

Bovine β -lactoglobulin levels in hydrolysed protein formulas for infant feeding

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Summary

A bovine milk protein, β -lactoglobulin (β LG), was measured by an enzyme-linked immunosorbent assay (ELISA) in seven different infant formulas based on protein hydrolysates from cows' milk whey or casein, and from bovine collagen and soy. β LG levels in the formulas were 1/100 to 1/4 800 000 lower than in cows' milk (CM). There was a great difference in the β LG level between the partly and the extensively hydrolysed formulas; the amount of β LG was 40 000-fold higher in the partial hydrolysates vs the extensively hydrolysed formulas. Residual β LG may have been responsible for the allergic reactions described in some children with cows' milk allergy (CMA) receiving these formulas.

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Introduction

CMA is common in infancy. Its incidence is about 2% [1,2]. Several different CM proteins, both in the casein and in the whey (albumin, α -lactalbumin, β LG, lactoferrin and immunoglobulins), can act as allergens [3,4]. β LG is probably the most important allergen in the whey [5,6]. Its concentration in CM is about 3.5 g/l [7]. β LG is not an endogenous component of human milk, but it is commonly present in the milk of mothers consuming dairy products [8–12]. Human milk contains IgA antibodies to CM proteins, which may play an important role in the exclusion and elimination of β LG [13]. However, few infants develop CMA during breast feeding [13,14]. CMA does not usually appear until infant formulas are introduced. These formulas contain the same amounts of milk proteins as CM [3]. Formulas labelled as 'hypoallergenic' have been developed, that are less antigenic and allergenic than CM. These are prepared by heat treatment of the protein source, bovine casein, whey or collagen proteins, and/or by acid or enzymatic hydrolysis [15,16]. The resulting mixture of amino acids and low molecular weight (MW) peptides is considered to be less, if at all, allergenic than the intact proteins [17].

We have used a sensitive enzyme immunoassay of β LG with the detection limit of 0.002 μ g/l, to evaluate the

degree of hydrolysis, i.e. the success in removing β LG in seven infant formulas based on different protein hydrolysates.

Materials and methods

β -lactoglobulin ELISA

The method for double antibody ELISA has been described in detail previously [18]. Briefly, affinity purified rabbit anti- β LG (0.34 mg/l in 0.05 mol/l carbonate buffer, pH 9.6) was used to coat microtitre plate wells (Immunoplate I, Nunc, Roskilde, Denmark). Plates were incubated 3 hr at room temperature followed by an overnight incubation at +4°. Plates were then washed four times and free binding sites blocked with 1.5% gelatine (Bio-Rad). Samples and standards (0.015–10 μ g/l) were tested in triplicate in several dilutions in phosphate buffer containing 0.5 mol/l NaCl, 0.1% Tween 20 and 3% polyethylene glycol 6000 (Macrogol). The plates were covered and incubated at +4° overnight and washed four times with washing solution. One hundred microlitres of the alkaline phosphatase-conjugated immunoglobulin fraction was used as a second antibody. After an overnight incubation at +4° and four times washing, 100 μ l of *p*-nitrophenol phosphate (1 g/l in 1 mmol/l diethanolamine buffer, pH 9.8, containing 0.5 mmol/l MgCl₂, was added as substrate. After exactly 30 min at room temperature the reaction was stopped with 50 μ l of 3 mol/l NaOH. Absorbances were measured at 405 nm against a

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