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Original article

Safety, growth, and support to healthy gut microbiota by an infant formula enriched with functional compounds

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SUMMARY

Background & aims: Safety and growth adequacy of infant formulae enriched by functional ingredients need stringent evaluation by means of randomized controlled trials (RCTs), therefore we performed a double-blind RCT to evaluate an infant formula enriched with galacto-oligosaccharides, beta-palmitate, and acidified milk vs. a standard infant formula.

Methods: Weight, length, head circumference and fecal bacteria (Bifidobacteria, BIF/Clostridia, CLO) were measured in healthy full term infants, at baseline – as before 21 days of life – at 60 and 135 days thereafter. A group of 51 neonates received the enriched formula (ENR), 59 the standard one (ST). Parents were trained to daily register gastrointestinal diseases.

Results: All the infants grew homogeneously increasing the anthropometric parameters and complying with WHO and Italian standards: the mean (SD) difference in daily weight between ENR and ST groups was −0.74 (1.13) g/day, corresponding to a 90% CI of −2.62 to 1.13 g/day, well within the postulated interval of equivalence of −3.9 to +3.9 g/day. A statistical improvement in BIF concentration in the microbiota of infants fed by ENR was recorded. There was no between-group change in $\log_{10}$CLO, but $\log_{10}$BIF increase was higher at T2 vs. T0 in ENR (treatment × time interaction = 0.71, 95% CI 0.08–1.34, $p = 0.028$) than in ST neonates. This corresponds to estimated mean (95% CI) values of 8.37 (8.04–8.69) $\log_{10}$-units for ENR vs. 8.08 (7.77–8.39) $\log_{10}$-units for ST neonates. Gastrointestinal effects were mild and similar, with no statistical difference between two groups.

Conclusion: Safety and growth ability of the enriched formula has been confirmed. A positive effect on neonatal gut microbiota, consisting of increased fecal BIF counts at T2 vs. baseline has been shown too. Nonetheless, larger RCTs are needed to estimate with greater precision the effective potential attributable to the enriched formula on neonatal microbiota, with particular reference to the mode of delivery.

1. Introduction

Human milk is a species-specific nourishment adapted to satisfy the particular nutritional requirements of the infant and it is also able to educate the off-spring's immune system to develop a first protection against pathogens and harmful antigens [1]. The World Health Organization (WHO) recommends exclusive breastfeeding up to 6 months of age and prolonged breastfeeding through the

Abbreviations: GE, gestational age; BIF species, Bifidobacterium; CLO species, Clostridium; MLMs, mixed linear models; MGLMs, mixed general linear models; MAR, missed at random; ESPGHAN, European Society for Paediatric Gastroenterology, Hepatology and Nutrition; WHO, World Health Organization; FDA, food and drug administration.

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complementary feeding period [2]. If in this period, human milk becomes unavailable to an infant, a new-generation cow milk formula closer to the gold standard of human milk should and can be used. Currently available cow milk formulae should be evaluated to see whether they provide the best alternative to human milk, with the aim of approaching the structural and functional effects observed with breastfeeding [3]. In the last years, a great variety of formulae intended to be the most possible similar to human milk, became available on the market adding bioactive/functional components to the traditional composition. Based on the recommendations of the ESPGHAN Coordinated International Expert Group, the nutritional safety and adequacy of infant formula should be scientifically demonstrated to support, *in primis*, infant's optimal growth and development [4]. Moreover Food and Drug Administration (FDA) and Health Canada defined the regulatory and research issues that are particularly critical in assessing the safety of the addition of new functional ingredients to infants food, stressing the need to utilize appropriate statistical or analytical approaches, as the basis for making judgements about safety [5]. For this purpose, evaluating the activity of the bioactive compounds, i.e. ingredients that are related to the growth of an appropriate neonatal microbiota, is important [6,7]. Colonization of the neonatal gut begins soon at birth and is influenced by the composition of the maternal microbiota during pregnancy, thus by the mode of delivery, and finally by the mode of feeding [8,9]. During a vaginal delivery, healthy women can immediately transmit their infants' gut favourable bacterial communities, but not in case of caesarean section delivered infants, when positive colonisation (i.e. by Bifidobacteria) is usually significantly delayed [8]. These differences in gut microbiota could still be evidenced in childhood, because the mode of the colonization in early infancy leads the further development of the immune system. Then, maternal milk contains functional substances that can drive gut colonisation towards helpful microbiota, i.e. Bifidobacteria. In fact, the microbiota composition of breast-fed is characterized by a higher proportion of Bifidobacterium species than formula-fed infants, as measured in 60–90% vs. 50% of the faecal bacterial community, and by a lower presence of Clostridium species in the first group [9,10].

This microbiota composition can help preventing many different diseases: from necrotising enterocolitis in preterm newborns, to the risk of cancer later in life [11–13]. For this purpose galacto-oligosaccarides and other functional compounds, similar to these of maternal milk, have been added to formulae. Triglycerides are the major source of energy in breast milk and formula: beta (or sn-2) palmitate fatty acid, has been added in the formula because yields a positional distribution of palmitic acid more closely resembling that of maternal milk, thus reducing faecal excretion of palmitic acid soaps, improving the assimilation of calcium–fatty acid complexes, softening stools, and positively influencing the microbiota profile [14]. Acidified milk can be included because of its capability to produce an acid milieu that can increase beneficial bacterial proliferation, can stimulate the gut associated immune system (GALT) and can create an acidic environment inhospitable to infectious organisms [15]. Acidified milk is obtained adding L-lactic acid-producing bacteria, but due to their inactivation, does not contain viable bacteria in the final product, however contains bacterial residual responsible of the cited effects. The primary aim of this double blind, randomized study was to evaluate the safety and growth ability (as equivalence) of an infant formula, enriched by the above cited functional ingredients (galacto-oligosaccharides, beta-palmitate and acidified milk), vs. a standard formula milk (identical, but without functional ingredients), in healthy full term newborn infants during a study period of 135 days. As secondary outcomes, the study evaluated the possible effect on infants faecal microbiota of the 2 different formulae, hypothesizing that the functional ingredients could beneficially drive the microbiota profile of the receiving infants and thus the gastrointestinal (GI) comfort, decreasing related symptoms. Both formulae are available on the market.

2. Patients and methods

This double-blind randomized, controlled trial, after the approval of the Bioethics Committee of our Institute and after informed consent was obtained from both the parents, took place in our Neonatal Unit. Inclusion criteria were: infants of both sexes born to natural or caesarean delivery, gestational age (GE) = 37–42 weeks, birth weight between 10th and 90th percentile according to the WHO Child Growth Standard [16], single birth, Caucasian race, switching from breastfeeding to complete formula milk before 21st day of life. Exclusion criteria were: infants with genetic and/or congenital diseases, receiving antibiotic therapy, requiring hospitalisation for longer than 7 days, having familial history for atopy, metabolic or chronic diseases, parents refusing to sign a written informed consent. After a preliminary visit, 148 healthy full term infants were screened for the study and 117 complying with the above mentioned criteria, were enrolled into the study, and allocated to randomly receive the formula milk supplemented with functional ingredients (n = 55 ENR group), or a standard formula (n = 62 ST group). Infants were randomized (by a computer generated sequence) to receive either the ENR or ST formula and were to consume the assigned formula exclusively until the introduction of complementary feeding. Because we lost to follow-up 4 neonates in ENR group and 3 in ST group (not showing on time at last control visit), 51 neonates in the ENR group and 59 in ST fully finished the study and relevant data were analysed, as reported in Fig. 1 by the Consort flow chart [17]. Twenty-two infants of the ENR and 16 of the ST group were born by caesarean section. The composition of the 2 formulae was identical, with the exception of the functional ingredients, galacto-oligosaccharides (7 g/L), beta-palmitate (palmitic acid is 60% of total fatty acids, whose 39% are esterified at the sn-2 position) and acidified milk (which represents 50% of the whole milk in the formula). They were provided in powdered form and could not be differentiated by smell, consistency, or any other characteristics. Neither the investigators nor the parents knew which product the infant was receiving; nutritional details of the 2 formulae are reported in Table 1. Heinz Italia S.p.A. (Latina, Italy) supplied ENR and ST formulae.

Infants were evaluated at the enrolment (T0), after 60 ± 5 days from T0 (T1), and at the end of the studied period 135 ± 5 days from T0 (T2). They were evaluated, within routinely paediatric visits, for body weight, length, head circumference and registering adverse events, in particular inquiring the parents about minor gastrointestinal issues: information were obtained by diaries filled in by the parents, after they were trained to recognize and measure them with the aid of semi-quantitative and/or illustrated scales. Gastrointestinal symptoms included stool frequency and consistency, bowel cramps, intestinal gas. In order to evaluate neonatal microbiota, on T0, T1 and T2 visits, faecal samples were collected by the parents in sterile boxes and frozen, then stored (−20 °C) by paediatrician until the samples were researched for Bifidobacterium (BIF), and Clostridium (CLO) species, as representative of the gut colonisation. Bacterial DNA was isolated using a commercial kit according to manufacturer protocol (Fast Prep Instrument-MP Biomedical Santa Ana, CA, USA). Total bacterial DNA was extracted by means of mechanical cell disruption (FastDNA Spin Kit Biomedical, Santa Ana, CA, USA). DNA concentrations were

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