

Short Communication

Acute allergic reaction to an iron compound in a milk-allergic patient

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A child allergic to cow's milk developed a mild systemic allergic reaction after the first dose of Ferplex 40® (iron proteinsuccinylate). Skin tests and *in vitro* studies were performed in the child, in three cow's milk-allergic controls and in a non-allergic control. Milk, casein and iron proteinsuccinylate (Ferplex 40®) were used for skin tests, specific immunoglobulin E (IgE) determination, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), immunoblotting and enzyme allergeo sorbent test (EAST) inhibition. A review of the drug information sheet and contact with the manufacturer were also performed. Although proteinsuccinylate is indeed a succinylated casein (each dose containing about 575 mg of casein) there was no indication of the milk protein content in the prescribing information provided by the manufacturer. Skin tests and specific IgE were positive in the case and in all allergic controls, except for EAST to iron proteinsuccinylate in one control. In EAST, iron proteinsuccinylate in solid phase was 100% inhibited by casein and casein in solid phase was inhibited 74% by iron proteinsuccinylate. SDS-PAGE of iron proteinsuccinylate showed a broad 46 kDa band and a blur of aggregated material. On immunoblot, the patient's IgE reacted to this heavily aggregated material and in the native casein extract recognized a 35-kDa band. The allergenicity of succinylated casein (proteinsuccinylate) among milk-allergic children is demonstrated. The protein source used in drug-protein conjugates should always be indicated by the manufacturer (as it should be in foods) to avoid potential risks to allergic patients.

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Iron proteinsuccinylate binds iron to a succinylated casein, improving digestive tolerance and increasing the absorption of iron (1–5). It was first introduced in Italy in 1986 and it is commercialized under different brand names in several European countries and in Latin America, and it has been available online in the USA for the last 3 yr. Despite the potential risk of administration of milk proteins to milk-allergic patients, we are not aware of any published case of allergic reaction to this compound in any patient.

Case report

A 4½-yr-old atopic child with fish intolerance but without evidence of allergic sensitization to fish was admitted to our hospital for control of milk allergy.

The patient had been diagnosed of cow's milk allergy in another hospital because of two episodes of immediate pruritic perioral erythema after the first and second intake of cow's milk when he was 5 months old. Positive skin

Table 1. Characteristics of the case and of the three milk-allergic control children

	Symptoms with milk	CAP milk (IgE U/ml)	EAST casein (IgE U/ml)	EAST Ferplex® (IgE U/ml)
Case: M, 4 yr 6 months	Contact urticaria	12.6	3.36	1.39
C-1: F, 2 yr 4 months	Urticaria, vomiting	19.1	7.43	4.3
C-2: M, 8 yr 10 months	Anaphylaxis	3.46	1.85	1.29
C-3: M, 15 months	Perioral symptoms, atopic dermatitis	3.82	2.87	<0.2

EAST, enzyme allerge sorbent test; M, male; F, female; C-1, C-2, and C-3 are controls. Perioral symptoms include immediate perioral erythema and pruritus.

prick tests and specific immunoglobulin E (IgE) to milk and milk proteins (casein, α -lactalbumin, β -lactoglobulin) were found at the age of 2. A milk challenge test was carried out, being positive with 5 ml of adapted formula. The child has always been on a strict cow's milk-free diet, but sensitivity persisted as evidenced by urticaria after accidental skin contact with milk.

In a routine blood test, mild anemia was diagnosed by his family pediatrician and Ferplex 40® (Italfarmaco S.A., Alcobendas, Spain) (iron proteinsuccinylate 800 mg suspension) was prescribed. Ten to fifteen minutes after the intake of the first dose, the patient began with palpebral angioedema, perioral erythema, dyspnea and rhinitis, which resolved in 2 h with injectable steroid treatment in an emergency room.

Methods

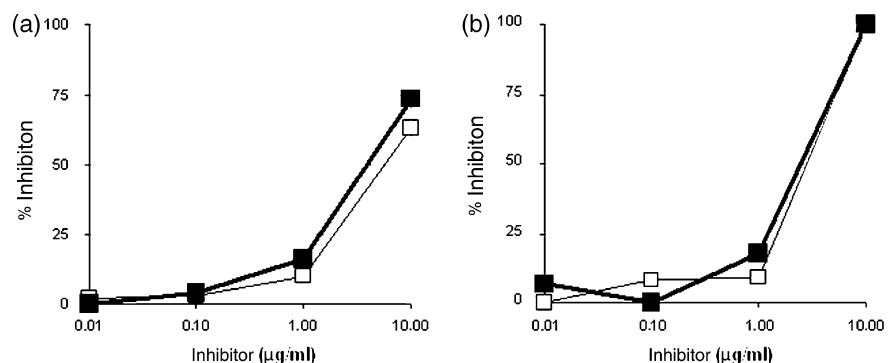
To investigate the reaction, several aspects were analyzed. First, the drug information sheet (6) was reviewed to investigate about the content of dairy proteins in the product. Second, the protein content in milk formula (7) was used to estimate the amount of milk proteins taken by the patient in the previous positive challenge. Finally, the manufacturer of Ferplex 40® was contacted to estimate the amount of protein administered to the patient during the adverse reaction. Then, *in vivo* skin prick tests (8) with whole milk extract, casein – both at 10 mg/ml (Laboratorios

Leti, Barcelona, Spain) – and Ferplex 40® 'as is' and an *in vitro* study [sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of Ferplex40®, specific IgE to milk and casein by CAP System (Pharmacia, Uppsala, Sweden) and immunoblot to casein and Ferplex 40®] were carried out. The relationship of previous sensitivity to casein and the observed sensitivity to iron proteinsuccinylate were investigated by means of an enzyme allerge sorbent test (EAST) on cyanogen bromide-activated cellulose disks and IgE inhibition assays, in serum samples from the patient and three control children with cow's milk allergy not previously exposed to the product (clinical description in Table 1). Serum from a non-atopic subject without milk intolerance was always included as a negative control in EAST and in immunoblot experiments.

Results

No indication about the protein used as source material of proteinsuccinylate was found on the information sheet. The manufacturer confirmed that proteinsuccinylate is actually a succinylated casein. Each vial of 15 ml contains about 575 mg of modified casein, an amount roughly equivalent to the amount of casein present in the same quantity of cow's milk (7), and 5–10 times the dose of casein that elicited a positive challenge with an adapted formula 2 yr before. Skin tests with whole milk, casein and iron proteinsuccinylate were positive in the index case

Fig. 1. Enzyme allerge sorbent test inhibition. (a) Casein disks (solid phase); (b) iron proteinsuccinylate disks (solid phase). ■, casein (inhibitor); □, iron proteinsuccinylate (inhibitor).



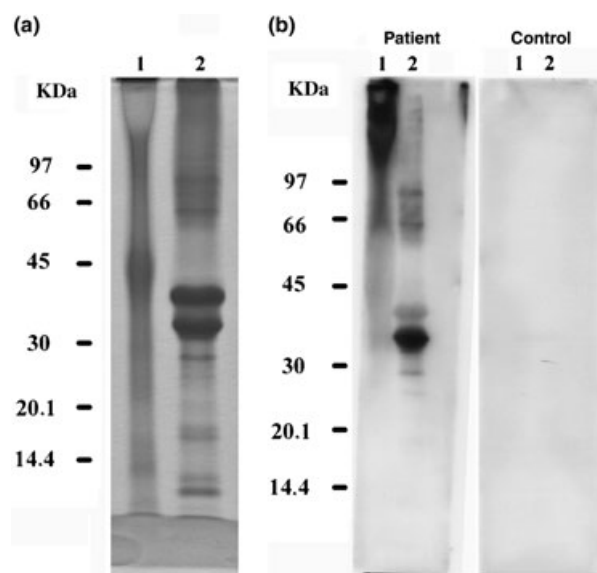


Fig. 2. (a) Fifteen percent sodium dodecyl sulfate-polyacrylamide gel electrophoresis stained with Coomassie blue. (b) Immunoglobulin E immunoblot from the index case (patient) and from a non milk-allergic patient (control). Lane 1: iron proteinsuccinylate; lane 2: casein.

and in the three milk-allergic controls. Both the patient and the milk-allergic controls displayed serum-specific IgE to milk and casein (Table 1). Immunoglobulin E to iron proteinsuccinylate was found in the patient and in two of the controls.

Enzyme allerge sorbent test inhibition experiments showed a high degree of cross-reactivity between casein and iron proteinsuccinylate. IgE binding to casein (Fig. 1a) was strongly inhibited by iron proteinsuccinylate (74% at 10 μ g/ml); in turn, casein completely inhibited IgE binding to proteinsuccinylate (Fig. 1b).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of iron proteinsuccinylate suggested a high degree of chemical modification when compared with purified cow's milk casein (Fig. 2a). The characteristic pattern of two main bands (i.e., α and β caseins) was replaced by a broad protein band at about 46 kDa and a blur of aggregated material. Similar differences were observed in immunoblots; patient's IgE bound strongly iron proteinsuccinylate as aggregated material while a discrete band pattern (mainly a 35-kDa band) was observed in purified casein (Fig. 2b). No IgE binding was detected with the serum from a patient without milk intolerance (Fig. 2b).

Discussion

The deficient absorption of iron, its poor digestive tolerance and its bad taste have been a

challenge for the treatment of anemia in children. Conjugation of iron to proteins has proved a valid strategy to, at least partially, overcome these problems. The binding of iron to succinylated milk proteins, as casein or albumin, has been one of these strategies (1, 9), but the only one of such complexes commercially available is iron caseinsuccinylate.

Ferplex 40® was introduced in the Spanish market in 1993 as iron proteinsuccinylate, without reference to its casein content. It has been recommended for use in the treatment of pediatric anemia, but, to date, no references to allergic reaction in milk-allergic patients have been published (PubMed and IME – Spanish medical database – search).

The product is an important source of casein, with a casein content similar to an equivalent dose of whole cow's milk. The allergenicity of succinylated casein has been demonstrated in this child and in three unexposed milk-allergic children as well as the cross-reactivity of succinylated casein with native casein. The capacity of inducing an allergic reaction after the first dose of the drug is documented in this paper.

For ethical reasons, none of the patients studied in the present work has been challenged with Ferplex40®. Despite this, the potential risk of allergic reactions to the product among milk allergic children is demonstrated.

As in food allergy, hidden sources of allergens should always be kept in mind when treating allergic patients. The potential hazards of the administration of native or modified proteins in drugs should not be neglected. Recently, the information sheet of an ovalbumin formulation of iron (Profer®, Tedec-Meiji Farma SA, Alcalá de Henares, Spain) has changed because of adverse effects in egg-allergic patients (C. Morales Amodeo, personal communication). Similar to our observation, these reactions strongly support that, when heterologous proteins are used in the manufacture of drugs, especially those proteins of recognized allergenic potential, the nature of the source protein should be clearly indicated on the patient information leaflet. This holds true not only for native proteins but also for modified ones.

Declaration

All laboratory procedures were performed at the R&D Department of IPI S.A. Neither the authors nor the company (IPI S.A.) have a commercial or any other kind of interest in the analyzed product.

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