2012 International Conference on Nutrition and Food Sciences
IPCBEE vol. 39 (2012) © (2012) IACSIT Press, Singapore

Propolis Inhalation Reduces Allergic Airway Inflammation in *Dermatophagoides Farinae* -Treated Mice.

Ryoji HIROTA[1+], Nlandu Roger NGATU[1], Hiroyuki NAKAMURA[2] and Narufumi SUGANUMA[1]

[1] Department of Environmental Medicine, Kochi Medical School

[2] Department of Environmental and Preventive Medicine, Kanazawa University Graduate School of Medical Science

Abstract. Propolis is one of the main flavonoids which is reported to inhibit the inflammatory response by suppressing the production of reactive oxygen species. The aim of this study was to evaluate whether propolis can inhibit *Dermatophagoides farinae* -induced airway hyperresponsiveness (AHR), eosinophilic infiltration and other histological changes in the lung, T helper (Th)2 cytokine production and airway remodeling in a mice model of asthma. Treatment with propolis significantly reduced the levels of IL-5, IL-13, eotaxin, MCP-1, and TGF-β_1 in bronchoalveolar lavage fluid. The goblet cell metaplasia, thickness of airway smooth muscle, and airway fibrosis were markedly decreased in propolis-treated mice. Furthermore, AHR to acetylcholine was significantly abrogated in propolis-treated mice. These results indicate that propolis has a potential to reduce airway remodeling and AHR in asthma model.

Keywords: Propolis; Asthma; *Dermatophagoides farinae*; Airway inflammation; Th2 cytokine

1. Introduction

Bronchial asthma is a chronic inflammatory disease characterized by airway obstruction in response to allergens, chronic eosinophilic airway inflammation, mucin hypersecretion, and non-specific airway hyper-responsiveness (AHR). Evidence reveals that these inflammatory responses are mediated by T-helper type 2 (Th2) cells, mast cells, B cells, and eosinophils [1, 2]. Upon challenge with various allergens, these inflammatory cells infiltrate into the airway and produce Th2 cytokines, such as IL-4, IL-5, and IL-13 [1]. Therefore, targeted therapies have been directed toward preventing Th2 responses.

By releasing cytokines, lipid mediators, reactive oxygen species and highly charged cytotoxic granular proteins, activated eosinophils contribute to airway inflammation and cause damage to the bronchial mucosa. Thus, eosinophils play a central role in airway inflammation in bronchial asthma.

Airway remodeling may begin at the initial stages of bronchial asthma, which may facilitate the continuation of tissue damage and the inflammatory response by altering the composition of extracellular matrix (ECM) in association with primarily eosinophils and transforming growth factor-beta (TGF-β) released by fibroblasts and other growth factors. Consequently, the thickening of the airway wall and the smooth muscle layer, and the alteration of the composition of the adventitial matrix are closely associated with the primary characteristic of asthma, airway hyperreactivity [3, 4].

In recent years, as the search for alternative treatments to replace conventional drugs that have major adverse effects is being promoted; for example, anti-oxidant food ingredients (plant polyphenols, probiotics,…) have been attracting attention [5, 6]. Reactive oxygen species (ROS) have been shown to play an important role in bronchial asthma [7-9]. We have reported that the administration of the antioxidant reagent N-acetyl-L-cysteine decreased diesel exhaust particles (DEPs)-induced eosinophil chemotaxis [10].

[+] Corresponding author. Ryoji Hirota. Tel.: +810888802407; fax: +810888802407.
E-mail address: hirotar@kochi-u.ac.jp.

Propolis is a resinous substance that bees collect from exudates of plants and used to seal holes in the beehive and has been reported to possess an anti-oxidant and anti-allergic effects in a number of in vitro and in vivo studies [11-13].

In this study, we examined the effectiveness of the ethanolic extracts of Brazilian green propolis in reducing airway inflammatory reactions and improving asthma symptoms in *Dermatophagoides farinae* (*Der f*) -treated airway inflammation model.

2. Materials and Methods

In order to evaluate anti-inflammatory effect of Brazilian green propolis on BALB/c mice, mice (5 weeks of age) were divided into three groups of ten.

DD group: after mice were exposed to 60% ethanol using a nebulizer as below, mice received intratracheal injection of 4 μg of *Der f* and plus 62.5 μg DEP in PBS solution (total volume 100 μl) on day 1, 2, 7, 8, 14, 15, 21, 22, 28 and 29.

DP group: mice were exposed to 10% propolis (1 mg/kg, once a day) using an ultrasonic nebulizer (NE-U12, Omron, Tokyo, Japan; output 0.8 ml/min) for 20 min in a Plexiglas exposure chamber (22.5 × 29.5 × 16.0 cm). After treatment with propolis, mice received intratracheal injection of 4 μg of *Der f* plus 62.5 μg DEP suspended in PBS on the same days in the same route.

The control group (C): mice were exposed to 60% ethanol using a nebulizer as above, and then instilled with PBS (100 μl).

At 48 h after the final challenge (day 31), all mice were recruited to AHR induction using acetylcholine (ACh), lung specimen sampling for histological examination, and blood sampling for measurement of serum *Der f*-specific IgG_1 level and cytokines levels in BALF.

3. Results and Discussion

It is well-known that airways exposure to allergen such as *Der f* in sensitive mice species induces an increase in the serum level of allergen specific IgG_1. In this experiment, significantly lower levels of *Der f* specific-IgG_1 were observed in the DP group ($p<0.01$) groups as compared with the DD group. Similarly, serum total IgE level in the DP group was lower as compared with the DD group, but not significantly ($p>0.05$).

To examine the effect of propolis on cells chemotaxis, i.e, recruitment of inflammatory cells into the airway, total and differential cell counts were performed in BALF. The DD group showed a significantly increase of number of total cells, macrophages, eosinophils, neutrophils and lymphocytes as compared with the control group ($p<0.001$). In addition, treatment with propolis significantly reduced the total number of inflammatory cells ($p<0.001$), macrophages ($p<0.05$), eosinophils ($p<0.001$) in BALF as compared with the DD group. The observed reduction in eosinophils chemotaxis into the airway was well-correlated with the histological changes in the lung parenchyma.

To examine the effect of propolis on cytokine production, the level of cytokines were measured in BALF. IL-5, IL-13, eotaxin, TGF-β_1 and MCP-1expressions were significantly reduced in DP as compared with the DD group (67%, 48%, 35%, 60% and 68%, respectively), whereas IFN-γ was significantly increased in DP as compared with the DD group (60%).

To evaluate the AHR in mice, ACh was administered intravenously and bronchoconstriction was measured using a bronchospasm transducer. An increase in the intensity of bronchoconstriction was observed in dose-dependent way, particularly in DD mice, while this process was markedly attenuated by propolis treatment in DP mice ($p < 0.01$). In addition, no significant difference was observed between DD and control mice ($p>0.05$).

To evaluate whether propolis affects the allergen induced histopathological changes, right lung specimens were stained with HE. Sections from the control mice displayed normal structure and no pathologic changes under a light microscope. *Der f* and DEPs challenges induced marked marked perivascular and peribronchial infiltration of eosinophils into the lungs of DD mice, a trait of allergic airway inflammation. However this process was reduced by propolis treatment in DP mice. To evaluate whether propolis affects mucin production in bronchial goblet cells, lung specimens were stained with PAS. A

marked goblet cells hyperplasia was observed in lung specimens from DD mice, while the number of those cells was reduced in DP mice.

To evaluate whether propolis affects airway remodeling from fibroblast proliferation, lung specimens were stained with MT; the airway fibrosis was significantly decreased in propolis treated DP mice ($p<0.05$). In addition, the smooth muscle thickness was also significantly decreased in the DP mice ($p<0.05$). These results indicate that treatment with propolis efficiently inhibited the infiltration of inflammatory cells, attenuated allergic airway inflammation and collagen deposition.

In conclusion, results from this study showed that propolis treatment inhibited the production of IgE, representative Th-2 cytokines, chemokines MCP-1 and TGF-β_1, allergen-specific IgG_1, IgE; which resulted in the inhibition of AHR, reduction of collagen deposition and the influx of inflammatory cells into the lungs of *Der f* and DEPs challenged mice.

These findings suggest that propolis may potentially be beneficial as a prophylactic and therapeutic agent for asthma. However, further research is to be performed in order to evaluate the beneficial effects of propolis in asthmatic human subjects.

4. Financial disclosure

This study was supported by Yamada research grant 2008.

5. References

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