약학석사학위논문

Preventive Effects of Korean Red Ginseng on Dextran Sulfate Sodium-induced Colitis and Colon Carcinogenesis in C57BL/6J Mice

실험적으로 유도된 마우스 대장염과 대장암에 대한
홍삼의 보호효과

2014년 2월

서울대학교 대학원
분자의학 및 바이오제약학과
신훈정

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지도교수 서영준

이 논문을 약학석사 학위논문으로 제출함

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신훈정의 약학석사 학위논문을 인준함

2014년 2월

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ABSTRACT

Preventive Effects of Korean Red Ginseng on Dextran Sulfate Sodium-induced Colitis and Colon Carcinogenesis in C57BL/6J Mice

Hoon-Jeong Shin

Under the supervision of Professor Young-Joon Surh

Department of Molecular Medicine and Biopharmaceutical Sciences

The Graduate School

Seoul National University

Korean Red Ginseng (KRG) exerts chemopreventive effects on experimentally induced carcinogenesis. However, the underlying molecular mechanisms remain largely unresolved. In this study, we investigated effects of KRG on dextran sulfate sodium (DSS)-induced colitis and azoxymethane (AOM) plus DSS-induced colon carcinogenesis in mice. Male C57BL/6J mice were fed diet containing 1% KRG or a standard diet more than one week before and throughout the experiment. The mouse colitis was induced by administration of 3%

DSS in drinking water for 1 week. DSS caused body weight loss, diarrhea, rectal bleeding and colon length shortening, and all these symptoms were ameliorated by KRG treatment. KRG inhibited DSS-induced expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) by suppressing activation of nuclear factor-kappa B (NF-κB) and signal transducer and activation of transcription 3 (STAT3). In another experiment, colon carcinogenesis was initiated by single i.p. injection of AOM (10 mg/kg) and promoted by 2% DSS. KRG administration relieved the symptoms of acute colitis and reduced the incidence, the multiplicity and the size of colon tumor. The up-regulation of COX-2, iNOS, cMyc and cyclin D1 by AOM plus DSS was inhibited by KRG treatment through prevention of NF-κB and STAT3 activation. These results suggest that KRG is a potential candidate for chemoprevention of inflammation-associated disorders in the colon.

Keywords : Korean red ginseng, colitis, colon cancer, COX-2, NF-κB, STAT3

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INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer death in the world and its incidence rate is increasing in Korea [1]. Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, is associated with the pathogenesis of CRC. Thus, patients with long-standing IBD have an increased risk of developing CRC than general population [2].

Cyclooxygenase-2 (COX-2) is up-regulated in inflamed colon tissue. The enzyme converts arachidonic acid to prostaglandin H_2 which is further converted to prostaglandin E_2 (PGE_2). NF-κB and STAT3 are major transcription factors that regulate expression of COX-2. NF-κB is sequestered in the cytoplasm by IκBα in resting cells. Activation of NF-κB is dependent on degradation of IκBα and phosphorylation of the p65 subunit. Activated NF-κB migrates into the nucleus to regulate the expression of multiple target genes [3]. STAT3 is activated through tyrosine phosphorylation. The phosphorylated STAT3, in turn, dimerizes and the dimer translocates to the nucleus, where it directly regulates gene expression [4]. Both NF-κB and STAT3 play a principal role in mediating the pro-inflammatory gene expression and their overactivation is implicated in inflammation-associated carcinogenesis.

Korean ginseng (*Panax ginseng* C.A. Meyer) has been used as a medicinal herb for thousands of years. One way to process raw ginseng is steaming, generating red ginseng. Korean red ginseng (KRG) is known to be beneficial for immunity, brain function, fatigue, diabetes, cancer, etc. The active components of KRG include saponins, polysaccharides, flavonoids, and volatile oils. Prolonged administration of KRG is known to have cancer preventive effects [5]. However, molecular mechanisms responsible for its chemopreventive effects have not been well elucidated yet.

In this study, we have investigated whether KRG could prevent dextran sulfated sodium (DSS)-induced colitis and azoxymethane (AOM) plus DSS-induced colon carcinogenesis in C57BL/6J mice and its underlying molecular mechanisms.

MATERIALS AND METHODS

Materials

DSS with an average molecular weight of 36,000-50,000 was obtained from MP Biomedicals, LLC (Solon, OH, US). AOM was obtained from Sigma-Aldrich (St Louis, MO, USA). KRG powder was supplied by Korea Ginseng Corporation (Seoul, Korea). COX-2 (murine) polyclonal antibody produced from rabbit was supplied by Cayman Chemical (Ann Arbor, MI, USA). Polyclonal rabbit anti-iNOS/NOS type II antibody was provided by BD Biosciences (Franklin Lakes, NJ, USA). Primary antibodies against cyclin D1, STAT3, pSTAT3, p65 and pIκBα were offered by Cell Signaling Technology, Inc (Danvers, MA, USA). Antibodies against pp65 and IκBα were obtained from Santa Cruz Biotechnology, Inc (Santa Cruz, CA, USA). Antibody against lamin B_1 was obtained from Invitrogen Corporation (Camarillo, CA, USA). Antibodies against actin and α-tubulin and Absiganl western blot detection kit were bought from Abclon. Horseradish peroxidase-conjugated anti-mouse and rabbit secondary antibodies were obtained from Zymed lavoratories (San Fransico, CA, USA). NF-κB oligonucleotide probe containing the consensus sequence (5'-AGT TGA GGG GAC TTT CCC AGG C-3', 3'-TCA ACT CCC CTG AAA

GGG TCC G-5') came from Promega (Madison, WI, USA). EPD and pico EPD Western blot detection kit were purchased from ELPIS (Republic of Korea). All other chemicals used in our experiments were of the purest.

Animal treatment

All the animal experiments were performed according to the approved guidelines of the Seoul National University (SNU-120629-1). Four-week-old male C57BL/6J mice were obtained from Central Lab Animal (Republic of Korea) and maintained on conventional housing conditions. After an acclimation for 7 days, mice were divided into groups as indicated below and fed experimental diet throughout the experiment (**Fig. 1**). Composition of experimental diet and content of ginseosides of Korean red ginseng powder are shown in Table 1.

Study 1 : DSS-induced colitis

Mice in the control group and the DSS group received control diet. Mice in the DSS+KRG group and the KRG alone group received control diet supplemented with 1% (w/w) Korean red ginseng (KRG) powder. DSS (3%, w/v) in drinking water was given for 1 week. After 7 days of DSS exposure, all mice were sacrificed.

Study 2 : AOM plus DSS-induced colon carcinogenesis

Mice in the control group and the AOM+DSS group received control diet and mice in the AOM+DSS+KRG group received control diet supplemented with 1% KRG powder. Mice in the AOM+DSS group and the AOM+DSS+KRG group were given single *i.p.* injection of azoxymethane (AOM, 10 mg/kg body weight) and exposed to 2% DSS in drinking water for 1 week, and then kept without any further treatment for 14 weeks.

Macroscopic assessment

Study 1 : DSS-induced colitis

During 7 days of DSS treatment, the body weight of mice was measured every day. Rectal bleeding and stool consistency were monitored and scored from 0 to 3 in a modified design depending on the severity of blood and diarrhea. Disease activity index (DAI) was determined as the sum of scores of rectal bleeding and stool consistency.

Study 2 : AOM plus DSS-induced colon carcinogenesis

Collected colon tissue was cut longitudinally and the colon tumors

were identified. After the measurement of the number and the size of tumors, visible tumors were excised, collected and weighed.

Histological examination

Specimens of distal parts of the colon were fixed with 10% phosphate buffered formalin, and embedded in paraffin and stained with hematoxylin and eosin (H&E).

Western blot analysis

Mouse colon parts were cut longitudinally, and washed with phosphate-buffered saline (PBS), and stored at -70°C until before use. Colon tissue was homogenized in the lysis buffer [cell lysis buffer (Cell Signaling Technology), 1 mM phenylmethylsulfonylfluoride (PMSF) and EDTA-free protease inhibitor cocktail tablet (Roche Applied Science)] followed by periodical vortex for 2 hours. Lysates were centrifuged at 13,000 rpm for 15 min at 4°C. Supernatants were collected and stored at -70°C. For western blot analysis, the total protein concentration was quantified using bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology). after mixing and heating with

sodium dodecyl sulfate (SDS) buffer, 20~30 μg of whole lysate protein samples was separated by SDS-PAGE and transferred to polyvinyliden difluoride (PVDF) membrane (Pall Corporation, USA) at 300 mA for 3 hours. The blots were blocked in 5% skim milk in TBST [Tris-buffered saline (TBS) with 0.1% Tween-20] for 1 hour at room temperature and incubated with primary antibodies in TBST at 4°C overnight. Blots were then washed with TBST for 30 minutes and incubated in horseradish peroxidase-conjugated secondary antibody in TBST for 1 hour at room temperature. Blots were washed again three times and transferred proteins were visualized with enhanced chemiluminescence detection kit and LAS-4000 image reader according to the manufacturer's instructions.

Fractionation of nuclear and cytoplasmic extracts

Nuclear and cytoplasmic extracts were prepared using the method described below. Colon tissue was homogenized in hypotonic buffer A [10 mM HEPES (pH 7.8), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol (DTT), 0.2 mM PMSF] and incubated for 1 hour on ice and 0.1% NP-40 was added right before centrifugation. After centrifugation at 13,000 rpm for 15 minutes at 4°C, the supernatants (the cytoplasmic extracts) were collected and stored at -70°C.

Precipitated pellets were washed with buffer A for 2 times to remove remaining cytoplasmic components. Then pellets were re-suspended in buffer C [20 mM HEPES (pH 7.8), 20% glycerol, 420 mM Nacl, 1.5 mM $MgCl_2$, 0.2 mM ethylenediaminetetraacetic acid (EDTA), 0.5 mM DTT, 0.2 mM PMSF] and incubated on ice for 1 hour with vortexing in every 5 minutes. After centrifugation at 13,000 rpm for 15 minutes at 4°C, the supernatants (the nuclear extracts) were collected and stored at -70°C.

Electrophoresis mobility shift assay (EMSA)

DNA binding activity of NF-κB was measured with EMSA using a DNA binding detection kit according to manufacturer's protocol (Gibco BRL; Grand island, NY, USA). In brief, T4 polynucleotide kinase transferred ^{32}P labeled γ-phosphate from ATP to NF-κB oligonucleotide. After purification with a G-50 micro column (GE Healthcare, UK), [γ-^{32}P] labeled probes were mixed with 10 μg of nuclear extracts and incubation buffer [10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM DTT, 1 mM EDTA, 4% glycerol and 0.1 mg/ml sonicated salmon sperm DNA]. All the samples were mixed with 2 μl 0.1% bromophenol blue loading dye after 50 minutes incubation and separated on 6% non-denatured polyacrylamide gel in a cold room. Finally, gels were dried

and exposed to X-ray films (Agfa Healthcare, Belgium).

Statistics

All values were expressed as the mean ± SD or the mean ± SE according to data type. Statistical significance was determined by the Student's *t*-test and $p < 0.05$ was considered to be statistically significant.

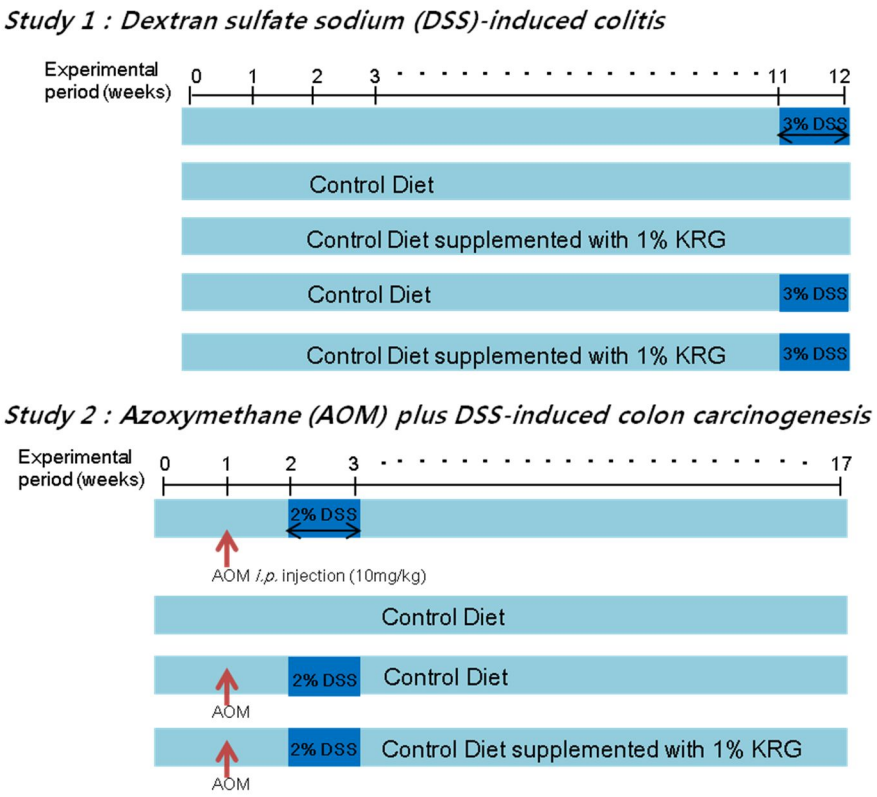

Figure 1. Experimental protocol for DSS-induced colitis and AOM plus DSS-induced colon carcinogenesis.

(A) Composition of experimental diets

Ingredient (g)	CD	CD+KRG
Casein(from milk)	200	200
Corn Starch	397.486	397.486
Sucrose	100	100
Dextrose	132	132
Cellulose	50	50
Soybean Oil	70	70
Mineral Mixture	35	35
Vitamin Mixture	10	10
TBHQ	0.014	0.014
L-Cystine	3	3
Choline Bitartrate	2.5	2.5
KRG powder	-	10
Kcal	4000	4000
Total	1000	1010

(B) Contents of ginsenosides in Korean red ginseng powder

Ginsenoside (mg/g)	
Rg1	2.71
Rb1	4.43
Rg3s	0.15
Re	1.38
Rc	1.69
Rb2	1.52
Rd	0.23
Rf	0.67
Rh1	0.16
Rg2s	0.19
Rg3r	0.09
Total	13.23

Table 1. Composition of experimental diets (A) and content of ginsenosides in KRG powder (B). (CD, control diet; CD+KRG, control diet supplemented with 1% KRG; TBHQ, t-butylhydroquinone.)

RESULTS

Study 1 : DSS-induced colitis

Macroscopic assessment and microscopic assessment

DSS is a sulfated polysaccharide and commonly used in animal models for inducing acute and chronic colitis. DSS increases the colonic mucosal permeability and activates inflammatory signaling pathways [6]. From the 4th day of 3% DSS exposure, the body weight of mice in the DSS only group was significantly decreased compared to the control group. KRG treatment inhibited body weight loss induced by DSS (**Fig. 2A**). DAI was scored according to the severity of bleeding and stool consistency. DAI score of mice in the DSS+KRG group was significantly lower than that of mice in the DSS only group (**Fig. 2B**). Moreover, DSS exposure for 7-days shortened the colon length of mice in the DSS only group, but KRG treatment abolished it (**Fig. 2C**). By H&E staining of distal colon, we demonstrated that DSS resulted in mouse colitis exhibiting symptoms of epithelial degeneration, crypt loss and inflammatory cell infiltration. KRG treatment inhibited DSS-induced mucosal damage of colon (**Fig. 2D**). These findings indicated that KRG treatment ameliorated DSS-induced

colitis.

Effects of KRG on the expression of pro-inflammatory enzymes

COX-2 and iNOS are inducible pro-inflammatory enzymes which are often overexpressed in inflammatory conditions. The Western blot analysis of colon revealed that DSS induced expression of COX-2 and iNOS, which was significantly reduced by KRG treatment (**Fig. 3**).

Effects of KRG on DSS-induced inflammatory signaling pathways

NF-κB and STAT3 are important transcription factors that up-regulate the expression of COX-2 and iNOS. DSS activated NF-κB signaling pathways by causing phosphorylation and degradation of IκBα (**Fig. 4**). Nuclear accumulation and phosphorylation of NF-κB p65 were also induced by DSS treatment (**Fig. 5A, 5B**). But, the activation of NF-κB signaling induced by DSS was inhibited in the mice treated with KRG. Further, we found that NF-κB-DNA binding affinity was less elevated in the DSS+KRG group than the DSS only group as determined by the gel shift assay (**Fig. 5C**). Moreover, KRG treatment decreased DSS-induced phosphorylation of STAT3 (**Fig. 6**). These results imply that KRG exerts anti-inflammatory effects on DSS-induced colitis by

blocking activation of NF-κB and STAT3 (**Fig. 7**).

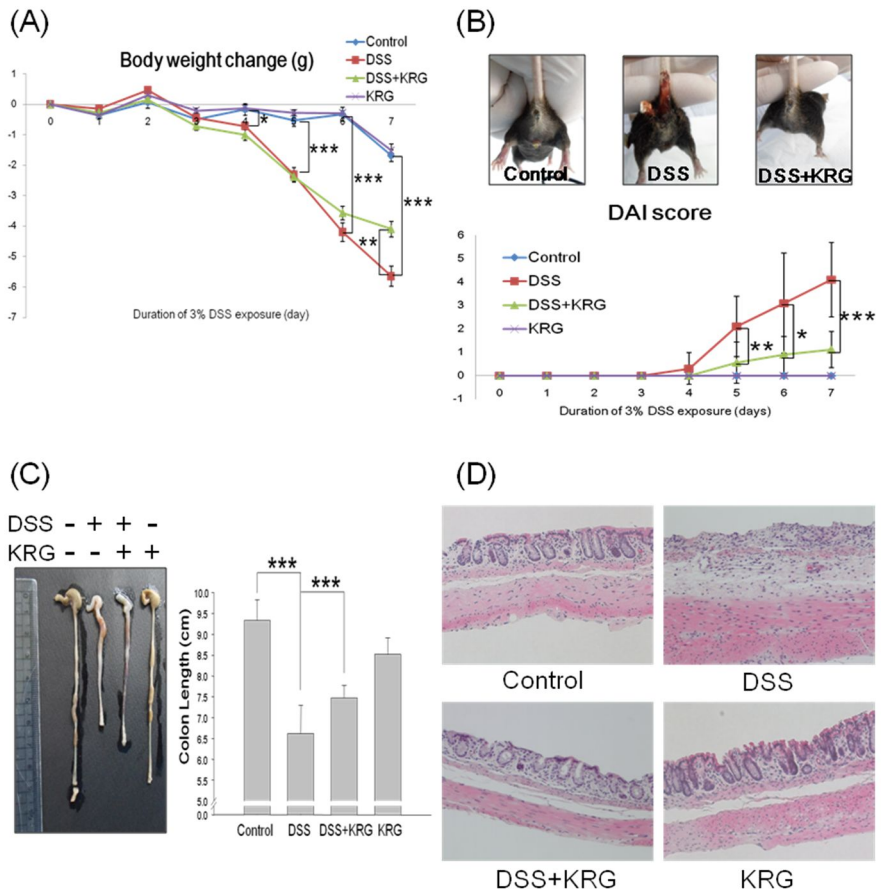

Figure 2. Macroscopic and microscopic assessment of mouse colitis. KRG ameliorated severity of colitis according to the body weight change (A), DAI (B) and the colon length (C). Microscopic observation revealed that DSS-induced mucosal damage of colon was attenuated by KRG treatment (D). Results are presented as means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

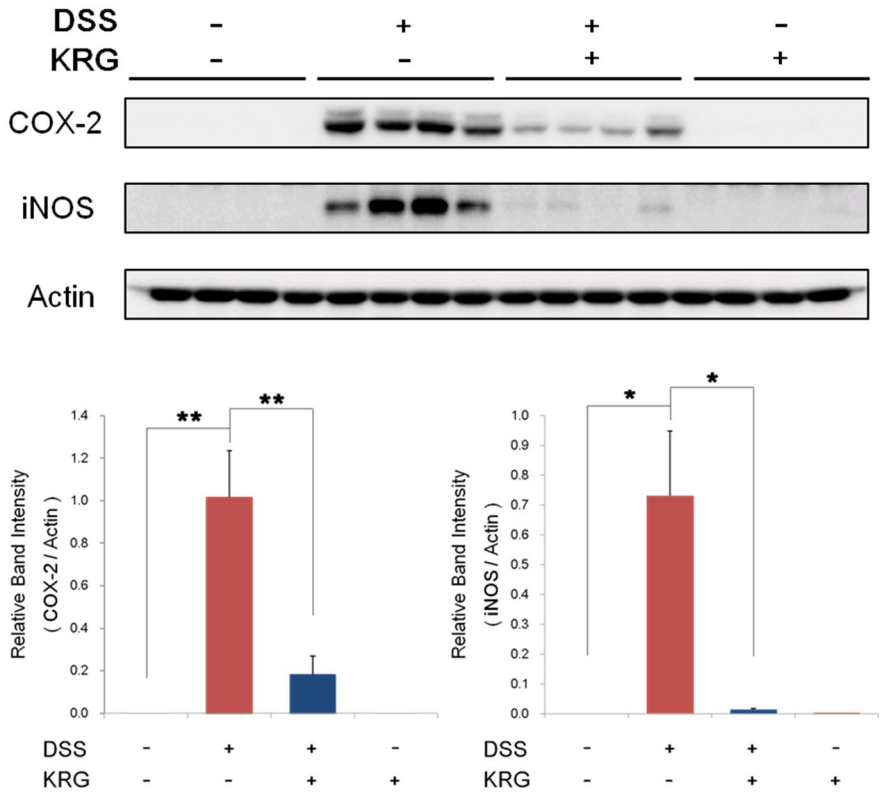

Figure 3. Inhibitory effects of KRG on DSS-induced COX-2 and iNOS expression. All mice were sacrificed after 7 days of DSS exposure and colon tissue was collected. Colon was cut longitudinally and divided equally. Inhibitory effects of KRG on DSS-induced COX-2 and iNOS expression were determined by Western blot analysis. Results are presented as means ± SE. $^{*}P < 0.05$ and $^{**}P < 0.01$.

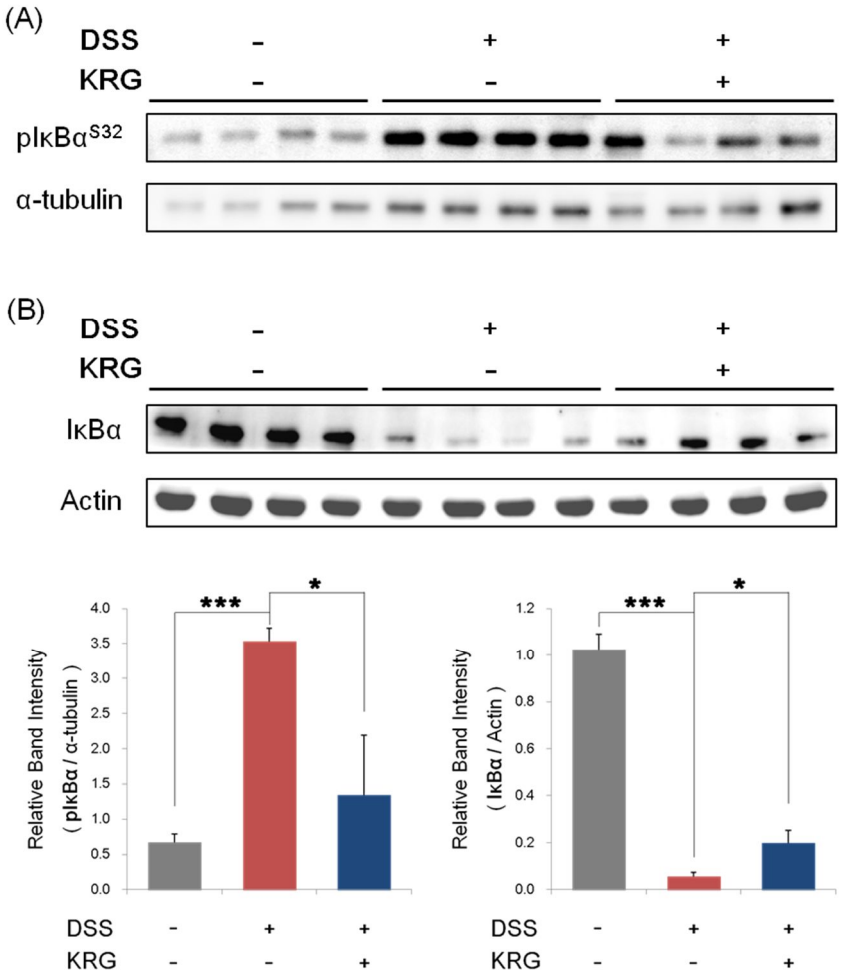

Figure 4. Inhibitory effects of KRG on DSS-induced IκBα degradation and phosphorylation. Inhibitory effects of KRG on DSS-induced IκBα phosphorylation (Ser32) were determined by Western blot analysis using cytoplasmic extracts (A). Inhibitory effects of KRG on DSS-induced IκBα degradation were determined by Western blot analysis (B). Results are presented as means ± SE. $^{*}P < 0.05$ and $^{***}P < 0.001$.

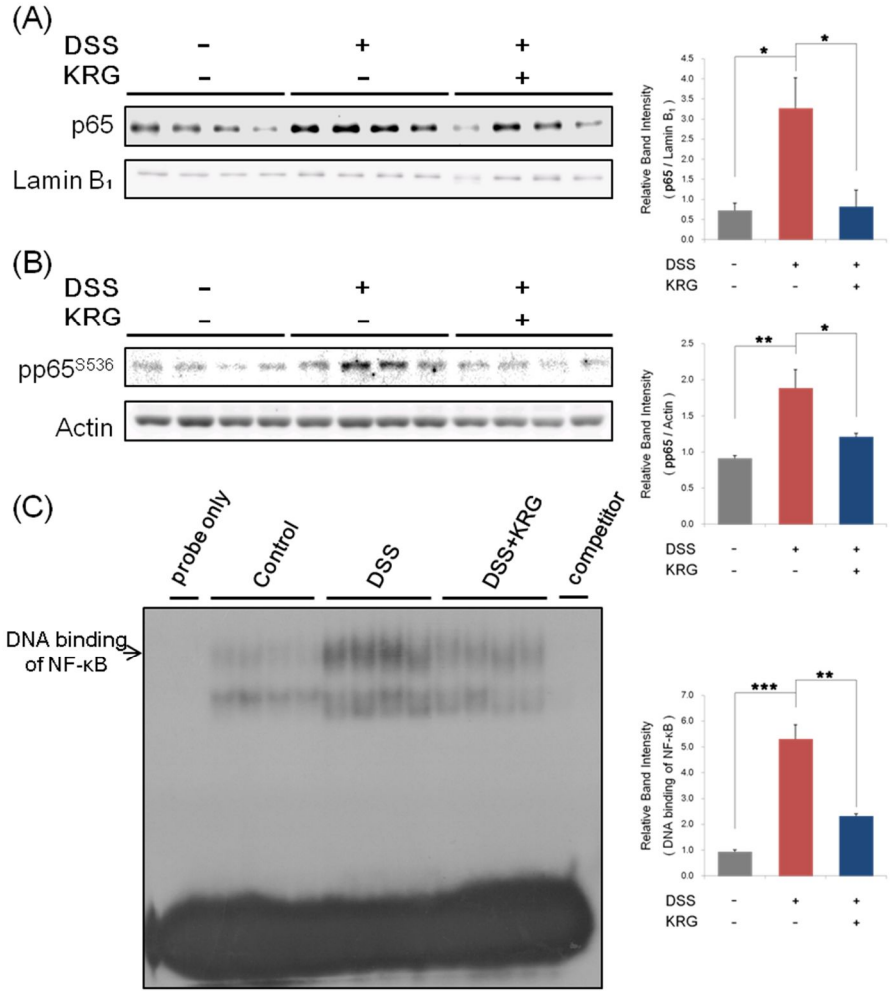

Figure 5. Inhibitory effects of KRG on DSS-induced phosphorylation, nuclear accumulation and DNA binding of NF-κB p65. Nuclear accumulation (A) and phosphorylation (Ser536) (B) of NF-κB p65 were determined by Western blot analysis (B). NF-κB-DNA binding activity (C) was determined by EMSA. Results are presented as means ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

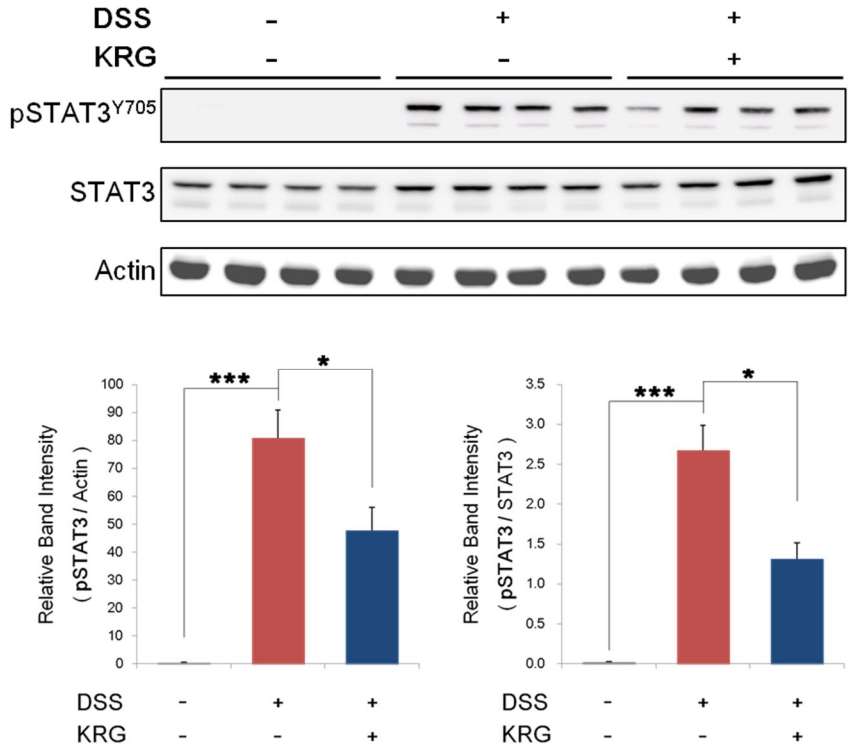

Figure 6. Inhibitory effects of KRG on DSS-induced STAT3 phosphorylation. Inhibitory effects of KRG on DSS-induced STAT3 phosphorylation (Tyr705) were determined by Western blot analysis. Results are presented as means ± SE. $^{*}P < 0.05$ and $^{***}P < 0.001$.

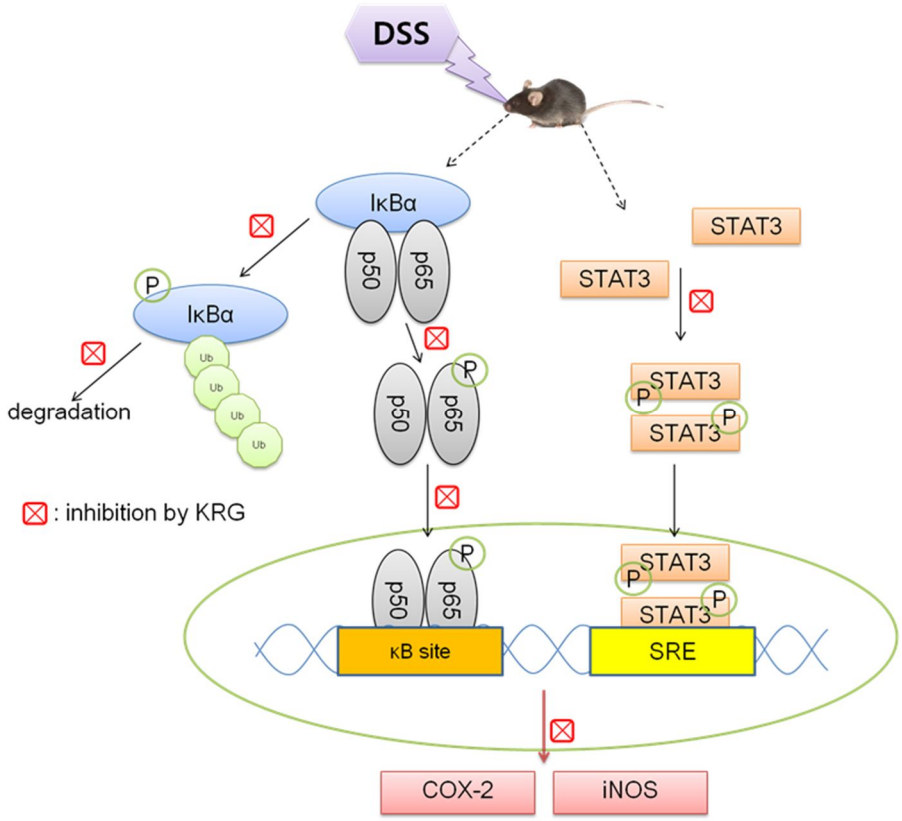

Figure 7. Molecular mechanisms by which KRG inhibits DSS-induced mouse colitis.

Study 2 : AOM plus DSS-induced colon carcinogenesis

Macroscopic assessment

During 7 days of 2% DSS exposure, the body weight and the DAI of mice in each group were checked. KRG treatment relieved the severity of colitis induced by DSS (**Fig. 8**). Our previous data indicated that a single injection of AOM following with DSS (inflammatory agent) treatment for 1 week causes rapid formation of colon cancer driven by colitis [7]. All mice were sacrificed 16 weeks after the AOM injection. Mice in the AOM+DSS group developed colon tumors. However, only 50% of mice in the AOM+DSS+KRG group had colon tumors. In addition, KRG treatment significantly reduced the tumor multiplicity and the total tumor weight (**Fig. 9**).

Effects of KRG on the proliferation and the inflammation of colon

cMyc and cyclin D1 are oncogenic proteins that stimulate cell proliferation. In tumor-free region of colon in the AOM+DSS group, cMyc and cyclin D1 were significantly up-regulated compared to the control group, and KRG treatment suppressed it (**Fig. 10A**). COX-2 and iNOS are inducible inflammatory enzymes that promote colon carcinogenesis. COX-2 and iNOS were constantly expressed in the

colon of the AOM+DSS group. KRG treatment inhibited the up-regulation of AOM plus DSS-induced iNOS and COX-2 expression (**Fig. 10B**).

Effects of KRG on activation of NF-κB and STAT3

NF-κB and STAT3 play a vital role in inflammation-associated carcinogenesis. Colons of the mice in the AOM+DSS group showed persistent activation of NF-κB and STAT3 via phosphorylation of IκBα with subsequent nuclear accumulation of NF-κB p65 and pSTAT3, respectively. KRG treatment inhibited all these events (**Fig. 11**).

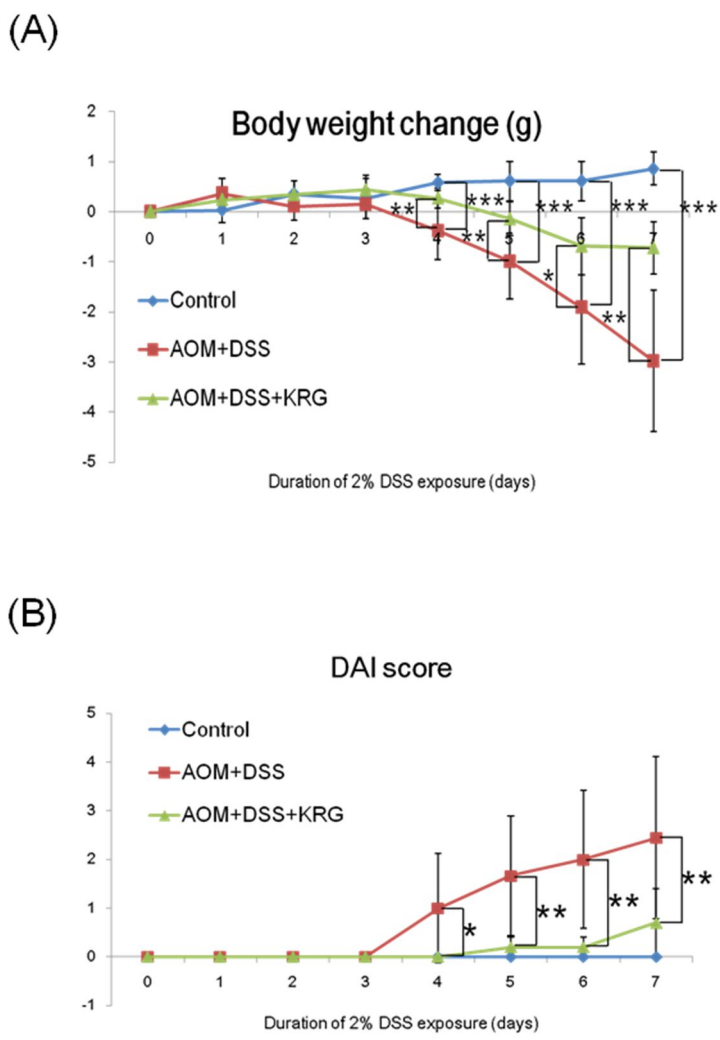

Figure 8. Macroscopic assessment of mouse colitis. Following *i.p.* injection of AOM, 2% DSS was provided in drinking water for 1 week. During 7 days of DSS exposure, the body weight (A) and DAI (B) were checked daily. KRG treatment relieved the symptoms of DSS-induced colitis. Results are presented as means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

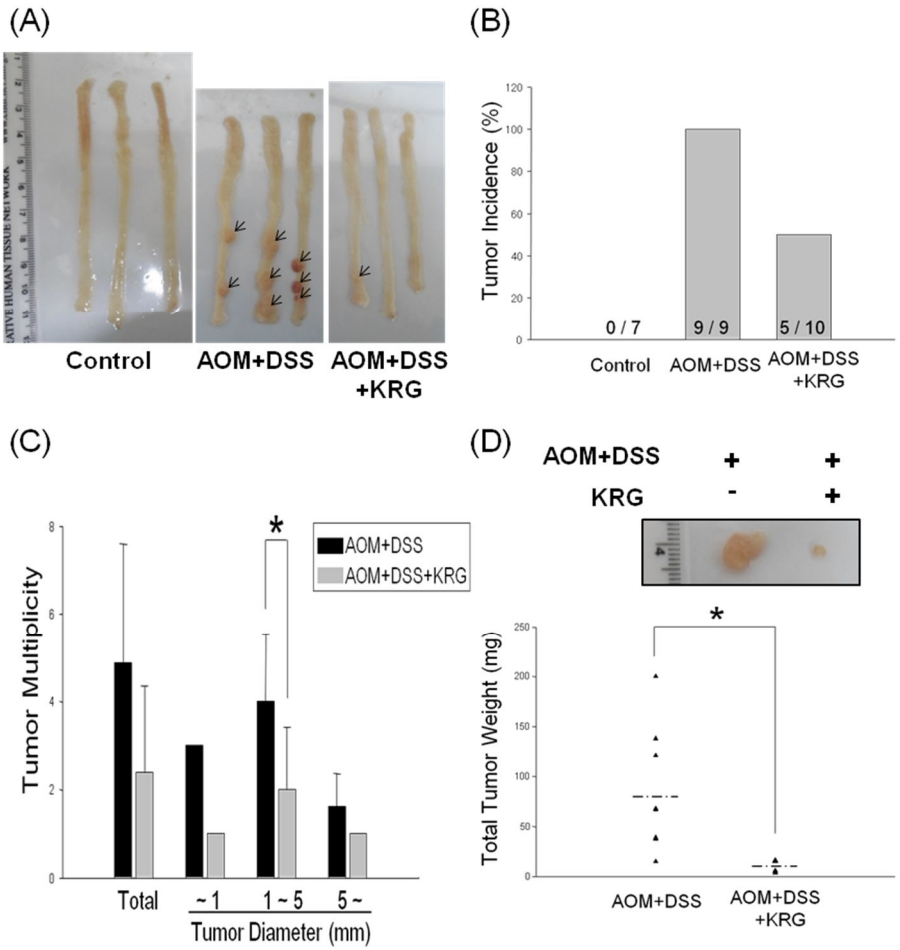

Figure 9. Macroscopic assessment of mouse colon tumor. All mice were sacrificed 16 weeks after the single *i.p.* injection of AOM (10 mg/kg) followed by exposure to 2% DSS for 7 days. KRG treatment inhibited AOM plus DSS-induced colon carcinogenesis (A). The tumor incidence (B), the number (C) and the total weight (D) of tumor were assessed. Results are presented as means ± SD. $^{*}P < 0.05$.

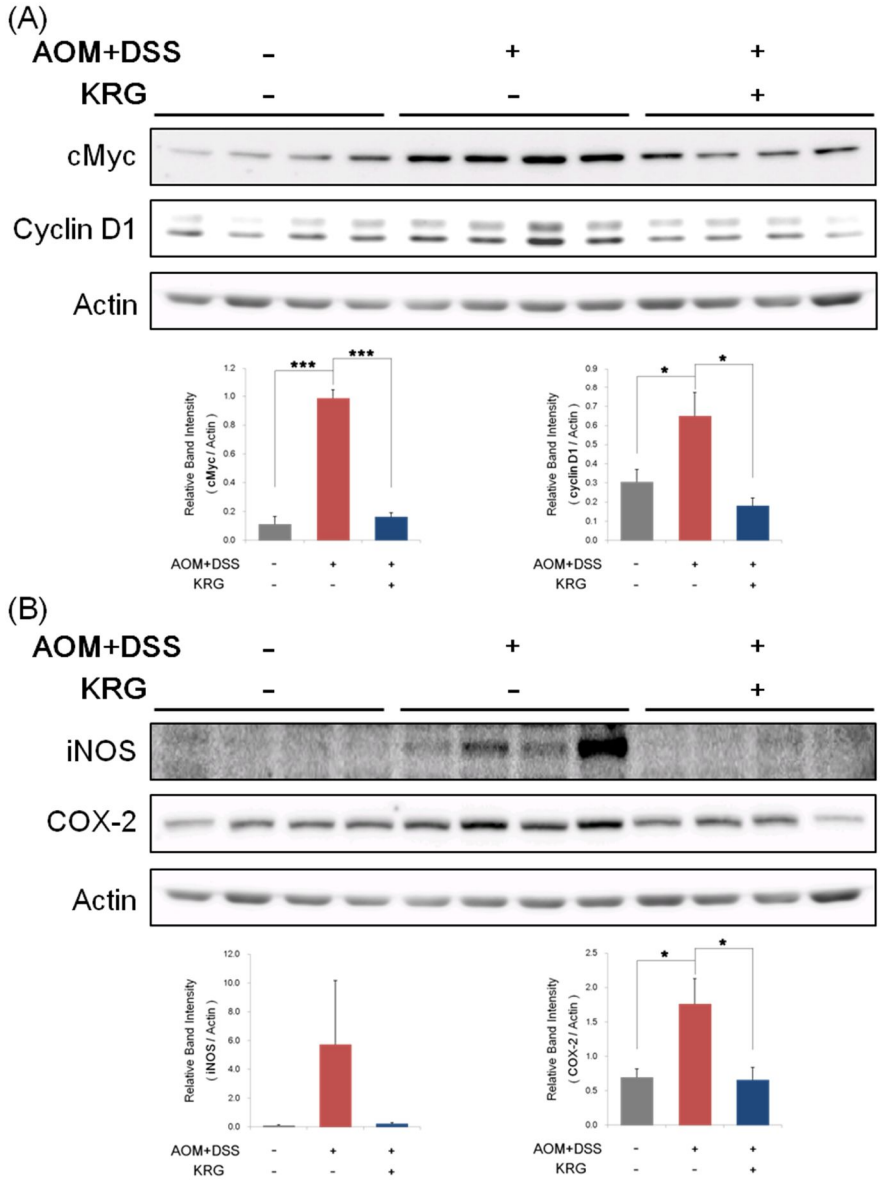

Figure 10. Inhibitory effects of KRG on AOM plus DSS-induced up-regulation of proliferation (A) and inflammation (B) markers. All mice were sacrificed at indicated time and colons were collected. After excision of all visible tumors, remaining colon tissue was subject to Western blot analysis. Results are presented as means ± SE. $^{*}P < 0.05$

and $^{***}P < 0.001$.

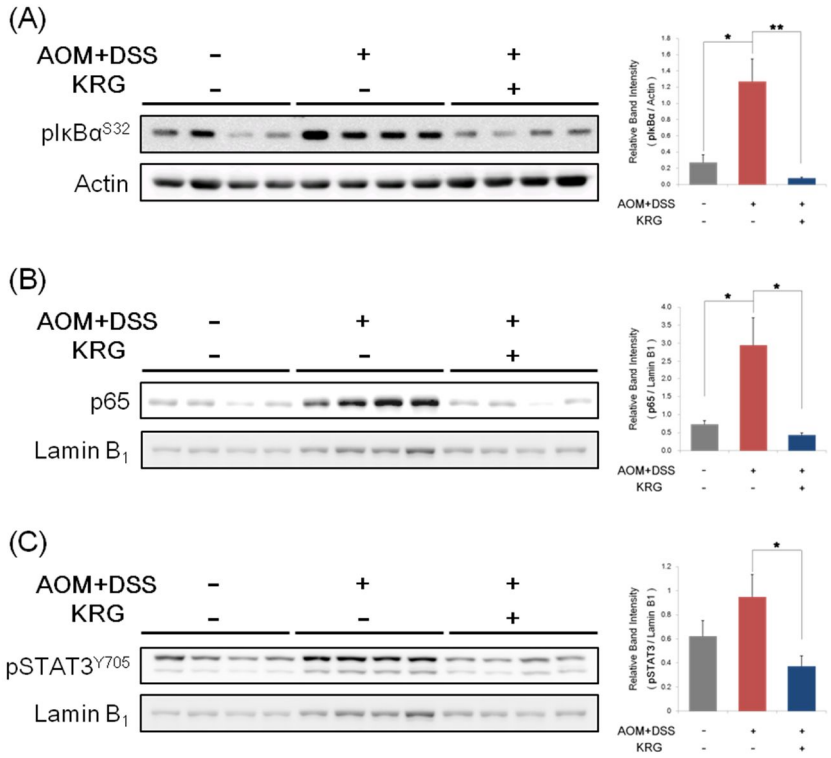

Figure 11. Inhibitory effects of KRG on AOM plus DSS-induced IκBα phosphorylation, nuclear accumulation of NF-κB p65 and pSTAT3. Inhibitory effects of KRG on AOM plus DSS-induced IκBα phosphorylation (Ser32) were determined by Western blot analysis (A). Inhibitory effects of KRG on nuclear accumulation of NF-κB p65 (B) and pSTAT3 (C) were determined by Western blot analysis. Results are presented as means ± SE. $^{*}P < 0.05$ and $^{**}P < 0.01$.

DISCUSSION

Chronic inflammation is closely related to the progression of cancer [8]. Inflammatory cells, such as macrophages and neutrophils, secrete various cytokines that activate NF-κB and STAT3 in epithelial cell. During chronic inflammation, constitutive activation of NF-κB and STAT3 induces over-expression of oncogenic proteins such as COX-2 and cMyc, respectively which can promote the processes of carcinogenesis. Hence, inhibition of chronic inflammation is an one way to prevent cancer.

KRG has been known for its preventive effects on various cancers [9]. However, its molecular mechanisms have not been elucidated well. In this study, we hypothesized that KRG is a possible anti-inflammatory agent in colon and investigated effects of KRG on colitis and colitis-associated colon carcinogenesis.

It has been reported that American ginseng has anti-inflammatory effects on experimentally induced mouse colitis through suppression of key inflammatory markers such as iNOS and COX-2 [10, 11]. Our results also indicated that KRG treatment ameliorated DSS-induced colitis through inhibition of expression of COX-2 and iNOS and activation of NF-κB and STAT3. However, it is not clear which

component of KRG is responsible for these effects. There are lots of active components in KRG including saponins and acidic polysaccharides. Ginseng saponins, which are called ginsenosides, are potent candidates that exert anti-inflammatory effects. A panaxdiol-type ginsenoside, Rg_3 inhibited expression of inflammatory mediators in phorbol ester-treated mouse skin [12] and LPS/IFN-γ-stimulated BV-2 cells [13]. Another type of ginsenosides, 20(S)-protopanaxatriol, inhibited the expression of iNOS and COX-2 through inactivation of NF-κB in LPS-stimulated macrophages [14]. Based on above research, ginsenosides might be the main components of KRG that is responsible for the inhibition of mouse colitis. Ginsenosides have structural similarity to steroids [15]. Thus, ginsenosides contained in KRG could bind to and activate a specific steroid receptor, which may account for the preventive effects of KRG on DSS-induced colitis.

Colon is exposed to lots of environmental factors. One considerable factor is the presence of intestinal bacteria. Imbalance of enterobacteria induces host immune responses [16] and provokes experimental colitis in rats and mice [17]. DSS-induced colitis begins with the penetration of luminal bacteria into colon epithelium [18]. Supplement of probiotics ameliorates experimental colitis by producing a balance in the microbial environment in mouse colon [19]. Mitsuoka reported that extracts of *Panax ginseng* inhibited the growth of various clostria and

enhanced growth of bifidobactierum in vitro [20]. It is possible that beneficial effects of KRG on the intestinal microflora may also contribute to mitigation of DSS-induced colonic inflammation processes.

Chronic inflammation promotes carcinogenesis in colon. DSS-induced colitis dramatically accelerates AOM-initiated colon carcinogenesis [21]. Single DSS exposure induces chronic colitis in C57BL/6 mice [22], and increases the risk of developing colon cancer. In our experiment, all of mice in the AOM plus DSS group developed colon tumors. Chronically inflamed colon tissue of mice in the AOM plus DSS group showed the constitutive activation of NF-κB and STAT3 and the up-regulation of cMyc, cyclin D1, iNOS and COX-2, which creates microenvironment for cancer development. KRG prevented the progression toward chronic colitis and subsequently reduced the incidence and the multiplicity of cancer. In addition, the total weight of tumors was significantly reduced by KRG treatment.

Ginsenosides and its active byproduct Compound K have been investigated with regard to their anti-cancer activity. It is reported that ginseng saponins exert anti-proliferative activity in prostate cancer cells [23] and pro-apoptotic activity on breast cancer cells [24]. Compound K, an active metabolic product of ginseng saponins formed by intestinal

bacteria, has been known to exert anti-cancer activity in breast cancer [25, 26], lung cancer [27] and leukemia cells [28]. Another constituent of KRG responsible for its anti-cancer activity is an acidic polysaccharide. It is reported that red ginseng acidic polysaccharide reduced the tumor weight in B16-F10 melanoma-transplanted mice [29].

In summary, we demonstrated that KRG prevented experimentally induced colitis and colon carcinogenesis by blocking NF-κB and STAT3 activation. Anti-carcinogenic activity of KRG results from the inhibition of chronic colitis which is fundamental to promotion of colon carcinogenesis. Therefore, KRG is a potential candidate for chemoprevention of inflammation-associated colon carcinogenesis.

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국문초록

한국홍삼은 암 예방효과가 있다고 보고 되고 있으나, 그 분자적 기전은 아직 명확히 밝혀져 있지 않다. 본 실험에서는 한국홍삼의 dextran sulfate sodium (DSS)에 의해 유도된 대장염과 azoxymethnae과 DSS에 의해 유도된 대장암에 대한 효과를 연구하였다. 수컷 C57BL/6J 마우스는 1% 한국홍삼분말이 포함된 사료나 정상사료를 전 실험기간에 걸쳐서 섭취하였다. 마우스 대장염은 3% DSS가 섞인 음용수를 1주간 마우스에게 노출시킴으로써 유도하였다. DSS는 마우스의 체중 감소, 설사, 직장 출혈과 대장 길이 감소를 유도하였고, 이러한 증상들은 한국홍삼 투여에 의해 완화되었다. 한국홍삼은 nuclear factor-kappa B (NF-κB) 와 signal transducer and activation of transcription 3 (STAT3)의 활성화를 억제함으로써 DSS에 의해 유도되는 cyclooxygenase-2 (COX-2)와 inducible nitric oxide synthase (iNOS)의 발현을 저해하였다. 다른 실험에서, 대장암 발생과정은 1회의 AOM (10 mg/kg) 복강 주사로 개시되고, 2% DSS에 의해서 촉진되었다. 한국홍삼투여는 급성 대장염의 증상들을 완화하였고 대장 종양의 발생률, 개수와 크기를 감소시켰다. 한국홍삼은 NF-κB 와 STAT3의 활성화 방지

를 통해서 AOM과 DSS에 의한 COX2, iNOS, cMyc과 cyclin D1의 up-regulation을 억제하였다. 이러한 결과들은 한국홍삼이 대장에서 염증과 관련한 질환들의 예방을 위한 잠재적인 후보임을 시사하는 바이다.

주요어 : Korean red ginseng, colitis, colon cancer, COX-2, NF-κB, STAT3

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