

Drugs of porcine origin—A risk for patients with α -gal syndrome?



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Clinical Implications

- Drugs of porcine origin such as pancrelipase and Enzynorm f contain α -gal and induce a strong response in skin tests and basophil activation tests in patients with delayed anaphylaxis to red meat. These drugs should be administered with caution in affected patients.

TO THE EDITOR:

The α -gal syndrome is characterized by a delayed allergic reaction to mammalian meat associated with the presence of specific IgE antibodies to galactose- α -1,3-galactose.¹ Sensitized patients are at risk upon ingestion of red meat, in particular pork kidney and innards.² However, diverse other products of mammalian origin have been shown to carry the α -gal epitope and to trigger allergic symptoms, for example, therapeutic mAbs of rodent origin, bovine-derived gelatin colloids, as well as gelatin contained in drugs and sweets.³ Polyclonal antibody preparations such as antivenoms derived from horses have been shown to carry the α -gal epitope,⁴ and a case of hypersensitivity has been reported recently in an α -gal-sensitized patient.⁵ The high gelatin content of some vaccines has been attributed to anaphylaxis upon vaccination in 2 α -gal-allergic patients,^{6,7} and the premature degradation of bioprosthetic heart valves has been associated with the presence of specific IgE in 2 patients.⁸

In the present study, we wanted to analyze the potential allergenicity of the porcine-derived drugs Creon (AbbVie, Inc, North Chicago, Ill), also known as pancrelipase or pancreatin, and Enzynorm f (Nordmark, Uetersen, Germany). Creon is a pancreas extract containing amylases, lipases, and proteases. It is used in the treatment of exocrine pancreatic insufficiency in the context of cystic fibrosis, pancreatitis, and pancreatic cancer. Pancreatin is also marketed under a number of different brand names such as Pancreaze and Zenpep. Enzynorm f contains pepsin as the main ingredient as well as hydrolyzed bovine blood proteins, and it is freely available in drug stores as support for gastric malfunction. Pepsin is approved by the Food and Drug Administration as an over-the-counter drug to support digestion, and it is available either as a single ingredient preparation in a few countries (Canada and Poland) or as a multi-ingredient preparation in many countries in the world.

The presence of α -gal could be demonstrated by immunoblot in protein extracts from both Creon and Enzynorm f (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). An α -gal-reactive band at about 27 kDa as

well as a diffuse reactivity in the low molecular weight range was detected in Creon. Enzynorm f showed a prominent band at 42 kDa corresponding to the main ingredient pepsin. Seventeen patients with α -gal syndrome were recruited in Luxembourg and Germany to evaluate the allergenic potential of both drugs. The study protocol was approved by the ethics committee of each medical center. The characteristics of the patients are presented in Table 1. Prick-to-prick skin tests with the drugs were performed by crushing the content of a Creon 10000 capsule or a pill of Enzynorm f, adding a few drops of saline, and applying the mixture to the skin. Prick-to-prick tests were also done with Gelafundin 4% (B. Braun Melsungen AG, Melsungen, Germany), raw pork and beef, as well as raw pork kidney. Skin tests with the drugs gave strong positive reactions. Enzynorm f, in particular, yielded significantly larger wheals than did pork kidney (Figure 1, A). Eleven controls with specific IgE to α -gal less than 0.1 kU_A/L did show either no reactivity or a maximum wheal of 3 to 3.5 mm (Figure 1, A).

To further investigate the allergenicity of both drugs, basophil activation tests were performed with pork kidney, Creon, and Enzynorm f. Experimental procedures are detailed in this article's Online Repository at www.jaci-inpractice.org. Both drugs were able to strongly activate basophils of patients with delayed allergy to red meat (Figure 1, B). Only 1 patient (P13) was below the threshold of 15% activated basophils for stimulation with Creon. None of the extracts was able to stimulate basophils from 2 atopic controls. Sensitivity of the basophils was assessed by the calculation of CD-sens. The parameter CD-sens is the inverse of the concentration eliciting a release in 50% of the basophils, multiplied by 100 (1/EC₅₀ × 100). Sensitivity of the basophils was significantly higher to Enzynorm f and Creon than to pork kidney (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org).

The specificity of the IgE-mediated reactions was confirmed by inhibition ELISA as previously described.² Human serum albumin carrying α -gal (Dextra Laboratories, Reading, UK) was coated to microtiter plates, and patient sera were diluted and added to the microplate with or without a prior overnight preincubation with Enzynorm f or Creon as inhibitor at 4°C. Detailed experimental procedures are described in this article's Online Repository at www.jaci-inpractice.org. Binding to α -gal could be reduced by 27% to 97% (median 83.5%) for Creon and by 60% to 99% (median 88.5%) for Enzynorm f, confirming the presence of α -gal epitopes in both drugs (see Figure E3 in this article's Online Repository at www.jaci-inpractice.org).

In the present study we showed that α -gal epitopes are present in the porcine-derived drugs Creon and Enzynorm f. Both drugs showed a strong reactivity in skin tests and basophil activation, pointing to a potential risk for α -gal-sensitized patients upon ingestion of these drugs. Reactions were even stronger than those to pork kidney, which is a potent trigger of allergic symptoms in patients with α -gal syndrome. For Creon, the recommended dose ranges from 500 to 2500 lipase units per kilogram of body weight per meal. For Enzynorm f, 1 to 2 tablets are recommended with each meal, with a maximum of 20 tablets per day corresponding to 5 g of pepsin. Upon oral intake of these

TABLE I. Clinical characteristics of patients with allergy to α -gal

Patient no.	Age (y)	Sex	Trigger of anaphylactic reaction	Delay in reaction	Symptoms	Anaphylaxis severity grade (Sampson classification)	Positive SPT result to meat	α -Gal sIgE (kU _A /L)	Total IgE (kU _A /L)
1	67	Female	Pork kidney	1-3 h	F, U, laryngeal AE, Dy	2	PK	3.2	86.4
2	54	Female	Pork kidney	1-4 h	Increased heart rate, F, W, Bm	1	PK, P, B	1.71	20.6
3	59	Female	Pork kidney	1 h	U, AE, Hy	4	PK, P, BK	1.13	34.3
4	52	Male	Pork kidney, beef meat, veal meat	1-6 h	U, AE, Hy, Brsp, S	5	PK, P, BK	88.7	1504
5	53	Male	Meat	3-6 h	U, AE	1	(PK and P: n.d.) BK	51.1	672
6	61	Male	Pork kidney, beef meat, pork meat	3-6 h	U, Hy, Brsp, S	5	PK, P, BK	1.4	82.8
7	61	Female	Pork kidney, pork meat	3-6 h	U, AE, Hy	4	PK, BK	2.41	46
8	13	Male	Pork meat	2-3 h	Ap, U, V, heart palpitations	3	P, PK	69	765
9	19	Male	Sport	1-4 h	U, nausea, and Ap (1 episode)	1	B, PK	5.52	80
10	29	Female	Hacked meat, ham, sausage	1-6 h	U, palpebral AE	1	B, PK	1.55	72
11	31	Female	Cheese burger or cheese	3-4 h	U, lingual AE	1	B, P, PK	2.02	18
12	46	Male	Hacked meat, sausage, cheese	3-4 h	U	1	B, PK	18	32
13	48	Male	Sausages	2-6 h	U, V, D, labial or laryngeal AE	2	B, P, PK	12	126
14	20	Male	Bolognaise or cheese	1-4 h	Ap, V, F	1	PK	13	143
15	62	Male	Infusion beef gelatine	Immediate	Perioperative anaphylaxis	4	PK	18	4993
16	27	Male	Beef meat and other red meats, exacerbation with alcohol	4-6 h	U, Dy, Wh	2	B, P, PK	66	373
17	32	Male	Beef and pork meat	3-6 h	Acute U, Dy, C, laryngeal dysphagia	2	B, P, PK	27	401

AE, angioedema; Ap, abdominal pain; B, beef; BK, beef kidney; Bm, bowel movement; Bp, breathing problems; Brsp, bronchospasm; C, coughing; D, diarrhea; Dy, dyspnea; F, faintness; Hy, hypotonia; nd, not done; P, pork; PK, pork kidney; S, syncope; sIgE, specific IgE; SPT, skin prick test; U, urticaria; V, vomiting; W, weakness; Wh, wheezing. Note: Total IgE and serum sIgE levels against α -gal (o215) were measured with an immunoenzymatic assay (ImmunoCAP; Thermo Fisher Scientific, Uppsala, Sweden).

drugs, allergic reactions are expected to be delayed, as for those described for gelatin-containing products.⁹ Because none of the patients of this study required medication with Creon or Enzy-norm f, it was not possible, for ethical reasons, to perform oral challenges with these drugs. The administration of Creon is also indicated in patients with exocrine pancreatic insufficiency due to different conditions such as chronic pancreatitis or pancreatectomy. These patients are usually older and may bear a higher risk of allergic reactions than young patients with cystic fibrosis because α -gal allergy is more prominent in adults.³ The World Health Organization pharmacovigilance database (Vigibase; <https://www.who-umc.org/vigibase/vigibase/>) was queried in November 2018 with the narrow MedDRA standardized medical query “hypersensitivity” and it retrieved 437 cases involving pancreatin. More than 20 marketed drugs containing

pancreatin were listed. Pancreatin was the only suspected drug in 268 cases, mostly originating from the United States (44%) and Europe (30%). Most patients were women (61%), and median patient age was 55 years. Besides rashes accounting for half of the cases, and 10% of unspecified hypersensitivity reactions, 96 reports (22%) were urticaria and 15 (3%) were angioedema. The standardized medical query “hypersensitivity” retrieved 8 cases involving pepsin, 6 of them originating from Asia, relating to skin rash in children who recovered after drug discontinuation. Unfortunately, no additional data were associated with these cases, and a link to meat allergy or α -gal syndrome can neither be established nor refuted.

Our data clearly showed that patients clinically reactive to red meat had a strong reactivity to both drugs in skin tests and basophil activation tests. In our study, we report a theoretical

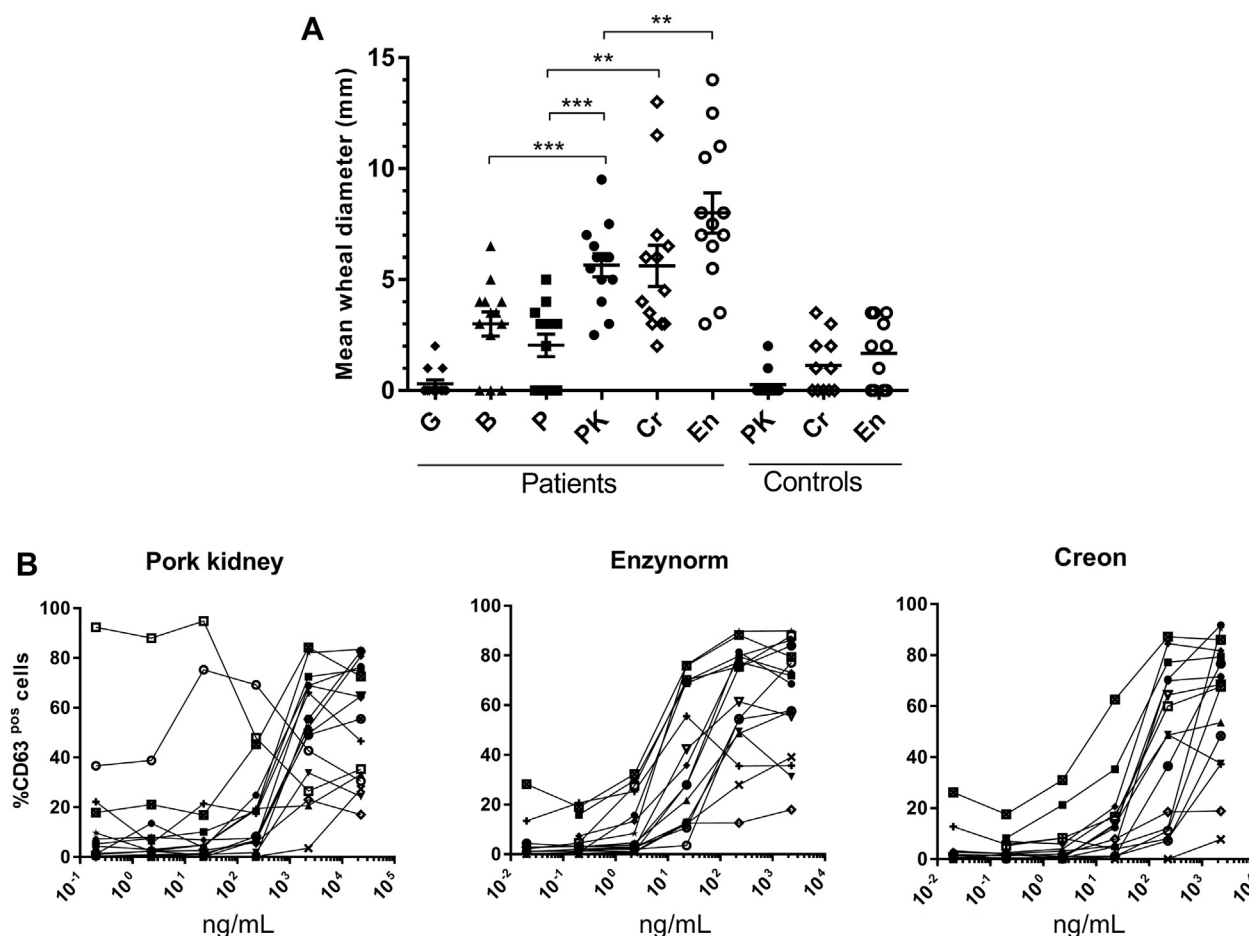


FIGURE 1. Porcine-derived drugs elicit positive skin reactions and basophil activation in patients with α -gal syndrome. **A**, Wheal diameter of prick-to-prick tests with Gelafundin (G), raw beef (B), pork (P), pork kidney (PK), Creon (Cr), and Enzynorm f (En). $**P < .01$ and $***P < .001$ for the comparison between groups using the paired t test. **B**, Patients' basophils were incubated with increasing concentrations of PK, Cr, and En. Relative cell counts of CD63⁺ basophils were determined by flow cytometry.

risk because no case has been reported so far. However, these drugs should be administered with caution in patients with α -gal syndrome until the actual risk has been cleared or established.

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ONLINE REPOSITORY

METHODS

Reagents

Gal- α -1,3-gal- β -1,4-GlcNAc human serum albumin (α -gal-HSA; NGP2334) was obtained from Dextra Laboratories (Reading, UK). The mouse monoclonal anti- α -gal IgM antibody, clone M86, was purchased from Enzo Life Sciences (Lausen, Switzerland). HSA was obtained from Sigma-Aldrich (Diegem, Belgium).

Basophil activation test

Basophil activation test was performed with whole blood from patients using the Flow CAST kit (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland) according to the manufacturer's instructions. Pork kidney extract was prepared as described.^{E1} Creon and Enzynorm f were prepared as follows. A Creon capsule was opened and 15-mg granules were dissolved in 0.5 mL PBS with a protease inhibitor cocktail (Roche, Mannheim, Germany). The coating of an Enzynorm f pill was washed away in 5 mL water; the rest of the pill was dissolved in 5 mL PBS with protease inhibitor cocktail by vortexing. Creon and Enzynorm f preparations were centrifuged for 5 minutes at 5000g and only the supernatant was used in basophil activation test. Relative cell counts of CD63⁺ basophils were determined by flow cytometry (FACSCanto flow cytometer, BD Biosciences, San José, Calif) upon stimulation with increasing amounts of protein (0.1 ng/mL to 100 μ g/mL). Basophils were gated as CCR3⁺/side scatter^{low}. Upregulation of the basophil activation marker CD63 was calculated on the basis of the percentage of CD63⁺ cells compared with the total number of identified basophils. CD-sens was determined as the inverse value of the concentration at half-maximal stimulation, multiplied by 100 (1/EC50 $\times$ 100).^{E2}

SDS-PAGE and immunoblot

Proteins were separated under reducing conditions using 10% SDS-PAGE and immunoblotting as described.^{E3} Pork kidney, Creon, and Enzynorm f were prepared as mentioned earlier. Proteins carrying α -gal were detected with a mouse monoclonal anti- α -gal IgM antibody diluted one-third in blocking buffer (Tris-buffered saline, 0.05% Tween-20; 3% BSA [Sigma-Aldrich]) followed by incubation with a rat monoclonal anti-mouse antibody labeled with alkaline phosphatase (Southern Biotech, Birmingham, Ala). Bands were visualized using nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (Promega, Leiden, The Netherlands).

ELISA inhibition

Specific IgE to α -gal was detected by coating α -gal-HSA overnight at a concentration of 2 μ g/mL to microtiter plates (NUNC MaxiSorp, Roskilde, Denmark). Unspecific binding was blocked by the addition of blocking buffer (1% HSA in PBS) for 30 minutes. Patient sera were diluted at one-fourth in blocking buffer and added to the wells. For inhibition assays, 100 μ g/mL of Creon or Enzynorm f was added to the serum and incubated overnight before adding to the microplate. Binding of specific IgE was detected by addition of biotin-labeled anti-human IgE (Southern Biotech) followed by incubation with alkaline phosphatase-labeled streptavidin (BD Biosciences, Erembodegem, Belgium) and color development with para-nitrophenyl phosphate (Sigma-Aldrich). Optical density was read at 405 nm.

Statistical analysis

Statistical analyses were carried out using GraphPad Prism 7.0 (GraphPad Software, San Diego, Calif). All statistical tests were performed 2-sided, and a significance level of 5% was used.

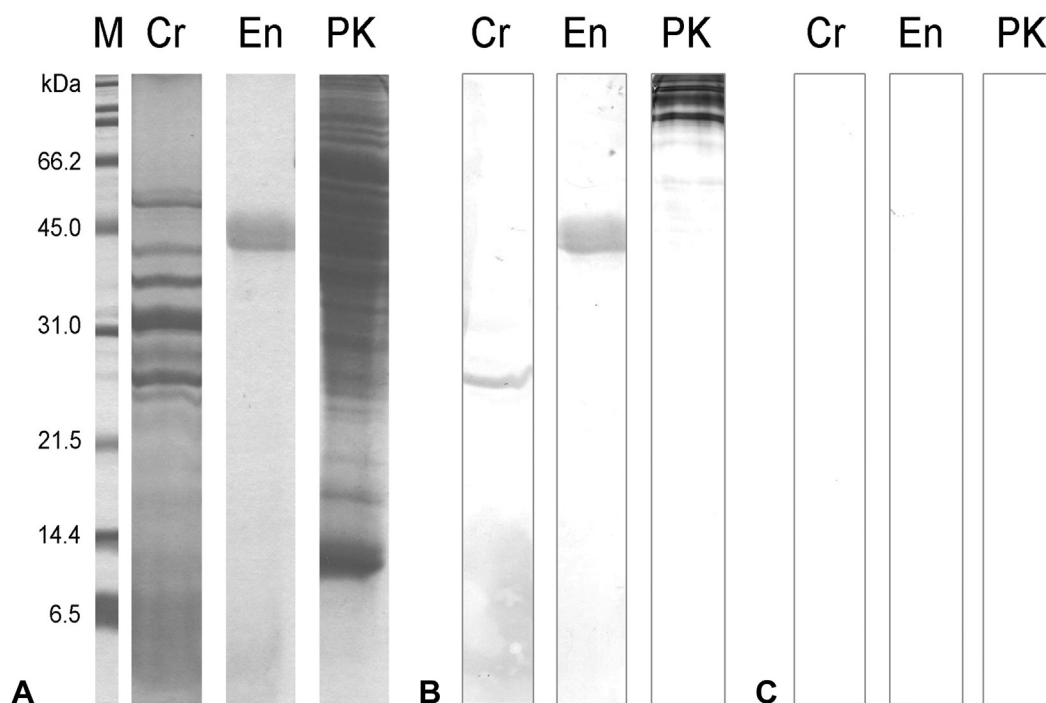


FIGURE E1. Porcine-derived drugs contain α -gal epitopes. **(A)** Coomassie-stained SDS-PAGE gel, **(B)** corresponding immunoblot developed with anti- α -gal antibody, and **(C)** negative control. *Cr*, Creon; *En*, Enzynorm f; *M*, molecular weight marker; *PK*, pork kidney.

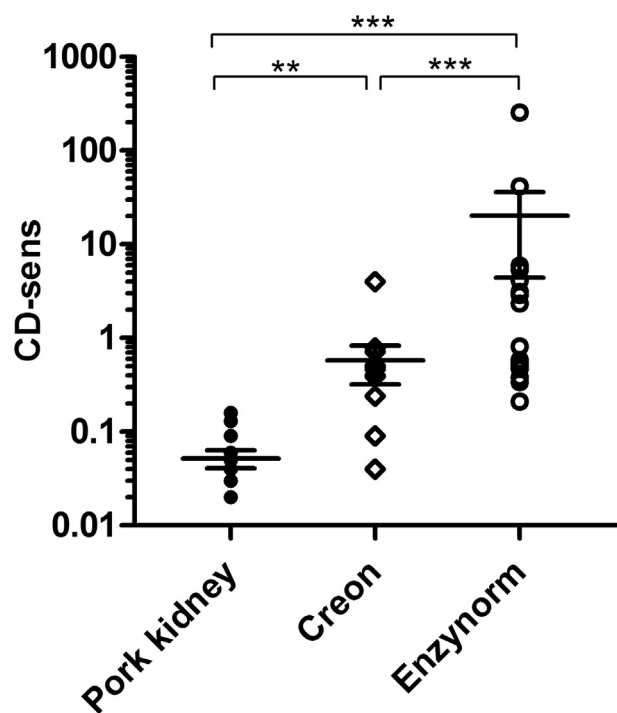


FIGURE E2. Basophils are significantly more sensitive to Creon and to Enzynorm f than to pork kidney extract. $**P < .01$ and $***P < .001$ for the comparison between groups using the Wilcoxon matched pairs test.

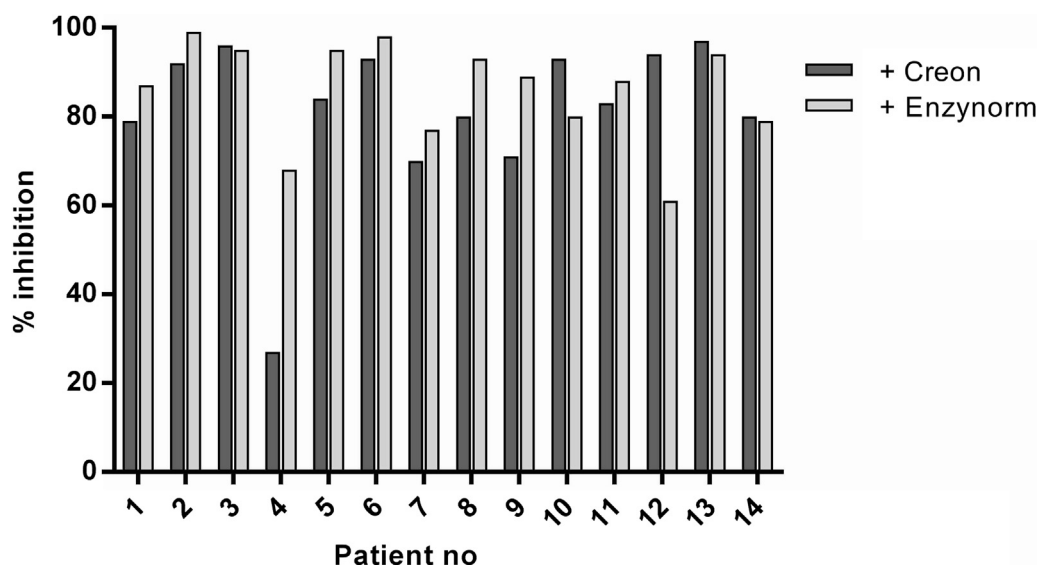


FIGURE E3. ELISA inhibition confirms IgE reactivity to porcine-derived drugs. Inhibition with 100 $\mu\text{g/mL}$ of each drug strongly reduced binding to $\alpha\text{-gal}$.

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