

## Negative Oral Provocation Test With Porcine Pancreatic Enzyme Plus Cofactors Despite Confirmed $\alpha$ -Gal Syndrome

Eberlein B<sup>1</sup>, Mehlich J<sup>1</sup>, Reidenbach K<sup>1</sup>, Pilz C<sup>1</sup>, Hilger C<sup>2</sup>, Darsow U<sup>1</sup>, Brockow K<sup>1</sup>, Biedermann T<sup>1</sup>

<sup>1</sup>Department of Dermatology and Allergy Biederstein, School of Medicine, Technische Universität München, Munich, Germany

<sup>2</sup>Department of Infection and Immunity, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg

J Investig Allergol Clin Immunol 2020; Vol. 30(6): 468-469  
doi: 10.18176/jiaci.0513

**Key words:**  $\alpha$ -Gal syndrome. Pancreatic extracts. Anaphylaxis. Cofactors.

**Palabras clave:** Síndrome de alfa-gal. Extractos pancreáticos. Anafilaxia. Cofactores.

We report the case of a 66-year-old patient who presented in August 2016 after a severe anaphylactic reaction. She had been stung by a small dark insect on her wrist while gardening in the morning and subsequently developed a common local insect sting reaction. The insect did not seem to be a wasp or bee, and it was not clear if she had received a tick bite. At noon she had eaten “sour” pork kidneys for lunch (fine strips soaked in milk, briefly fried and seasoned with salt, pepper, and vinegar). An hour and a half later, she complained of dizziness and an itchy rash all over her body, followed by difficulty swallowing and shortness of breath. She collapsed on the way to her family doctor 1 hour later, and emergency medical care was provided, including administration of prednisolone, clemastine, and inhaled adrenalin. She was monitored for several hours in hospital but was discharged the same day.

In 2009, she underwent pancreas head resection (Whipple procedure) for pancreatic carcinoma. Since then, she has had been receiving medication with Creon 25000 (twice daily) and acetylsalicylic acid (ASA, 100 mg). She remembered having had a few tick bites about 20 years ago and regularly consumed innards, although she had never experienced symptoms, and tolerated other mammalian food (eg, pork meat, salami, ham, and milk).

Skin prick testing was performed in 2016 with pork meat, pork kidney, beef meat, beef kidney (each raw and cooked), gelatin, cow milk, cetuximab (500  $\mu$ g/mL), Gelafundin 4%, Creon 10000 pancreatic powder (Mylan Healthcare GmbH), an atopy series (grass, birch, Dermatophagoides pteronyssinus, cat), part of an idiopathic anaphylaxis series (celery, soy lupine flour, gluten, hydrolyzed wheat protein), and spices. Intradermal testing was performed with Gelafundin 4% (undiluted) and bee and wasp venom.

Testing was positive for pork and beef kidney (raw and cooked), cetuximab, Creon, hydrolyzed gelatin (prick), Gelafundin, and bee and wasp venom at a concentration of 0.1 mg/mL (intradermal).

sIgE was determined to  $\alpha$ -gal, bee venom, wasp venom, rApi m 1, rApi m 2, rApi m 5, rApi m 10, rVes v 1, rVes v 5, and CCD MUXF3 (cross-reactive carbohydrate determinants). The  $\alpha$ -gal sIgE value was 3.65 kU<sub>A</sub>/L (<0.10 kU<sub>A</sub>/L). sIgE to all insect venom allergens was negative. Total IgE and tryptase were in the normal range.

In 2016, oral provocation tests were performed with hydrolyzed gelatin (0.5 g, 1 g, 5 g) and pork kidney (3 g, 5 g, 10 g). Hydrolyzed gelatin was tolerated, but the patient developed generalized erythema, throat tightness, and dyspnea 2 hours after consumption of 10 g of pork kidney.

A basophil activation test (BAT) was performed in 2016 with a pork kidney extract (22.7 mg/mL to 0.00022 mg/mL), alpha-Gal-HSA (1000 ng/mL to 0.32 ng/mL) (BAG2-GAL; Bühlmann Laboratories AG), and Creon (2227 ng/mL to 0.0227 ng/mL) according to previous reports [1,2]. All substances activated the basophils.

In 2018, oral provocation tests were performed with 3 capsules of Creon 25000 (first day), ASA (1000 mg) and 3 capsules of Creon 25000 (second day), and, finally, ASA, alcohol (20 mL), physical exercise, and Creon 25000 (third day). No symptoms were recorded. sIgE to  $\alpha$ -gal showed a value of 0.77 kU<sub>A</sub>/L (<0.10 kU<sub>A</sub>/L).

In 2018, the BAT performed 1 day after the oral provocation test series with Creon was positive to alpha-Gal-HSA and negative to Creon and bee and wasp venom. The BAT performed 1 month later was once again positive to Creon (Supplementary Table).

This case shows that Creon, an  $\alpha$ -gal-containing porcine pancreas extract can be tolerated at higher doses than usual and despite cofactors by patients with  $\alpha$ -gal syndrome. This is particularly relevant in cases where the drug is necessary to treat exocrine pancreatic insufficiency. Two similar cases have been described [3], although this is the first case in which cofactors, which are well-known amplifiers of reactions to  $\alpha$ -gal [4], were also tested with the  $\alpha$ -gal-containing drug. The allergy work-up enabled us to rule out hymenoptera allergy as a cause or cofactor of the anaphylaxis.

Several factors may enable patients to tolerate Creon despite sensitization. Provocation tests have generally shown that sensitivity to  $\alpha$ -gal can be quite variable: some patients react only to pork kidney but routinely tolerate muscle meat, even in the presence of cofactors, whereas others, eg, patients with mastocytosis, experience biphasic immediate and delayed severe reactions [5-7]. Higher amounts of  $\alpha$ -gal determinants in kidney than in meat may explain these observations. In the case we report, the patient also tolerated hydrolyzed gelatin. We do not know how much  $\alpha$ -gal is present in Creon. In a recently published study [1] and in the present case, basophil activation was more pronounced with Creon than with pork kidney extract, thus pointing to a relevant amount. Inhibition experiments also revealed that the absolute reduction in  $\alpha$ -gal sIgE binding by Creon could be reduced by up to 97% (median, 83.5%), thus confirming specific recognition of  $\alpha$ -gal epitopes in Creon [3]. In the present case, the eliciting dose of pork kidney was 10 g, and the total amount of Creon ingested was 0.9 g (gel capsules), which might be below the threshold for eliciting symptoms.

In the context of Creon protein extracts [1],  $\alpha$ -gal may not be absorbed in the gastrointestinal tract. In vitro studies

suggest that only lipid-bound  $\alpha$ -gal is able to cross the intestinal monolayer. In vivo,  $\alpha$ -gal could only be detected in the blood when carried by chylomicrons—but not when bound to proteins—3 to 6 hours after consumption of beef [8].

Induction of tolerance to  $\alpha$ -gal in Creon—but not in pork kidneys—through the long-term intake of Creon for pancreatic insufficiency would also be conceivable. The fact that the BAT result was negative after a 3-day, high-dose course of Creon argues for a desensitization protocol that is exclusive to Creon, because basophil activation by alpha-Gal-HSA was not reduced at this time point. Specific desensitization to Creon would argue for a contribution of the protein environment to the  $\alpha$ -gal epitope or a diversity of carbohydrates near the epitope, as suggested elsewhere [9]. Desensitization to  $\alpha$ -gal-containing cetuximab in a rapid protocol resulted in  $\alpha$ -gal sIgE becoming negative [10].

In conclusion, it appears that there is a clinically relevant difference in vivo between oral intake of  $\alpha$ -gal-containing pancreatic powder and  $\alpha$ -gal-containing food. Since positive skin test and BAT results probably do not yield sufficient information to confirm clinical relevance and clinical reactions to cofactors cannot be excluded, provocation tests (with cofactors) are essential if drugs containing  $\alpha$ -gal have to be administered for therapeutic reasons. Based on the extensive testing performed, the patient in the present case was recommended to avoid large amounts of red meat and innards, but to continue taking Creon.

#### Funding

The authors declare that no funding was received for the present study.

#### Conflicts of Interest

BE, MJ, and TB received nonfinancial support from BÜHLMANN Laboratories AG.

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■ Manuscript received March 23, 2020; accepted for publication May 11, 2020.

**Bernadette Eberlein**

Klinik und Poliklinik für Dermatologie und Allergologie am  
Biederstein  
Biedersteiner Str. 29  
80802 München  
E-mail: bernadette.eberlein@tum.de