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Freund's complete adjuvant로 유발시킨 rat 류마티스성 관절염에 대한 빈소산의 치료 효과

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ABSTRACT

Therapeutic Effects of *Binsosan*(檳蘇散) on Adjuvant-induced Rheumatoid Rats

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실험목적 : 빈소산은 11가지 생약으로 구성된 복합 한약 처방으로 관절염을 포함한 다양한 염증성 질환의 치료제로 사용되어 왔으나, 관절염에 대한 직접적인 효력평가는 찾아 보기 힘들다. 따라서 본 실험에서는 빈소산 추출물이 Freund's complete adjuvant (FCA)로 유발된 rat 류마티스성 관절염에 미치는 치료 효과를 dexamethasone (15mg/kg, 복강 투여) 의 효과와 비교 평가하였다.

실험방법 : 류마티스성 관절염은 FCA (10mg in 1ml paraffin oil 0.1ml/rats)를 좌측 후지에 피내 투여하여 유발하였다. 실험동물은 Wistar 랫트를 사용하였고, FCA 투여 14일 후 유사한 무릎관절 둘레를 나타내는 류마티스성 관절염 유발 rat와 정상 rat 및 실험군을 그룹당 9마리씩 나누었다.

실험동물은 100 또는 200mg/kg의 빈소산 추출물을 FCA 투여 14일 후부터 14일간 경구 투여하였으며, dexamethasone은 15mg/kg 농도로 복강 투여한 다음, 희생하여, 체중, 연골내 collagen 함

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량 및 chondroitin sulphate, heparin sulphate 및hyaluronic acid와 같은 뼈내glycosaminoglycan 함량의 변화를 각각 관찰하였다. 실험결과는 항염 효과가 이미 입증되어 있는 dexamethasone 15mg/kg 복강 투여군과 비교하였다.

결 과 : FCA 투여는 현저한 체중, 연골내collagen 함량 및 chondroitin sulphate, heparin sulphate 및hyaluronic acid와 같은 뼈내 glycosaminoglycan 함량의 감소와 함께 유발 관절 둘레 및 조직내 prostaglandin E_2의 증가와 같은 전형적인 류마티스성 염증을 초래하였으나, 이러한 류마티스성 관절염 소견은 dexamethasone 및 모든 용량의 빈소산 추출물 투여에 의해 현저히 억제되었으며, 특히 빈소산 투여군에서는 투여 용량 의존적인 감소가 인정되었다.

결 론 : 이상에서 빈소산 추출물은 투여 용량 의존적인 prostaglandin E_2억제를 매개하여 FCA 유발 류마티스성 관절염에 대한 치료 효과를 나타내는 것으로 관찰되었다. 따라서 새로운 관절염에 대한 치료제로서 개발 가능성이 있을 것으로 판단된다. 한편 빈소산 추출물은 주로 prostaglandin E_2억제작용에 의해 항염 효과를 나타내는 것으로 관찰되었으나, 금후 다른 작용기전에 대한 연구와 빈소산의 구성성분 중 유효 성분 규명을 위한 실험이 수행되어야 할 것으로 판단된다.

Key word : Freund's complete adjuvant (FCA), Rheumatoid arthritis, 빈소산(檳蘇散, bīng sū sǎn)

Ⅰ. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease associated with long-term disability and premature mortality[1]. The effects of chronic inflammation of rheumatoid arthritis on cartilage metabolism have received attention primarily at the level of the local erosive destruction of cartilage in diarthroidal joint cartilage collagen and bone destruction which are the hallmark ofwell-established rheumatoid arthritis[2].

Adjuvant arthritis in rats is a widely used experimental model of chronic inflammation with manifestations resembling clinical disorders such as rheumatoid arthritis and ankylosing spondylitis. Although the knowledge of the molecular events associated with the pathological manifestations of adjuvant arthritis is limited, it is apparent that some of the most significant modifications of the disease are due to the metabolic changes in connective tissue matrix components[3].

During the process of inflammation, the hydrolytic enzymes like glycohydrolases were released from invading macrophages, neutrophils and tissue cells such as synoviocytes and chondrocytes. Enhanced activities of sialyltransferase and galactosyltransferase in serum and liver of inflamed rats were demonstrated. Since the inflammatory process of

adjuvant arthritis is a systemic disease and causes alterations in the metabolism of connective tissue macromolecular components involving many organs, the changes occurring in the metabolism of connective tissue macromolecules such as glycoproteins, proteoglycans, glycosaminoglycans and collagen in arthritic disease are of considerable importance from the point of view of the incidence of rheumatoid arthritis[4].

Treatment decisions in rheumatoid arthritis are individualized and depend largely on the disease activity at the time presentation. Many drugs are clinically used for the treatment of rheumatoid arthritis, but therapeutic effects are restricted by their side effects Hormone can cause osteoporosis[5] methotrexate can inhibit bone marrow proliferation and cause leukopenia and thrombocy- topeni1a[6] and leffunomide can lead to gastrointestinal dysfunction, skin rash, allergic reaction, loss of body weight, and reversible baldness[7]. Dexamethasone is well-known glucocorticoid, and they are the most widely used anti-inflammatory drugs as reference drug on development of the new anti-inflammatory drugs[8-10].

As the increase of the concern in the functional food and well being in life, the demands and consumption of functional food originated form natural sources are increased[11]. Medicinal plants playa key role in health care in man and many plants are claimed to possess anti-arthritic activity[12]. Traditional herbal medicine, *Binsosan* (BSS) consisted of11 kinds of medicinal herbs and it has been used in treatment of lower limb arthritis (inclouding RA) induced by wind and humidity[13].

We, therefore, hypothesizedthat BSS will be showed favorable effectsagainst adjuvant-induced rheumatoid arthritis. There are no scientific evidences that BSS have pharmacological effects on rheumatoid arthritis.

In the present study, the effects of BSS were evaluated on the FCA-induced rheumatoid arthritis rats at 100 and 200mg/kg levels comparing with intraperitoneal treatment of dexamethasone 15mg/kg as reference drug. Rheumatoid arthritis was induced by intradermal injection of FCA (10mg in 1ml paraffin oil 0.1ml/rats).

II. MATERIALS & METHODS

1. Animals and Husbandry

Ninety male albino rats of Wistar rats (6-wk old upon receipt, SLC, JAPAN) were used in this study. Animals were allocated four per polycarbonate cage in a temperature (20~25℃) and humidity (40~45%) controlled room. Light : dark cycle was 12hr : 12hr and feed (Samyang, Korea) and water were supplied free to access. Animals were acclimatization for 10 days (body weights were showed 177 ~ 192g after 10 days of acclimatization). Seventy-two rats were FCA injected and 18 rats were saline injected. About half of FCA or saline injected rats were selected after 14 days of treatments based on ankle circumferences and body weights. All animals were treated according to the Guide for the Care and Use of Laboratory Animals by Institute of Laboratory Animal Resources, Commission on Life Science, National Research Council, USA on 1996, Washington D.C. Nine rats per group was selected after 14 days of FCA or saline treatments based on ankle circumferences and body weights, and 5 groups were divided as

below(Table 1).

1) EXPERIMENTAL GROUPS

(1) Intact control

Saline injected vehicle control group

(2) FCA control

FCA-induced rheumatoid arthritis vehicle control group

(3) Reference drug

Dexamethasone 15mg/kg intraperitoneally administered, FCA-induced rheumatoid arthritis group

(4) BSS treated group

Two different dosages (100 and 200mg/kg) of BSS extracts orally dosed, FCA-induced rheumatoid arthritis groups.

Table 1. Experimental Groups and Test Articles Used in This Study

Groups (Routes of administration)		Dose
Intact group	Intact control (intraperitoneally)	Saline only
FCA-induced rheumatoid arthritis groups*	FCA control (intraperitoneally)	Saline only
	Dexamethasone (intraperitoneally)	15mg/kg
	BSS extracts (Orally)	
	Lower dosage	100mg/kg
	Higher dosage	200mg/kg

*Rheumatoid arthritis are induced by intradermal administration of Freund's complete adjuvant (FCA; 10mg in 1ml paraffin oil, 0.1ml/rats); All test articles and dexamethasone were dosed at a volume of 5ml/kg, once a day for 14 days from 14 days after FCA treatment; BSS, *Binsosan*.

2. Test article and Formulation

Aqueous BSS extractswere prepared by routine methods using rotary vacuum evaporator (Lab. Camp, Korea) and programmable freeze dryer (IlShin Lab., Korea) from 11 kinds of BSS herbal components listed in Table 2, which were purchased from Cho-Heung Pharmaceutical Ind. Co. (Daegu, Korea) after confirming the morphology under voucher.

From 400g (10 folds of each 11 kinds of components) of BSS, 61.16g(yield 15.29 %) of lyophilized aqueouspowder of BSS extracts were acquired and used in this study. Powders of lyophilized BSS extract are deep brown powder. The extracted aqueous powders were stored in a refrigerator at -20ºC to protect from light and degeneration. The appearances of BSS extracts in vehicle are clear deep brown solution in distilled water, and it is well soluble upto 40mg/ml concentration levels. Dexamethasone(Sigma, St. Louis, MO, USA) listed below was used as Reference drug in this study.

Table 2. Composition of *Binsosan* Used in This Study

Herbs	Korean	Amounts (g)
Atractylodis Rhizoma	[창 출]	8
Cyperi Rhizoma	[향부자]	4
Perilla Folium	[소 엽]	4
Fraxini Cortex	[진 피]	4
Chaenomelis Fructus	[목 과]	4
Arecae Semen	[빈 랑]	4
Ostericii Rhizoma	[강 활]	4
Achyranthis Bidentatae Radix	[우 슬]	4
Glycyrrhizae Radix	[감 초]	2
Allii Radix	[총 백]	1
Zingiberis Rhizoma Recen	[생 강]	1
Total		40

All herbal components of BSS used in this study was purchased from Cho-Heung Pharmaceutical Ind. Co. (Daegu, Korea)

BSS extracts were dissolved in distilled water and dosed by oral gavage using a sonde attached to 3 ml syringes containing test article at dosage

of 100 or 200mg/kg in a volume of 5ml/kg, once a day for 14 days from 14 days after FCA treatment. Dexamethasone wasintraperitoneally dosed in a suspension of saline at 15mg/kg dose levels, once a day for 14 days from 14 days after FCA treatment. In intact and FCA controls, saline was intraperitoneally injected as same methods and frequencies as test articles or reference drug. The administered dosage and schedule of these drugs were shown in Table 2.

3. Induction of Rheumatoid Arthritis

To induce rheumatoid arthritis, FCA containing 10mg of heat killed *Mycobacterium tuberculosisin* 1ml paraffin oil (0.1ml; Sigma, USA) was injected into the left hind paw of rat interadermally according to the method of Ramprasath et al[12]. In intact control, only saline was intradermally injected instead of FCA.

4. Measurement of Body Weights

Changes of body weight and its gains were calculated at one day before FCA treatment, at FCA treatment and 7, 14 (start day of test article or dexamethasone administration), 21 days after FCA treatment at sacrifice. To reduce the individual differences from initiation of study, 3 different body weight gains were calculated as follow:

EQUATION 1. Body Weight Gains (g)

1) Body weight gains during induction periods (Day 0 ~ Day 14)

2) Body weight gains after test article treatments periods(Day 14 ~ Day 28)

3) Body weight gains throughout experimental periods (Day 0 ~ Day 28)

5. Measurement of Ankle Circumferences

One hour before the treatments of the test article or reference drugs, the circumference of both hind ankles was measured using the cotton thread method[14] and recorded at FCA treatment day, 14, 21 and 28 days after FCA treatment. The difference between the intact ankle and induced ankle was calculated as follow:

EQUATION 2. Difference in Ankle Circumference (cm)

= Circumference of induced ankle - circumference of intact ankle

6. Measurement of Inflammatory Tissue Prostaglandin E_2 Levels

Prior to sacrifice, all rats were anesthetized with ethyl ether (Duksan Pure Chemical., Korea). Both the left inflammatory feet were removed around ankle and stored in buffer containing normal saline for 60 min. Then the buffer was centrifuged at 3000 rpm for 10 min and 0.1 ml supernatant was carefully transferred to a fresh tube. After adding 2ml 0.5 mol/L KOH (Sigma, USA) - methanol, the mixture was incubated at 50℃or 20 min, and then diluted to 20 ml with methanol. The prostaglandin E_2 level in the mixture was determined with an ultraviolet spectrophotometer (Model 22, Angstrom Advanced Inc., MA, USA) at 278 nm[15] and expressed as ng/paw.

7. Assays for Cartilage Collagen Contents

Collagen was estimated in terms of hydroxyproline in the knee joint (ankle) cartilage of rats. For the estimation of hydroxyproline, the procedure of Neuman and Logan[16-7] with the modification of Leach[18] was followed. The hydroxyproline was calculated and then multiplied by a constant factor 7.46 to convert percentage of hydroxyproline

to percentage of collagen/100 mg cartilage.

8. Assays for Bone Glucosaminoglycan Contents

The glucosaminoglycans were extracted following the method of Takeuchi et al[19]. The levels of glycosaminoglycan components namely heparan sulphate, chondroitin sulphate and hyaluronic acid were measured in joint bones[20] as mg/g defatted tissues. The optical density was measured at 650 nm for hyaluronic acid, 478 nm for heparan sulphate and 480 nm for chondroitin sulphate with an ultraviolet spectrophotometer (Model 22, Angstrom Advanced Inc., MA, USA).

9. Statistical Analyses

Multiple comparison tests for different dose groups were conducted. Variance homogeneity was examined using the Levene test. If the Levene test indicated no significant deviations from variance homogeneity, the obtained data were analyzed by one way ANOVA test followed by least-significant differences (LSD) multi-comparison test to determine which pairs of group comparison were significantly different. In case of significant deviations from variance homogeneity were observed at Levene test, a non-parametric comparison test, Kruskal-Wallis H test was conducted. When a significant difference is observed in the Kruskal-Wallis H test, the Mann-Whitney U-Wilcoxon Rank Sum W test was conducted to determine the specific pairs of group comparison, which are significantly different. In addition, the changes between intact and FCA controls were calculated to detecting the severitiesof subacute hepatic damages induced, and the changes betweenFCA control and test article or reference dosing groups were also calculated to help the understanding of the efficacy of test materials as follows:

EQUATION 3. Percentage Changes Compared with Intact Control (%)

= ((Data of FCA control - Data of intact control)/Data of intact control) × 100

EQUATION 4. Percentage Changes Compared with FCA Control (%)

= ((Data of test article or reference dosing groups - Data of FCA control)/Data of FCA control) × 100

III. RESULTS

1. Changes on the Body Weights and Gains

Changes on the body weight and gains during 3 different periods after FCA and test substance administration were summarized in Table 3 and Fig. 1.

Significant ($p<0.01$) decreases of body weight was detected from 14 days after FCA injection throughout experimental periods and the body weight gains during induction periods of FCA-induced rheumatoid arthritis, test article treatment periods and during experimental periods were also significantly ($p<0.01$) decreased in FCA control compared to that of intact control, respectively. The body weights of dexamethasone-treated rats were significantly ($p<0.01$) decreased from 21 days after FCA treatment (7 days of dexamethasone administration), and body weight gains during test article treatment periods and experimental periods were also significantly ($p<0.01$) decreased as compared with intact or FCA controls, respectively. However, significantly ($p<0.01$ or $p<0.05$) increases on the body weight were detectedin the both dosages of BSS extracts-treated groups compared to that of FCA control

from 21 days after FCA treatment (7 days after BSS extracts treatments), respectively. Consequently, body weight gains during test article treatment periods and experimental periods were also significantly ($p<0.01$) increased compared to that of FCA control, respectively. Body weight gains during induction periods of FCA-induced rheumatoid arthritis in dexamethasone and two different dosages ofBSS extracts treated groups were quite similar to that of FCA control, respectively(Table 3 and Fig. 1).

Table 3. Changes on the Three Different Periods of Body Weight Gains in Intact and FCA-induced Rheumatoid Arthritis Rats

Groups	Body weight gains during:		
	Induction periods of arthritis (14 days)	After treatment of test substances (14 days)	whole experimental periods (28 days)
Controls			
Intact	22.895.71	20.335.27	43.224.89
FCA	-3.333.94*	-19.896.64*	-23.226.63*
Dexamethasone	-3.563.50*	-30.897.64*†	-34.448.55*†
BSS treated as			
100mg/kg	-3.222.64*	4.335.22*†	1.115.46*†
200mg/kg	-2.893.10*	-8.004.12*†	-10.894.76*†

n=9; (Mean S.D., g); FCA, Freund's complete adjuvant; BSS, *Binsosan* aqueous extracts; * $p<0.01$ compared with intact control at each checking periods; † $p<0.01$ and compared with FCA control at each checking periods.

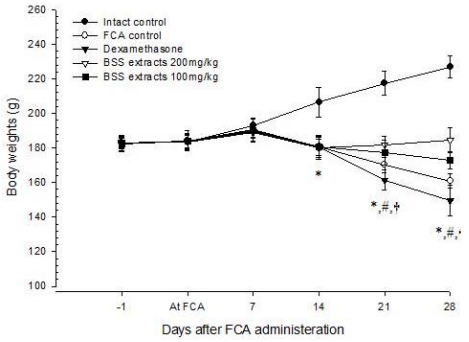

Fig. 1. Body Weight Changes in Intact and FCA-induced Rheumatoid Arthritis Rats

Note that significant(*$p<0.01$) decreases of body weight were detected in all FCA-treated rats as compared with intact control from 14 days after FCA treatment, respectively. However, significant (#$p<0.01$ or $p<0.05$) increases of body weight were detected in 100 and 200mg/kg of BSS extracts administered groups from 21 days after FCA administration (7 days after start of BSS extracts administration), respectively. In case of dexamethasone administered rats, significantly († $p<0.01$) decreases on body weights were detected from 21 days after FCA administration (7 days after start of dexamethasone injection); Mean ± SD of 9 rats, g; FCA, Freund's complete adjuvant; BSS, *Binsosan*.

2. Changes on the Ankle Circumferences

Changes on the ankle circumferences after FCA and test substance administrations were summarized in Fig. 2 ~ 4.

Although no meaningful changes were detected in non-FCA administered ankle circumferences of both different dosages of BSS extracts treated rats, FCA control rats as compared with intact control rats at each checking times, significant ($p<0.01$) decreases on the intact ankle circumferences were detected in dexamethasone treated rats from 7 days after administration as compared with FCA and intact control rats, respectively (Fig. 2).

Significant ($p<0.01$)increases of induced ankle circumferences were detected in FCA control as

compared with intact control from 14 days after FCA treatment. Consequently, the differences between intact and induced ankle circumferences were also significantly ($p<0.01$) increased as compared with intact control from 14 days after FCA treatment. However, these increases on induced ankle circumferences and differences between intact and induced sides were significantly ($p<0.01$) decreased as compared with FCA control from 7 days after dexamethasoneand both BSS extracts treatment, respectively(Fig. 3 and 4).

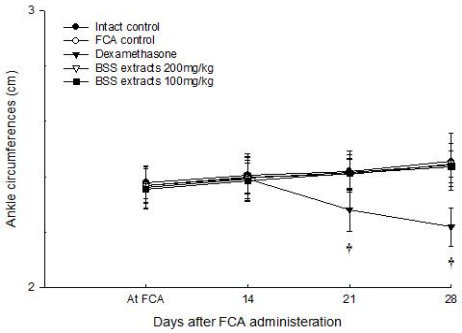

Fig. 2. Changes on Intact Ankle Circumferences on Intact and FCA-induced Rheumatoid Arthritis Rats

Although significantly († $p<0.01$) decreases in intact ankle circumference were detected in dexamethasone treated mice from 7 days after dexamethasone administration, no meaningful changes were detected between intact control and remainder FCA treated groups; Mean ± SD of 9 rats, cm; FCA, Freund's complete adjuvant; BSS, *Binsosan*.

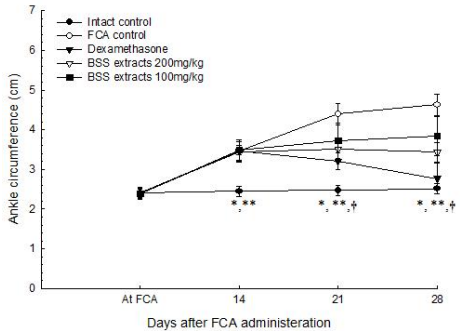

Fig. 3. Changes on Induced Ankle Circumferences on Intact and FCA-induced Rheumatoid Arthritis Rats

Note that significant (*$p<0.01$) increases on induced ankle circumferences were detected in all FCA treated ratsas compared with intact control from 14 days afterFCA treatment throughout experiments except for dexamethasone at a termination (** $p<0.01$ as compared with intact control rats and FCA treated rats except for dexamethasone administrated rats, they were not shown significances). However, they were significantly († $p<0.01$) decreased in dexamethasone and both different dosages of BSS extracts administrated rats from 7 days after administration as compared with FCA control rats, respectively Mean ± SD of 9 rats, cm; FCA, Freund's complete adjuvant; BSS, *Binsosan*.

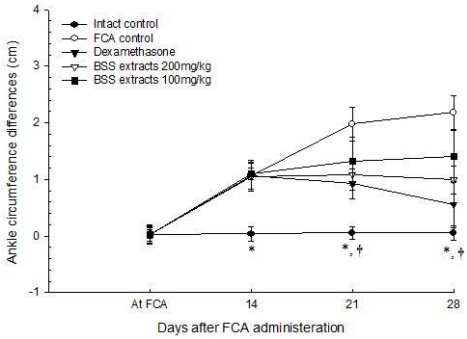

Fig. 4. Changes on the Differences Between Intact and Induced Ankle Circumferences on Intact and FCA-induced Rheumatoid Arthritis Rats

Note that significant (*$p<0.01$) increases on the differences between intact and induced ankle circumferences were detected in all FCA treated rats as compared with intact control from 14 days after FCA treatment throughout experiments. However, they were significantly († $p<0.01$) decreased in dexamethasone and both different dosages of BSS extracts administrated rats from 7 days after administrationas compared with FCA control rats, respectively Mean ± SD of 9 rats, cm; FCA, Freund's complete adjuvant; BSS, *Binsosan*.

3. Changes on the Inflammatory Tissue Prostaglandin E_2 Levels

Changes on the inflammatory tissue prostaglandin

E_2 contents after FCA and test substance administrations were summarized in Fig. 5. In the present study, inflammatory tissue prostaglandin E_2 contents were expressed as ng/ paw.

Significant ($p<0.01$) increases on the tissue prostaglandin E_2 contents were detected in FCA control compared to that of intact control at sacrifice. However, the increases of tissue prostaglandin E_2contents were significantly ($p<0.01$ or $p<0.05$) inhibited by the treatments of dexamethasone and both different dosages of BSS extracts. In BSS extracts treated groups, dose-dependent inhibitions on the FCA-induced rheumatoid arthritis related tissue prostaglandin E_2content elevations in the present study.

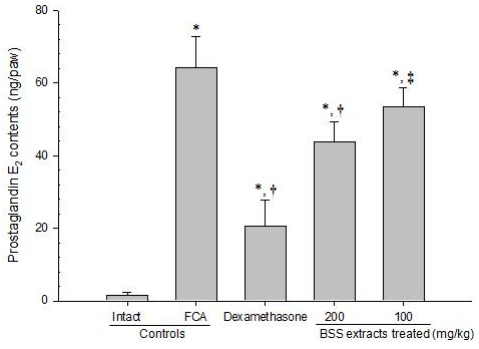

Fig. 5. Changes on the Inflammatory Tissue Prostaglandin E_2 Contents in Intact and FCA-induced Rheumatoid Arthritis Rats

Note that significant (*$p<0.01$) increases on the inflammatory tissue prostaglandin E_2 contents were detected in all FCA treated ratsas compared with intact control at a termination. However, they were significantly († $p<0.01$) decreased in dexamethasoneand 200mg/kg of BSS extracts administrated rats at a termination as compared with FCA control rats, respectively. In 100mg/kg of BSS extracts administered rats also showed significantly (‡ $p<0.05$) decreased inflammatory tissue prostaglandin E_2 contentsas compared with FCA control rats; Mean ±SD of 9 rats, ng/paw; FCA, Freund's complete adjuvant; BSS, *Binsosan*.

4. Changes on the Cartilage Collagen Levels

Changes on the cartilage collagen contents after FCA and test substance administrations were summarized in Fig. 6. In the present study, collagen contents were expressed as hydroxyproline mg/100mg cartilages.

Significant ($p<0.01$) decreases on the cartilage collagen contents were detected in FCA control compared to that of intact control at sacrifice. However, the decreases on the cartilagecollagen contents were significantly ($p<0.01$) inhibited by the treatments of dexamethasone and both different dosages of BSS extracts. In BSS extracts treated groups, dose-dependent inhibitions on the FCA-induced rheumatoid arthritis related cartilage collagen content decrease in the present study.

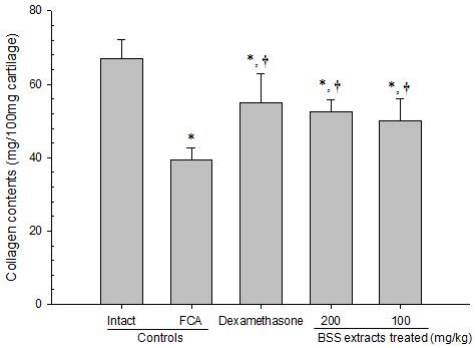

Fig. 6. Changes on the Cartilage Collagen Contents in Intact and FCA-induced Rheumatoid Arthritis Rats

Note that significant (*$p<0.01$) decreases on the cartilage collagen contents were detected in all FCA treated rats as compared with intact control at a termination. However, they were significantly († $p<0.01$) increased in dexamethasone and both two different dosages of BSS extracts administrated rats at a termination as compared with FCA control rats, respectively Mean ± SD of 9 rats, hydroxyproline mg/100mg cartilage; FCA, Freund's complete adjuvant; BSS, *Binsosan*.

5. Changes on the Bone Chondroitin Sulphate Contents

Changes on the bone chondroitin sulphate contents after FCA and test substance administrations were summarized in Fig. 7. In the present study, bone chondroitin sulphate contents were expressed as mg/g of defatted tissues.

Significantly ($p<0.01$) decreases on the bone chondroitin sulphate contents were detected in FCA control compared to that of intact control at sacrifice. However, the decreases on the bone chondroitin sulphate contents were significantly ($p<0.01$) inhibited by the treatments of dexamethasone and both different dosages of BSS extracts. In BSS extracts treated groups, dose-dependent inhibitions on the FCA-induced rheumatoid arthritis related bone chondroitin sulphate content decreases in the present study.

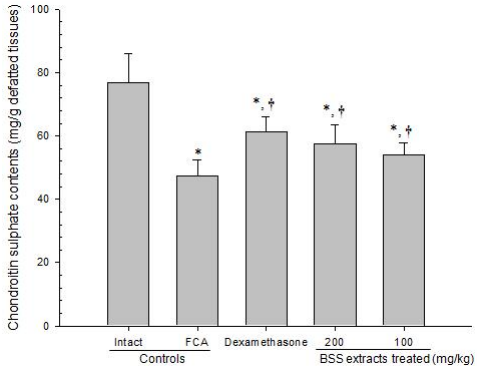

Fig. 7. Changes on the Bone Chondroitin Sulphate Contents in Intact and FCA-induced Rheumatoid Arthritis Rats

Note that significant (*$p<0.01$) decreases on the bone chondroitin sulphate contents were detected in all FCA treated rats as compared with intact control at a termination. However, they were significantly († $p<0.01$) increased in dexamethasone and both two different dosages of BSS extracts administrated rats at a termination as compared with FCA control rats, respectively Mean ±SD of 9 rats, mg/g defatted tissues; FCA, Freund's complete adjuvant; BSS, *Binsosan*.

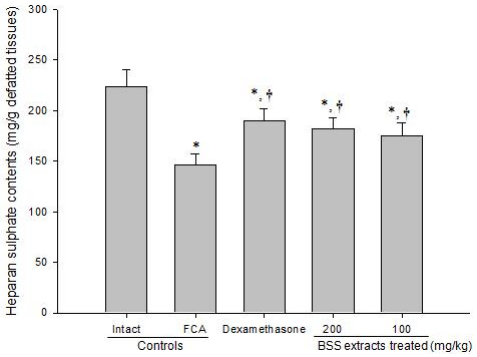

Fig. 8. Changes on the Bone Heparan Sulphate Contents in Intact and FCA-induced Rheumatoid Arthritis Rats

Note that significant (*$p<0.01$) decreases on the bone heparan sulphate contents were detected in all FCA treated rats as compared with intact control at a termination. However, they were significantly († $p<0.01$) increased in dexamethasone and both two different dosages of BSS extracts administrated rats at a termination as compared with FCA control rats, respectively Mean ± SD of 9 rats, mg/g defatted tissues; FCA, Freund's complete adjuvant; BSS, *Binsosan*.

6. Changes on the Bone Heparan Sulphate Contents

Changes on the bone heparan sulphate contents after FCA and test substance administrations were summarized in Fig. 8. In the present study, bone heparan sulphate contents were expressed as mg/g of defatted tissues.

Significantly ($p<0.01$) decreases on the bone heparan sulphate contents were detected in FCA control compared to that of intact control at sacrifice. However, the decreases on the bone heparan sulphate contentswere significantly ($p<0.01$) inhibited by the treatments of dexamethasone and both different dosages of BSS extracts. In BSS extracts treated groups, dose-dependent inhibitions on the FCA-induced rheumatoid arthritis related bone heparan sulphate content decreases in the present study.

7. Changes on the Bone Hyaluronic Acid Contents

Changes on the bone hyaluronic acid contents after FCA and test substance administrations were summarized in Fig. 9. In the present study, bone hyaluronic acid contents were expressed as mg/g of defatted tissues.

Significantly ($p<0.01$) decreases on the bone hyaluronic acid contents were detected in FCA control compared to that of intact control at sacrifice. However, the decreases on the bone hyaluronic acid contents were significantly ($p<0.01$) inhibited by the treatments of dexamethasone and both different dosages of BSS extracts. In BSS extracts treated groups, dose-dependent inhibitions on the FCA-induced rheumatoid arthritis related bone hyaluronic acid content decreases in the present study.

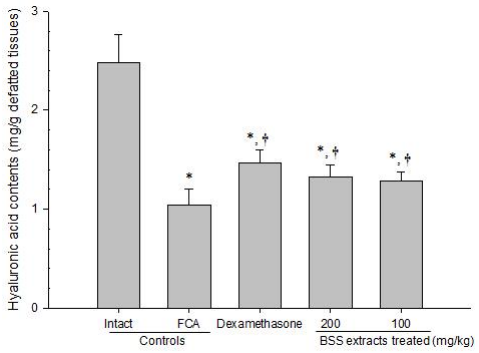

Fig. 9. Changes on the Bone Hyaluronic Acid Contents in Intact and FCA-induced Rheumatoid Arthritis Rats

Note that significant (*$p<0.01$) decreases on the bone hyaluronic acid contents were detected in all FCA treated rats as compared with intact control at a termination. However, they were significantly († $p<0.01$) increased in dexamethasone and both two different dosages of BSS extracts administrated rats at a termination as compared with FCA control rats, respectively Mean ± SD of 9 rats, mg/g defatted tissues; FCA, Freund's complete adjuvant; BSS, *Binsosan*.

Ⅳ. DISCUSSION

Traditional herbal medicine, *Binsosan* (BSS) consisted of 11 kinds of medicinal herbs. In this study, the prescription of BSS is according to 'Donguibogam(東醫寶鑑)[13]' of Heo Jun and based on the book 'Uihak-ipmun(醫學入門)[21]' of Li Chan. Accordingly it has been usedin the treatment of lower limb diseases induced by wind and humidity[13]. In addition, Cyperi Rhizoma[22], Perilla Folium[23-4], Fraxini Cortex[25], Chaenomelis Fructus[26-7], Ostericii Rhizoma[28-30], Achyranthis Bidentatae Radix[31], Glycyrrhizae Radix[32] and Zingiberis Rhizoma Recen[33] have been shown anti-inflammatory activities. BSS have been shown analgesic, sedative, antipyretic and anti-inflammatory actions[34]. We, therefore, hypothesized that BSS will be showed favorable effects against adjuvant-induced rheumatoid arthritis.

In the present study, the effects of BSS were evaluated on the FCA-induced rheumatoidarthritis rats at 100 and 200mg/kg levels comparing with intraperitoneal treatment of dexamethasone 15mg/kg as reference drug.

Rheumatoid arthritis wasinduced by intradermal injection of FCA (10mg in 1ml paraffin oil 0.1ml/rats). Each of 9 rats showing regular ankle circumferences per group was selected after 14 days after FCA treatment to confirm the induction of rheumatoid arthritis, and 100 or 200mg/kg of BSS extracts were orally dosed once a day for 14 days from 14 days after FCA treatments. Dexamethasonewas intraperitoneally administered 15mg/kg, once a day for 14 days from 14 days after FCA treatments. Experimental animals were sacrificed after 14 days of continuous

oral administration of BSS or intraperitoneal administration of dexamethasone, and changes on the body weight, ankle circumferences, inflammatory tissue prostaglandin E_2 levels and cartilage collagen, glucosaminoglycans compositions - chondroitin sulphate, heparinsulphate and hyaluronic acid in the present study.

As results of FCA-injection caused classic rheumatoid arthritisin injected side paws, featuring decreases on the body weights, cartilage collagen contents and bone glucosaminoglycans - chondroitin sulphate, heparansulphate and hyaluronic acid contents, with increases on the ankle circumferences and inflammatory tissue prostaglandin E_2 levels. However, these changes from FCA-induced rheumatoid arthritis were clearly reduced by treatment of dexamethasone and both two different dosages of BSS extracts. In BSS extract treated groups, clear dose-dependent inhibitions were detected as compared with FCA control, respectively.

In the present study, decreases on the body weight changes and three different periodsof body weight gains were detected in FCA treated rats as compared with intact control rats similar to that of previous study[1]. The inhibition of adjuvant-induced rheumatoid arthritis related body weight decreases by treatment of BSS extracts detected in the present study are considered as indirect evidence that BSS extracts can be ameliorated the adjuvant-induced rheumatoid arthritis. Dexamethasone is well-known glucocorticoid, andhas been showed potent anti-inflammatory effects[8,9]. However, it has been showed various systemic side effects including body weight loss[35], and the significant body weight decreases were also detected in the present study as compared with intact and FCA control from 7 days after dexamethasone treatment.

The ankle circumferences injected with adjuvant has been widely used for studying the extent of the disease. They reflected the edematous changes in adjuvant-induced rheumatoid arthritis. Inhibition of paw edema in adjuvant-induced rheumatoid arthritic rats is a hallmark for anti-inflammatory drug action[1,12].In the present study, BSS extracts dose-dependently inhibits the increased ankle circumferences induced by FCA treatments and these are direct evidences that BSS has anti-inflammatory activities.

Prostaglandin $E_2$is generally regarded as an inhibitory or regulatory prostanoid, with ability to modulate both bronchoconstriction and airway inflammation[36]. Prostaglandin E_2 is an inflammatory mediator and is the major product of the enzymatic activity of cyclic oxidase isozymes (COX_2), which can induce normal tissue to produce inflammatory medium that will promote synthetic prostaglandin and damage body tissues[37]. Our study showed that BSS extracts were effective for decreasing the prostaglandin E_2 level. Since increasing prostaglandin E_2can cause inflammatory reactions, such as local congestion, edema, and pain in rheumatoid arthritis[36,38], the significant decrease of prostaglandin E_2 in rheumatoid rats may be due to inhibition of COX_2 activity following the administration of BSS extracts. The ability of BSS extracts to inhibit the levels of prostaglandin E_2 along with its anti-edematous activity suggest that BSS might be potentially useful in counteracting various inflammations including rheumatoid arthritis.

During the process of inflammation, the hydrolytic enzymes like glycohydrolases were released from

invading macrophages, neutrophils and from tissue cells such as synoviocytes and chondrocytes. Enhanced activities of sialyltransferase and galactosyltransferase in serum and liver of inflamed rats were demonstrated. Since the inflammatory process of adjuvant arthritis is a systemic disease and causes alterations in the metabolism of connective tissue macromolecular components involving many organs, the changes occurring in the metabolism of connective tissue macromolecules such as glycoproteins, proteoglycans, glycosaminoglycans and collagen in arthritic disease are of considerable importance from the point of view of the incidence of rheumatoid arthritis[4].

Among the connective tissue proteins, collagen is most affected in adjuvant arthritis. The development of arthritis is accompanied by marked changes in metabolic turnover of collagen. The changes in collagen metabolism manifest the systemic damage produced by adjuvant arthritis. Lymphocyte and monocyte generate interleukin-1 β in the synovium, which together with monocyte derived tumour necrosis factor α stimulate the release of collagenase and prostaglandin E_2 that degrade collagen[39]. In RA, the damage to connective tissue and the consequent severity of the lesions are the direct consequences of sustained hydrolase production and release from macrophages[40]. Activation of macrophages is associated with increased release of a variety of proteolytic and lysosomal enzymes including collagenase, gelatinase, elastase acid phosphatase and cathepsin-D, which can directly act on cellular membrane causing damage[41]. As a result of pathological destruction of collagen in bone and cartilage crosslinks, mature collagen isresorbed more rapidly. This causes a rise in their excretion. In rheumatoid arthritis, apart from the crosslink resorption at the site of inflamed joints, there may be increased resorption due to general bone loss associated with disease activity[42]. In the present study, the cartilage collagen contents were markedlydecreased as progress of FCA-induced rheumatoid arthritis. The dose-dependent inhibitions on the decreases of cartilage collagen contents induced by FCA treatments by treatmentof BSS extracts detectedin the present study are considered as direct evidences that BSS has anti-rheumatoid activities.

It has been well established that the initial breakdown of the proteoglycans by lysosomal glycohydrolases such as β-glucuronidase and β-N-acetyl glucosaminidase cause the opening up of collagen fibres for the attack by collagenolytic enzymes[43]. The extracellular matrix of cartilage contains several proteoglycans such as chondroitin sulphate, heparin sulphate, keratin sulphate and dermatan sulphate[44]. In arthritic condition, chondroitin sulphate and heparan sulphate have been detected in the blood in significant amount and are also prominent in many non-collagenous connective tissues. These results further confirm the cartilage degradation during the course of arthritis[45].

Hyaluronic acid is a high molecular weight polysaccharide produced in the joint primarily by type B synoviocytes. The synthesis of hyaluronic acid is increased by joint inflammation. Thus, increased hyaluronic acid production in the inflamed joint leads to spill over of hyaluronic acid into circulation. In adjuvant arthritis, serum hyaluronic acid was found to correlate with degree of synovial involvement. Studies have suggested that the level of serum hyaluronic acid

correlates with joint inflammation and may predict future joint destruction[46].As course of FCA-induced rheumatoid arthritis, bone glycosaminoglycans - chondroitin sulphate, heparin sulpate and hyaluronic acid were markedly decreased. The dose-dependent inhibitions on the decreases of bone glycosaminoglycans contents induced by FCA treatments by treatmentof BSS extracts detectedin the present study are considered as direct evidences that BSS inhibited the deconstruction of bony tissues.

Base on the results, it is, thus, concluded that BSS extracts showed clear therapeutic effects on the FCA-induced rheumatoid arthritis. The anti-arthritic activities of BSS extracts may be mediated by the prostaglandin E_2 and consequently, the edematous changes and deconstruction of joints like, collagen and glycosaminoglycans were inhibited in the present study. Hence, it needs further development for exploit as a therapeutic agent. Multiple mechanisms may interplay in its anti-inflammatory effectsand further research on the mechanism of action of BSS is underway.

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