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Immunomodulatory effect of *Juglans sinensis*, *Psoralea corylifolia*, Cheong-a-hwan extract and cyclosporine A on Th1(IFN-γ)/Th2(IL-4) cytokine balance, eosinophil accumulation in a murine model of asthma

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Abstract

The immune balance controlled by T helper 1 (Th1) and T helper 2 (Th2) is crucial for immunoregulation and its imbalance causes various immune diseases including allergic disorders and asthma. It has been recently proposed that asthma may result from an imbalance between T helper 1 (Th1) and T helper type 2 (Th2) cells. *Juglans sinensis* Dode (family, Juglandaceae), *Psoralea corylifolia* Linn. (family, Fabaceae), and Cheong-a-hwan (herbal prescription composed of *J. sinensis*, *P. corylifolia*), which has been used widely in Korean traditional medicine, has been shown to exhibit various biological activities but its immunoregulatory activities have not been well studied. We investigated these activities of *J. sinensis*, *P. corylifolia* and Cheong-a-hwan on Th1/Th2 cytokine production using an ovalbumin-induced asthma animal model.

Our results have shown that *J. sinensis*, *P. corylifolia* and Cheong-a-hwan and CsA have profound inhibitory effects on the accumulation of eosinophills into airways and blood. Also, they upregulated the production of OVA-specific Th1 cytokine (IFN-γ) and downregulated OVA-specific Th2 cytokine (IL-4) in culture supernatant of spleen cells. These results indicate that *J. sinensis*, *P. corylifolia* and Cheong-a-hwan extracts may be a potential novel therapeutic agent for asthma by modulating the relationship between Th1/Th2 cytokine balance.

Keywords: IL-4; IFN-γ; Eosinophil; Asthma; *Juglans sinensis*; *Psoralea corylifolia*

1. Introduction

Bronchial asthma results from excess production of T helper 2 (Th2) cytokines (IL-4, IL-5, IL-13) relative to T helper 1 (Th1) cytokine (IFN-γ) (Factor, 2003). Th1 cells are characterized by the prevalent production of IL-2, IFN-γ, and TNF-β, without IL-4, IL-5, IL-9, and IL-13 production. By contrast, Th2 cells are characterized by the prevalent production of IL-4, IL-5, IL-9, and IL-13 in the absence of IFN-γ. Eosinophils have a crucial role in the pathogenesis of allergic diseases. Clinical and experimental studies have established eosinophilia as a marked sign of allergic disorders including asthma (Rothenberg, 1998). Th2 cells and their secreted products initiate a cascade of events that begins with the recruitment and activation of eosinophils, macrophages, and other inflammatory cells. The recruited eosinophils produce IL-3, IL-5, IL-6, IL-13, granulocyte-monocyte-cell stimulating factor (GM-CSF), tumour necrosis factor (TNF), major basic protein, and eosinophil cationic protein (Epstein, 2006).

IL-4 can directly induce airway hyperresponsiveness, airway and blood eosinophilia in asthmatic patients (Shi et al., 1998). The role of Th1 cytokine IFN-γ in asthma is still a matter of debate: in an earlier study Krug and coworkers described an increased frequency of IFN-γ + T cells in bronchoalveolar lavage fluid from asthmatic compared with control subjects (Krug et al., 1996), and other investigators have shown an inhibitory effect of IFN-γ on pulmonary allergic responses. IFN-γ downregulated the secretion of certain Th2 cytokines. The local administration of aerosolized recombinant IFN-γ in mice prevented antigen-induced eosinophil infiltration into the trachea and normalized airway function (Nakajima, Iwamoto, & Yoshida, 1993).

In our preliminary study, several medicinal herbs have shown to promote immunity in different ways. *Juglans sinensis*

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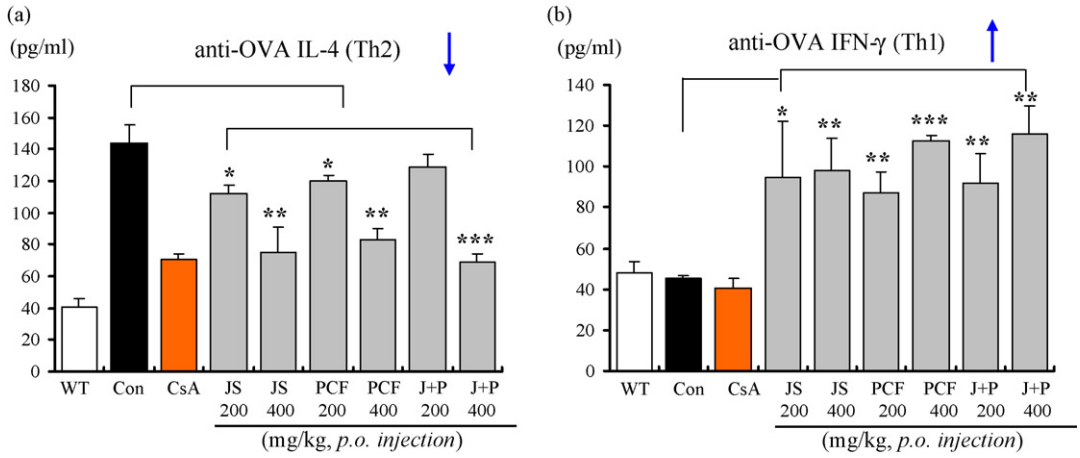

Fig. 1. Immunomodulatory effects of JS, PCF, CAH on OVA-specific Th1/Th2 cytokines production in spleen cells. After 48 h of culture, supernatant from the splenocytes of JS, PCF, CAH fed mice were analyzed the levels of IL-4 and IFN-γ in each samples (described in Section 3). WT: normal C57BL/6 mice; Con: ovalbumin inhalation (control group); CsA: OVA + CsA (10 mg/kg); JS: OVA + JS (200, 400 mg/kg); PCF: OVA + PCF (200, 400 mg/kg); CAH (JS + PCF): OVA + CAH (200, 400 mg/kg). The results are expressed the mean ± S.E. ($N = 5$). Statistically significant value compared with control group data by *t*-test ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

Dode (family, Juglandaceae), *Psoralea corylifolia* Linn. (family, Fabaceae), and Cheong-a-hwan (Cheong-a-hwan; herbal prescription composed of *J. sinensis*, *P. corylifolia* by a ratio of 50:50 based on Korean traditional medicinal formulae) are medicinal herbs and prescription that have been used for treating airway inflammatory deseases (including asthma) in Korea.

They have shown to augment specific cellular and humoral immune response. *P. corylifolia* Linn. has been used traditionally as medicine in China and recommended for the treatment of stomachic, deobstruent, anthelmintic, diuretic, vitiligo and also certain skin diseases, e.g., leucoderma, psoriasis and leprosy (Kotiyal & Sharma, 1992; Zhu, 1998). *P. corylifolia* seed extract has been found to stimulate the immune system in mice (Latha, Evans, Panikkar, & Jayavardhanan, 2000). *J. sinensis* exerts the beneficial effect against mercury chloride-induced acute renal failure and its effect may be due to antioxidant action (Ahn, Song, Kim, & Kim, 2002).

Few reports, however, have addressed the immunoregulatory effects of Cheong-a-hwan, *P. corylifolia* Linn. and *J. sinensis* on Th1/Th2 cytokine balance. Therefore, the aim of this study is to evaluate the immunomodulatory effect of Cheong-a-hwan, *P. corylifolia* Linn. and *J. sinensis* and to understand the effects of eosinophil migration.

2. Results and discussion

2.1. Immunomodulation of OVA-specific Th1/Th2 cytokines production in spleen cells

Spleen cells were isolated from experimental murine model of asthma (in Section 3) and stimulated in the absence JS, PCF, CAH and CsA.

To study whether JS, PCF, CAH were related to Th1/Th2 cytokine balance, after 48 h of culture, supernatant from the splenocytes of JS, PCF, CAH fed mice were analyzed the levels of IL-4 and IFN-γ in each samples.

As shown in Fig. 1, IL-4 productions in spleen were suppressed by JS, PCF, CAH dose dependently. On the contrary, JS, PCF, CAH dose dependently enhanced the secretion of IFN-γ levels.

2.2. Inhibitory effects of JS, PCF, CAH and CsA on eosinophil accumulation in BALF, blood

JS, PCF, CAH and CsA also attenuated the decrease of eosinophils in BALF induced by repeated i.n. administration of allergen (OVA inhalation 500 μg/ml; on two or three consecutive days). The number of eosinophils was significantly different among these three groups. In OVA-exposed group, the number of eosinophils was significantly increased, but in JS, PCF, CAH treated group, the number of it were significantly decreased in comparison with the OVA-exposed group (Figs. 2 and 3).

JS, PCF and CAH are well known herb medicines and prescription used in oriental medicine for treatment anti-inflammatory and many allergic diseases. They were prepared by the routine method of water decoction, which is in accordance with the method used in clinical application.

Airway inflammation is associated with the infiltration of eosinophils, lymphocytes into the airways and increased numbers of these cells have been found in the bronchoalveolar lavage fluid (BALF) and bronchial biopsies from asthmatics (Kay, 1991). It has been suggested that eosinophils contribute to several of the clinical features of allergic asthma, including tissue damage and airway hyperresponsiveness (Bousquet et al., 1990). Th2 cytokines cause recruitment of eosinophils to the airway. Also, Th2 cytokine IL-4 plays a role in the pathogenesis of allergy and asthma (Mosmann & Sad, 1996) and Interferons are Th1-type cytokines that have a role in inhibiting IgE production and Th2-cell proliferation and function (Busse & Rosenwasser, 2003).

Biological balance appears to be needed between IFN-γ and IL-4 producing Th1 and Th2 subsets so that health can be maintained and autoimmune disease can be prevented.