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Anaphylactic reaction to galactose-derived oligosaccharides residues from lactose used as a drug excipient

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To the editor:

Inhaled steroids are currently the most widely used and recommended medicines for the management of persistent asthma because of their high local and anti-inflammatory efficacy.[1] There are three main presentations of the drug: aerosol spray (hydrofluoroalkane – HFA - as a propellant), liquid solution and dry powder inhaler. Dry powder devices are often preferred over HFA sprays in patients over 6 years of age, both for environmental issues and for the intrinsic advantages of the technique, such as ease of use and greater uptake and metabolism of the drug.[1,2]

As with many pediatric medications, many of the steroids presented in dry powder inhaler have lactose as excipient. This substance has the sole function of stabilizing the pharmaceutical preparation, but it has been associated with the occurrence of important adverse reactions in patients at risk.[3] Some case reports demonstrated hypersensitivity reactions due to contamination of lactose with cow's milk proteins, such as casein and β-lactoglobulin.[2]

Recently, non-protein food allergens with important clinical repercussions have been discovered, for example, galactose-alpha-1,3-galactose (alpha-gal), an oligosaccharide expressed on glycoproteins and non-primate mammalian glycolipids. The production of alpha-gal-specific IgE and its causal relationship with anaphylactic reactions after ingestion of red meat is well known, but its relation with milk is not well established.[4]

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This report aims to describe a case of hypersensitivity reaction after the use of dry powder inhalation medication containing lactose as excipient, possibly associated with galactose-derived oligosaccharide residues.

An 8-year-old boy was referred to the pediatric allergy and immunology department of a university hospital due to cow's milk protein allergy, as well as having asthma and allergic rhinitis. After severe anaphylaxis, with glottis edema, following the ingestion of cow's milk for the first time at 3 months of age, he was diagnosed with cow's milk protein allergy and required hospitalization at the Intensive Care Unit. Further investigation demonstrated casein-specific IgE:> 100 kU/L and β lactoglobulin-specific IgE: 2,3 kU/L both dated from March 30, 2016. Total serum IgE level was 400 on June 9, 2016. The patient's family was then advised to exclude cow's milk and dairy products from his diet, which he did.

For the management of asthma and rhinitis, budesonide nasal spray, loratadine and budesonide dry powder inhaler were prescribed. The mother reported that after the use of two consecutive doses of inhaled budesonide, the child showed pallor and dyspnea, with improvement after use of short-acting β2-agonist. Continuous use of budesonide nasal spray was maintained without any complaint.

Considering the clinical condition of the patient, who had weekly respiratory symptoms with a frequent need for rescue medication, and a pulmonary function test which characterized uncontrolled asthma (Forced Expiratory Volume in First Second / FEV1 = 73% and Intermediate Forced Expiratory Flow at 25-75% of the pulmonary volume/ FEF 25- 75% = 56%), there was a need for the use of inhaled steroids between asthmatic attacks, according to the Global Initiative for Asthma GINA.[1] To evaluate the possibility of safe use of budesonide dry powder inhaler for this patient, we chose to perform a challenge test with the hypothesis of a possible hypersensitivity reaction to the drug.

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A placebo dose (one capsule without content) was initially performed, without any clinical repercussion. After the first dose of the medication (budesonide dry powder inhaler with lactose as excipient), the patient complained of lingual and labial pruritus, associated with pallor. A decrease in oxygen saturation from 97% to 91% was identified, with a rapid recovery, without requiring oxygen therapy. After 10 minutes, the child was again asymptomatic and remained so throughout the observation period. The challenge test was therefore considered positive.

The patient was then instructed to use continuous HFA-beclomethasone dipropionate and to avoid the use of any medication containing lactose as excipient. Seven days after the positive challenge test, the child was reevaluated and no late allergic symptoms or adverse reaction to HFA-beclomethasone dipropionate were reported.

In order to identify if there was contamination of the lactose (used as excipient of budesonide dry powder inhaler) with cow's milk proteins, a study of the medication used in the challenge test was performed using the mass spectrometry method.

The sample of medication (SP1) used in the child was compared with four other samples: two capsules of the same medication, dosage form and manufacturer, but from different manufacturing batches (SP2 and SP3); a budesonide nasal spray from the same manufacturer (SP4), and a negative control medication (HFA-beclomethasone dipropionate), from a different provider. (SP5).

An aliquot equivalent to 200 µg of each active ingredient from samples was diluted in methanol:water (1:1) to a final volume of 1 mL. After homogenization, samples were sent to high-resolution mass spectrometry (HRMS), in which they were directly infused into an ESI-LTQ-XL Orbitrap Discovery mass spectrometer (Thermo Scientific, Bremen, Germany). Data acquisition was carried out for positive ions, survey scan mode, in the mass range of 50-2000 *m/z*. Operating conditions were as follows: 6 units of sheath gas, ESI source at 4 kV,

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flow rate of 10 μL.min^{-1} and capillary temperature of 280ºC. Spectral data were processed using XCalibur software (Thermo Scientific, San Jose, CA). All samples were acquired in quintuplicates.

HRMS data was then submitted to statistical treatment using partial least squares discriminant analysis (PLS-DA) through the online platform Metaboanalyst 3.0.[5] Spectral data were submitted to range scaling for data normalization prior to the analysis. PLS-DA provided a score plot (Figure 1) that shows clear clusters of samples, separating the medication used in the child (SP1) from the other two samples from different batches (SP2 and SP3) and, as expected, from the other dosage form and the negative control. For the election of biomarkers that are specific for SP1, a heatmap was built with features based on the variable importance in projection (VIP) scores, and four of the most relevant features for SP1 were elected and submitted to database search in METLIN (Scripps Center for Metabolomics and Mass Spectrometry, La Jolla, CA) for matches, based on mass accuracy (error ≤ 2 ppm). Out of these, three had molecules that are compatible with galactose-derived oligosaccharide residues that may exhibit allergenic potential:

1. Galabiose (*m/z* 707.2216), an oligosaccharide that has an alpha-gal-like structure, differing only in the glycosidic bond.

2. A series of Gal-ceramides (Gal-Cer) isomers (*m/z* 1397.9243 and *m/z* 1572.9022), which may be derived from milk.

3. Phosphatidylinositol bisphosphate (PIP2), a common lipid class found in milk that assisted in corroborating the origin of the components.

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No traces of milk proteins, either casein or lactoglobulines, were found. The identified ions are provided in Table 1. Due to the impossibility of dosing galabiose-specific IgE or galacto-oligossacharides (GOS), alpha-gal-specific IgE concentration was determined (ImmunoCAP method) and the result was 0.11 kU/L (low sensitization).

This study was approved by the ethics committee and informed consent was obtained from the patient's family.

This is, to our knowledge, the fourth pediatric case reported in the worldwide literature on hypersensitivity reaction after use of inhaled lactose-containing excipient medication; the first case related to the use of budesonide dry powder inhaler. It is, however, the first case report to describe an anaphylaxis after inhalation of galactose-derived oligosaccharide residues from the contamination of lactose used as an excipient in the formulation of an asthma medication.

In 2004, Nowak et al described the case of an 8-year-old child with a diagnosis of asthma and allergy to cow's milk, who had anaphylaxis after inhaling a dry powder medication containing a combination of fluticasone and salmeterol. In this case the contamination of the excipient with cow's milk protein fractions (β-lactoglobulin and casein) was demonstrated by the ELISA method and the specific labeling with monoclonal antibodies against milk proteins method.[2] Robles et al published the second pediatric case in 2014 which described a hypersensitivity reaction after using this same medication. In this case there was no characterized anaphylaxis and the contamination of the formulation used with cow's milk proteins was not tested.[6]

There are two other cases related to this topic: an adult allergic to cow's milk protein who presented exacerbation of asthma and atopic dermatitis after using inhaled formoterol dry powder inhaler containing lactose as an excipient[7], and one case described in

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2016 by Morikawa et al. regarding a 6-year-old girl who presented an anaphylactic reaction after inhalation of laninamivir (a medication used in the treatment of influenza).[8]

It is known that food allergy is now one of the main allergic manifestations present in childhood. Cow's milk is the main food triggering the disease, affecting between 1 and 5% of children.[9] It has been observed in recent years the increase in the incidence and prevalence of food allergy, as well as other allergic manifestations such as asthma, suggesting an association in the pathophysiology, epidemiological and genetic factors of these diseases. Children with food allergy are 2 to 4 times more likely to have another allergic manifestation, such as asthma, allergic rhinitis and atopic dermatitis than children who are not allergic to food.[10] Despite this well-known relationship, many of the medications for asthma and other allergic diseases use lactose as an excipient, a fact that exposes patients sensitized to cow's milk at greater risk of allergic reactions.

The case reported in this study, unlike the cases already described in the literature, describes a situation in which a patient, who underwent a cow's milk exclusion diet throughout life, had anaphylaxis after inhaling galactose-derived oligosaccharides residues from degradation of the lactose used as excipient of an asthma medication.

The role of oligosaccharide galactose-alpha-1,3-galactose (alpha gal), a component of glycoproteins and glycolipids of non-primate mammals, as food allergens has been studied for approximately 10 years. The manifestations of anaphylaxis resulting from the production of alpha-gal-specific IgE after exposure to the monoclonal antibody Cetuximab are well known. It has also been shown that the bite of the *Amblyomma americanum* tick species promotes individual sensitization to alpha-gal, inducing the production of specific IgE for such epitope, leading to late anaphylactic reactions after ingestion of red meat.[11,12]

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In general, alpha-gal specific IgE mediated reactions occur in older children, not necessarily sensitized to cow's milk protein. The presence of the alpha-gal portion in mammalian milk, such as cow and goat, has already been evidenced through positive immunoassay results. Thus, in the investigation of cow's milk allergy, alpha-gal-specific IgE should be considered as an auxiliary test, and cross-reactivity between meat and cow's milk should be considered.[4,13]

In this study, mass spectrometry revealed the presence of Galabiose, which is an oligosaccharide that has an alpha-gal-like structure, differing only in the glycosidic bond. Whereas alpha-gal is a [Gal(α1-3) Gal] species, galabiose is a [Gal(α1-4) Gal] saccharide. Although selected with the assistance of METLIN database, since HRMS is a technique unable to distinguish between structural isomers, it is not possible to determine accurately the glycosidic bond type. Hence, we hereby propose that this species may be considered and understood as a potential allergen in milk and its derivatives. Current literature brings galabiose as an adhesion molecule for bacterial fimbriae in infection mechanisms linked to immune response in humans, but its potential as an allergen has not yet been discussed.[14] Moreover, Soh and colleagues (2015) have discussed the characteristics and allergenicity potential of galacto-oligossacharides (GOS), a class that comprises short-length molecules with 2 to 6 saccharide units.[15] Thus, due to its election as a marker in the SP1 medication that leads to an anaphylactic condition reported herein, we therefore propose galabiose as a potential immunoreactive compound in individuals with milk allergy. Similarly, the other series of Gal-Cer presented both alpha-Gal and GOS-derived moieties in their structure. Although chemically bonded to a ceramide, these saccharides in the structure may be released under poor drug storage. For example, considering inadequate temperature and humidity conditions, a microenvironment may be formed inside the drug capsule that leads to the formation of Gal-Cer and galabiose byproducts, thereby releasing alpha-Gal and other GOS-

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derivatives *in situ* upon cleavage. This results in an effective allergen delivery mechanism in lung circulation, even considering a potentially high threshold for these species.

The main limitation of our report is the absence of measurement of specific IgE to galabiose and Gal-Cer. The technique for such measurement is not available in our scientific environment. However, we evaluate that the low sensitization to alpha-gal may be a surrogate marker, due to cross-reactivity between similar molecules. Therefore, the different patterns of linkage of the galactose moieties between the molecules of Galabiose and alpha-gal may justify the weak IgE response to alpha-gal. Furthermore, as discussed, there may be potential of alpha-gal to be formed *in situ*, even in small quantities, due to its presence in the structure of the described Gal-N-modified ceramides.

Considering the increasing prevalence of cow's milk allergy and asthma in the pediatric population and the frequent use of inhaled corticosteroids in this age group, the awareness of hypersensitivity reactions to components of lactose, frequently used as excipient of these medications, is of critical importance. To our knowledge, this is the first report suggesting a potential role of Galabiose and Gal-cer as food allergens derived from lactose in a pharmaceutic compound. We propose a new line of research focused on the role of galactose-derived oligosaccharide residues as milk food allergens.

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Fig. 1. Score plots generated by PLS-DA based on samples from mass spectrometric data. It is possible to observe a clear clustering of the budesonide medication given to the patient (SP1), isolating it from the other two samples of the same product, but from different batches (SP2 and SP3), which clustered together. The product with a different dosage form (SP4) and the negative control (beclometasone dipropionate, SP5) are clustered independently from the other samples, as expected.

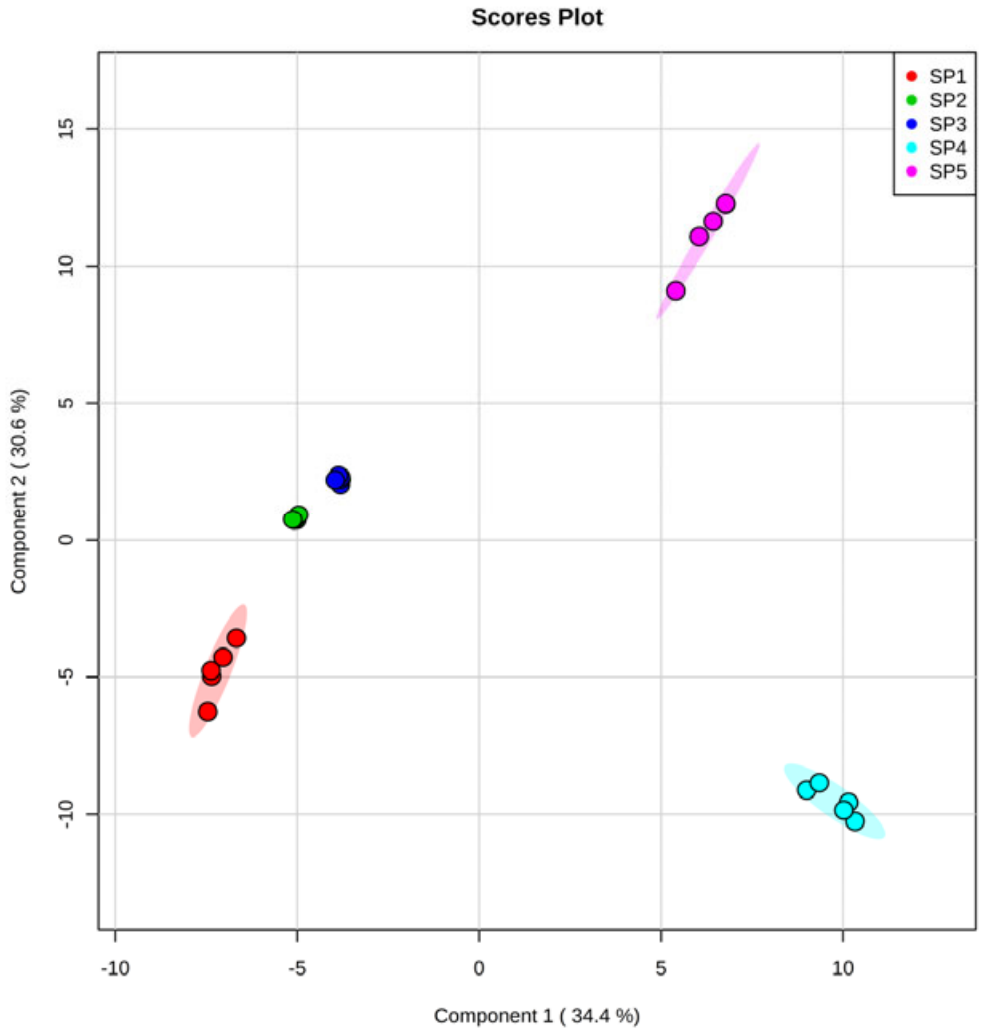

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Table 1. Biomarkers for the SP1 medication, identified by PLS-DA analysis.

Exp. Mass	Theor. Mass	Error (ppm)	Ion adduct	Identified compound*
707.2214	707.2216	0.3	$[M+Na]^+$	Galabiose
1397.9259	1397.9243	-1.1	$[M+CH_3OH+H]^+$	GalNAcbeta1-3Galalpha1-4Galbeta1-4Glcbeta-Cer(d18:1/26:1) and/or GlcNAcbeta1-3Galalpha1-4Galbeta1-4Glcbeta-Cer(d18:1/26:1) and/or Galalpha1-3(GalNAcbeta1-4)Galbeta1-4Glcbeta-Cer(d18:1/26:1) and/or Galalpha1-3(GlcNAcbeta1-6)Galbeta1-4Glcbeta-Cer(d18:1/26:1) and/or GlcNAcbeta1-3Galalpha1-3Galbeta1-4Glcbeta-Cer(d18:1/26:1) and/or GalNAcbeta1-3Galalpha1-3Galbeta1-4Glcbeta-Cer(d18:1/26:1) and/or Galbeta1-3GalNAcbeta1-4Galbeta1-4Glcbeta-Cer(d18:1/26:1) and/or Galbeta1-3GlcNAcbeta1-3Galbeta1-4Glcbeta-Cer(d18:1/26:1) and/or Galbeta1-4GalNAcbeta1-3Galbeta1-4Glcbeta-Cer(d18:1/26:1)
1572.9022	1572.9001	-1.3	$[M+H-H2O]^+$	NeuGcalpha2-3Galbeta1-4GlcNAcbeta1-3Galbeta1-4Glcbeta-Cer(d18:1/20:0) and/or Galbeta1-3GalNAcbeta1-4(NeuGcalpha2-3)Galbeta1-4Glcbeta-Cer(d18:1/20:0) and/or NeuGcalpha2-3Galbeta1-3GalNAcbeta1-4Galbeta1-4Glcbeta-Cer(d18:1/20:0)
1001.4539	1001.4557	1.8	$[M+H-H2O]^+$	PIP2(18:1/18:3) and/or PIP2(18:3/18:1)

* Isomers are indicated as “species 1 and/or species 2…”, etc.

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