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Antiallergic effect of *Trigonella foenum-graecum* L. extracts on allergic skin inflammation induced by trimellitic anhydride in BALB/c mice

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ABSTRACT

Ethnopharmacological relevance: Fenugreek (*Trigonella foenum-graecum* L.) has a wide variety of therapeutic properties for allergic and inflammatory diseases and is used as a traditional functional food, but its antiallergenic mechanism in these diseases is yet to be clearly elucidated.
Aim: In the present study, we investigated the antiallergic activity of fenugreek extract using trimellitic anhydride (TMA)-induced contact hypersensitivity (CHS) mice in vivo and ovalbumin (OVA)-immunized BALB/c mice *ex vivo* as represented model of T-helper (Th) 2-induced allergy.
Materials and methods: BALB/c mice were administered 250 mg/kg body weight (BW) of fenugreek extract for 7 days after sensitization and challenge treatment with 2–5% TMA. Ear thickness were noted, and the infiltration of eosinophils and mast cells was investigated by hematoxylin and eosin (H&E) and toluidine blue (TB) staining. The supernatants from homogenized ear and splenocytes were used for cytokine determination using ELISA. In addition, splenocytes from OVA-immunized BALB/c mice were treated with fenugreek extract *ex vivo*. The levels of cytokines present in the supernatants were determined by ELISA. The mRNA expression of T-box transcription factor 21 gene (T-bet), GATA-binding protein 3 (GATA-3), interferon (IFN)-γ, and interleukin (IL)-4 were evaluated by real-time RT-PCR.
Results: Fenugreek extract was found to reduce ear thickness as well as the infiltration of eosinophils and mast cells. In homogenized ear, the production of IL-4, IL-5, IL-13, and IL-1β was suppressed. To determine the mechanism by which fenugreek extract inhibits allergic skin inflammation, detailed studies were conducted revealing that fenugreek extract prevented differentiation into Th2 cells in the splenocytes of OVA-induced allergic mice, resulting from suppressing the secretion of IL-4 and mRNA expression of GATA-3, an IL-4 transcription factor. In earlier phase, these extracts enhanced the secretion of IFN-γ, the mRNA expression of T-bet, an IFN-γ transcription factor, and the number of IFN-γ-producing $CD4^+$ T cells.
Conclusions: These results indicate that fenugreek extract cures Th2-induced allergic skin inflammation by enhancing Th1 differentiation. These data suggest that fenugreek extracts may prove to be an useful therapeutic agent on allergic inflammatory diseases as traditional use as well as Th2-mediated allergic response.

1. Introduction

Allergic dermatitis (AD) is characterized by allergic skin inflammation. Among the various types of AD, contact dermatitis is induced by an allergic response to a multitude of chemical substances brought on by environmental contamination. It is important to consider the balance of Type 1T helper (Th1) cells/Type 2T helper (Th2) cells cytokines in patients with allergic diseases such as AD. Th1 cells secrete interferon (IFN)-γ and require T-box expressed in T cells (T-bet) for efficient immune responses against intracellular pathogens (Szabo et al., 2003) and tumors (Goedegebuure and Eberlein, 1995). Th2 cells produce interleukin (IL)-4 and require GATA-binding domain-3 (GATA-3) for immunity against helminths and other extracellular pathogens (Zheng and Flavell, 1997) or for the production of antibodies (Lee et al., 2000; Ting et al., 1996). IL-4 promotes the development of mast cells. Mast cells are recognized as key effector cells in immunoglobulin (Ig) E-associated Th2-type immune responses. Allergen-provoked mast cells trigger the release of allergic inflammatory mediators, including histamine via degranulation

Abbreviations: AD, allergic dermatitis; Ag, antigen; APC, antigen-presenting cell; CHS, contact hypersensitivity; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; H&E, hematoxylin and eosin; HRP, horseradish peroxidase; IFN, interferon; Ig, immunoglobulin; IL, interleukin; mAb, monoclonal antibody; OVA, ovalbumin; TB, toluidine blue; Th, T-helper; TMA, trimellitic anhydride

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(Galli et al., 2008). Some studies have demonstrated that IFN-γ suppresses the production of IL-4 from Th2 cells as well as the production of IgE by B cells (Finkelman et al., 1988; Gajewski and Fitch, 1988).

Several studies suggest the contribution of immunologic mechanisms to pathogenesis of AD. In the initiating phase of AD progression, Th2 cells producing IL-4, IL-5, and IL-13 play a critical role (Leung and Soter, 2001; Spergel et al., 1999). The development of dermatitis is thought to be caused primarily by the overproduction of Th2-mediated cytokines and IgE, as well as by the defective production of IFN-γ (Chen et al., 2004; Sandoval-López and Teran, 2001; Vestergaard et al., 2008). Many therapeutic trials have been conducted to evaluate agents that may modulate dermatitis, but prolonged drug use of such compounds has been found to cause a variety of side effects. Recently, natural herbs have been suggested to be potential alternative therapeutics for the treatment of dermatitis due to their proven safety and immune-regulatory effects. There are many studies on the use of natural herbs as improved therapies for the treatment of dermatitis (Koo and Arain, 1998; Kotani et al., 2000).

Fenugreek (*Trigonella foenum-graecum* L.) is a member of the legume family. Its seeds and leaves are used not only as food, but also as an ingredient in traditional medicine. Fenugreek is most commonly used as a spice in curries, seasoning blends, chutneys, pickles, and teas. The plant also has a wide variety of therapeutic properties and is used as a traditional functional food. Pharmacological studies have shown that the extract of this herbal plant exhibits immunomodulatory effect (Bin-Hafeez et al., 2003) as well as antidiabetic, antihypertensive, and cholesterol-lowering effects (Madar and Stark, 2002). In Ayurvedic and Unani systems of medicine, fenugreek is used for the treatment of allergic and inflammatory disease such as epilepsy, paralysis, gout, dropsy, chronic cough and piles (CCRUM). However, the inhibitory effect of fenugreek extract on Th2 cell-mediated allergic inflammatory response remains unclear.

In this study, we reveal that the administration of fenugreek extract restrained the Th2 cell-mediated AD in a trimellitic anhydride (TMA)-induced contact hypersensitivity (CHS) model. Moreover, to determine the antiallergic inflammatory mechanisms of fenugreek extracts, we investigated the inhibitory effect of fenugreek extracts on Th2 differentiation. Our results strongly suggest that the mechanism underlying these effects on OVA-induced Th2 differentiation was considered to promote Th1 differentiation by the production of IFN-γ, which is dependent on the mRNA expression of T-bet and IFN-γ.

2. Materials and methods

2.1. Sample preparation

The fenugreek used in this study was purchased from Kyungdong Oriental medicine market (Seoul, Korea), and identified by Professor Y. Bu, Department of Herbal Pharmacology, Kyung Hee University. The specimen (KFRI-SL-98) has been kept in functional materials research group, Korea Food Research Institute. The ground fenugreek seeds underwent reflux extraction twice in 70% ethanol using a Soxwave100 apparatus. The ethanol extract was dried under a vacuum in a rotary evaporator. The concentrated extract was finally lyophilized, yielding a dried powder that was kept at 4 °C until needed. The dried ethanol extract (EtOH extract) was dissolved in saline (Sigma-Aldrich, St. Louis, MO) and DMSO prior to use. This fraction was used in all the *in vivo* and *ex vivo* mouse studies. TMA (Sigma-Aldrich, St. Louis, MO) was dissolved in an acetone/isopropyl myristate (4:1) solution. Prednisolone was purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Animals

Female BALB/c mice, weighing approximately 18–20 g, were purchased from OrientBio Inc. (Kyeonggi, Korea). The 4-week-old mice were housed in an air-conditioned room (23 ± 2 °C) with a 12 h light/dark cycle. They were allowed free access to food and tap water. All animal experiments were performed in accordance with the guidelines for the animal use and care of the Korea Food Research Institute.

2.3. Schedules for mice sensitization, challenges with TMA, and sample treatment in vivo

The induction of CHS using TMA was performed as previously described (Schneider et al., 2009). A schematic of the experimental procedure is shown in Fig. 1A. For the induction of CHS, mice

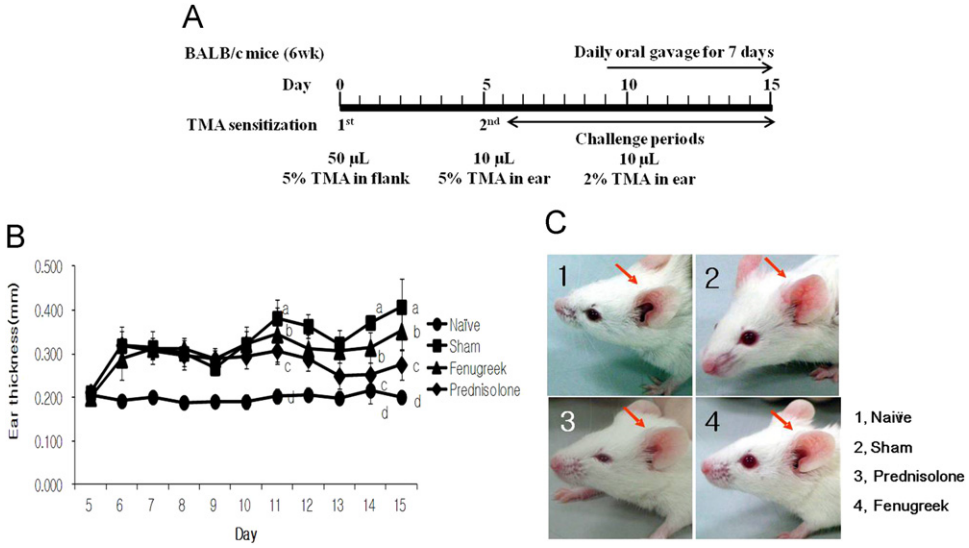

Fig. 1. Experimental protocol and inflammation parameter for trimellitic anhydride (TMA)-induced allergic response in mice. Experimental protocol (A). Ear thickness (B) and photographs (C) were taken after the last treatment. Data are shown as the mean ± SD. Data were analyzed using ANOVA followed by Tukey post hoc test. Data are statistically different ($P < 0.05$) among those columns with different symbols. The order of value is termed as order of alphabet.