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Forsythia suspensa extract alleviates hypersensitivity induced by soybean β-conglycinin in weaned piglets

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ABSTRACT

Aim of the study: Soybeans are known to stimulate food allergies; however, current therapies are lacking in alleviating hypersensitivity. The present study investigated whether *Forsythia suspensa* extract could attenuate purified soybean β-conglycinin-induced anaphylactic reactions in weaned piglets.
Materials and methods: Eighteen 14-day-old piglets were sensitized and boosted by oral administration of purified β-conglycinin. *Forsythia suspensa* extract was supplemented in the diet after the initial sensitization and continued for the remainder of the experiment. Piglets were challenged on day 28, and anaphylactic symptoms, passive cutaneous anaphylaxis, plasma histamine and intestinal microbial flora were analyzed. T-cell proliferative responses and cytokine production were also determined.
Results: Forsythia suspensa extract alleviated β-conglycinin-induced anaphylactic symptoms by markedly reducing anaphylactic antibodies, mast cell degranulation, and histamine release while improving intestinal microbial flora. Furthermore, *Forsythia suspensa* extract significantly suppressed β-conglycinin-induced T lymphocyte proliferation and IL-4 synthesis.
Conclusion: Forsythia suspensa extract protected β-conglycinin-sensitized piglets from anaphylactic reactions. *Forsythia suspensa* extract may provide a novel effective therapy for soybean allergy.

1. Introduction

Soybean is a major protein source for humans and animals alike; however, soybean has also been reported to contain inflammatory allergens (Cordle, 2004). Clinical allergic reactions caused by the ingestion of soybean products range from mild gastrointestinal distress to life-threatening asthma and death (Foucard and Malmheden, 1999). β-Conglycinin, a primary storage protein that accounts for about 30% of total storage protein in soybeans (Utsumi, 1992), has been found to bind IgE in subjects with soy allergies (Rozenfeld et al., 2002; Mittag et al., 2004).

Currently, strict avoidance of soybean products is the only way to prevent allergic reactions; however, in practice this is difficult to achieve due to the widespread use of soybean products in many foods. Anti-IgE therapy to alleviate food allergies has shown only modest and transient benefits (Leung et al., 2003). Conventional allergen-specific immunotherapy is also currently dissatisfactory because of adverse side effects and poor maintenance of tolerance afterward (Nelson et al., 1997). As the occurrence of food allergies increases, it is of great importance to find safe, effective and long-lasting therapies to alleviate allergic reactions to specific foods. Increasing attention is being given to the use of traditional Chinese medicines to find additional therapies to alleviate food allergies. Li et al. (2001) and Srivastava et al. (2005) reported that the Chinese herbal medicines named 'Food Allergy Herbal Formula-1' and 'Food Allergy Herbal Formula-2' blocked anaphylactic reactions in mice allergic to peanuts. These studies suggest that herbal medicines may be used as novel therapies to combat food allergies.

Forsythia suspensa Vahl (Oleaceae) is a climbing plant, which is widely distributed throughout China, Korea and Japan. The extract of the dried fruit from this plant has been widely used as an antipyretic, antidotal and anti-inflammatory agent for the treatment of infections, such as acute nephritis, erysipelas and ulcers (Guo et al., 2007a). Moreover, crude extracts exhibit resistance to hepatic injury, antibacterial, antiviral, antioxidant and anti-inflammatory activities (Ozaki et al., 2000; Guo et al., 2007a; Wang et al., 2008). A number of compounds including phenylethanoid glycosides, lignans, flavonoids, terpenes and volatile oils have been isolated from *Forsythia suspensa*. Among these, the first 3 com-

Abbreviations: cAMP, cyclic adenosine monophosphate; CBB, Coomassie Brilliant Blue; ELISA, enzyme-linked immunosorbent assay; FSE, *Forsythia suspensa* extract; IFN-γ, interferon-γ; IgE, immunoglobulin E; IL-4, interleukin-4; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NRC, National Research Council; PBS, phosphate buffered saline; PCA, passive cutaneous anaphylaxis; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SEM, standard error of mean; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-thiogalactopyranoside.

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pounds were found to be responsible for the various biological effects of the plant extract (Piao et al., 2008).

Liu (2008) reported that phillyrin isolated from *Forsythia suspensa* could enhance immunological function and alleviate delayed hypersensitivity in mice. However, the role of *Forsythia suspensa* extract (FSE) in alleviating the adverse effects from food allergies has not yet been investigated. The physiological and immunological similarities between pigs and humans have made the pig an important large-animal model for biomedical research (Murtaugh, 1994; Helm et al., 2002). Previous studies found that soybean β-conglycinin induced immediate hypersensitivity in early weaned piglets (Hao et al., 2009), while FSE promoted growth and well-being without any adverse effects. Therefore, the objective of the current study was to further investigate whether oral administration of FSE could alleviate soybean β-conglycinin anaphylaxis in pigs.

2. Materials and methods

2.1. Preparation of β-conglycinin

Purified β-conglycinin suspension was donated from the Food Institute of China Agricultural University (Patent number, 200410029589.4, China). After lyophilization, protein content was determined by the Kjeldahl method and β-conglycinin purity was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie Brilliant Blue R-250 staining (Laemmli, 1970). The protein concentration contained within the gel was determined using a Syngene GeneGenius gel documentation and analysis system (Syngene, Cambridge, United Kingdom) (Guo et al., 2007b). The degree of purity was measured to be >85%.

2.2. Extract preparation from Forsythia suspensa

Fructus forsythiae used in this study was purchased from Beijing Tong Ren Tang Pharmacy, China, and were authenticated by Xiang-Lan Piao, which was dried fruit of *Forsythia suspensa* (Thunb.) Vahl. A voucher specimen was deposited at State Key Laboratory of Animal Nutrition, China Agricultural University, China (Voucher No. FS 0801). Dried and powdered fruit of *Forsythia suspensa* (500 g) was extracted with 2500 mL of 80% methanol and sonicated for 1 h twice. The combined extracts were and concentrated under reduced pressure below 45 °C on a rotary evaporator. The solution was freeze-dried to give a powdered (62.2 g). The crude extract was re-dissolved in water, extracted with CH_2Cl_2 three times and n-BuOH four times in succession. The CH_2Cl_2 fractions and the n-BuOH fractions were combined, respectively. The two fractions were dried on a rotary vaporization (Büchi, Rotavapor R-124, Flawil, Switzerland; Wang et al., 2008) to give, respectively, 26.4 g solid residue from the CH_2Cl_2 and 21.8 g from the n-BuOH. An aliquot (20 g) from CH_2Cl_2 was loaded on a silica gel column (Qingdao Chem., 200–400 mesh, 600 g) with $CHCl_3$–MeOH as eluants with increasing polarity from 100:1 to 2:1 $CHCl_3$–MeOH ratio. Repeated silica column chromatography of the fraction yielded 4 compounds. They were identified, based on their mass spectral, ^1H NMR, and ^{13}C NMR data, as phillygenin (16.7 mg), phillyrin (81.7 mg), forsythialan A (41.3 mg) and forsythoside A (16.5 mg) (Lu et al., 2010).

2.3. Animals, diets and experimental design

Eighteen newly weaned barrows (Large White × Landrace; initial body weight 4.42 ± 0.08 kg; all 14 days of age) were selected from a commercial pig farm (Beijing, China) and transported to China Agricultural University. All piglets were individually housed in 1.3 m × 1.2 m pens with slotted stainless steel floors. A mechanically ventilated nursery room was supplied with 16 h light/8 h dark, and maintained at 25–27 °C. Water and feed were available *ad libitum*. Piglets were fed a soybean- and peanut-free diet, using skimmed milk powder and casein as major protein sources. The basal diet was formulated to meet the nutrient requirements suggested by the NRC (1998) and contained no antibiotics (Table 1). Animal experiments were conducted at the Ministry of Agriculture Feed Safety and Bio-availability Evaluation Center (Beijing, China). All procedures were approved by the China Agricultural University Animal Care and Use Committee.

Table 1
Composition and nutrient level of the basal diet.

Ingredient	Composition (%)
Corn	60.50
Skimmed milk powder	4.00
Whey powder	14.00
Casein	13.50
Spray dried porcine plasma	4.50
Limestone	0.90
Dicalcium phosphate	1.30
Salt	0.30
1% Premix[a]	1.00
Chemical analysis	
Crude protein	22.74
Lysine	1.61
Calcium	0.92
Phosphorus	0.75
Calculated DE, MJ/kg	14.59

[a] 1% premix provided per kilogram of the complete diet: vitamin A, 10,000 IU; vitamin D_3, 1,500 IU; vitamin E, 30 IU; vitamin K_3, 2.5 mg; vitamin B_1, 1.5 mg; vitamin B_2, 10 mg; vitamin B_6, 10 mg; vitamin B_{12}, 0.05 mg; folic acid, 1 mg; biotin, 0.5 mg; niacin, 30 mg; pantothenic acid, 20 mg; Cu, 20 mg; Fe, 100 mg; Zn, 110 mg; Mn, 40 mg; Se, 0.3 mg; and I, 0.54 mg.

Piglets were randomly allocated into three treatment groups (n = 6 for each group) according to litter and body weight; and housed individually. β-Conglycinin was dissolved in phosphate-buffered saline (PBS, pH 7.4), 50 mL/piglet. Group 2 was sham group, in which piglets were sensitized by means of oral gavage with 2 g purified β-conglycinin daily from day 0 to day 6. Following initial sensitization, piglets were boosted orally with 8 g β-conglycinin on day 21, and were then challenged with 12 g β-conglycinin (divided into 2 doses administered 30–40 min apart) on day 28. Piglets in group 3 had the same immunization schedule as group 2, and were treated with FSE (100 mg/kg) in the diet after the first sensitization and continued everyday thereafter. Naive piglets served as controls and were gavaged with equal volumes of PBS using the same schedule. 3 h after the second challenge dose, all piglets were slaughtered with an intracardial injection of sodium pentobarbital (50 mg/kg body weight) followed by jugular exsanguination. Sera from all groups of piglets were collected 1 day before β-conglycinin challenge and stored at −80 °C.

2.4. Assessment of systemic anaphylactic markers

Anaphylactic markers were evaluated 30–40 min after the second challenge dose by three investigators using a scoring system previously described (Helm et al., 2002) with minor modifications: (0) no symptoms; (1) scratching and rubbing around the nose and head; (2) coughing, gagging, evidence of stomach contractions, reduced activity, tendency to lie down, easily stimulated to move, minor rashes; (3) vomiting, lethargy/malaise, tremors, diarrhea, convulsions, reduced activity that could not be stimulated by provocation; (4) wheezing, respiratory distress and symptoms requiring epinephrine; and (5) death.