Original Article

Preparation and Analysis of Peanut Flour Used in Oral Immunotherapy Clinical Trials

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What is already known about this topic? It is well established that allergens are the active ingredients in immunotherapy; however, to our knowledge, there are no published reports of allergen characterization for investigational peanut oral immunotherapy products.

What does this article add to our knowledge? Here we report the relative allergen content, microbial load, and stability of these parameters in peanut flour used in oral immunotherapy. The article informs potential investigators of the necessary steps and procedures required by the Food and Drug Administration to use peanut flour for oral immunotherapy under Investigational New Drug applications.

How does this study impact current management guidelines? The study highlights key parameters that must be addressed when considering implementing clinical trials using a food source to desensitize allergic subjects, as is done in oral immunotherapy.

BACKGROUND: Oral immunotherapy (OIT) is an investigational therapeutic approach for the treatment of food allergies. Characterization of the drug product used in oral immunotherapy trials for peanut allergy has not been reported.

OBJECTIVE: To quantify relative amounts of the major peanut allergens and microbial load present in peanut flour used in OIT trials and assess whether these parameters change over a 12-month period. We also anticipate that this report will serve as a guide for investigators seeking to conduct OIT trials under Food and Drug Administration–approved Investigational New Drug applications.

METHODS: Densitometric scanning of Ara h 1 and Ara h 2 resolved on SDS-PAGE gels was used to assess allergen content in peanut flour extracts. Microbial testing was conducted on peanut flour under US Pharmacopeia guidelines for the presence of *Escherichia coli*, salmonella, yeast, mold, and total aerobic bacteria. In addition, aflatoxin was quantified in peanut flour. Reported results were obtained from 4 unique lots of peanut flour.

RESULTS: Relative amounts of the major peanut allergens were similar between different lots of peanut flour and remained stable over a 12-month period. *E coli* and salmonella were absent from all lots of flour. Yeast, mold, total aerobic bacteria, and aflatoxin were within established US Pharmacopeia guidelines on all lots tested and remained within the criteria over a 12-month period.

CONCLUSIONS: Peanut flour used as a drug product contains the major peanut allergens and has low levels of potentially harmful microbes. Both these parameters remain stable over a 12-month period. © 2017 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2017;■:■-■)

Key words: *Peanut allergy; Peanut oral immunotherapy; Peanut flour; Drug product; Stability testing; Microbial testing; Ara h 1; Ara h 2*

Allergy immunotherapy has been practiced for more than 100 years and is the only disease-modifying treatment for allergic disease.[1] In the United States, subcutaneous immunotherapy is used to treat environmental allergies to various allergenic sources

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Conflicts of interest: J. P. Berglund has received consultancy fees from the University of North Carolina and Etubics Corporation. B. Burnett is employed by Duke University. A. W. Burks has received research support from the Food Allergy & Anaphylaxis Network, National Institutes of Health (NIH), and Wallace Research Foundation; has received personal fees from Food Allergy Research and Education for being Chairman (2013-2015) and a member of the research advisory board; was a board member for the NIH Allergy, Immunology, and Transplantation Research Committee Review Panel, NIH Hypersensitivity, Autoimmune, and Immune-mediated Diseases Study Section, and World Allergy Organization; has received consultancy fees from Aimmune Therapeutics, Epiva Biosciences, Genentech, Merck, Regeneron Pharmaceuticals, Stallergenes, Sanofi US Services, and Valeant Pharmaceuticals North America; received personal fees as an independent contractor for pharmaceutical product development; and is a minority stockholder of Allertein. M. Kulis has received research support from NIH-National Institute of Allergy and Infectious Diseases and the Department of Defense and is employed by the University of North Carolina at Chapel Hill. The rest of the authors declare that they have no relevant conflicts of interest.

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Abbreviations used
FDA- Food and Drug Administration- OIT- oral immunotherapy

including pollens, pet dander, and house dust mite to prevent allergic rhinitis and asthma symptoms.[2] Subcutaneous immunotherapy is also effective for more severe allergic conditions such as anaphylaxis to stinging insect venoms. Recently, the US Food and Drug Administration (FDA) approved sublingual immunotherapy for grass and ragweed pollen allergies.[3] However, there is still no FDA-approved immunotherapy for food allergy.

Patients allergic to foods are instructed to strictly avoid the food they are allergic to and must be prepared to treat accidental ingestions and reactions with emergency medication, including epinephrine.[4,5] Subcutaneous immunotherapy was studied as a treatment for peanut allergy in the late 1980s, but this approach was abandoned because of a high rate of severe reactions.[6,7] In the past decade, researchers began to administer food allergens by the oral, sublingual, and epicutaneous routes in an attempt to provide a safe and efficacious therapy (reviewed in Pesek and Jones[8]). Evidence from several studies demonstrated that oral immunotherapy (OIT) for peanut, egg, and milk allergy was often well tolerated and highly effective in a large portion of subjects.[9-15] OIT appears to modify the immune responses to a greater extent than sublingual immunotherapy, leading to desensitization in a higher proportion of subjects.[16,17] OIT is typically administered in 3 phases: initial escalation, build-up, and maintenance. The initial escalation phase involves several low doses of protein (micrograms to milligrams) given over the course of a few hours. Then, subjects undergo the build-up phase by increasing the dose amount approximately every 2 weeks. After several months, subjects reach the maintenance dose (300-4000 mg of protein depending on the study protocol) and ingest it daily for many months or years.

As per FD&C Act (201(g)(1)), a drug is defined as any article that is "intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals." or "… a substance (other than food) intended to affect the structure or any function of the body." Used within the context of OIT clinical trials, the peanut product is administered with the intention of providing a treatment to peanut-allergic subjects by modulating their immune response to the allergen and therefore meets the definition of a drug. However, because peanut flour is not an FDA-approved drug, as per 21 CFR 312.2(b), OIT trials are not eligible for the Investigational New Drug exemption status. To comply with the regulatory requirements of conducting OIT clinical studies, an Investigational New Drug application outlining the characteristics of the peanut flour as well as a detailed description of the manufacturing process is needed. Manufacturing of the peanut flour as an investigational drug product occurs in a Good Manufacturing Practices–compliant environment under Standard Operating Procedures detailing the process. In addition, the stability of allergenic peanut proteins and microbial growth must be documented.

Within this article, we present the tests that we have conducted on the peanut flour used in clinical studies of the treatment of peanut allergy in children and adults. Before subject administration, the product was subjected to bioburden testing where levels of aerobic/anaerobic bacteria, yeast, and mold were assessed. Because of the specific nature of the peanut flour, the product was also tested for *E coli*, salmonella, and aflatoxin. Standardizing a drug product for OIT is of paramount importance so that subjects will receive equivalent amounts of allergens even when switching between different lots of the product. Therefore, appropriate testing has to be conducted before releasing the product. Here, we report our findings related to allergen content, bioburden, and stability of peanut flour used in OIT clinical trials.

TABLE I. FDA requirements for orally delivered drugs

Microbial Assessment	Nonaqueous preparation	Aqueous preparation
Escherichia coli (in 1 g or 1 mL)	Absent	Absent
Total aerobic microbial count (cfu/g or cfu/mL)	10^3	10^2
Total combined yeasts/molds count (cfu/g or cfu/mL)	10^2	10^1
Salmonella (in 1 g or 1 mL)	Absent	Absent
Aflatoxin in peanut products	<15 ppb	<15 ppb

METHODS

Drug manufacturing process

The overall process of drug manufacturing occurs in several stages: (1) bulk peanut flour is received; (2) the bulk flour is tested for compliance with established US Pharmacopeia guidelines for the presence of microbes (see Table I); (3) the bulk flour is examined for the presence of Ara h 1 and 2 and their consistency in relative quantity to a reference standard and the previous lot of peanut flour; (4) finally, the peanut flour is used to manufacture drug product doses. For the initial treatment under the OIT protocol (referred to as the initial escalation phase), the doses of the peanut flour as an investigational drug product are too low weight to be successfully administered with reproducible accuracy as a flour product. Therefore, a peanut liquid drug product is produced by extracting the flour in PBS and used to administer very low doses of allergen in the range of 0.1 to 0.8 mg. The peanut extract is filter sterilized, prepared at a concentration of 10 mg/mL, and stored frozen until use. The flour itself is manufactured into drug product doses ranging from 1.5 to 4000 mg of peanut protein by weighing out doses on an analytical balance into a vessel. The bulk flour and manufactured drug doses are kept refrigerated to prevent microbial growth and preserve protein integrity; nevertheless, the possibility of protein degradation or microbial growth exists and must be studied.

Peanut flour

Lightly roasted, partially defatted, 12% fat peanut flour was purchased from Golden Peanut Company (Alpharetta, Ga) in 50-lb bags. Upon receipt, the product was broken down into smaller bags (~10 lb each) and kept refrigerated at 2°C to 8°C. Each lot comes with a Certificate of Analysis provided by the Golden Peanut Company, including results for physicochemical properties and microbiological testing. Here, we report findings from 4 unique lots purchased between July 2014 and January 2016.

Extraction of soluble peanut proteins from lightly roasted peanut flour

Peanut flour was mixed with PBS at a 1:4 ratio (weight/volume). The suspension was stirred for 1.5 hours while maintaining a constant pH of 8.5 using 6 molar NaOH, then centrifuged at 30,000*g*