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EARNEST

EARly Nutrition programming- long term follow up of Efficacy and Safety Trials and integrated epidemiological, genetic, animal, consumer and economic research

Instrument: Integrated Project

Thematic Priority 5.4.3.1: Food Quality and Safety

Final public report on activity 6.1.2.

Title of activity: Clinical study in infants

Period covered from 15.04.2005 to 14.10.2010

Start date of project: 15.04.2005 Duration: 5,5 Years

Organisation Name of Lead Contractor for this report: ORDESA

Clinical study in infants

Participating partners: Ordesa, Beneo-Orafti, VDH

Objective

The objective of this demonstration activity was to investigate the effects of prebiotic inulin-type fructans such as oligofructose-enriched inulin on immunological-health related outcomes in infants. This was done by conducting a clinical study in newborn infants, who were followed up until the age of one year, to evaluate the effects of oligofructose-enriched inulin on the prevention of infections in infants.

Background information on oligofructose-enriched inulin

Inulin and oligofructose are are natural food components, storage carbohydrates in many plants. Inulin and oligofructose for food ingredient use is extracted out from chicory (Cichorium intybus), due to its high fructan content (>15%). Inulin is a mixture of oligo- and polysaccharides which are composed of fructose units connected by ß (2-1) links. Almost every molecule is terminated by a glucose unit. It is represented as G-Fn (with G, glucose; F, fructoses; n, number of fructose units joined by a β(2-1) bounds). The degree of polymerisation (DP) of chicory inulin ranges mainly between 2 and 60. Oligofructose is obtained through partial enzymatic hydrolysis of the inulin chains and the DP in Beneo-Orafti products ranges from 2-8. Long chain inulin is obtained by physical separation of the fraction with the largest degree of polymerisation. Oligofructose-enriched inulin is a mixture of oligofructose and long chain inulin, it is a mixture of carefully selected chain lengths (Orafti ®Synergy-1, BENEO-Orafti, Belgium).

Inulin and oligofructose are non-digestible and reach unmodified the colon where they are selectively fermented thus allowing specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefit upon the host well-being and health. Several health aspects of inulin and oligofructose have been addressed in intervention trials in infants as well as in adults, like a positive modification of the gut flora (“prebiotic effect”) and modulation of immune defense. A prebiotic effect of inulin and oligofructose in infants was confirmed by several intervention trials as well as beneficial effects on stool regularity (stool frequency and consistency) and reduced need for antibiotic prescription.

Study design and Methods

We conducted a multicenter, randomised, double-blind, placebo-controlled study in healthy term infants to evaluate the effects of oligofructose-enriched inulin on the prevention of infections in infants. Inclusion criteria were: healthy term infants with ages between 0 and 4 months, who are exclusively fed infant formula at study entry.

Infants were randomized to receive either an infant formula supplemented with 0.8 g/100 ml of oligofructose-enriched inulin (supplied by Beneo-Orafti, Belgium) or a standard formula with no prebiotics added. Both formulas had the same energy supply and the same protein and total fat content.

Infant follow-up was carried out up to the age of 12 months. Study visits with pediatricians were scheduled at 2, 4, 6, 9 and 12 months of age of the infants. Among variables recorded were number of days with fever, number and type of infections episodes (respiratory, gastrointestinal, other types), and data related with product tolerance and digestibility. Data were collected by pediatricians in Case Report Forms as well as in Diaries filled in by parents each month throughout all the study period. Data collected in parent's diaries were checked by the pediatricians at study visits.

In addition, stool samples were collected at 2, 6 and 12 months of age for analysis of microbial composition, calprotectin and total DNA. Saliva samples were collected at same study times for analysis of IgA and other cytokines.

Study progress

181 infants were recruited in different clinical centres of Spain and Belgium. During follow-up, 33 infants were lost for different reasons. Follow-up of the infants was completed in October 2010.

Stool samples collected at time 2, 6 and 12 months and stored at -20ºC have been sent in different batches to Beneo for analysis of microbial composition, and to VDH for analysis of calprotectin and total DNA. On the other hand, saliva samples collected throughout the clinical study have been sent to Granada for analysis of several cytokines and IgA.

In parallel, a study was undertaken in order to validate the usefulness of detection of human DNA in faeces as a marker of epithelial cell desquamation in 6 month old infants. Human DNA is present in both human epithelial cell and granulocytes, but calprotectin is only present in neutrophils. Combining data from human DNA and

calprotectin concentration in faeces may prove useful for the estimation of epithelial cell desquamation by subtracting the contribution of neutrophils. Faecal calprotectin was 87,70± 116,4 μg/g (mean ± SD), with a median of 51,3 and a range of 9,5 and 516,8 μg/g. In addition, very low concentration of short DNA fragments in infant stools were found. These results suggest that in children detection of human DNA in faeces is not a useful as a marker of epithelial cell desquamation. Low levels of human DNA in infants may reflect mainly to the increased presence of neutrophils.

Statistical analysis

A statistical database has been developed in Macro system and data from collected Case Report Forms have been introduced and checked by study monitor. Data collected in the Parents's diaries every month, containing information about infant behaviour, formula tolerance and infections, are being introduced in the statistical database in order to complete data entry. Study results including microbial analysis of faecal samples will be available in the next months.

Conclusions

Follow-up of infants participating in the clinical study to evaluate the effects of oligofructose-enriched inulin in term infants has been finished. Data entry and demographic pooled data analysis has been performed, whereas results per intervention group will be available in the next months.