## 마우스 천식 모델에서 ovalbumin으로 증가된 기도과민반응에 대한 장생도라지 사포닌 분획의 효과

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Effects of saponins isolated from the roots of *Platycodon grandiflorum* on ovalbumin-induced airway hyperresponsiveness in a mouse model of asthma

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## **Objectives**

We investigated protective effect of the saponins isolated from the roots of *Platycodon grandiflorum* (Changkil saponins, CKS) on airway hyperresponsiveness in a mouse asthma model immunized and challenged with ovalbumin (OVA).

## Materials and Methods

- 1. Materials: Chicken egg ovalbumin(Grade II) and Aluminum Hydroxide gel were obtained from Sigma Chemical Co. (St. Louis, MO), Giemsa solution from Fluka, Enzyme-linked immunosorbent assay (ELISA) from R&D Systems and BD Biosciences. CKS was supplied by Jang Saeng Doraji Co., Jinju, South Korea.
- 2. Animals and treatments: Female ICR mice at 6 weeks of age were obtained from Daehan Biolink co., Ltd (Chungbuk, Korea). For sensitization, the asthma control group and CKS treated groups received 50  $\mu$ g of OVA with 1 mg of aluminum hydroxide in 100  $\mu$ L of PBS, whereas the normal group received 100  $\mu$ L of PBS. Each group consisted of five animals. CKS in saline was administered intragastrically (i.g.) at 1 and 2 mg/kg, whereas control or OVA group was injected equal to saline on 12 to 16 days. Animals were challenged with OVA on final days by inhalation of 1 mg/ml OVA in PBS
- 3. Collection of BAL (bronchoalveolar lavage) fluid and differential cell count: The right lung was lavaged with PBS. The BAL fluid was centrifuged and the pellet was re-suspended with PBS. Total and differential cell counts were performed using microscopy.
- 4. Measurement of serum inflammatory cytokines levels: The level of cytokines in BAL fluid were measured by a sandwich ELISA using OptEIA Set mouse IL-4, IL-5, MCP-1 and IgE and DuoSet mouse TNF-alpha, INF-gamma and IL-13 according to the manufacturer's instructions.

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5. Histological studies: For histological examination, 4 µm sections of fixed embedded tissues were cut on a microtome, placed on glass slides, deparaffinized and stained with H&E for general morphology. Pulmonary histological changes were calculated five point score by three independent examiners. A score was: 0, no inflammatory cells; 1, minimal accumulation of inflammatory cells; 2, moderate accumulation of inflammatory cells; 3, severe accumulation of inflammatory cells 4, extreme accumulation of inflammatory cells. Airway mucus production was evaluated the Alcian blue-PAS for infiltrated goblet cells. Infiltrated goblet cells were calculated a five point score by three independent examiners. A score was: 0, no goblet cells; 1, minimal infiltration of goblet cells; 2, moderate infiltration of goblet cells; 3, severe infiltration of goblet cells 4, extreme infiltration of goblet cells.

## Results

- 1) Saponins isolated from the roots of *Platycodon grandiflorum* (CKS) significantly decreased the OVA-induced lung weights, total cells and eosinophils in BAL fluid.
- 2) CKS significantly inhibited the elevated cytokines level (IL-4, IL-5, IL-13, and TNF-alpha), OVA-specific IgE level, and chemokine (MCP-1) production in BAL fluid.
- 3) CKS attenuated OVA-increased infiltration of inflammatory or goblet cells into the peribronchial tissues.