Food-Induced Anaphylaxis: Visualization of Airway Contractility with Allergen Exposure in Precision Cut Lung Slices from a Donor with History of Fatal Anaphylaxis to Cashew.

Adam Price, MD[1,2]; Stacie Jones, MD[1, 2]; Richard Kurten, PhD[1, 2]; Claire Putt[1]; Suzanne House[1]; Joshua Kennedy, MD[1, 2]

[1]University of Arkansas for Medical Sciences, [2]Arkansas Children's Research Institute, Little Rock, AR

Arkansas Children's HOSPITALS • RESEARCH • FOUNDATION archildrens.org

UAMS UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES uams.edu

Abstract

Rationale: Peanuts and tree nuts account for the majority of anaphylaxis attributable deaths. Uncontrolled asthma is the single most important risk-factor for fatal anaphylactic events. We hypothesized that exposure to peanut or cashew allergen in post-mortem lung tissue from a donor with history of food allergy would cause airway contractility.

Methods: Precision cut lung slices (PCLS) were prepared from a food allergic and a nonallergic donor. Baseline photomicrographs measuring airway cross-sectional area were taken before and after 15-minute exposure to carbachol and histamine. PCLS with at least 20% contraction to histamine/carbachol were then exposed to 1mg/mL peanut or cashew. Images of the airways were obtained at 20-, 60-, and 360-minutes post-exposure. Serum was obtained from donors for specific IgE.

Results: The food allergic donor was 11yo with history of fatal anaphylaxis to cashew. The non-allergic donor was 20yo with no significant medical history. The allergic donor was positive for IgE to peanut (11.7kUA/L) and cashew (4.13kUA/L), but testing was negative in the non-allergic donor. In the allergic donor, 6/8 PCLS demonstrated peaked contractile responses to peanut at 60-minutes (mean % contraction [SD]; 71.4% [19.5%]) compared to 2/8 cashew exposed airways (mean % contraction [SD]; 13.2% [8.7%]). Despite airway responsiveness to contractile agonists, no airways responded to peanut in the non-allergic donor PCLS.

Conclusions: In the susceptible individual with asthma, peanut allergen exposure correlates with a quantifiable decrease in lung airway diameter. The diminished responses seen to cashew despite positive specific IgE tests represent donor anergy, given that the fatal anaphylaxis culprit antigen was cashew.

Introduction

IgE-mediated food allergy is one of the most common chronic medical conditions of childhood, affecting up to 10% of the population worldwide.[2] Reactions that are mediated by IgE are typically rapid onset, presenting with clinical symptoms within minutes to a few hours after exposure.[1] The most feared of systemic reactions is anaphylaxis, which can produce life-threatening cardiopulmonary collapse. The lifetime prevalence of anaphylaxis in the United States is estimated to be between 0.5-2%[7] with peanuts and tree nuts accounting for many of these reactions. In terms of risk factors, the most significant risk factor for a fatal anaphylactic event is uncontrolled asthma.[11] *Mullins et al* showed that in Australia from 2000-2013, 68% of the known anaphylactic fatalities (attributable to food ingestion) had a history of asthma.[11]

Representing a well-established technique[4,5], precision-cut lung slices (PCLS) provide a unique opportunity to learn about the relationship between asthma and food-attributable anaphylaxis. PCLS contain all the cells of the lower respiratory tract, including epithelial cells, smooth muscle cells, fibroblasts, macrophages, and mast cells.[4] Bronchoconstriction is primarily mediated by mediators released by mast cells and basophils, including histamine, leukotrienes, and prostanoids. Iconically, histamine binds to the H1 receptor on airway smooth muscle cells and induces an allergen-specific immediate airway constriction defined as the early airway response.[4] Recently, Delgado et al. showed that passive sensitization to dust mite in PCLS from donors with history of cancer caused bronchoconstriction after to the dust mite allergen.[4]

We questioned whether airways in PCLS from a food-allergic (FA) donor with known positive serum specific IgE and a history of asthma would respond to food allergens to which they were sensitized with bronchoconstriction post-mortem. Based on the literature, we hypothesized that there would be marked bronchial constriction in PCLS sensitized with peanut and cashew in PCLS from a donor with history of fatal anaphylaxis to cashew.

Methods

Preparation of Human Precision Cut Lung Slices

- Donor human lungs were obtained from the National Disease Research Interchange or the Arkansas Regional Organ Recovery Agency within 12-36 h of surgical collection and processed as previously described.
- Airways were identified in 15mm thick lung sections, cored and cut into 0.6mm thick, 12.5mm diameter slices.
- Slices were cultured with continuous agitation at 37°C, 5% CO2 in culture medium supplemented with antibiotics.

Measurement of Airway Cross-Sectional Area

- Baseline airway cross-sectional areas were measured by obtaining photomicrographs with a CCD camera using ImageJ. PCLS's were then exposed to 0.2μM carbachol (CCh) and histamine. After 15 minutes on an orbital shaker, the airway cross-sectional area was re-measured and compared to the baseline image in order to calculate the percent airway occlusion. The PCLS's were then exposed to 4μM forskolin (FSK) in order to restore maximal relaxation.
- PCLS's with at least 20% contraction were then exposed to 1 mg/mL of peanut or cashew protein for 15 minutes on an orbital shaker. Repeat micrographs were obtained at 20-, 60-, and 360-minutes post-exposure. After each measurement, the PCLS's were returned to the orbital shaker.
- The percent of airway contraction was then calculated for each time interval in both the food allergic and control (non-allergic) donors.

Measurement of Donor Serum IgE

- Blood obtained from both the food-allergic donor and control donor was collected and sent by the National Disease Research Interchange or the Arkansas Regional Organ Recovery Agency along with the donor lungs.
- Samples from both donors were analyzed for the concentration (kUA/l) of serum specific IgE on Phadia ImmunoCAP 100.

Results

Table I. Demographics.

	Food-Allergic (FA) Donor (hl237)	Non Food-Allergic Donor (Control) (hl238)
Age	11 years	20 years
Race/Sex	H / F	B / M
Height/Weight/BMI	60'' / 42 kg / 18.1	72'' / 82 kg / 24.5
Past Medical History	Food allergy, Asthma	None
Medications	Unspecified inhaler	None
Cause of Death	Anaphylaxis (presumed Cashew)	Head Trauma

Table II. Allergen Specific IgE Testing.

	Food-Allergic (FA) Donor Concentration (kUA/l)	Non Food-Allergic (Control) Donor Concentration (kUA/l)
f2 (Milk)	2.21	0.11
f13 (Peanut)	11.7	0.21
f20 (Almond)	0.13	<0.10
f201 (Pecan)	<0.10	<0.10
f202 (Cashew)	4.13	<0.10
f245 (Egg)	0.63	<0.10
f422 (Peanut r Ara h1)	0.12	<0.10
f423 (Peanut r Ara h2)	10.8	<0.10
f424 (Peanut r Ara h3)	<0.10	<0.10

Results

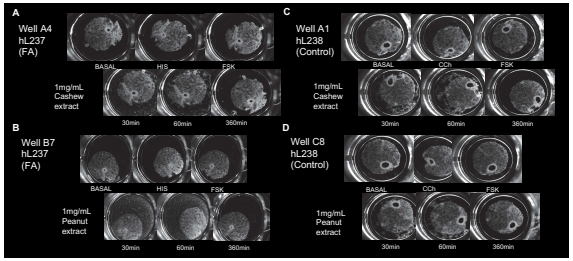

Figure 1: Airway Responsiveness to Food allergens in PCLS from a FA donor. FA donor showed no airways responsiveness to cashew (A) but had a significant response to peanut at all time points(B) (representative photomicrographs). (C) (D) Control donor did not show responsiveness to either food allergen.

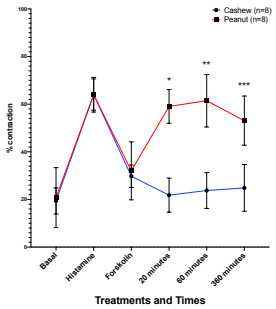

Figure 2: Mean airways responsiveness to food allergens in PCLS from FA donor. Airways significantly contracted to peanut (Red) (n=8) ($p<0.05$) but not to cashew (Blue) (n=8) at 20, 60, and 360 minutes post exposure. This finding occurred despite history of fatal anaphylaxis to cashew and positive serum IgE to cashew- likely representing anergy to this allergen.

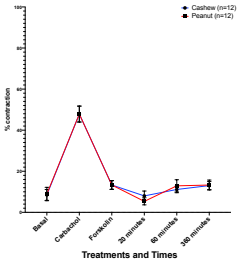

Figure 3: Mean airways responsiveness to food allergens in PCLS from control (non-allergic) donor. Airways did not respond to either peanut (Red) (n=12) or cashew (n=12) compared to basal measures. This finding occurred despite evidence that the airways were contractile (carbachol responsive).

Conclusions

- The mean percentage of airway diameter contraction in the FA donor was significantly increased as compared to the control donor and to basal tone for the FA donor.
  - In the FA donor with a reported fatal anaphylaxis to cashew, there was a mean diameter contraction of 71.4% and 13.2% when exposed to peanut protein and cashew protein, respectively.
  - In the control donor, no airways responded to peanut or cashew, despite airway responsiveness to contractile agonists carbachol and forskolin.
- Peak airway contraction of the food allergic donor occurred within 60 minutes of exposure to the allergen and persisted at 360 minutes of continuous exposure.
- Considering the history of fatal anaphylaxis to cashew, the absent response to cashew (despite positive serum IgE) may represent anergy (Figure 4).

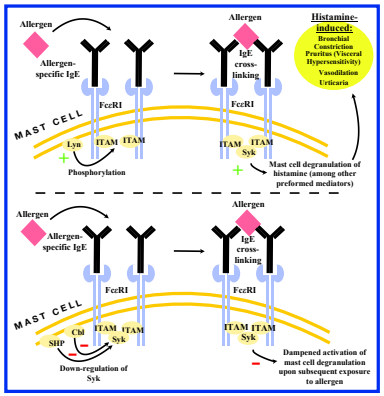

Figure 4: Serum IgE cross-linking with the FcεRI receptor on mast cells leads to degranulation and secretion of preformed mediators that lead to bronchial reactivity after ingestion of food allergen. Post-systemic degranulation leads to down regulated pathway activation via Syk-mediated signaling, causing anergy.

References

1. Anvari, Sara, et al. "IgE-Mediated Food Allergy." Clinical Reviews in Allergy & Immunology, vol. 57, no. 2, 29 Oct. 2018, pp. 244–260, 10.1007/s12016-018-8710-3.
2. Bartnikas, Lisa M., and Scott H. Sicherer. "Fatal Anaphylaxis: Searching for Lessons from Tragedy." The Journal of Allergy and Clinical Immunology: In Practice, vol. 8, no. 1, Jan. 2020, pp. 334–335, 10.1016/j.jaip.2019.11.005.
3. de Castro, Rodrigo Orlandini. "Regulation and Function of Syk Tyrosine Kinase in Mast Cell Signaling and Beyond." Journal of Signal Transduction, vol. 2011, 2011, pp. 1–9, 10.1155/2011/507291.
4. Delgado, Sharon Jiménez, et al. "Disruptive Anti-IgE Inhibitors Prevent Mast Cell–Dependent Early Airway Response in Viable Atopic Lung Tissue." Journal of Allergy and Clinical Immunology, vol. 145, no. 2, Feb. 2020, pp. 719-722.e1, 10.1016/j.jaci.2019.11.002.
5. Liu, Guanghui, et al. "Use of Precision Cut Lung Slices as a Translational Model for the Study of Lung Biology." Respiratory Research, vol. 20, no. 1, 19 July 2019, 10.1186/s12931-019-1131-x.
6. Motosue, Megan S., et al. "Risk Factors for Severe Anaphylaxis in the United States." Annals of Allergy, Asthma & Immunology, vol. 119, no. 4, Oct. 2017, pp. 356-361.e2, 10.1016/j.anai.2017.07.014.
7. Mullins, R. J., et al. "Increases in Anaphylaxis Fatalities in Australia from 1997 to 2013." Clinical & Experimental Allergy, vol. 46, no. 8, 31 May 2016, pp. 1099–1110, 10.1111/cea.12748.
8. Platts-Mills, Thomas A.E., et al. "IgE in the Diagnosis and Treatment of Allergic Disease." Journal of Allergy and Clinical Immunology, vol. 137, no. 6, June 2016, pp. 1662–1670, 10.1016/j.jaci.2016.04.010.
9. Pouessel, Guillaume, et al. "Food-induced Fatal Anaphylaxis: From Epidemiological Data to General Prevention Strategies." Clinical & Experimental Allergy, vol. 48, no. 12, 26 Nov. 2018, pp. 1584–1593, 10.1111/cea.13287.
10. Soriano, Joan B, et al. "Global, Regional, and National Deaths, Prevalence, Disability-Adjusted Life Years, and Years Lived with Disability for Chronic Obstructive Pulmonary Disease and Asthma, 1990–2015: A Systematic Analysis for the Global Burden of Disease Study 2015." The Lancet Respiratory Medicine, vol. 5, no. 9, Sept. 2017, pp. 691–706, 10.1016/s2213-2600(17)30293-x.
11. Turner, Paul J., et al. "Fatal Anaphylaxis: Mortality Rate and Risk Factors." The Journal of Allergy and Clinical Immunology: In Practice, vol. 5, no. 5, Sept. 2017, pp. 1169–1178, 10.1016/j.jaip.2017.06.031.

Funding: This work was funded by NIH NIAID (1K08AI121345-01A1), NIH NCATS (KL2TR000063, UL1TR000039), NIH NIGMS (P20GM121293), Arkansas Biosciences Institute, Marion B Lyon New Scientist Development Award, and UAMS Clinician Scientist Program.