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Anti-inflammatory Effects of Co-treatment of Jingyoganghwaltang and Cheongsimhwan on Croton Oil Induced Hemorrhoid Model in Rats

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ABSTRACT

Objectives : Hemorrhoids are one of the most common diseases in humans. Jingyoganghwaltang (JG) and Cheongsimhwan (CS) have been used for treating hemorrhoids in Korean traditional clinical practice. The present study was designed to evaluate the traditional effects of JG and CS on the experimental hemorrhoid model in rats.

Methods : Hemorrhoids are closely related to inflammation. Accordingly, we examined the nitric oxide (NO) production in macrophage cell line in order to evaluate the anti-inflammatory effect. The expression levels of inflammation related genes including IL-1 beta, IL-6, INOS, and TNF-alpha were examined via a real-time quantitative PCR. Croton oil-induced hemorrhagic animal model was used to test the *in vivo* efficacy against hemorrhoids. The rectal tissues were weighed and the inflammatory proteins were measured to confirm the anti-inflammatory effects.

Results : JG and CS have a statistically significant effect on inhibition of NO production and on the reduction of inflammatory gene expression such as IL-1 beta, IL-6, INOS, and TNF-alpha. The synergistic effects of co-treatment of JG and CS were found out in the IL-6 gene expression. The in vivo study using croton oil-induced hemorrhoid model in rat was performed to check the co-treatment effects. As a result, the co-treatment reduced the inflammation of the rectal tissue and decrease the inflammation related protein productions including ICAM1, MMP2 and MMP9.

Conclusions : These results suggest that JG and CS co-treatment demonstrated anti-inflammatory effects in croton oil-induced hemorrhoid model in rat.

Key words : Jingyoganghwaltang, Cheongsimhwan, Hemorrhoids, Croton oil, Anti-inflammatory effects

Ⅰ. INTRODUCTION

Hemorrhoids are one of the most common diseases in humans. About half of the people older than age 50 have symptomatic hemorrhoids. Hemorrhoids are characterized by the swelling of veins and tissues above the vascular structures of the anal canal[1,2]. Hemorrhoids are associated with itching, pain and bleeding, but the exact mechanism is unknown. The hemorrhoids are divided into 1-4 phases. In the first and second phases, mainly non-surgical treatment such as dietary manipulation or anti-hemorrhoidal medication. In many cases including the first and second phases, hemorrhoids could be treated effectively with diet, good hygiene, and topical medications. These non-surgical treatment such as dietary manipulation or anti-hemorrhoidal medication can cure or alleviate symptoms. Surgery is used for the third and fourth

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period, however, the surgical operation tends to be avoided by patients due to the postoperative pain or discomfort[3].

In traditional Korean medicine, Jingyoganghwaltang and Cheongsimhwan have been prescribed to treat hemorrhoids. Jingyoganghwaltang is known to have the efficacy of strengthening Eum (Yin in Chinese) principle, removing damp Gi, cleansing the bowels and cooling the blood, and treatment for itching and pain in the anus. Cheongsimhwan is known to have the fever-reducing efficacy and adjust the pathogenic wind and damp pathogen and is used to treat the heat of the heart[4]. Jingyoganghwaltang and Cheongsimhwan have been used for treating hemorrhoids separately or in combination in Korean traditional clinical practice. However, there have been no reports of inflammatory efficacy or anti-hemorrhoidal effects through animal experiments.

The present study was designed to investigate the effect of Jingyoganghwaltang and Cheongsimhwan co-treatment *in vitro* and its effects on croton oil-induced hemorrhoid model in rat.

Ⅱ. Materials and Methods

1. Preparation of samples

Jingyoganghwaltang and Cheongsimhwan were prepared as described in table 1. Each drug was purchased from Omniherb (Yeongcheon, Korea) and HMAX (Chungbuk, Korea) and confirmed by one of the authors (Prof. KH Leem), and a voucher specimen was kept in the College of Korean Medicine, Semyung University. The composition of Jingyoganghwaltang and Cheongsimhwan were based on Donguibogam. Three hundred g of Jingyoganghwaltang and Cheongsimhwan in 3,000 ㎖ distilled water was heated in a heating extractor for 3 hours, respectively. The extracts were filtered and concentrated by using the rotary evaporator. Jingyoganghwaltang extracts (JG) and Cheongsimhwan extracts (CS) were lyophilized by using freeze dryer.

Table 1. Compositions and extracting yields of Jingyoganghwaltang and Cheongsimhwan

Name	Fomula	Weight ratio (g)	Yield (%)
Jingyoganghwaltang	Glycyrrhizae Radix et Rhizoma	1.875	26.7
	Osterici Radix	5.625	
	Angelicae Tenuissimae Radix et Rhizoma	1.125	
	Ephedrae Herba	1.875	
	Saposhnikoviae Radix	2.625	
	Asari Radix et Rhizoma	0.750	
	Cimicifugae Rhizoma	1.875	
	Bupleuri Radix	1.875	
	Gentianae Macrophyllae Radix	3.750	
	Carthami Flos	0.750	
	Astragali Radix	3.750	
Cheongsimhwan	Hoelen cum Pini Radix	18.75	21.5
	Poria Sclerotium	18.75	
	Coptidis Rhizoma	37.50	

2. Cell culture

The mouse macrophage-like cell line RAW264.7 were purchased from the American Type Culture Collection (Rockville, MD, USA) and maintained using Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Grand island, NY, USA) containing 10% v/v fetal bovine serum (heat-inactivated, Invitrogen, Grand island, NY, USA) and 100 U/ml penicillin/streptomycin (Sigma-Aldrich, New York, NY, USA) in an incubator with a humidified atmosphere of 95% air and 5% CO_2, at 37℃.

3. Nitric oxide assay

Nitric oxide (NO) levels in the culture supernatants were measured by the Griess reaction. In brief, RAW264.7 cells were seeded in a 96-well plate in a density of 1.0×10^4 cells/well and incubated for 24 h. The next day, the medium was changed to DMEM without FBS. The following day, Blank (phosphate-buffered saline, PBS only), Control (lipopolysaccharide, henceforth referred as LPS, 100 ng/㎖, LPS only), JG (LPS 100 ng/㎖ + JG 20, 100, and 200 ㎍/㎖), CS (LPS 100 ng/㎖ + CS 20, 100, and 200 ㎍/㎖) and MIX (LPS

100 ng/㎖ + (JG + CS) 20, 100, and 200 ㎍/㎖) samples were treated and incubated for 24 h. MIX is a mixture of JG and CS in the same ratio. For example, MIX 100 ㎎/㎖ was prepared by dissolving 50 ㎎ of JG and 50 ㎎ of CS in 1 ml of DW. The next day, 0.1 ㎖ of culture supernatants from each samples were mixed with the same volume of the Griess reagent. Absorbance values were measured at 540 nm with ELISA reader (Synergy2, Biotek, VT, USA).

4. Realtime quantitative PCR

RAW264.7 cells were seeded in 100-mm culture dishes (5.0×10^5) and incubated 24 h. The next day, the medium was changed to DMEM without FBS. After 24 h, the cells were treated with Blank (PBS only), Control (LPS only), JG, CS, and MIX samples and incubated for 24 h. Total RNAs were extracted using RNeasy Protect Mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Then cDNA was synthesized from mRNA using QuantiTect Reverse Transcription kit (Qiagen, Valencia, CA, USA). Realtime quantitative PCR was performed using QuantiTectTM SYBR Green PCR kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. In brief, an initial denaturation of 10 min at 94℃ was followed by cycles of 10 s at 94℃, 15 s at 58℃, 20 s at 72℃. The PCR primer sequences are shown in Table 2. Analyses were performed using Rotor-Gene Q (Qiagen, Valencia, CA, USA) and gene expression values were calculated based on the comparative $\Delta\Delta$ CT method according to the manufacturer's protocol.

IL-1 beta, interleukin-1 beta; IL-6, interleukin-6; INOS, inducible nitric oxide synthase; TNF-alpha, tumor necrosis factor-alpha; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Table 2. Primer sequences realtime quantitative PCR

mRNA		Template
IL-1 beta	Sense	5′ -GAA ATG CCA CCT TTT GAC AGT G-3′
	Antisense	5′ -CTG GAT GCT CTC ATC AGG ACA-3′
IL-6	Sense	5′ -GAG TTG TGC AAT GGC AAT TCT G-3′
	Antisense	5′ -GCA AGT GCA TCA TCG TTG TTC AT-3′
INOS	Sense	5′ -ACA TCG ACC CGT CCA CAG TAT-3′
	Antisense	5′ -CAG AGG GGT AGG CTT GTC TC-3′
TNF-alpha	Sense	5′ -CCC TCA CAC TCA GAT CAT CTT CT-3′
	Antisense	5′ -GCT ACG ACG TGG GCT ACA G-3′
GAPDH	Sense	5′ -GCC ATT TGC AGT GGC AAA GTG G-3′
	Antisense	5′ -GAT GGG CTT CCC GTT GAT GAC AAG C-3′

5. Hemorrhoid animal model

The experimental procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee at Semyung University (Jecheon, Korea; approval no. smecae 14-05-01). Six weeks old male SD rats of approximately 200 g were purchased from Samtako (Osan, Korea). All rats were housed in the same animal holding room with standard laboratory animal environment, under a 12 h light/dark cycle in a controlled environment: temperature, 21 ± 2℃ and humidity, 50 ± 20%. The rats were randomly divided into 3 groups: blank, control, and co-treatment group, n = 8, respectively. 100 ㎎/㎏ of mixture of JG and CS were administered to the co-treatment group for 3 days. The mixture (100 ㎎/㎏) was prepared by dissolving 50 ㎎ of JG and 50 ㎎ of CS in 5 ㎖ of DW and the solution was administrated at the volume of 5 ㎖/㎏ of animal weight with sonde. The hemorrhoid model of rats induced by croton oil was established by the modified method of the paper published by Nishiki[5]. Briefly, a cotton swab was immersed in 0.1 mL of an inducing agent (deionized water: pyridine: ethyl ether: 6% croton oil / ethyl ether (1: 4: 5: 10)) and applied to the rat anus for 60 seconds. Four hours later, recto-anus tissue of 20 ㎜ in length was isolated after the rats were euthanized. The weights of rat body and recto-anus were measured.

6. Western blot

The recto-anus tissues were washed twice with PBS

and incubated in −20℃ with 300 ㎕ of PRO−PREP (Intron biotechnology, Seongnam, Kyungi, Korea) containing phosphatase inhibitor cocktail 2 and 3 (Sigma−Aldrich, New York, NY, USA) for 20 min. The tissues were homogenized by pipetting and centrifuged at 13,000 rpm for 5 min at 4℃. Protein concentrations were measured using DC Protein Assay kit (Bio−Rad, Hercules, CA, USA). Briefly, five microliters of standards and protein samples were transferred to 96−well plate and 25 ㎕ of alkaline copper tartrate solution contains Reagent S. Then 200 ㎕ of dilute Folin Reagent was added and incubated. After 15 min, the protein concentrations were measured at 750 ㎚ using an ELISA reader (Synergy 2, BioTek, VT, USA). Proteins were denatured with 5 × sample buffer and boiled for 5 min then fractionated by electrophoresis through a 10% SDS polyacrylamide gel at 100 V for 2 h, and the proteins were transferred onto PVDF membranes at 100 V for 60 min. Each membrane was blocked with TBST buffer (10 mM Tris−HCl, pH 7.4, 150 mM NaCl, 0.1% Tween−20) containing 5% BSA for 1 h and then incubated with primary antibodies (anti−ICAM1, anti−MMP2 and anti−MMP9 antibodies raised in rabbit) in TBST buffer containing 1% BSA at 4℃ overnight. The membranes were washed 3 times with TBST buffer and further incubated with anti−rabbit IgG secondary antibodies conjugated with horseradish peroxidase for 2 h. Each membrane was filmed by chemiluminescent imaging system (Fusion SL2, Vilber Lourmat, Marne−la−Vallée Cedex, France), and analyzed using Bio1d software (Vilber Lourmat, Marne−la−Vallée Cedex, France).

7. Statistical analysis

The results were expressed as mean ± SEM. One−way ANOVA followed by Tukey's post hoc test was performed for statistical analysis (GraphPad prism ver. 6), and p−values of less than 0.05 ($p<0.05$) indicated significant differences. All *in vitro* experiments were performed with triplicate independent samples.

Ⅲ. RESULTS

1. The effects of JG and CS on the production of nitric oxide

Nitric oxide production in RAW 264.7 cells stimulated with LPS only showed 18.4 ± 0.9 ㎛. JG treatment (20, 100, and 200 ㎍/㎖) showed the 17.8 ± 0.8 ㎛, 17.7 ± 0.8 ㎛, and 17.7 ± 0.8 ㎛ of nitric oxide production, respectively. There was no statistical difference JG−treated groups compared with LPS−treated group (Figure 1A). CS treatment (20, 100, and 200 ㎍/㎖) showed the 22.7 ± 1.1 ㎛, 22.3 ± 0.9 ㎛, and 21.1 ± 0.9 ㎛ of nitric oxide production, respectively. LPS−treated group showed 23.1 ± 1.2 ㎛. Two hundred ㎍/㎖ treatment significantly decreased nitric oxide production (Figure 1B). JG and CS co−treated MIX group (20, 100, and 200 ㎍/㎖) showed the 18.8 ± 0.9 ㎛, 18.4 ± 0.7 ㎛, and 17.9 ± 0.9 ㎛ of nitric oxide production, respectively. LPS−treated group showed 20.4 ± 0.6 ㎛. Both 100 and 200 ㎍/㎖ treated groups showed the statistical differences compared with LPS−treated group (Figure 1C).

2. The effects of JG and CS on the gene expressions of IL−1 beta, IL−6, INOS, and TNF−alpha in RAW 264.7 cells

RAW 264.7 cells were treated with samples and IL−1beta, IL−6, INOS, and TNF−alpha gene expression were measured by quantitative realtime qPCR.

IL−1beta gene expressions in RAW264.7 cells stimulated with LPS showed 3.3 ± 0.3 fold and 100 ㎍/㎖ of JG, CS, and MIX treated groups showed 4.0 ± 0.4, 1.8 ± 0.7, and 0.4 ± 0.3 of IL−1b gene expressions. IL−1b gene expressions were significantly decreased in CS and MIX treated groups (Figure 2A).

IL−6 gene expressions in RAW264.7 cells stimulated with LPS showed 16.7 ± 1.9 fold and 100 ㎍/㎖ of JG, CS, and MIX treated groups showed 18.4 ± 0.3, 20.8 ± 2.4, and 11.9 ± 1.1 of IL−6 gene expressions. IL−6 gene expressions were significantly decreased in MIX treated group (Figure 2B).

INOS gene expressions in RAW264.7 cells stimulated with LPS showed 2.5 ± 0.1 fold and 100 ㎍/㎖ of JG, CS, and MIX treated groups showed 1.6 ± 0.2, 1.5 ± 0.1, and 2.0 ± 0.1 of INOS gene expressions. INOS gene expressions were significantly decreased in JG, CS, and MIX treated groups (Figure 2C).

TNF−alpha gene expressions in RAW264.7 cells stimulated with LPS showed 2.4 ± 0.2 fold and 100 ㎍/㎖ of JG, CS, and MIX treated groups showed 2.0 ± 0.1, 1.3 ± 0.1, and 1.8 ± 0.1 of TNF−alpha gene expressions. TNF−alpha gene expressions were significantly decreased in CS and MIX treated groups (Figure 2D).

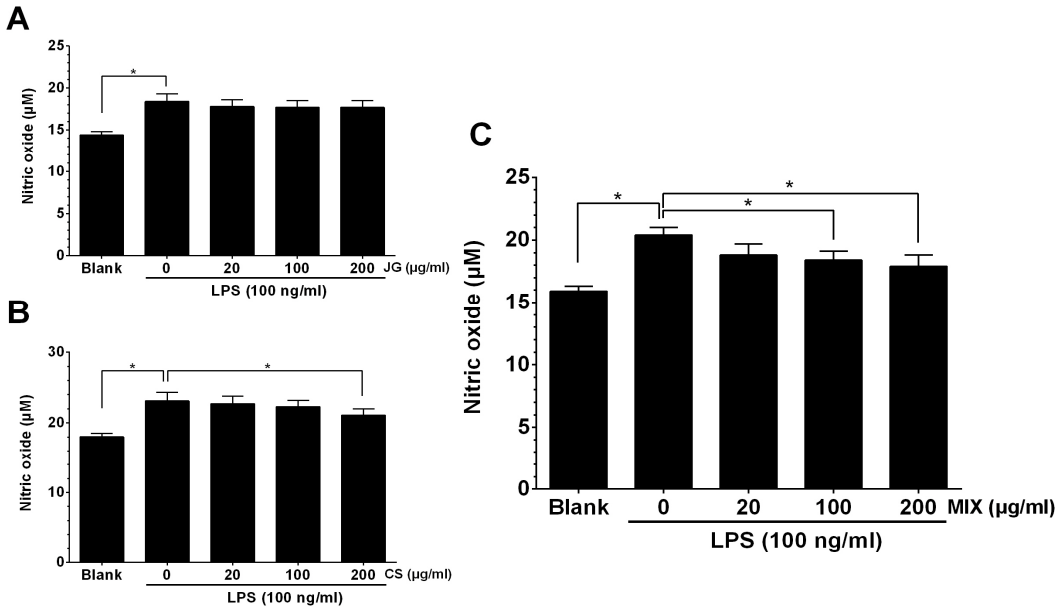

Figure 1. The effects of extracted Jingyoganghwaltang (JG), Cheongsimhwan (CS), and mixture of JG and CS (MIX) on the production of nitric oxide in RAW 264.7 cells. The production of nitric oxide was assayed from culture medium of cells treated with 20, 100, and 200 ㎍/㎖ of JG, CS, and MIX for 24h. NO production was determined by the Griess reaction. Data are presented as the means ± SEM of triplicate experiments (*p<0.05). A: The production of nitric oxides in JG treated cells; B: The production of nitric oxides in CS treated cells; C: The production of nitric oxides in MIX treated cells.

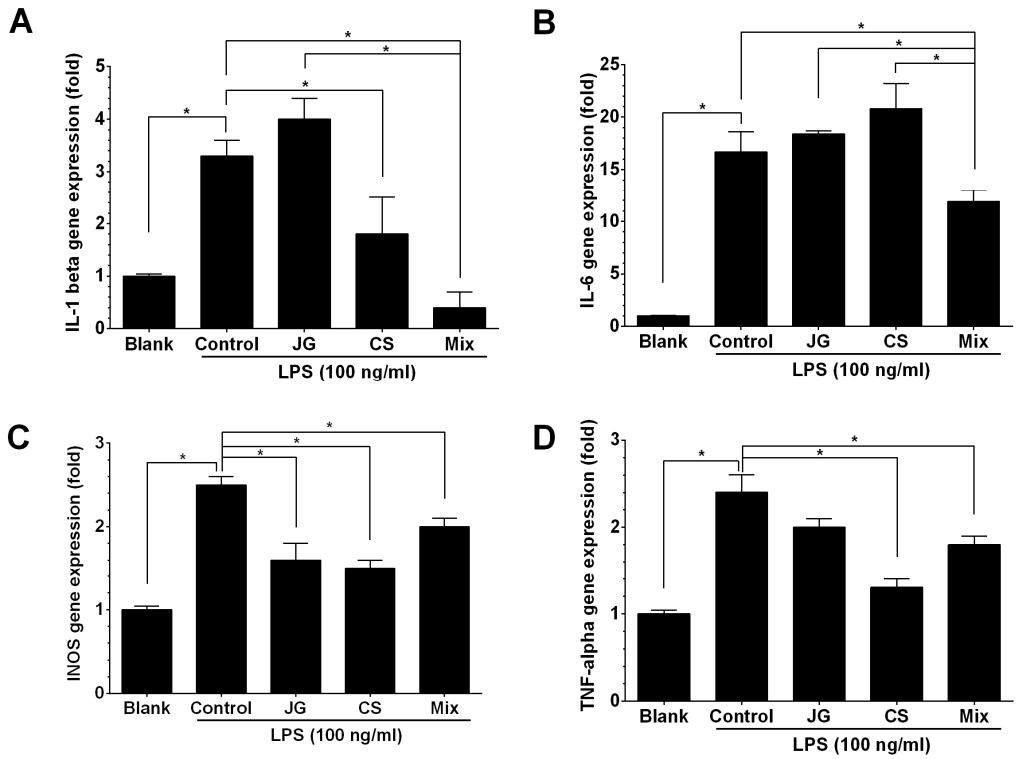

Figure 2. Effects of JG, CS, and MIX (JG and CS) on the gene expression of IL-1 beta (A), IL-6 (B), INOS (C), and TNF-alpha (D). RAW 264.7 cells were treated with 100 ㎍/㎖ of JG, CS, and MIX for 24h. The mRNA level of IL-1 beta, IL-6, INOS, and TNF-alpha was evaluated by realtime qPCR. GAPDH was used as an internal control for realtime qPCR. Data are presented as the means ± SEM of triplicate experiments (*p<0.05).

3. The effects of JG and CS co-treatment on the recto-anus tissue weight in hemorrhoid animal model

Hemorrhoids were induced to all the groups, except normal blank group, by applying croton oil preparation. After 4 hours, recto-anus tissue were taken, weighed, and preserved for western blot experiments. The croton oil treatment made the inflammation and the weight of the recto-anus tissue was increased. The co-treatment group significantly reduced the weight of retro-anal tissue to 86.4±2.9% (compared to 100±5.4% of croton oil treated control group) (Figure 3).

4. The effects of JG and CS co-treatment on the recto-anus tissue weight in hemorrhoid animal model

In the present experiment, ICAM1, MMP2, and MMP9 protein expression in the hemorrhagic tissue was measured by western blot analysis (Figure 4A). As a result, JG and CS co-treatment significantly reduced the ICAM1 expression to 71.2 ± 4.3% compared to control group (Figure 4B). The MMP2 expression was significantly reduced to 80.5 ± 6.2% compared to control group (Figure 4C). The MMP9 expression was significantly reduced to 54.0 ± 9.9% compared to control group (Figure 4D).

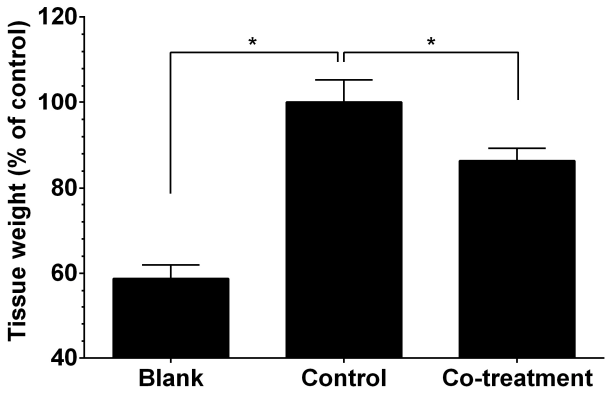

Figure 3. Effects of co-treatment of JG and CS on the recto-anus tissue weight of croton oil-induced hemorrhoid model in rat. Data are presented as the means ± SEM (*p<0.05).

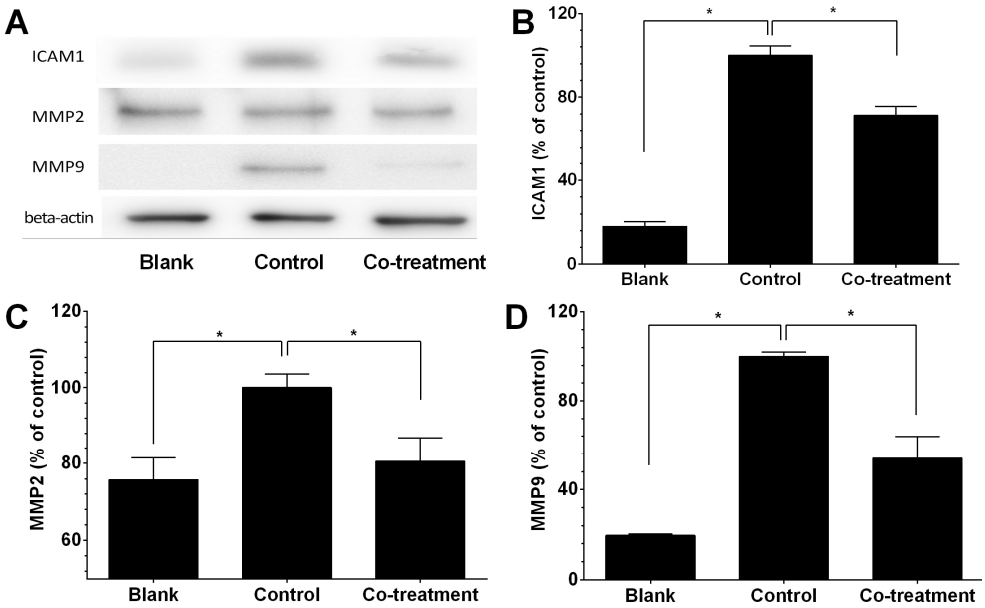

Figure 4. Western blots analysis of ICAM1, MMP2, and MMP9 expression in the recto-anus tissue of croton oil-induced hemorrhoid model in rat. A: Western blot analysis; B, C, and D: Densitometric quantification of ICAM1, MMP2, and MMP9 from western blot, respectively. Data are presented as the means ± SEM (*p<0.05).

Ⅳ. DISCUSSION

In oriental medicine, it is common to use a combination of prescriptions. For the treatment of hemorrhoids, liquid medicine (Jingyoganghwaltang) and pills (Cheongsimhwan) are used in combination[4]. The one of main therapeutic mechanisms of those prescriptions is known as anti-inflammatory activity. In order to observe the synergistic effect when administered together, anti-inflammatory *in vitro* experiments using RAW264.7 cells were carried out.

When two drugs were administered together, effect was augmented (Figure 1). In particular, the 100 ㎍/㎖ group of JG or CS showed no statistical significance when administered alone, but the co-treatment of two drugs was statistically significant (Figure 1).

Inflammation is one of the typical symptoms of hemorrhoids. In hemorrhoids, severe inflammatory reactions are observed in the blood vessel wall and surrounding tissues[6]. It is known that IL-1beta, IL-6, INOS, and TNF-alpha genes play an important role in the inflammatory response. RAW 264.7 cells were treated with samples and IL-1beta, IL-6, INOS, and TNF-alpha gene expression were measured by realtime quantitative qPCR.

IL-1beta is a well-known inflammation-related factor, and is associated with inflammation, autoimmune apoptosis, and pain[7]. IL-1beta gene expressions were significantly decreased in CS and MIX treated groups. The inhibitory effect was further enhanced in the MIX treatment group than in the CS alone treatment group (Figure 2A).

IL-6 is synthesized early in the inflammatory response due to infection and tissue damage and contributes to the defense, but it also causes pathological reactions such as chronic inflammation and autoimmunity[8]. JG and CS treatment groups did not change IL-6 gene expression, but MIX group showed a statistically significant decrease in IL-6 gene expression (Figure 2B).

INOS is a direct result of the inflammatory response. It is produced by bacterial products and cytokines. And it acts as a mediator of NO regulation and pro-inflammatory responses[9]. INOS gene expressions were significantly decreased in JG, CS, and MIX treated groups (Figure 2C).

TNF-alpha was initially known as a tumor necrosis factor, but is now known to be a major factor in inflammatory responses. TNF-alpha is involved in a series of cellular responses including cell death, survival, differentiation, proliferation and migration. And it affects a variety of inflammatory diseases including rheumatoid arthritis, inflammatory bowel disease and psoriasis[10]. TNF-alpha gene expressions were significantly decreased in CS, and MIX treated groups (Figure 2D).

Co-treatment reduced the expression of almost all inflammatory genes (Figure 2). Especially, JG and CS co-treatment further reduced IL-1beta and IL-6 gene expression (Figure 2A and B). Taken together, it was found that JG and CS, when administered together, resulted in further reduction of NO production and further reduction of inflammatory gene expression. Therefore, we decided to use the co-treatment of JG and CS in the hemorrhagic animal model induced by croton oil.

Croton oil has been widely used as an acute inflammation model in experimental animals as an inflammatory agent. Nishiki et al. Used croton oil as an inflammatory hemorrhoid model by inducing inflammation in the anus. In this experiment, the method of Nishiki et al. was modified to evaluate the anti-hemorrhoid efficacy of the samples in the hemorrhoid animal model[5,11]. As a result, co-treatment group showed reducing the inflammation and the weight of retro-anal tissue of drug treated group was significantly decreased (Figure 3).

ICAM is induced by IL-1 beta or TNF-alpha and is closely related to vascular endothelial cell adhesion of leukocytes causing acute inflammatory responses[12]. MMP2 and MMP9 have been shown to be involved in tumor cell proliferation and angiogenesis, and in particular, recent studies have shown that they are over expressed in tumor and inflammatory responses[13].

In the present acute inflammatory hemorrhoid model induced by croton oil, the expressions of ICAM1, MMP2, and MMP9 in the hemorrhagic tissue were measured. As a result, the co-treatment group showed the significantly decreased contents of ICAM1, MMP2 and MMP9 compared with croton oil-stimulated control group (Figure 4).

In this study, we tried to confirm the academic basis of JG and CS used in Korean traditional medicine for hemorrhoids. It was confirmed that JG and CS have the statistically significant effect on inhibition of NO production and reduction of inflammation related factors such as IL-1 beta, IL-6, INOS, and TNF-alpha. And the anti-hemorrhoid efficacy of co-treatment of JG and CS on the croton oil-induced hemorrhagic animal model was performed and the statistically significant effects were found out. And co-treatment of JG and CS significantly reduced the expression levels of the intercellular adhesion molecule and matrix metalloproteinase proteins such as ICAM1, MMP2 and MMP9. These results suggest that treatment of Jingyoganghwaltang and Cheongsimhwan has the anti-inflammatory effects in croton oil-induced hemorrhoid model in rat.

Ⅴ. CONCLUSION

Hemorrhoids are one of the most common diseases in humans. JG and CS have been used for the medical treatment of hemorrhoids in Korean traditional clinical practice. In this study, we observed anti-inflammatory effects of JG and CS in RAW264.7 cells and *in vivo* effects on croton oil induced hemorrhoid model in rat. The conclusion which can be drawn from this study are these.

1. Both 100 and 200 ㎍/㎖ of the JG and CS co-treated MIX group significantly decreased nitric oxide production.

2. The JG and CS co-treated group significantly decreased the expressions of IL-1beta, IL-6, INOS, and TNF-alpha genes.

3. The co-treatment significantly decreased the weight of retro-anal tissue of hemorrhoids induced by croton oil.

4. The co-treatment significantly decreased contents of ICAM1, MMP2 and MMP9 in the hemorrhagic tissue.

These results could provide a mechanistic explanation for the anti-inflammatory effects and hemorrhoids treatment effects of JG and CS.

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REFERENCES

1. Kaidar-Person O1, Person B, Wexner SD. Hemorrhoidal disease: a comprehensive review. J Am Coll Surg. 2007 ; 204(1) : 102-17.
2. Chong PS, Bartolo DC. Hemorrhoids and Fissure in Ano. Gastroenterol Clin North Am. 2008 ; 37(3) : 627-44.
3. Clinical Practice Committee, American Gastroenterological Association. American Gastroenterological Association medical position statement: Diagnosis and treatment of hemorrhoids. Gastroenterology. 2004 ; 126 : 1461-2.
4. Heo J. Donguibogam. Seoul:Bubin Publishers. 2012 : 911-2.
5. Nishiki K, Nishinaga K, Kudoh D, Iwai K. Croton oil-induced hemorrhoid model in rat: comparison of anti-inflammatory activity of diflucortolone valerate with other glucocorticoids. Nihon Yakurigaku Zasshi. 1988 ; 92(4) : 215-25.
6. Lohsiriwat V. Hemorrhoids: from basic pathophysiology to clinical management. World J Gastroenterol. 2012 ; 18(17) : 2009-17.
7. Ren K, Torres R. Role of interleukin-1beta during pain and inflammation. Brain Res Rev. 2009 ; 60(1) : 57-64.
8. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014 ; 6(10) : 1-16.
9. Hämäläinen M1, Lilja R, Kankaanranta H, Moilanen E. Inhibition of iNOS expression and NO production by anti-inflammatory steroids. Reversal by histone deacetylase inhibitors. Pulm Pharmacol Ther. 2008 ; 21(2) : 331-9.
10. Bradley JR. TNF-mediated inflammatory disease. J Pathol. 2008 ; 214(2) : 149-60.
11. Azeemuddin M, Viswanatha GL, Rafiq M, Thippeswamy AH, Baig MR, Kavya KJ, Patki PS, Shyam R. An improved experimental model of hemorrhoids in rats: evaluation of antihemorrhoidal activity of an herbal formulation. ISRN Pharmacol. 2014 ; 2014 : 530931.
12. Frank PG, Lisanti MP. ICAM-1: role in inflammation and in the regulation of vascular permeability. Am J Physiol Heart Circ Physiol. 2008 ; 295(3) : H926-7.
13. Roy R, Yang J, Moses MA. Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J Clin Oncol. 2009 ; 27 : 5287-97.