Report

Rat Hypersensitivity Study

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Introduction

This experimentation was performed at the request of FDA to answer the question of whether the small amount of denatured shrimp protein still present in the NOCC preparation would present a hazard to individuals with shrimp allergies. The concern was that there is the potential for anaphylactic responses in these individuals upon exposure to NOCC intraperitoneally. To assess this, we used a rat model of Type 1 immediate hyper sensitivity where a shrimp extract was made and injected, subcutaneously, into rats. To test for the development of the characteristic antibody response which would yield Type 1 hypersensitivity, we utilized the Passive Cutaneous Anaphylaxis test, which indicates the presence of reaginic antibody. The basis of this test is to obtain antibody bearing serum from the sensitized animals and inject it into the skin of other, naïve animals to passively achieve local sensitization. Once sensitivity was confirmed, we challenged with the NOCC solution or with a control shrimp extract. Because it might take time for a potential allergen to be liberated from the NOCC solution, the animals were observed closely for three days.

Methods

The shrimp extract was prepared by homogenizing 39.91g of previously frozen shrimp (head removed) in 50 mL ddH20 using a polytron tissue homogenizer. After this, a further 50 mL ddH20 was added. After multiple centrifugations at increasing g force to remove particulates, the extract was filter sterilized and aliquated in 1.5 mL microfuge tubes. A protein assay was performed and the extract was found to contain 344 mg/mL soluble protein.

The extract was injected subcutaneously into 10 mail Sprague Dawley rats (250g) using alum as an adjuvant. Three weeks later, blood was sampled by saphenous vein puncture into heparinized hematocrit tubes and serum isolated. This was then injected (total injection volume of 100µl) at dilutions of ¼, 1/8, 1/16, and 1/32 intradermally into the shaved back of anesthetized naïve male SD rats. Injected rats were left for 24 hours to facilitate arming of local skin mast cells by the reaginic antibody in the serum. Challenge was by intravenous injections (penile vein) of 1 mg shrimp extract and 0.5% Evans blue in file volume of 1 mL. PCA circles were read, with a circle of at least 5 mm being considered positive.

When all of the animals showed positive PCA titres of at least 1/16, the experiment with the NOCC challenge was performed. In this case, 5 mL of sterile 2% NOCC solution was injected into the animals by intraperitoneal injection using an 18 gauge needle. Lower quadrant injection was used to eliminate the possibility of injection in to the gastro-intestinal tract. As a control, 5 mg of shrimp extract was made up to 5 mL in PBS and injected in the same manner.

To blind the experiment, one rat was randomly chosen from each cage (2 rats per cage) and injected with NOCC. This animal was marked on the underbelly so that it could be identified later, but the marking could not be seen during the observation stage. After challenge, the animals were observed for 30 minutes

for the characteristics of mild rodent anaphylaxis (hunched body, non-ambulation, and breathing abnormalities). At this level of sensitization, no deaths were expected. The animals were then observed hourly for 5 hours for the next three days.

Results

The development of hypersensitivity in such models requires care in the level of priming allergen since multiple injections are often needed and the risk of anaphylaxis is present at each injection time point. Thus we chose to use relatively low level priming doses and this required four injections to achieve a robust PCA response in all animals.

At 30 minutes, none of the sensitized animals challenged with NOCC showed any adverse signs (of anaphylaxis). In contrast, all of the shrimp extract challenged animals showed obvious signs of reaction to the challenge. All of the 5 animals had hunched bodes within 30 minutes of the challenge. None of the shrimp-challenged animals exhibited normal ambulation. Two of the five shrimp-challenged animals showed signed of obvious disturbance in breathing.

Rat #	Group	Observation at 30 min
1	Control	Hunched; non-ambulatory; difficulty breathing
2	NOCC	normal
3	Control	Hunched; non-ambulatory
4	NOCC	normal
5	Control	Hunched; non-ambulatory
6	NOCC	normal
7	Control	Hunched; non-ambulatory; rapid breathing
8	NOCC	Normal
9	Control	Hunched; non-ambulatory
10	NOCC	normal

When the animals were observed for 5 hours there was some improvement in the control animals and no change in the NOCC treated animals. By the 24 hour post-challenge, all animals exhibited normal behavior and this continued for the duration of the experiment (to 72 hours post challenge).

Conclusions

We conclude from this experimentation that the residual shrimp protein in NOCC will not induce hazardous anaphylaxis in an animal model and, by inference, in humans. The mass of NOCC (100 mg), and hence, the antigen load, delivered to the rats was such that it would exceed the dosage (adjusted for body mass) that would be expected to be delivered to humans by a factor of 6.

Whether these results are because the level of residual allergen is too low to induce the response or whether the allergen is degraded by the techniques required to produce NOCC is unclear. However, it is our opinion that the latter of these possibilities is almost certainly true. The conditions under which NOCC is derived are not conducive to protein stability.

These data provide evidence that NOCC can be safely used in individuals with shrimp allergies.