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REGULAR ARTICLE

Intestinal permeability in different feedings in infancy

Gemma Colomé[1], Carlos Sierra (carlos.sierra.sspa@juntadeandalucia.es)[1], Javier Blasco[1], Mª Victoria García[2], Esteban Valverde[2], Elisa Sánchez[3]

1.Unidad de Gastroenterología y Nutrición Infantil, Hospital Materno-Infantil
2.Servicio de Farmacia, Hospital Materno-Infantil
3.Departamento de Bioestadística, Facultad de Medicina, Málaga, Spain

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Correspondence
Dr. Carlos Sierra Salinas Unidad de Gastroenterología y Nutrición Infantil. Hospital Materno-Infantil Avda Arroyo de los Ángeles s/n 29011, Málaga, Spain.
Tel: +34951292191 |
Email: carlos.sierra.sspa@juntadeandalucia.es

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Abstract

Aim: To determine the intestinal permeability (IP) as a marker of intestinal epithelial integrity in formula-fed infants compared with healthy breast-fed infants.

Methods: IP was measured in 57 healthy infants less than 4 months old. A dual sugar test with lactulose and mannitol was performed. Three urinary ratios were established: lactulose/mannitol (L/M), lactulose/creatinine (L/C) and mannitol/creatinine (M/C). Five groups were studied: breast-fed (n = 11), prebiotic supplemented formula (n 17), nucleotides supplemented formula (n = 9), LC-PUFA supplemented formula (n = 9) and LC-PUFA and nucleotides supplemented formula (n = 11).

Results: We have not found any difference in IP between breast-fed and formula-fed infants nor when the different formulas are compared with each other. The indirect information of the paracellular pathway by the ratio L/C and the transcellular route by the ratio M/C reflects some difference when the ingredients added are fructooligosaccharides and galactooligosaccharides, expressing a higher degree of lactulose permeation with respect to mannitol. When LC-PUFA supplementation was evaluated a lesser ratio of L/C was found, expressing intestinal barrier related to a process of epithelial tight.

Conclusions: The most important factor in the maintenance of the integrity of epithelial barrier function is probably the delivery of nutrients in the gastrointestinal tract. The role of the different ingredients added should be clarified.

INTRODUCTION

Human milk contains agents that produce profound effects upon the function and integrity of the gastrointestinal tract. Infant formulae provide the nutrients needed for adequate growth but not all of the components of human milk can be duplicated in these formulae. Some of the bioactive constituents of human milk have traditionally been considered non-essential nutrients. However, recent studies indicate they are essential during development. Such nutrients include oligosaccharides, nucleotides and long-chain polyunsaturated fatty acids (LC-PUFA). Intestinal epithelial cells not only regulate the absorption of nutrients but also provide barriers to foreign agents. The pattern and timing of the so-called gut closure varies as a function of neonatal maturity and intestinal development at birth.

Maturation of the gastrointestinal tract is a dynamic process that is still continuing at birth and could be assessed by the measuring intestinal permeability (IP). This is high at birth and decreases progressively during the first week of life (1,2). Several factors, including postnatal age and feeding method, could play a role in the maturation process of the functional integrity of the small intestine mucosa resulting in a decrease in IP. In preterm infants IP is higher than in healthy term infants. However, there is no difference if measured within 2 days after birth and at a postnatal age of 4–7 days (3).

AIM

The objective of this study is to evaluate the effect on IP related of different infant formula. The changes in IP are measured in infants less than 4 months old who had been exclusively breast-fed or artificially fed with different normal infant formulae.

PATIENTS AND METHODS

The study was approved by the local medical ethical committee and informed parental consent was obtained for each infant before the start of the study. Fifty-seven healthy infants were included (18–120 days). All infants were born after an uncomplicated labour and showed appropriate weight for age and height. No infant with strong family history of atopic (more than one first-degree family member with an already known allergy) was included. All of them were fed according to the mothers' decision (breast or different *cow's milk* formula). The infants were divided into five groups according to breast fed or the type of *cow's milk* formula: (i) Breast-fed (n = 11), (ii) Infants receiving a formula supplemented with prebiotics (n = 17) (neither nucleotides nor LC-PUFA added), (iii) Infants receiving a formula

supplemented with nucleotides (n = 9) (neither prebiotics nor LC-PUFA added), (iv) Infants receiving a formula supplemented with LC-PUFA (n = 9) (no prebiotics and no nucleotides added), (v) Infants fed with a formula supplemented with LC-PUFA and nucleotides (n = 11) (no prebiotics added).

Study Design

A sugar permeability test (SPT) with lactulose and mannitol was performed by a single oral load method. Fasting before the test was not mandatory. The test solution prepared by the hospital pharmacy contained 250 mg lactulose and 100 mg mannitol per 5 ml water. In both breast and formula-fed infants the SPT was put into the mouth with a syringe. Urine was collected by parents in a plastic bag for 6 hour. The samples were stored at −20 °C with 0.1 ml 10 % sodium merthiolate as the preservative. The concentrations of lactulose and mannitol were measured by gas chromatography as previously described (4,5). Urine creatinine concentration was measured using a Dimension Boehring analyser. Three urinary ratios were expressed. The lactulose/mannitol ratio (L/M), informative index of IP, is not influenced by variables such as gastric emptying time, intestinal peristalsis, and glomerular filtration rate, which may interfere with the urinary elimination of each probe (6). The lactulose/creatinine ratio (L/C) could reflect changes in the paracellular area, where lactulose is thought to permeate the intestinal mucosa. The mannitol/creatinine (M/C) ratio expresses the transcellular route.

STATISTICAL ANALYSIS

Data are given as mean value ± SD. Two-sample *t* test and ANOVA were used to test the significance of the results. Comparisons between groups were made using the Mann-Whitney test. Significance was established at p less than 0.05.

RESULTS

The age of the infants was 72.50 ± 30.52 days (mean ± SD) (breast-fed group 64.54 ± 31.61 and formula-fed group 74.41 ± 30.30).

The data obtained in the breast-fed group and in all formula-fed infant groups showed no significant differences in the L/M, L/C and M/C (Table 1).

The influence of the different ingredients (prebiotics, LC-PUFA, nucleotides) was studied comparing each formula group. These results showed no significant difference in the L/M ratio, thus expressing no change in the IP (Table 2).

Infants fed with a standard formula supplemented with prebiotics (fructooligosaccharides and galactooligosaccharides) expressed a higher L/C (p = 0.024) and M/C ratio (p = 0.039) than no supplemented infant formula. We found greater differences in the infant group fed with formula containing LC-PUFA with lower L/C ratio (p = 0.002) and M/C ratio (p = 0.004) than the non–LC-PUFA–supplemented infant formula.

When all the nucleotide supplemented infant formulae were compared with the formula without added nucleotide, there was no difference in the L/C and M/C ratios.

When we compared infants fed with a formula with nucleotides and LC-PUFA with the rest of the formulae, no change was found in IP. The L/C ratio shows the lowest value of the study (p = 0.001) with more statistical power than the decrease of the M/C ratio (p = 0.006).

DISCUSSION

We used the single load method according to van Elburg (3) for measuring IP because it has the advantage that just a small dose of the sugar solution needs to be given. The ratio of lactulose and mannitol in urine depends less on complete urine collection for 6 h, which is in accordance with the results of Akran (7), who found no difference in intestinal permeability between a 2-h urine collection and a 5-h collection in adults. In our study, complete urine collection was achieved in 40% of the infants, probably because of scarce adherence of the collection bag, and for this reason we used the simple ratio lactulose/mannitol and not the percentage of the excretion of lactulose and mannitol. Measurement of the ratio of these markers in urine is less influenced by variables such as gastric emptying and renal excretion.

The relationship between feeding in healthy infants less than 4 months and changes in IP has been studied by the SPT showing no difference between breast-fed and formula fed infants. Previous studies provide conficting data with regard to neonatal IP, but it is considered to be normal an increase in IP for sugar probes during the first 2 days of life (3) or 7 days of life (8) finding that it could be related to the process of gut closure. The IP probably decreases faster in breast-fed infants than in those fed with infant formula as Catassi (2) described at 1 month of life. We did not find any differences between breast-fed and formula fed-infants.

No significant differences ocurred with L/M, L/C and M/C indicating a similar intestinal maduration. Probably,

Table 1 Results of intestinal permeability in healthy infants (mean ± SD)

	n	L/M	L/C	M/C
Group I (breast-fed)	11	0.313 ± 0.188	2.591 ± 1.871	8.604 ± 3.255
All infant formula-fed	46	0.311 ± 0.196	3.168 ± 3.121	11.377 ± 11.414
Group II (prebiotics)	17	0.331 ± 0.222	4.921 ± 3.957	18.019 ± 15.187
Group III (nucleotides)	9	0.341 ± 0.250	3.558 ± 1.916	13.055 ± 7.266
Group IV (LC-PUFA)	9	0.296 ± 0.152	2.168 ± 1.926	7.950 ± 6.
Group V (nucleotides + LC-PUFA)	11	0.268 ± 0.149	1.751 ± 0.880	7.950 ± 6.

Table 2 Results of ratios L/M, L/C and M/C in infant formula-fed compared according to different functional ingredients (mean ± SD)

	n	L/M	L/C	M/C
Prebiotics infant formula	17	0.331 ± 0.222	4.921 ± 3.957	18.019 ± 15.187
p		n.s.	0.024	0.039
No prebiotics infant formula	29	0.299 ± 0.183	2.442 ± 1.731	9.374 ± 5.982
LC-PUFA infant formula	20	0.281 ± 0.147	1.939 ± 1.419	7.718 ± 4.605
p		n.s.	0.002	0.004
No LC-PUFA infant formula	26	0.334 ± 0.227	4.450 ± 3.411	16.301 ± 13.050
Nucleotide	20	0.301 ± 0.199	2.565 ± 1.675	10.016 ± 5.991
p		n.s.	n.s.	n.s.
No nucleotide infant formula	26	0.319 ± 0.198	3.968 ± 3.604	14.533 ± 13.536
Nucleotides + LC-PUFA	11	0.268 ± 0.149	1.751 ± 0.880	7.529 ± 3.291
p		n.s.	0.001	0.006
Rest of the formulae	35	0.324 ± 0.209	3.863 ± 3.233	14.529 ± 12.148

the most important factor in the maintenance of functional and structural intestinal integrity is the simple delivery of nutrients to the gastrointestinal tract. In spite of the intestinal microflora changes according to the feeding, the degradation of non-absorbed lactulose and mannitol doesnot seem to have influence in the results . In fact, there are no appreciated differences in the IP between breast-fed and formula-fed infants.

When we studied the differences between the formulae, the L/M ratio–index of intestinal permeability–showed no change. Moreover the L/C was higher in the group of infants who were fed with the formula supplemented with prebiotics when compared with the formula without pre-biotics, and to a lesser degree, the ratio M/C was also higher in prebiotic group. Although the IP did not change, these findings could probably reflect changes in the paracellular area, where lactulose permeates the intestinal mucosa.

Human milk oligosaccharides are often regarded as a model for the addition of oligosaccharides from a prebiotic nature to infant formula even though the biologic role of human milk oligosaccharides appears to be far more complex than the roles of the simple oligosaccharides presently added to formulae.

No differences were found with regard to nucleotide-supplemented formula in relation with non–nucleotide-supplemented infant formula. All the ratios (L/M, L/C, M/C) showed similar results. There are several publications indicating that supplemental nucleotides enhance immune status in formula-fed infants when assessed by antigen-specific antibody response to immunization. It seems that supplemental nucleotides cause shifts in the development of *T*-cell populations, improve infant humoral immune status, and may help to provide some of the immunologic benefits associated with breast-feeding (9). No other effects on intestinal function have been described.

The comparison of LC-PUFA supplemented with non-supplemented formulae showed no change in IP either. The ratio L/C was lower than in the group of infants fed with non-supplemented with LC-PUFA formula ($p = 0.002$). The ratio M/C was also lower in LC-PUFA added formula ($p = 0.004$). Probably tightening up of the junction complexes and a loss of pinocytotic activity would lead to a reduction of lactulose permeation of the intestinal mucosa in a higher proportion than the changes in the surface area; so this could explain a lower value of L/M which showed no statistical difference. It is hypothesized that the decrease in lactulose permeation in infants fed with LC-PUFA–supplemented formula is part of the maturation process of the intestinal barrier related to a process of epithelial tight. It seems that the effect of dietary fatty acids and the lipid-induced changes in mucosa permeability appear to be a function of the fatty acid chain length (10). An earlier study determining the protective effect of *docosahexaenoic acid* (DHA) against methotrexate-induced intestinal damage in mice showed that daily administration of DHA reduced intestinal permeability, possibly resulting from the effects observed in the structure of intestinal mucosa (11). This protective effect has been also described in 5-fluoracil-induced intestinal injury in rats (12).

The ESPGHAN Committee of Nutrition assembled in the International Expert Group states in its medical position paper that infant formulae should only contain components in such amounts that serve a nutritional purpose or provide any other benefit. On this basis, the Committee recommends that the addition of new ingredients should be made possible if the safety, benefits and suitable for nutritional use by infants have been established by generally accepted scientific data (13).

In conclusion, no significant difference was found in the IP between breast-fed and standard cow's milk formula fed neither comparing each formula group nor according to the different ingredients added such as prebiotics, LC-PUFA and nucleotides. Probably the most important factor after the gut closure in the maintenance of integrity of epithelial barrier functional is the delivery of nutrients into the gastrointestinal tract and not the characteristics of these nutrients. However the addition of optional ingredients in infant formula could influence the functional integrity of the intestinal mucosa.

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