



PROBIOTIC WITH LYSOZYME IN THE COMPLEX TREATMENT OF PREMATURE INFANTS WITH INFECTIOUS AND INFLAMMATORY DISEASES

Kushnareva Maria Vasilievna¹ and Shcherbakova Eleonora Grigoryevna²

¹Academician Yu.E.Veltishchev Research Clinical Institute of Pediatrics, N.I.Pirogov Russian National Research Medical University of the Ministry of health of the Russian Federation, Moscow, Russian Federation

² Medical Academy of Postgraduate Educations, Ministry of Health of the Russian Federation, Moscow, Russian Federation

ARTICLE INFO

Article History:

Received 8th September, 2017

Received in revised form 20th

October, 2017

Accepted 16th November, 2017

Published online 28th December, 2017

Key words:

probiotic, microbiota, intestines, infants, Bifilis.

ABSTRACT

Background: Disturbances in the composition of the microbiota of an intestine and intestinal dysfunctions at premature newborns with infectious-inflammatory diseases.

Methods: Probiotic preparation Bifilis, comprising bifidobacteria and lysozyme, was used in the complex treatment of 45 premature infants (gestational age at birth was from 28 to 37 weeks) with infectious and inflammatory diseases complicated by intestinal disorders on the background of antibiotic therapy. Bifilis was administered for 2 weeks at 2.5 doses 2 times a day to newborn infants and at 5 doses 2 times a day – to infants older than 1 month. Intestinal microflora of all children was studied before and after the treatment course with Bifilis, and 22 infants were tested on sIgA and lysozyme concentrations in coprofiltrates.

Results: All infants had severe intestinal microbiocenosis disturbances prior to probiotic therapy. After the treatment course, the improvement of the clinical state and intestinal microflora was found in all children; among them, 10 infants was diagnosed with normobiocenosis and 22 infants – with grade 1 dysbiosis; the rate of sIgA and lysozyme detection increased by 3 and 2 times, respectively, and their concentration increased by more than 6 times.

Conclusions: Bifilis is an effective tool for intestinal disorders treatment in premature infants, including babies with very low birth weight and with infectious and inflammatory diseases. The probiotic normalizes the intestinal microbiota and increases the concentration of lysozyme and sIgA in the intestine.

Copyright © 2017 Kushnareva Maria Vasilievna and Shcherbakova Eleonora Grigoryevna. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Probiotic preparations comprising bifidobacteria and lactobacilli (Bac-Set Baby, Primadophilus, Bifidumbacterin, Bifidumbacterin forte, Probiophore, Acipol, Linex *et al.*) are routinely used for prevention and therapeutic correction of intestinal dysbiosis in preterm infants of the first year of life [1,2,3,4]. Over the last years, the probiotic preparation Bifilis (syn. Vigel, bifilys) has become widely used in pediatric practice, and has shown good effectiveness in intestinal infections and chronic gastroenterological disorders [1,2,5,6]. Probiotic preparation Bifilis is a complex of live lyophilized bifidobacteria and lysozyme. One (1) bottle of the preparation includes: bifidobacteria lyophilized - at least 10 million, lysozyme - 10 mg. This corresponds to “5 doses” of a probiotic. The particular action of Bifilis is specified by its complex composition. Bifidobacteria have a high capacity to

colonize intestinal mucosa and antagonistic activity against pathogens; they normalize parietal digestion and increase resistance of the body to infections [1,5]. Lysozyme as a component of Bifilis, is a factor in the biological defense, as it can be found in many body fluids in large quantities. It has anti-inflammatory action and antibacterial activity against gram-positive microorganisms, enhances immune defense, stimulates regenerative processes; increases the growth of bifidobacteria [1,5,6,7]. In spite of the fact that Bifilis become widely used in pediatrics, in fact, there are no researches devoted to its effectiveness in premature infants, including very low body weight infants with infectious and inflammatory diseases receiving massive antibacterial therapy. In this paper, we present our experience with using Bifilis in such children.

Purpose

To determine the clinical and microbiological efficacy of Bifilis probiotic preparation, its action on local intestinal immunity in premature infants with infectious and inflammatory diseases complicated by intestinal disorders.

MATERIAL AND METHODS

Bifilis was used in the complex treatment of 45 premature infants with gestational age at birth from 28 to 37 weeks. Among them, 27 were extremely preterm and very preterm very low birth weight (900 - 1500 g) infants (16 babies aged 1 to 3 weeks and 11 babies- 1 to 2.5 months). 18 infants have birth weight from 1620g to 2530g (12 infants aged 2 to 4 weeks and 6 infants – aged 1 to 3 months). 11 babies received a 2-3-week treatment course with Bifidumbacterin before prescribing of Bifilis. The infants under one month of age received 2.5 doses of Bifilis 2 times a day, and the infants older than one month - 5 doses 2 times a day, for 2 weeks. Treatment with Bifilis was carried out during antibacterial therapy with beta-lactam antibiotics of 2 - 4 generation, Netromycin, Amikacin, Rifampicin, Metrogl and Lincomycin due to pneumonia (in 16 infants), tracheobronchitis (in 12), omphalitis (in 11), otitis (in 2), purulent conjunctivitis (in 4). In the course of antibacterial therapy, all children with infectious and inflammatory diseases have gastrointestinal disorders - possetting, severe meteorism, intestinal colic, poor weight gain. There were more-frequent bowel movements; feces had the appearance of non-homogeneous foamy, watery mass of green color with undigested elements, sometimes - with mucus and a large number of leukocytes.

Stool microbiology test was performed before and after Bifilis treatment course. Bacteria culture test included the quantification of a wide range of pathogenic bacteria (Enterobacteriaceae, Staphylococcus spp., Streptococcus spp., Pseudomonas spp., Candida spp., Lactobacterium spp., Bifidobacterium spp.) [3,8]. 22 bottlefeeding infants receiving breast milk substitutes were tested on sIgA and lysozyme concentrations in coprofiltrates. The sIgA level was studied using a reagent sets REF K275 and REF K276 "XEMA" for the sandwich enzyme-linked immunosorbent assay (ELISA) before and after Bifilis treatment course. Lysozyme concentrations was estimated by modified technique of Kagramanova K.A. and Yermol'yeva Z.V. by measuring the lysis of acetone powder of the Micrococcus lysodeiktitikus cell walls (NCTC 2665) [9].

RESULTS

Tables 1 and 2 show the results of the stool microbiology test in infants before and after a two-week Bifilis treatment course.

Intestinal microflora tests before and after the treatment course revealed impaired intestinal micro biocenosis in all infants. Only 6 children had grade I dysbiosis (violations only in the aerobic component), and the majority (39 children - 87%) had combined altered aerobic and anaerobic components, which corresponded to grades II and III dysbiosis. Impaired intestinal micro biocenosis were characterized by massive growth (up to lg8 -lg10) of hemolytic E. coli with altered enzymatic properties (in 10 infants), lactose negative Escherichia coli (in 5 infants), Klebsiella pneumoniae (in 15 infants), Enterobacter cloacae and Proteus vulgaris (in 1 and 1 infants), Entrococcus with hemolytic properties (in 14 infants), Staphylococcus aureus (in 10 infants), Staphylococcus epidermidis with

hemolytic properties (in 7 infants). Candida fungi in the amount of lg6 - lg8 were found in the intestine of 12 infants. 37 babies had 2 or 3 facultative bacteria with high capacity to colonization (associations of two species of enterobacteria or enterobacteria with coccal microflora and/or Candida spp.). In the intestine of 8 infants, one opportunistic bacterial pathogen was predominant. No infant has a normal level of Lactobacterium spp. Normal amount of Bifidobacterium spp. was found only in 6 newborns (13%). Decreased amount of Bifidobacterium spp. was revealed in 39 infants (83%). After the treatment course with Bifilis, positive changes in the intestinal biocenosis were found in all children. By this time, normobiocenosis was revealed in 10 infants, and 22 infants had mild I grade dysbiosis. More severe gut microbiota disturbance corresponding to the II and III grade of dysbiosis were found only in 13 infants (29%). Bifidobacterium spp. put in appearance in 10 children (absence before treatment); titers increased 10-1000 times - in 26 infants. Bifidobacterium spp. titers remained unchanged in 7 babies. These anaerobes were not detected in 2 babies. Absence of Bifidobacterium spp. in 2 children and decreased amount of Bifidobacterium spp. in 8 children at the end of the Bifilis treatment course is associated with the active antibiotic therapy.

Lactobacterium spp. was found in feces in most infants (43 of 45 infants, 96%) after treatment with Bifilis. In 25 of these infants, the number of these microorganisms has increased 10-100 times. The number of lactose positive E. coli increased 3-10 times in 25 children. There was complete elimination of S. aureus in 6 babies, hemolysing S. epidermidis - in 5 infants and haemolysing Enterococcus spp. - in 6 children. Other infants had a decreased level (by 2-10 times) of Gram-positive cocci - almost normal level. Decreased level (by 10 times) of Candida fungi was found only in 6 children. Anti-mycotic drugs were additionally prescribed to all infants with increased level of Candida spp. The activity of Bifilis against the bacterial family Enterobacteriaceae was lower. Thus, complete elimination of hemolysing E. coli was found in only 6 of 10 patients, and Klebsiella pneumoniae - in 7 of 15. In other infants, these microorganisms, as well as Proteus vulgaris, Enterobacter cloacae and lactose-negative E. coli were predominant in biocenosis, although decrease in their number (compared with baseline level) was usually observed after Bifilis treatment course. In this regard, these infants consistently received a course of one of the specific bacteriophages (pyo-phage, Klebsiella and E.coli-Proteus bacteriophage) for 7-10 days and then - Bifilis for 2 weeks after antibacterial drug withdrawal.

To date, the general, nonspecific mechanisms of bacterial resistance to the action of lysozyme have been studied, and known protein inhibitor of lysozyme and the features of their synthesis regulation by a bacterial cell have been described. This applies to opportunistic bacterial gram-negative pathogens [5,7]. The use of biopreparation with lysozyme is not sufficient to rapid eradication; it is required to prescription of pharmacological agents with targeted action {bacteriophages, preparations of specific immunoglobulins - lactoglobulin, KIP (Immunoglobulin complex preparation (TRC), Kipferon, selective decontamination with antibiotics in some cases}. At the same time, Gram-positive cocci, as a rule, retain a sensitivity to lysozyme [5,6,7,9,10].

The results of the study of the concentration of sIgA and lysozyme in feces are presented in Table 3.

Table 1 Distribution of infants according to the degree of intestinal dysbiosis before and after a two-week treatment with probiotic

The survey period of infants	norm nP		Number of infants with impaired intestinal microbiocenosis						Total infants	
			I degree		II degree		III degree		n	%
			n	%	n	%	n	%		
Before treatment with bifilysis	0	0	6	13	23	51	16	36	45	100
After treatment with bifilysis	10	22,2	22	49	11	24,4	2	4,4	45	100

Notes. 1. n is the number of babies. 2. Here and in Table 3, the percentage of small numbers is given for comparison of indicators before and after treatment with bifilysis.

Table 2 The content of Bifidobacterium spp. and Lactobacterium spp. in feces in 45 premature babies before and after a two-week course of treatment with bifilysis

The survey period of infants	Number of infants with Bifidobacterium spp. in the following titer (lg in 1 g of feces)					
	< lg6	lg6	lg7	lg8	lg9	lg10
Before treatment with bifilysis	12	5	12	10	5	1
After treatment with bifilysis	2	0	7	4	16	16
Number of infants who have Lactobacterium spp. in the following titer (lg in 1 g of feces)						
The survey period of infants	< lg4	lg4	lg5	lg6	lg7	lg8
Before treatment with bifilysis	20	19	6	0	0	0
After treatment with bifilysis	2	10	15	12	3	3

Table 3 The detection rate and concentration of sIgA and lysozyme in feces in 22 infants before and after treatment with bifilysis

The survey period of infants	Concentration		Number of infants who have sIgA and lysozyme found in feces			
	sIgA mr/100 r	Lysozyme	sIgA		Lysozyme	
			n	%	n	%
Before treatment	1,9±0,4	1,0±0,22	6	30	11	50
After treatment	12,6±3,2*	6,1±1,6*	19	90	22	100

Notes. 1. "n" is the number of babies with sIgA or lysozyme detected. 2. * - the reliability of the difference in the indicator in different periods of the survey (p≤0,05).

As shown in Table 3, sIgA and lysozyme were found in feces not in all infants before treatment with Bifilis. However, after treatment with the probiotic, the rate of sIgA and lysozyme detection increased significantly - by 3 and 2 times, respectively, and their concentration increased more than 6 times, that had a positive action on the intestinal microflora. The clinical effect of Bifilis was quite good. Its use was accompanied by a significant improvement in the overall clinical state by 5-8 days of treatment in most infants (in 38 of 45. 84%), and by 10-12 days - in other children. Stool normalization on the 2nd - 5th day of treatment occurred in 20 infants, on 6th - 8th day - in 10 and on 12th - 14th days - in 5 infants. In the remaining 10 children, stool was normalized only after additional treatment with bacteriophages and Bifilis. There was also an improvement in the clinical state of the infants with partial or complete stopping of edema syndrome and a significant improvement in the neurological status. Improvement in motor activity and appetite, a stable body weight growth and the elimination of infectious inflammatory foci were found in all infants. An increase in the amount of mucus in the feces during the first 2-3 days of Bifilis treatment course without worsened general state was observed in 3 infants. This may occur due to the individual sensitivity of the intestinal mucosa to the components of the preparation. The remaining infants (93%) had no negative clinical dynamics.

CONCLUSION

Thus, Bifilis is an effective tool for intestinal disorders treatment in premature infants, including babies with very low birth weight and with infectious and inflammatory diseases. The probiotic quickly restores an anaerobic component of gut microbiota (bifidobacteria), stimulates the growth of lactobacilli and lactose-positive E. coli, and increases concentration of lysozyme and sIgA in intestinal lumen. Also, Bifilis rapidly eliminates Gram-positive cocci, sensitive to the

antibacterial action of lysozyme. The therapeutic action of Bifilis against lactose-negative enterobacteria is lesser, but it is significantly increased when this probiotic used in combination with specific bacteriophages.

References

- Deshpande G, Jape G, Rao S, Patole S. Benefits of probiotics in preterm neonates in low-income and medium-income countries^ a systematic review of randomized controlled trials. *BMJ Open*. 2017 Dec 7;7(12):e017638. doi: 10.1136/bmjopen-2017-017638.
- Fatheree NY, Liu Y, Taylor CM, Hoang TK, Cai C, Rahbar MH, Hessabi M *et al*. Lactobacillus reuteri for Infants with Colic: A Double-Blind, Placebo-Controlled, Randomized Clinical Trial. *J Pediatr*. 2017 Dec; 191:170-178.e2.doi: 0.1016/j.jpeds.2017.07.036. Epub 2017 Sep 29.
- Xu HB, Jiang RH, Sheng HB Meta-analysis of the effects of Bifidobacterium preparations for the prevention and treatment of pediatric antibiotic-associated diarrhea in China. *Complement Ther Med*. 2017 Aug; 33:105-113. doi: 10.1016/j.ctim.2017.07.001. Epub 2017 Jul 6.
- Blokhin B.M., Prokhorova A.D. Modern aspects of probiotics use in pediatric practice. *Pediatratriaj*; 2016: 95: 4: 223-229. http://pediatratriajournal.ru/files/upload/mags/353/2016_4_4696.pdf
- Kushnareva M.V., Shcherbakova E.G., Keshishian E.S., Semenov A.V. Efficiency of using the probiotic "Linex for children" in infants with acute respiratory viral diseases. *Ros Vestn perinatal I pediat* 2014; 59: 5: 109-112.
- Scherbakova E. G. Lysozyme containing compositions in the treatment and nutrition of children. Storage and processing of agricultural products 2006; 4: 52-56.

7. Andryuschenko S. V., Perunova N. B., Bukharin O. V. Molecular mechanisms of interaction of bacteria with lysozyme and their role in microsymbionts advances in modern biology 2015; 135: 5: 453-463.)
8. Kuvaeva IB, Ladodo KS. Microecological and immune disorders in children. 1991; Moscow, "Medicina": 240.
9. Labinskaya A.S. Microbiology of the equipment for microbiological studies. Handbook. Moscow: Medicine 1978; 164-166 (in rus).
10. Alimova D.M., Kamilov K. P. Factors of nonspecific resistance and their clinical value at patients with recurrent aphthous stomatitis. *European Science Review* 2016; 11: 1: 33-35.
