectally injected with TNBS. Based on these results, TNBS may initiate production of ROS, which may then be important factors in initiating and perpetuating colonic inflammation. Thus, ROS may play an important role in activation of the transcription factor, NF-κB, in TNBS-induced colitis. Therefore, we screened a lipid peroxidation inhibitor, soyasaponin I, from soybean and investigated its anticolitic effect in mice.

Soybeans, which contain 0.085% soyasaponin I (*30*), are orally given (daily 10−100 g) as food supplements in Japan and Korea. We orally treated mice with 10 and 20 mg/kg of soyasaponin I to mice to evaluate its anticolitic effect, considering human daily intake of soybean and the bioavailability of chemicals between mice and humans. Orally administered soyasaponin I reduced the TNBS-induced level of lipid peroxidation in colons of mice. Thus, soyasaponin I reduced malondialdehyde and 4-HNE levels and restored superoxide dismutase and catalase activity as well as glutathione content. Based on these results, induction of lipid peroxidation associated with superoxide production may be an early critical event in colitic mice.

Table 2. Inhibitory Effect of Soyasaponin I on Malondialdehyde (MDA) Content, 4-Hydroxy-2-nonenal (4-HNE) Content, Glutathione Content (GSH), and Superoxide Dismutase (SOD) and Catalase Activities in TNBS Chronic Colitis with Relapse[a]

		content			activity	
group	dose (mg/kg)	MDA (μM/mg)	4-HNE (ng/mL)	GSH (μg/mL)	SOD (U/mg)	catalase (mol/min/mg)
normal		5.7 ± 5.5	3.5 ± 2.8	3.0 ± 0.3	9.5 ± 2.4	7.4 ± 3.6
control (TNBS)		$28.3 \pm 5.9^{\#}$	$17.5 \pm 4.0^{\#}$	$0.9 \pm 0.2^{\#}$	$2.6 \pm 1.9^{\#}$	$2.0 \pm 1.2^{\#}$
soyasaponin I	10	$10.7 \pm 9.9^{*}$	15.7 ± 1.6	$2.4 \pm 0.3^{*}$	$6.9 \pm 0.1^{*}$	$5.6 \pm 2.3^{*}$
soyasaponin I	20	$8.3 \pm 4.9^{*}$	9.1 ± 6.4	$2.7 \pm 0.2^{*}$	$7.2 \pm 0.6^{*}$	$5.7 \pm 1.0^{*}$
sulfasalazine	50	$8.7 \pm 6.1^{*}$	9.8 ± 1.7	$2.1 \pm 0.1^{*}$	$6.7 \pm 0.5^{*}$	$6.6 \pm 2.6^{*}$

[a] The test agents were orally administered once a day for 3 days prior to TNBS treatment. The mice were anesthetized with ether and killed 3 days after TNBS treatment. All values are the mean $\pm$ SD ($n = 10$). #Significantly different vs normal group in each column of mice ($P < 0.05$). *Significantly different vs TNBS-treated control group in each column of mice ($P < 0.05$).

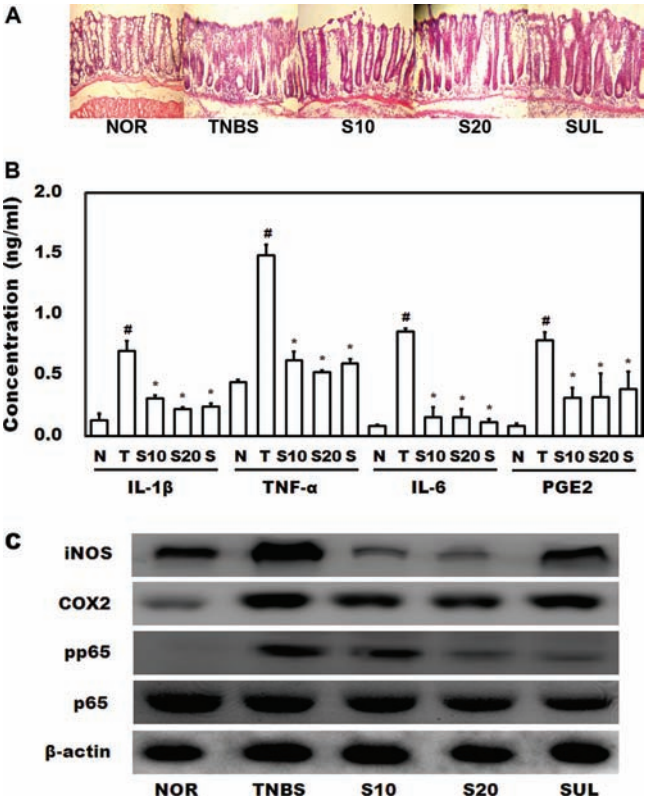

Figure 6. The inhibitory effect of soyasaponin I in TNBS-induced colitic mice. (**A**) Histological photograph. The colons were stained with hematoxylin−eosin and then assessed by light microscopy. (**B**) Effect on proinflammatory cytokine expression. IL-1β, TNF-α, IL-6 and PGE2 were measured by ELISA. (**C**) iNOS and COX-2 expression and NF-κB activation were measured by immunoblot analysis. The test agents (NOR, normal group treated vehicle alone; CON, TNBS-treated control group; S10, 10 mg/kg of soyasaponin I with TNBS; S20, 20 mg/kg of soyasaponin I with TNBS; SUL, 50 mg/kg sulfasalazine with TNBS) were orally administered to mice daily for 3 days prior to TNBS treatment. The mice were anesthetized, their colons were collected, and inflammatory markers were assessed. All values are the mean $\pm$ SD ($n = 10$). #Significantly different vs normal group ($p < 0.05$). *Significantly different vs control group ($p < 0.05$).

Soyasaponin I also inhibited the production of PGE2 and NO in the colons of TNBS-treated mice as well as the expression of COX-2 and iNOS, which produce PGE2 and NO, respectively. Soyasaponin I also inhibited the expression of proinflammatory cytokines, such as IL-1β, IL-6 and TNF-α, as well as NF-κB activation. Based on these findings, soyasaponin I may ameliorate colitis by inhibiting NF-κB.

Various inflammatory diseases involve overexpression of proinflammatory cytokines, such as TNF-α and IL-1β, and inflammatory mediators such as NO and PGE2 via NF-κB and MAPK pathways in macrophages (*31*, *32*). Therefore, we investigated the anti-inflammatory effects of soyasaponin I in mouse peritoneal macrophages. Soyasaponin I reduced expression levels of COX2 and iNOS in LPS-treated peritoneal macrophages. Soyasaponin I also inhibited the degradation and phosphorylation of IκBα, as well as the LPS-induced translocation of the p65 subunit of NF-κB into the nucleus. However, p-JNK, p-ERK and p-p38 were barely changed after addition of soyasaponin I. Therefore, the anti-inflammatory effects of soyasaponin I were regulated not via the MAPK pathway but via the NF-κB pathway, and soyasaponin I consequently inhibited the production of LPS-induced inflammatory mediators via the inhibition of NF-κB activation.

Based on these findings, soyasaponin I may ameliorate colitis by scavenging lipid peroxides produced by TNBS as well as by inhibiting NF-kB activation. Therefore, soybeans may prevent and improve colitis.

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