Evaluation of a Mouse Model of Food Allergy for Determination of Oral Elicitation Threshold Doses for Hazelnut

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Invited Speaker, ILSI, HESI, IFBiC, Biotechnology Update Symposium 2013, Arlington, VA

May 8, 8-2 p.m.

Presentation overview

- Part-I
  - Food allergy: why is this a problem of critical significance?
  - How to predict food allergenicity?

- Part-II
  - Transdermal sensitization/oral elicitation (TS/OE) mouse model of food allergy
    - Development
    - Characterization

- Part-III
  - Evaluation of TS/OE mouse model to determine oral elicitation threshold doses for hazelnut
  - Overall conclusions and the future direction

  - Questions? Comments?

How to predict food protein allergenicity?

- Many approaches described in the literature; but none is an absolute predictor by itself
  - Molecular/database approach
  - In vitro, Ex-vivo (Digestion assays, Basophil histamine release)
  - Animal models (Dog, Rat, Mice)

- We have used the approach of developing a mouse model of food allergy that:
  - Simulates the life-threatening anaphylaxis observed in human near-fatal/fatal reactions

Birmingham et al (2007) Int Arch Allergy Immunol. 144(3):203-10; Parvataneni et al (2009) Int J Immunogent, 36(5):261-7

Gonipeta et al (2010) Int Arch Allergy Immunol.152 (3): 219-225

Gonipeta et al (2013) ISRN Allergy, Vol 2013, ID 509427, 7

Gonipeta et al (2013) *Crit Rev Food Science & Human Nutrition* (In Press)

A key concept question in food allergy

Can clinically significant sensitization to food protein result from transdermal exposure to food protein?

Can we test this possibility without using alum or cholera toxin adjuvant in mice?

Part-II

Transdermal sensitization/Oral elicitation (TS/OE) mouse model of food allergy

Hypothesis & Approach

Tree nut (Hazelnut)

Saline control

Transdermal exposure protocol

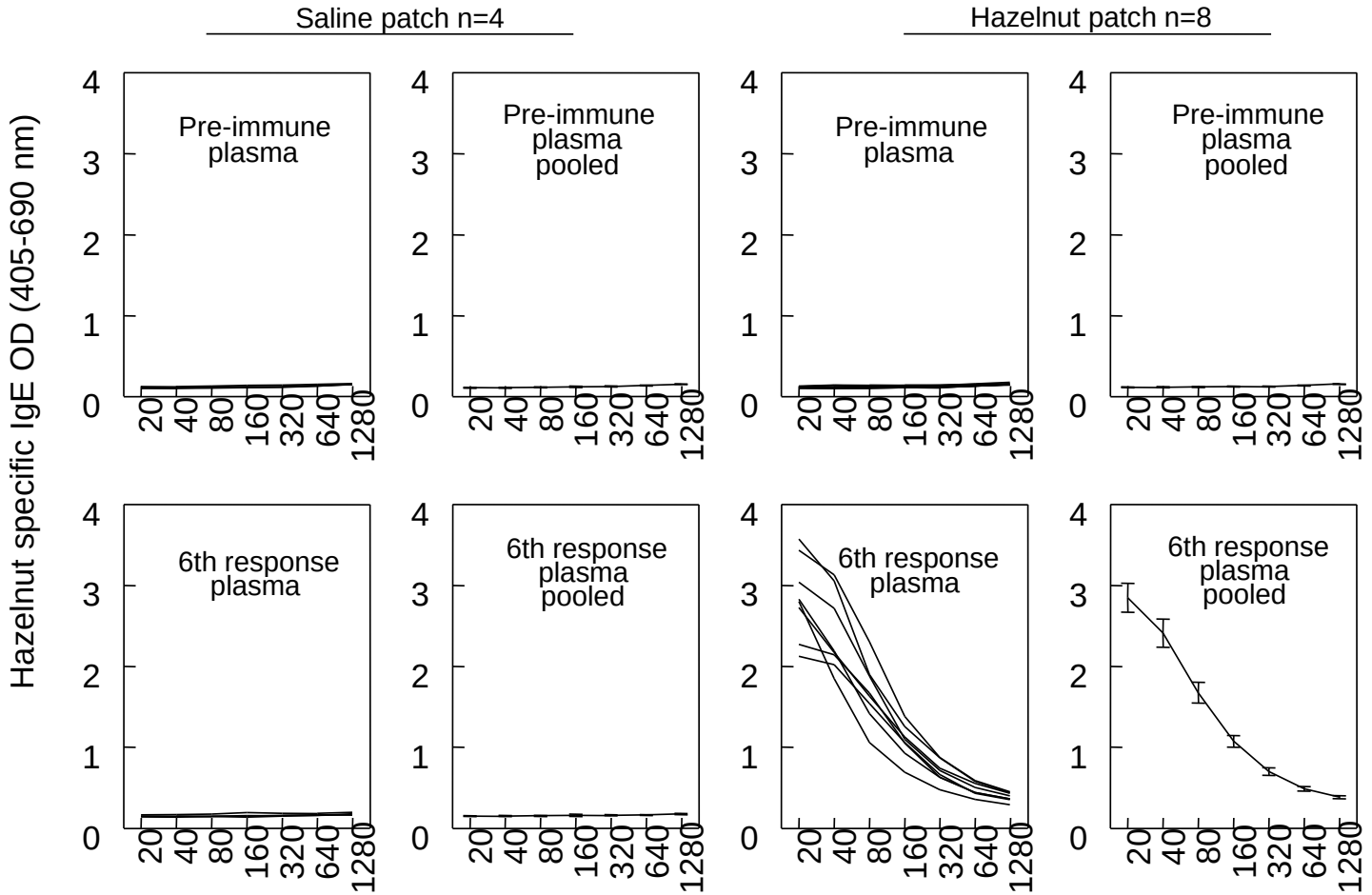
(Dose response; Time-course analysis)

Study IgE antibody response to hazelnut using the optimized IgE assay we had developed

(Birmingham et al *J Immunol Methods*, 2003)

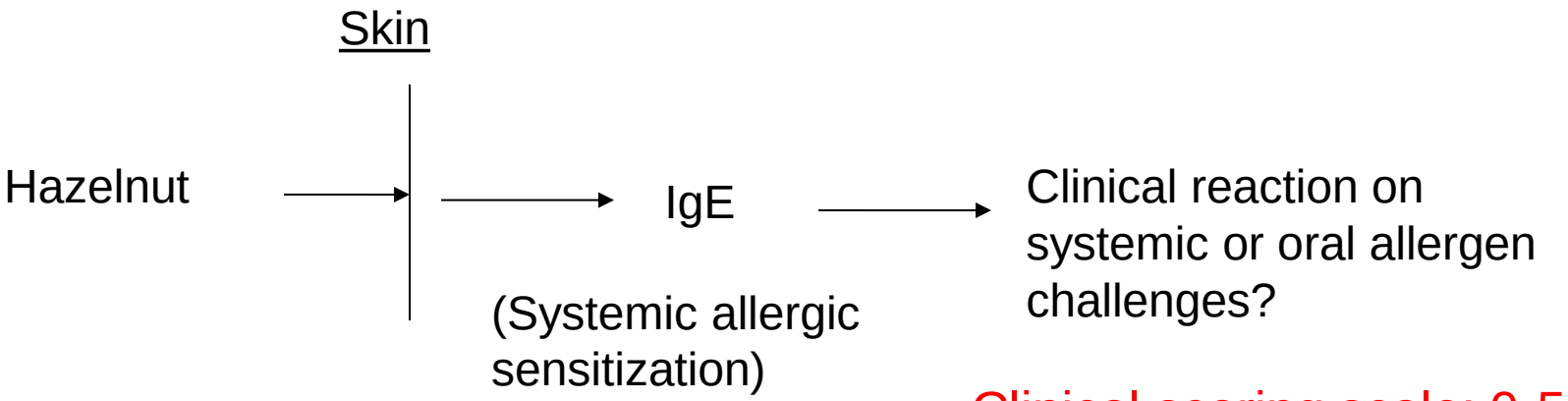
Hypothesis: Transdermal exposure to HN results in systemic IgE antibody response (i.e., allergic sensitization)

Transdermal exposure to hazelnut results in robust IgE Ab response in mice



Birmingham et al 2005; Birmingham et al (2007) Int Arch Allergy Immunol. 144(3):203-10)

Does transdermal exposure to hazelnut clinically sensitizes mice for systemic anaphylaxis?



Clinical scoring scale: 0-5

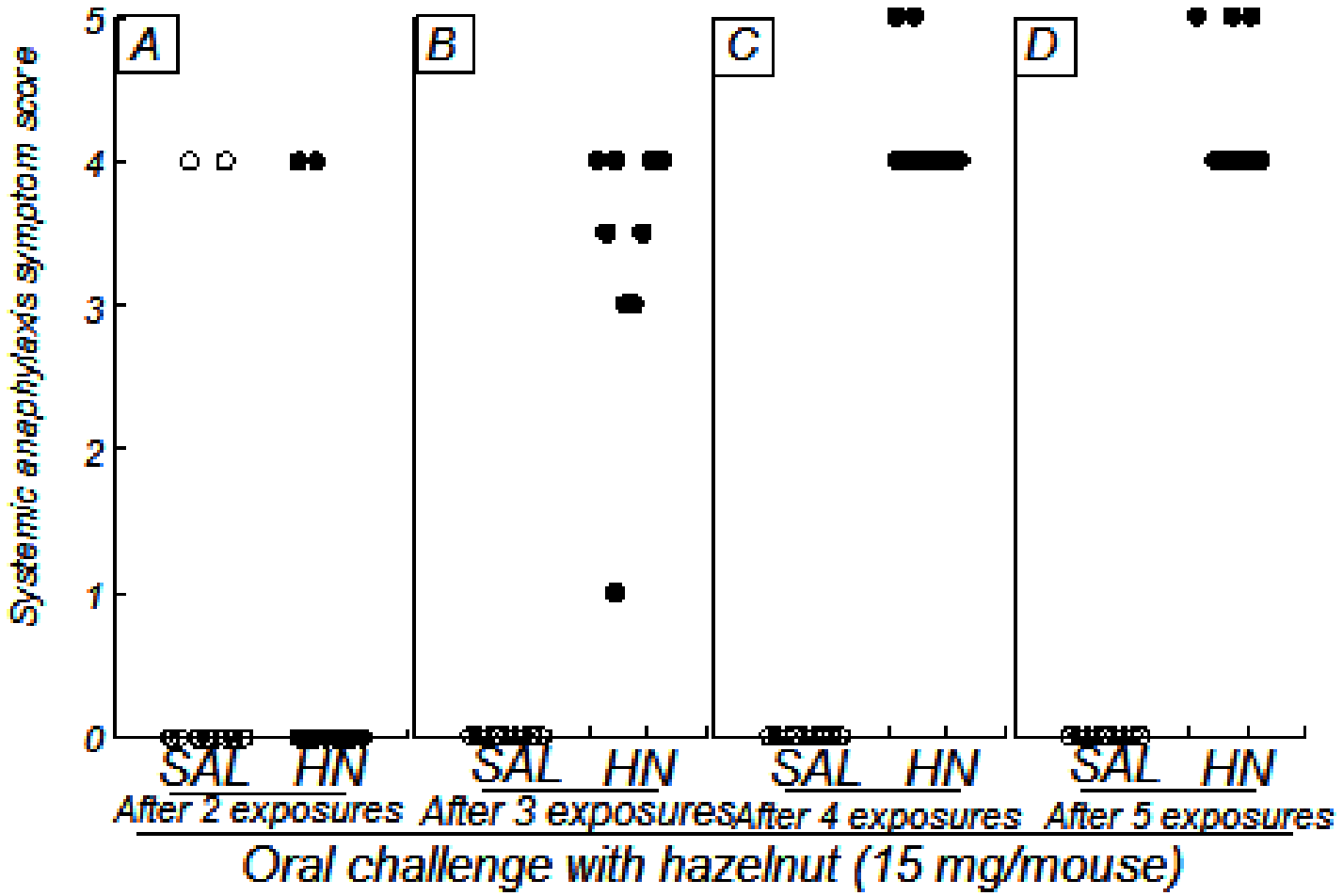
Rectal Temperature Drop (Hypothermia Reaction)

Clinical symptom scoring of systemic anaphylaxis

Score	Symptoms
0	No symptoms
1	Scratching and rubbing around the nose and head
2	Puffiness around the eyes and mouth, diarrhea, pilar erecti, reduced activity, and/or decreased activity with increased respiratory rate
3	Wheezing, labored respiration, cyanosis around the mouth and the tail
4	No activity after prodding, or tremor and convulsion
5	Death

(**Li XM et al J Allergy Clin Immunol. 2000 Jul;106(1 Pt 1):150-8.)**

Transdermal exposure to hazelnut protein clinically sensitize mice for systemic anaphylaxis to oral hazelnut challenge

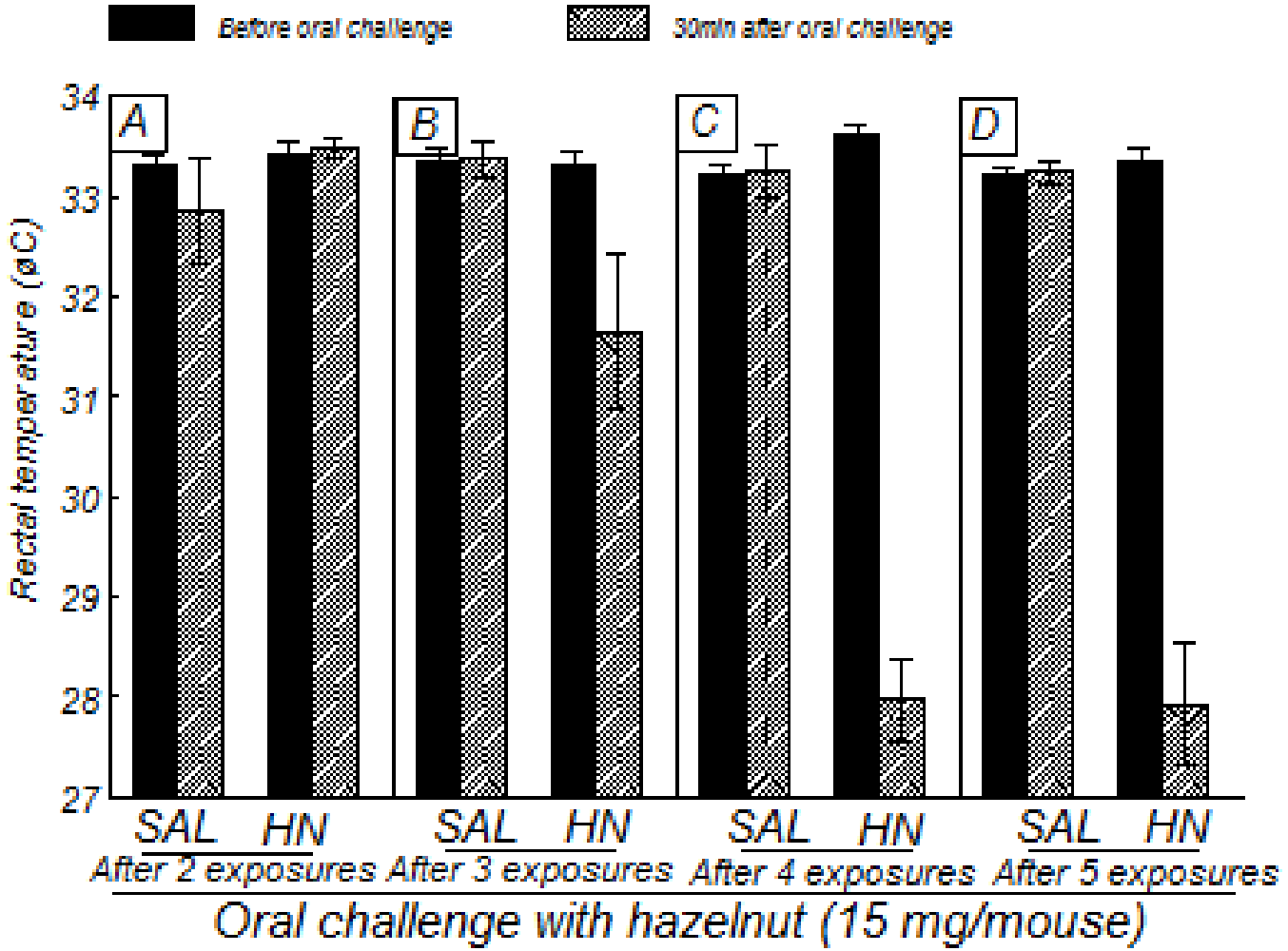


Do mice undergoing systemic anaphylaxis exhibit hypothermia?

- Hypothermia is a classical surrogate quantifiable marker of systemic anaphylaxis used in mouse models

(*Strait, R. T et al 2002 J Allergy Clin Immunol 109:658-668)*

Oral challenge with hazelnut induces immediate (30 min post-challenge) hypothermia in transdermally sensitized mice



Conclusions

- Transdermal exposure to hazelnut is sufficient to sensitize BALB/c mice for systemic anaphylaxis in response to i.p., and oral hazelnut protein challenge
- Clinical symptoms are associated with a rapid hypothermia—a quantifiable readout of systemic anaphylaxis
- The TS/OE mouse model has been validated for other allergenic foods such as: milk, egg, fish, shellfish, sesame and cashew nut
- Kidney bean, pinto bean, pigeon pea, blueberry, vanilla extracts elicit little/nice/no IgE responses but not significant hypothermia reactions
- Oral challenge induces intestinal anaphylaxis as evidenced by histopathology changes
- Transdermal exposure to hazelnut protein sensitizes BALB/c mice for systemic anaphylaxis in a *Stat6 and IL4* dependent manner

Part-III

Predicting life-threatening systemic reactions: determination of oral elicitation threshold doses for hazelnut

(work in progress)

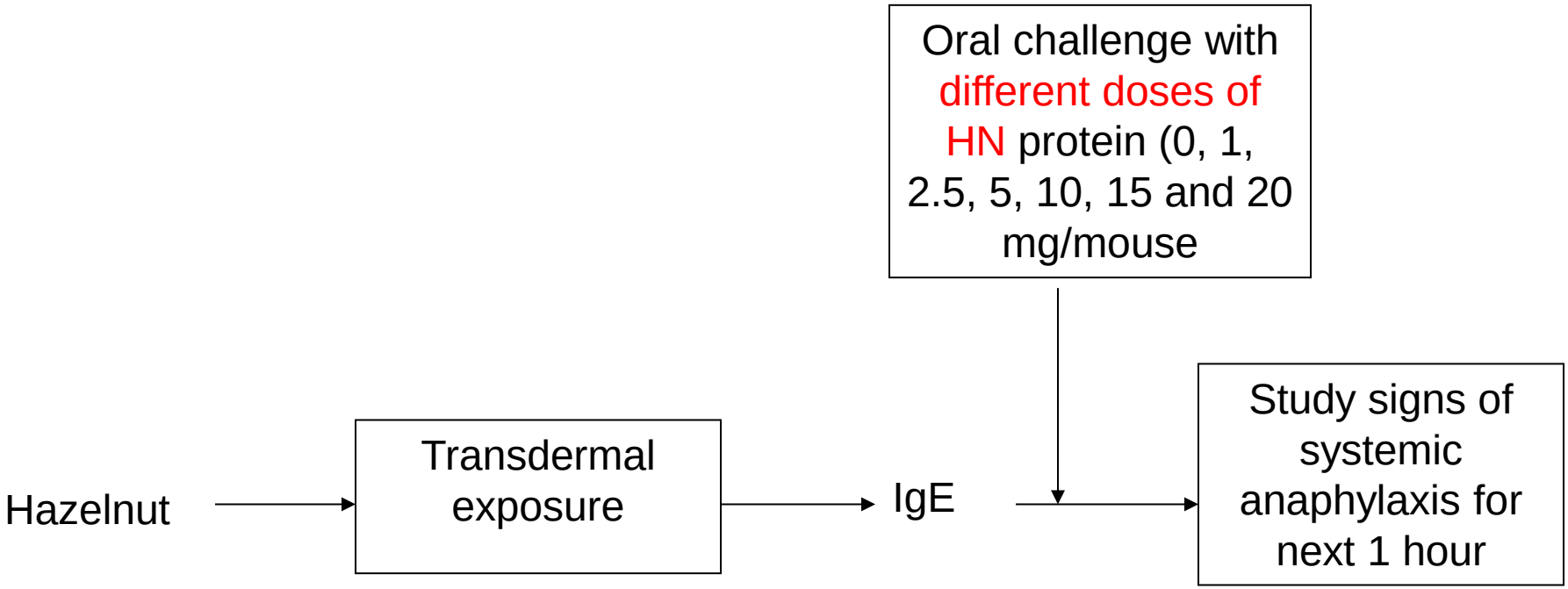
Why one needs to determine threshold doses for food allergens?

- To predict and prevent life-threatening reactions to food allergens among sensitized subjects

- To assist regulatory agencies (US FDA, US EPA, USDA etc) and food industry to set science-based acceptable standards/threshold limits for cross-contamination with allergens

- Current regulations are based on “zero tolerance” policy

Approach: oral threshold dose study



Quantify hypothermia responses

Test dose-response hypothesis?

Establishing dose-response curves to estimate threshold oral elicitation dose

(work is in progress)

- **NOAEL**

(No Observed Adverse Effect Level)

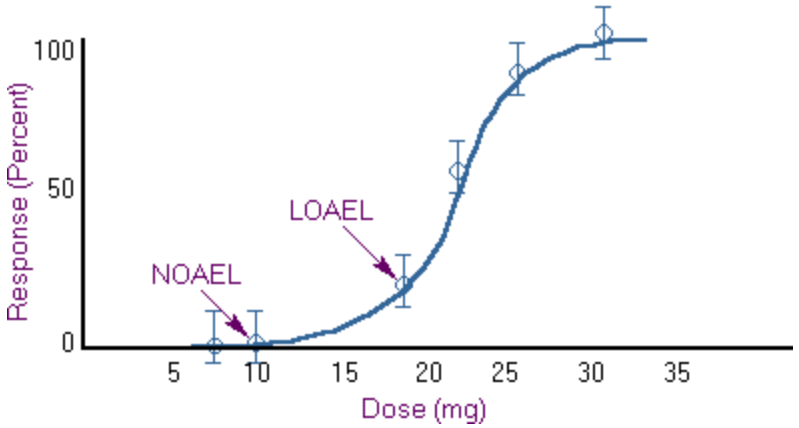
Greatest concentration or amount of a food protein, which causes no significant allergic reaction upon oral exposure in TS/OE model

- **LOAEL**

(Lowest Observed Adverse Effect Level)

Lowest concentration or amount of a food protein, which causes a significant allergic reaction distinguishable from normal (control) mice upon oral exposure in TS/OE model

(Defined based on definitions of IUPAC Compendium of Chemical Terminology, Second Edition, 1997)



Establishing Thresholds in Three Types of Exposure Models: *Proof-of-Principle* Studies

1) Homogeneous sensitization followed by repeated oral challenges with different doses of HN

2) Homogeneous sensitization followed by single oral challenge with different doses in different groups

3) Heterogeneous sensitization followed by heterogeneous oral exposures with different doses of HN

To simulate human exposure conditions that are generally heterogeneous

Establishing Threshold Dose in Humans: How to Translate Mouse Dose to Humans?

FASEB J. 2008 Mar;22(3):659-61. Epub 2007 Oct 17.

Dose translation from animal to human studies revisited.

Reagan-Shaw S, Nihal M, Ahmad N.

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Abstract

As new drugs are developed, it is essential to appropriately translate the drug dosage from one animal species to another. A misunderstanding appears to exist regarding the appropriate method for allometric dose translations, especially when starting new animal or clinical studies. The need for education regarding appropriate translation is evident from the media response regarding some recent studies where authors have shown that resveratrol, a compound found in grapes and red wine, improves the health and life span of mice. Immediately after the online publication of these papers, the scientific community and popular press voiced concerns regarding the relevance of the dose of resveratrol used by the authors. The animal dose should not be extrapolated to a human equivalent dose (HED) by a simple conversion based on body weight, as was reported. For the more appropriate conversion of drug doses from animal studies to human studies, we suggest using the body surface area (BSA) normalization method. BSA correlates well across several mammalian species with several parameters of biology, including oxygen utilization, caloric expenditure, basal metabolism, blood volume, circulating plasma proteins, and renal function. We advocate the use of BSA as a factor when converting a dose for translation from animals to humans, especially for phase I and phase II clinical trials.

Conclusions and Future Direction

- It is possible to establish percent responder curves to determine oral elicitation threshold doses for hazelnut protein in mice using this model
- This *proof-of-principle* study shows that oral elicitation doses can be determined for food allergens, at least for hazelnut now (and that they are unlikely to be “zero” for hazelnut)
- We have previously validated this model for egg, milk, cashew nut, sesame, fish and shellfish
- Therefore, in principle, these data demonstrate that this model may be applied for determination of oral elicitation threshold doses for these allergenic foods

Questions? Comments?

Thank you for inviting me and for listening!

Interested in funding? Will be happy to discuss (after the presentation)!