The Characteristics of Kiwi Fruit Allergy

Final Report TO7038

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Executive Summary

THE CLINICAL AND IMMUNOLOGICAL CHARACTERISTICS OF KIWI FRUIT ALLERGY IN THE UK
TO7038

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This study was conducted in response to a perceived increase in the prevalence of kiwi fruit allergy, and followed on from FSA funded TO7025. Information concerning kiwi fruit allergy was sparse at the start of this study, and we therefore started with a descriptive analysis of the problem in the UK, as well as clinical studies to investigate the use of clinical investigations in the diagnosis of kiwi fruit allergy.

The aims of the study were:

1. Using kiwi fruit as a model, to provide an illustration of the way in which post-marketing surveillance should be conducted to detect allergy to newly introduced foods.

2. Full characterisation of all syndromes (ie.. oral allergy syndrome, systemic reactors) associated with kiwi allergy, including elaborating on cross-reactivity where it occurs. To characterise the major IgE binding proteins in the different syndromes and show whether the patterns are different.

3. To identify clinical and biological differences between Hayward and Zespri Gold^{TM} kiwi fruit.

Approach

Two hundred and seventy three patients with self-reported kiwi fruit allergy completed questionnaires, 46 of whom volunteered to participate in clinical investigations, and 117 supplied sera for *in vitro* studies. Information from the questionnaires was used to phenotype the patients, and provided the data required to make recommendation for aim 1. The WHO Decision Tree for predicting allergenicity was used to see if kiwi fruit would be detected as a potentially allergenic food. Extracts of kiwi fruit protein were used to identify allergens in the fruit, and proteins were identified using MALDI TOF. Allergen binding by patient specific IgE was confirmed by ELISA and Western blotting. Further characterisation of the allergens included digestion of kiwi fruit using simulated gastric fluid, followed by SDS-PAGE and Western blotting of the kiwi fruit protein extract. Cross reactivity with other allergens (grass pollen, birch pollen, peanut, house dust mite) was investigated by inhibition ELISA and inhibition Western blots.

Key findings

The results of this study confirmed that kiwi fruit should be considered a significant food allergen, capable of causing severe reactions, particularly in young children. DBPCFC confirmed allergy to kiwi fruit in 53% of the subjects tested, who had a previous history suggestive of kiwi allergy. Skin testing with fresh fruit has good sensitivity (93%), but poor specificity (45%) in this population. CAP sIgE and a commercially available skin test solution were both much less sensitive (54%; 75%) but had better specificity (90%; 67%). Patients with allergy to pollens were as likely to have severe kiwi allergy as those

without. Indeed, inhibition immunoblots and ELISAs showed that cross reactivity with pollens is not common in this UK population.

Using kiwi fruit as a model, we have shown that when a new food is introduced onto the market, its consumption may be low and associated problems will consequently be low. Additionally, the demographics of people consuming a food type will change with time. When kiwi fruit was first introduced it was a novelty food used as a garnish. It is now commonly eaten by infants and young children, as well as adults. It is therefore necessary for post-marketing surveillance to occur for a prolonged period (ie. decades) after the introduction of a new food. The clinicians of the Paediatric Allergy Clinic in Southampton first started to recognise kiwi fruit as an allergenic concern in the late 1990s. Our study would suggest that by this stage a significant number of UK citizens had developed allergy to the fruit. Under the current system even a large allergy clinic will not identify a problem until a large critical mass of the population have developed allergy.

Western blotting to kiwi fruit protein extract revealed twelve proteins which were bound by IgE. A protein with a molecular weight of 38 kDa was the major allergen in this population, recognized by 59% of the population. No IgE bound to actinidin (Act c1), in kiwi protein extract, purified native or recombinant forms of actinidin during Western blotting. Pooled sera bound to kiwi protein extract but not purified actinidin on ELISA. Pre-incubating sera with actinidin did not inhibit IgE binding to kiwi protein extract on immunoblot or ELISA. This confirms that kiwi fruit contains multiple allergens, but actinidin, previously reported as the major kiwi fruit allergen, is not one of them in this large study population.

There were no proteins visible on SDS-PAGE stained with Coomassie following digestion of the kiwi protein extract. However, IgE binding demonstrated that allergenic epitopes persisted in the digested extract. The methodology suggested for predicting allergenicity by digesting the allergens and staining the SDS-PAGE, as recommended by the WHO/FAO is therefore inadequate. This was the first study to demonstrate that patients with oral allergy syndrome react to digestion labile epitopes, in contrast to patients with systemic symptoms whose IgE binds to digestion stable epitopes. Pepsin digestion of kiwi fruit *in vitro* was effected by minor changes in acidity, presumably affecting the sensitization capacity of the proteins.

We have shown that cross reactivity of kiwi proteins with epitopes in grass pollens, birch pollens, house dust mite and carbohydrate determinants is of little clinical relevance in this UK population. This is in contrast to findings from studies in European populations.

This was the first study to report the immunogenicity and clinical allergenicity of gold kiwi fruit, demonstrating that people with kiwi fruit allergy are at risk of developing allergy to gold fruit.

Technical Evaluation

- Post-marketing surveillance is necessary if we are to detect allergy to new foods at an early stage. The surveillance will need to be long term.
- WHO Decision Tree successfully identified kiwi fruit as having a high probability of being allergenic.
- The methods recommended for determining pepsin digestibility detected that kiwi fruit proteins are rapidly digested but failed to identify the allergenicity of the digested

products. Additionally, protein digestion needs to be determined at different pHs in order to reflect the variations within the population. The higher pH of gastric juice in infants may partially explain the increased severity of reactions in this population. Patients on antacid medication may also be at risk.

- The labelling of proteins as major allergens after one report, from one population is clearly inappropriate. Actinidin has been named as a major allergen, but not one of our patients are allergic to this protein.
- Gold kiwi fruit is likely to cause allergic reactions in patients with kiwi fruit allergy. There is extensive cross-reactivity between the allergens in the two fruits.

GLOSSARY

AP	Atopic pool of sera
BSA	Bovine serum albumin
DBPCFC	Double blind placebo controlled food challenge
ESI	Electrospray ionization
FEIA	Fluorescent enzyme immunoassay
HDM	House dust mite
KAP	Kiwi allergic pool of sera
MALDI-TOF	Matrix assisted laser desorption ionization time of flight
MES	Morpholinoethanesulfonic acid
MOPS	Morpholinopropanesulfonic acid
MS	Mass spectrometry
NAP	Non-atopic pool of sera
OAS	Oral allergy syndrome
PBS	Phosphate buffered saline
RAST	Radio Allergo Sorbent Test
SDS-PAGE	sodium dodecylsulphate-polyacrylamide gel electrophoresis
SLIT	Sublingual swallow allergen immunotherapy
TBS	Tris buffered saline
TBS-T	Tris buffered saline with 0.5% tween-20
ELISA	Enzyme linked immunosorbent assay
MS-MS	Tandem mass spectometry
kDa	Kilo dalton
IEF	Isoelectric focusing
PVDF	polyvinylidinedifloride
BLAST	Best local alignment search tool
LDS	Lithium dodecyl sulphate
SGF	Simulated gastric fluid
GI	gastrointestinal
TCR	T cell receptor
Treg	Regulatory T cells
T_h	Helper T cells
WHO	World Health Organization
FAO	Food and Agriculture Organization of the United Nations
GM	Genetically modified
kU_A/L	Kilounits of antibody per litre
LREC	Local Research Ethics Committee
OD	Optical Density
w/v	Weight per volume
pI	Isoelectric point
PAS	Periodic acid-Schiff