Inhibitory Effect of *Coicis Semen* Composition on Inflammatory Responses in the Collagen-induced Arthritis Mouse Model

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This study was performed to investigate possible mechanisms underlying possible effect of *Coicis Semen* composition (CSC) on inflammatory diseases using in vivo model of RA in the mice. Results are summarized as follows. In production of inflammatory cytokines, INF-γ in the spleen and IL-6 in the serum were decreased by CSC treatment. TNF-α in serum was significantly decreased, IL-4 in the spleen was significantly increased by CSC treatment. In production of rheumatoid factors, IgM and IgG were significantly decreased by CSC treatment. The present data suggest that CSC treatment can improve pathological damage by CIA. So we expect that CSC should be used as a effective drugs for not only rheumatoid arthritis but also another autoimmune disease. Therefore we have to survey continuously in looking for the effective substance and mechanism in the future.

Key words : *Coicis Semen* composition, Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic multisystemic diseases showing the characteristic feature of persistent inflammatory synovitis, usually in the peripheral joints. Major symptoms include joint swelling, pain and stiffness, weakness, deformity, malaise and others. RA affects about 1% of the population, in female to male ratio of 2.5:1[1].

Herbal medication has recently attracted RA patients in Western as well as Asian societies. Studies of herbal medication on efficacy and toxicity are growing, and their studies involve inflammatory cytokines IL-1β, TNF-α[2].

Here in the present study, a herbal prescription *Coicis Semen* composition (every abbreviation from now on CSC) was examined whether it has any attenuating effects on RA pathological responses in collagen-induced arthritis model in the mice[3]. CSC is composd several herbal drugs (see Table 1). *Coicis Semen* promotes diuresis to eliminate wetness-evil and the other drugs effects anti-inflammatory diseases. Determination of levels of inflammatory cytokines in collagen-induced RA model showed significant changes in cytokines and improvement of inflammatory features in RA tissues by CSC administration.

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Materails and Methods

1. Materials

1) Animals

DBA/1J mice (6 week old) obtained from Charles River Co. (Japan), were used in the present study. The animals were maintained in a conventional system at 12 hr of day light (200~300 Lux) and 12 hr of dark condition in 22±2℃. The animals were fed with food pellets and water, and adjusted at least 2 weeks before the experiment.

2) Drugs

The CSC used in this study was purchased from Daejeon University Oriental medicinal hospital and the composition of a pack is as follows.

Table 1. Prescription of *Coicis Semen* composition (CSC)

Korean Name	Herbal nomenclatures	Amount (g)
Euiin (薏苡仁)	*Coicis Semen*	20
Sanyak (山藥)	*Dioscoreae Rhizoma*	4
Haingin (杏仁)	*Armeniacae amarum Semen*	4
Gobon (藁本)	*Ligustici Rhizoma*	4
Mahwang (麻黃)	*Ephedrae Herba*	4
Nabokja (蘿蔔子)	*Oryzae Fructus germinatus*	4
Gamcho (甘草)	*Glycyrrhizae Radix*	4
Ganghwal (羌活)	*Notopterygii Rhizoma*	2
Gilgyung (桔梗)	*Platycodi Radix*	2
Changchul (蒼朮)	*Atractylodis Rhizoma*	2
Maikmundong (麥門冬)	*Liripis Tuber*	4
Omija (五味子)	*Schizandrae Fructus*	2
Total amount		58 g

2. Methods

1) Drug extraction

Three packs of dried CSC were dissolved in 2,000㎖ distilled water, extracted for 3 hr. After filtration using the rotary evaporator (Büchi B-480, Switzerland), the purified powder 21.4g was obtained by using the freeze dryer (EYELA FDU-540, Japan), and kept at -84℃ until use. The powder obtained was diluted with proper concentration before use.

2) Drug administration

DBA/1J mice were divided into normal animal groups (Wild type-every abbreviation from now on WT), Collagen-induced arthritis (every abbreviation from now on CIA) in the mice is control group (every abbreviation from now on CT), a group CIA-CT with MTX administration (0.3㎎/㎏) is positive control(every abbreviation from now on MTX), and a group CIA-CT with CSC administration (200 ㎎/㎏, 400 ㎎/㎏) is experimental group. There were six animals in each group and 0.1 ㎖ of saline were daily injected into Wild type and Control groups, 0.1 ㎖ of CSC into the CSC group.

3) Generation of Rheumatoid arthritis mouse model

DBA/1J mice were divided into WT, CT, MTX and a group with CSC administration(200 ㎎/㎏, 400 ㎎/㎏). CT, MTX and CSC groups were subcutaneous injected into the dorsal portion of the tail with 100 ㎍ of bovine type II collagen and 0.1㎖ complete Freund's adjuvants, and induced boosting responses by injecting the same dose at 21 days later.

4) Analysis of cytokines and rheumatoid factors (RF)

Serum concentrations of IL-4, IL-6, IFN-γ, TNF-α and rheumatoid factors (RF) IgG3, IgG2b and IgM were measured by enzyme-linked immuno-sorbent assay (ELISA, Endogen, USA). 100 ㎕ of serum (1:100 dilution) was dispensed into each well, and reacted for 1 hr at room temperature. After washing twice with buffer, cells were treated with 100 ㎕ of HRP-conjugated avidin antibody for 1 hr, added 100 ㎕ of TMB substrate and incubated in the dark for 30 min. Then, the reaction was stopped with 50 ㎕ of the stop solution treatment, and used for spectrophotometric measurement of protein concentration at 450 nm.

5) Data analysis

The results are presented as the mean±standard error mean(SEM). Statistical analysis was performed by Student's T-test ($p<0.05$).

Results

1. Effects of CSC on the cytokine production

1) INF-γ and IL-4 production in the spleen

Levels of INF-γ were 1432.0±44.7(pg/㎖) and 1021.0±32.5 (pg/㎖) with 200 ㎎/㎏ and 400 ㎎/㎏ respectively, indicating significant decreases by CSC treatment in a dose-dependent manner. Levels of IL-4 were 59±9.5(pg/㎖) and 75±7.6(pg/㎖) with 200 ㎎/㎏ and 400 ㎎/㎏ respectively, indicating increases by CSC treatment in a dose-dependent manner(Table 2).

Table 2. The Effects of CSC on the Production of INF-γ and IL-4 in Spleen of CIA Mice

Group	INF-γ (pg/ml)	IL-4 (pg/ml)
WT	864.0±10.5	48±5.4
CIA-CT	765.0±43.5	31±3.8
MTX	690.0±9.8	44±4.6
CSC 400 ㎎/㎏	1021.0±32.5***	75±7.6***
CSC 200 ㎎/㎏	1432.0±44.7***	59±9.5**

: Statistically significant value compared with CIA-CT data by T test (**p<0.01, ***p<0.001).

2) TNF-α and IL-6 production in the serum

Levels of TNF-α were 121.1±12.1(pg/㎖) and 100.6±9.8 (pg/㎖) with 200 ㎎/㎏ and 400 ㎎/㎏ respectively, indicating significant decreases by CSC treatment in a dose-dependent manner. Levels of IL-6 were 86.4±10.5(pg/㎖) and 88.5±11.0 (pg/㎖) with 200 ㎎/㎏ and 400 ㎎/㎏ respectively, indicating some decreases by CSC treatment(Table 3).

Table 3. The effects of CSC on the production of TNF-α and IL-6 in the serum of CIA Mice

Group	TNF-α (pg/ml)	IL-6 (pg/ml)
WT	60.3±8.9	12.6±4.5
CIA-CT	175.2±26.7	110.2±17.6
MTX	77.9±11.2	58.3±7.7
CSC 400 ㎎/㎏	100.6±9.8**	88.5±11.0
CSC 200 ㎎/㎏	121.1±12.1*	86.4±10.5

: Statistically significant value compared with CIA-CT data by T test (*p<0.05, **p<0.01).

2. Effects of CSC on rheumatoid factor production

1) IgM rheumatoid factor

Levels of IgM rheumatoid factor were 58.2±4.8 (pg/㎖) and 49.7±7.6 (pg/㎖) with 200 ㎎/㎏ and 400 ㎎/㎏ respectively, indicating significant decreases in a dose-dependent manner compared with CIA-CT(Table 4).

Table 4. The effects of CSC on total IgM rheumatoid factor level in serum of CIA mice

	Group	Product(pg/ml)
IgM level	WT	7.2±1.8
	CIA-CT	78.8±8.4
	MTX	32.5±6.7
	CSC 400 ㎎/㎏	49.7±7.6**
	CSC 200 ㎎/㎏	58.2±4.8*

: Statistically significant value compared with CIA-CT data by T test (*p<0.05, **p<0.01).

2) IgG rheumatoid factor

Levels of IgG rheumatoid factor were 38.7±8.3(pg/㎖) and 36.5±4.8 (pg/㎖) with 200 ㎎/㎏ and 400 ㎎/㎏

respectively, indicating significant decreases compared with CIA-CT(Table 5).

Table 5. The Effects of CSC on Total IgG Rheumatoid Factor Level in Serum of CIA Mice

	Group	Product(pg/ml)
IgG level in serum	WT	5.4±1.2
	CIA-CT	55.9±6.9
	MTX	18.6±3.4
	CSC 400 mg/kg	36.5±4.8*
	CSC 200 mg/kg	38.7±8.3*

: Statistically significant value compared with CIA-CT data by T test (*p<0.05).

Discussion

Rheumatoid arthritis(every abbreviation from now on RA) is a chronic multisystemic disease with a variety of systemic manifestation. RA appears to develop from a deregulated immune response leading to progressive synovial inflammation and joint destruction[4].

RA appears to develop from a deregulated immune response leading to progressive synovial inflammation and joint destruction. Here, precipitating factors for the development of RA have not been elucidated fully, though T-cell involvement has been strongly implicated[5].

Cytokines, such as interleukin-1 and tumor necrosis factor-α[6-8], and, recently reported, activation-induced, T cell-derived, chemokine-related cytokine/lymphotactin[9] and macrophage migration inhibitory factor[10] have been found in high levels in RA patients. These endogenous compounds stimulate synovial tissue effector functions, including proliferation, metalloproteinase expression, adhesion-molecular expression, secretion of other cytokines, and prostaglandin production, all of which may have a role in RA pathogenesis[3,11]. Matrix metalloproteinases cause cartilage and bone degradation by means of extracellular matrix remodeling and degradation. Aberrant adhesion molecule expression leads to uncontrolled binding of T cells to synovial type B cells, resulting in excess release of matrix metalloproteinases. RF is a group of autoantibodies (IgM, IgG, and IgA) that recognizes the Fc portion of IgG. It is known that normally RF aids in the clearing of immune complexes, the processing of antigens by B cells, and the development of an early antibody repertoire[12], and then increases in RF production commonly are seen prior to the clinical onset of RA factors[4].

In oriental medicine, CSC has been broadly used for the treatment of several inflammatory diseases, and the present data strengthen and provide insight into understanding possible molecular mechanism. Since there are several sites of actions of CSC on the RA tissues, mechanistic inference on how CSC exerted anti-inflammatory and anti-immune activity is not possible. CSC used in the current study are the extracts composed of 12 different herbal drugs. Informations on a certain active ingredients from these drugs are not available, emphasizing the importance of chemical analysis for active component identification.

In the present study, possible effects of CSC on inflammatory responses was investigated in vivo using CIA mice model. increases in inflammatory cytokines such as IFN-γ, serum TNF-α, IL-6 and serum IgM and IgG rheumatoid factors of RA provide an evidence that CIA model was properly established in this study[13-16].

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Then the treatment of CSC with dosages of 200 mg/kg and 400 mg/kg significantly changed immune cell population, cytokines in most cases.

In this study, Production of inflammatory cytokines in the spleen INF-γ, and serum TNF-α and IL-6 were decreased in CSC-treated animal group compared with CIA negative control group. Production levels of IL-4 in the spleen were increased by CSC treatment.

Treatment of CSC at 200 or 400 mg/kg significantly decreased these cytokines, suggesting possible anti-inflammatory function of CSC in vivo. Production of rheumatoid factors IgG and IgM were decreased in CSC-treated animals compared with CIA control animals. In most of the cases, levels of inflammatory cytokines in CSC-treated animals were close to those of MTX-treated positive control.

It was recently shown that CSC treatment in FLS cells isolated from synovial tissues similar effective in alleviating inflammatory responses in relation to RA. Together with current data, CSC may be clinically relevant for the treatment of RA. The present data suggest that the possible function of CSC might alleviate inflammatory responses by regulating the production of inflammatory cytokines. It should however be noted that actions of CSC in relation to the regulation of CIA are multifunctional; total and T cell population as well as several key cytokine levels. As mentioned above, studies using activated FLS cells down-regulated inflammatory and immune responses as similarly observed in the present, suggesting potential role of anti-inflammatory and anti-immune function of CSC in activated inflammatory cells such as synoviocytes. Final important observation from the current study was the recovery of activated cells in the inflammatory tissues. Further long-term study would be useful for determining functional

recovery in cells or tissues in the affected area.

Taken together, the present findings strongly suggest that oriental medicinal drug CSC might have attenuating effect on the inflammatory progression in several tissues undergoing RA. Further investigation is required to confirm present results in other experimental animal models to further understand underlying molecular mechanisms.

Conclusion

The present study was performed to investigate possible mechanisms underlying possible effect of *Coicis Semen* composition (CSC) on inflammatory diseases using in vivo model of RA in the mice. Results are summarized as follows.

In production of inflammatory cytokines, INF-γ in the spleen and IL-6 in the serum were decreased by CSC treatment. TNF-α in serum was significantly decreased, IL-4 in the spleen was significantly increased by CSC treatment. In production of rheumatoid factors, IgM and IgG were significantly decreased by CSC treatment.

The present data suggest that CSC treatment can improve pathological damage by CIA. So we expect that CSC should be used as a effective drugs for not only rheumatoid arthritis but also another autoimmune disease. Therefore we have to survey continuously in looking for the effective substance and mechanism in the future.

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