

DO PROBIOTICS EXERT BENEFICIAL EFFECTS ON THE MANIFESTATIONS OF AUTISM SPECTRUM DISORDERS?

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ABSTRACT

Autism spectrum disorders are characterized by a spectrum of symptoms that include reduced social skills and social isolation, repetitive behavior, a predilection for routines and abnormal responses to sensorial stimuli. There are different phenotypes of autism; therefore, this would appear to suggest that there are different causes triggering the disorders. A range of symptoms associated with the gastrointestinal tract such as dysbiosis, increased intestinal permeability and differences in microbiome composition has been found in some of these individuals. Clinical evidence also supports the idea that probiotics interfere in the central nervous system and in the host's behavior by restricting stress response and anxiety. From the point of view of microbial endocrinology, the microbiome involves specific pathways in which microorganisms affect behavior, thus enabling a new approach to the treatment of mental diseases through modulation of the microbiome gut-brain axis. Probiotics may be useful for restoring microbial balance, relieving gastrointestinal problems and minimizing immunological abnormalities.

Keywords: Autistic Disorder; Probiotics; Dysbiosis; Microbiome.

OS PROBIÓTICOS EXERCEM EFEITOS BENÉFICOS SOBRE OS TRANSTORNOS DO ESPECTRO DO AUTISMO?

RESUMO

Transtornos do espectro do autismo são caracterizados por um espectro de sintomas que incluem a redução habilidades sociais e isolamento social, comportamento repetitivo, uma predileção por rotinas e respostas anormais a estímulos sensoriais. Existem diferentes fenótipos no autismo, sugerindo a existência de diferentes causas que desencadeiam o transtorno. Uma variedade de sintomas associados com o trato gastrointestinal, tais como disbiose, aumento da permeabilidade intestinal e diferenças na composição microbioma foi encontrada em alguns destes indivíduos. Evidências clínicas também apoiam a ideia de que os probióticos interferem no sistema nervoso central e no comportamento do hospedeiro, restringindo resposta ao estresse e ansiedade. Do ponto de vista da endocrinologia microbiana, o microbioma envolve vias específicas que afetam o comportamento do hospedeiro, permitindo assim uma nova abordagem para o tratamento de doenças mentais, através da modulação do eixo microbioma intestino-cérebro. Probióticos podem ser úteis para restaurar o equilíbrio microbiano, aliviando problemas gastrointestinais e minimizando anormalidades imunológicas.

Palavras-chave: Transtorno Autístico; Probióticos; Disbiose; Microbioma.

INTRODUCTION

Autism spectrum disorders (ASD) are a range of neural development disorders, the prevalence of which is increasing, with 1 in every 91 children in the United States now being affected by the condition⁽¹⁾. Up to the present time, the Brazilian Ministry of Health has no conclusive data on the incidence of ASD in Brazil; however, estimates suggest that two million individuals are affected⁽²⁾. The disorders are characterized by a spectrum of symptoms

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that include reduced social skills and social isolation, repetitive behavior, a predilection for routines and abnormal responses to sensorial stimuli. Treatments currently available include behavioral⁽³⁾ and pharmacological interventions for the associated comorbidities or symptoms⁽⁴⁾. Since the underlying causes of ASD have yet to be defined, studies on the etiology of the disorder are focusing on specific areas that include the environment, genetics and epigenetic factors⁽⁵⁾. The common objective shared by these different initiatives is to acquire further information and design effective strategies for the prevention and treatment of the disorders.

There are different phenotypes of ASD; therefore, this would appear to suggest that there are different causes triggering the disorders. Nevertheless, little is known about the etiology except in rare cases in which genetic abnormalities are identifiable. Experimental approaches aimed at gaining new insights into the etiology are focusing more and more on subgroups of individuals who share a defined set of characteristics and this focus enables specific factors associated with certain aspects of ASD to be more easily identified. A range of symptoms associated with the gastrointestinal tract such as dysbiosis, increased intestinal permeability⁽⁶⁾ and differences in microbiome composition⁽⁷⁾ has been found in some of these individuals.

Currently, the use of lactic acid-producing bacteria referred to as probiotics is considered to be of the utmost importance as support in the treatment of gastrointestinal and autoimmune diseases⁽⁸⁾.

Based on these data, the objective of this paper is to provide an update on the therapeutic use of probiotics in treating the symptoms of ASD.

GUT MICROBIOTA

The composition of the gut microbiota is affected by temporal and spatial factors. The human fetal intestine is sterile; however, colonization begins immediately after birth and is modified according to the type of delivery, breastfeeding, the use of artificial formulae, the composition of the diet and the use of antibiotics⁽⁹⁾. The intestinal flora of breastfed infants is generally colonized by lactic acid-producing bacteria and by bifidobacteria, which are considered beneficial⁽¹⁰⁾. Breast milk is a complex biofluid composed of oligosaccharides, lactose (6-8%), lipids and proteins. In their free forms, oligosaccharides, which are indigestible to infants, act as receptors for pathogenic bacteria, hampering the binding of these microorganisms to the mucous membrane, thus preserving bowel function⁽¹¹⁾. Biochemical

analyses of these oligosaccharides have revealed around 200 different molecules, varying in size and stability as a function of their anomeric configuration (α or β anomers), sugar residue and type of binding⁽¹²⁾. By breastfeeding, mothers provide these sugars as a substrate for the microbiota, stimulating the development of the infant gut flora⁽¹³⁾. Bailey and Coe (1999) emphasized the importance of this association when they found that, in rhesus monkeys, interrupting mother-infant bonding altered the composition of the babies' gut microbiota as a result of the strong emotional stress, thereby increasing their vulnerability to disease. The microflora of formula-fed infants is less stable and *Bacteroides*, *Clostridium* species and *Enterobacteriaceae* are often present⁽¹⁵⁾. Introducing these formulae at an early age, in addition to altering the composition of the microbiome, promotes colonization by anaerobic microorganisms such as the *Clostridium coccoides*, *Prevotella* and *Atopobium* groups during the first three months of life⁽¹⁶⁾.

Among the various roles played by the host microflora, their direct effects on digestion and metabolism are the most important, since they affect the availability of nutrients. Their effect on digestion occurs through the release of energy resulting from the metabolism of oligosaccharides⁽⁹⁾, from the production of short-chain fatty acids⁽¹⁷⁾, and in modulating absorption. In addition to these important mechanisms, the gut microbiota also contributes to the production of vitamins such as biotin, riboflavin, pantothenic acid, ascorbic acid, thiamine and folates⁽¹⁸⁾.

With respect to defending the organism, the normal intestinal flora competes with pathogens for receptors and nutrients, produces antimicrobial compounds⁽⁹⁾, strengthens the intestinal epithelial barrier, acts on motility, regulates the immune system by stimulating IgA secretion and limits bacterial penetration^(19,20).

Hooper *et al.* (2012) reported that dysbiosis resulting from antibiotic treatment is sufficient in itself to lead to inflammation of the bowel. This imbalance may either trigger or perpetuate the inflammatory state that characterizes gastrointestinal diseases associated with chronic or recurrent inflammation⁽²²⁾. In addition, pathogenic bacteria are able to invade the tight junctions between the epithelial cells and disrupt intestinal barrier function, resulting in the translocation of bacteria and conduction of the inflammatory immune response⁽²³⁾.

Dysbiosis also has a negative effect on the central nervous system (CNS) through various intertwined pathways that form the gut-brain axis, involving the following processes

- 1) Synthesis of a neuropeptide⁽²⁴⁾;
- 2) Modulation of the brain-derived neurotrophic factor⁽²⁵⁾;

- 3) Bacterial growth in the pylorus and adjacencies (*Helicobacter pylori*)⁽²⁴⁾;
- 4) Modulation of local and peripheral inflammation, with depletion of butyric acid-producing bacteria possibly contributing to inflammation⁽²⁴⁾. In addition, there is evidence linking an abnormal pro- and anti-inflammatory cytokine profile with schizophrenia⁽²⁶⁾, bipolar disorder⁽²⁷⁾ and depression⁽²⁸⁾.
- 5) Reduction of antioxidant production and the absorption of essential nutrients such as polyunsaturated fatty acids, vitamins and amino acids, and increase of lipid peroxidation and of the synthesis of ammonia, phenols, indoles, sulfides and amines, which are harmful to the organism⁽²⁹⁾.
- 6) Changes in intestinal permeability, with an increased flow of circulating toxins⁽²⁴⁾;
- 7) Modifications of the function of the autonomic nervous system⁽²⁴⁾.

According to Nikolov *et al.* (2009), the prevalence of gastrointestinal symptoms in ASD is considerable, with symptoms such as diarrhea, constipation, vomiting, acid reflux, abdominal pain and discomfort, gases and atypically fetid feces, associated with increased irritability, anxiety and social isolation, being common. The existence of a specific gastrointestinal pathology in ASD remains controversial; however, data suggest an association with gut microbiota because changes have been found in the composition of the microbiota of these individuals when compared to a control group⁽⁷⁾. The genera *Clostridium*, *Bacteroides* and *Desulfovibrio* are commonly found in individuals with ASD, whereas the presence of *Firmicutes* and *Bifidobacterium* species is reduced in this population⁽³⁰⁾. Intestinal permeability disorders have also been documented, with a similar picture being found in the first-degree relatives of ASD patients⁽³¹⁾.

In a discussion on the possible etiologies involved in the condition, Lázaro *et al.* (2014) reported the hypothesis of oxidative stress combined with a link between intestinal bacteria and intestinal permeability⁽³³⁾. In this proposed model, the pathogenesis of ASD would be a consequence of the inter-relationship in the deficiencies of sulfur metabolism and an imbalance in gut microbiota, principally with a proliferation of *Clostridium* and/or *Desulfovibrio* species, together with a reduction in the amount of bifidobacteria. The consequences resulting from dysfunctional bowel mucosa are also related to autism since they facilitate the absorption of toxins, bacterial products, proinflammatory cytokines and neurotransmitters that would reach blood circulation and penetrate the blood-brain barrier, leading to neuroinflammation⁽³⁴⁾. Such alterations could contribute to the clinical manifestations of ASD. Giving strength to this hypothesis, Sandler *et al.* (2000) noted that

when treatment based on antibiotics was administered there was an improvement in gastrointestinal symptoms and in cognitive function in autistic children.

PROBIOTICS

The World Health Organization defines probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”⁽⁸⁾. These effects, however, vary in accordance with the quantity, species and, in particular, with the strain selected, based on the genetic differences and the nature of the interaction between the microorganism and the host⁽³⁶⁾.

The strains belonging to the genera *Bifidobacterium* and *Lactobacillus* are the most commonly used. These microorganisms act by limiting the number of pathogenic bacteria, stabilizing the intestinal microflora, reducing bowel permeability and pH⁽³⁷⁾, strengthening barrier function through the tight junctions and stimulating immune responses⁽³⁸⁾.

Clinical evidence also supports the idea that probiotics interfere in the CNS and in the host’s behavior by restricting stress response and anxiety in addition to changing the mood of patients with irritable bowel syndrome⁽³⁹⁾. Although the mechanism of action has yet to be completely clarified, some probiotics reduce inflammatory cytokines and oxidative stress and improve the patient’s general nutritional status⁽⁴⁰⁾.

Together with probiotic therapy, the host’s dietary pattern is also a factor of the utmost importance. In addition to modulating the microbiome by altering the availability of nutrients, the profile of the diet affects the composition of the microbiota⁽⁴¹⁾. The production of short-chain fatty acids can also be modified by the amount of fiber in the diet. Diets rich in saturated fats and poor in fiber increase the quantity of microorganisms tolerant to acids and bile salts (*Alistipes*, *Bilophila* and *Bacteroides*), increase plasma endotoxin levels⁽²⁴⁾ and reduce the levels of *Firmicutes* that hydrolyze polysaccharides⁽⁴²⁾.

Although the interactions of the brain, bowel and microbiome are multifactorial and have yet to be completely clarified, the vagal system is known to function as a communication channel between the microbiota and the brain⁽⁴³⁾. In view of the previously mentioned scientific evidence, the microbiome is believed to be important to the CNS, both for the promotion of health and for the genesis and maintenance of the pathological state. The gut-brain axis and the microbiome interact directly and indirectly and may be associated with a new concept of integrative physiology in which associations occur between the neural,

immune and endocrine systems, and between nutrients and immune markers of both the CNS and the gastrointestinal tract⁽¹⁹⁾.

The mechanisms used by the pathogens to modify the host's behavior were recognized decades ago; however, evidence is accumulating of a direct interaction with the neurophysiological system in a non-invasive manner. The capacity of the microorganism to produce and recognize neurochemical compounds with a similar structure to that of those produced by the host's nervous system explains the way in which they may affect behavior through a non-infectious and possibly non-immune-mediated pathway⁽⁴⁴⁾. From the point of view of microbial endocrinology, the microbiome involves specific pathways in which microorganisms affect behavior, thus enabling a new approach to the treatment of mental diseases through modulation of the microbiome gut-brain axis⁽⁴⁴⁾. Studies conducted to investigate these influences have demonstrated that production of cytokines and inflammatory mediators involves known neuronal targets both in the CNS and in the enteric nervous system (Meissner's and Auerbach's plexuses).⁽⁴⁵⁾ The fact that bacteria produce neuroendocrine hormones suggests that the interaction of the microbiome may go well beyond the bacterial neuroendocrine interactions that occur in infectious diseases.

Another point that merits attention is the constant communication between the enteric nervous system and the CNS. In addition to the nerve connections, the chemosensitivity of the luminal epithelium may respond and transmit information on bacterial metabolites such as neuroactive compounds⁽⁴⁶⁾.

Although recent studies have focused on the effect of the microbiota on the CNS, other data have also suggested an inverse effect. The autonomic nervous system and the hypothalamic-pituitary-adrenal axis, which connects the nervous system to the viscera, may modulate the physiology of the bowel, altering, for example, motility, secretion and epithelial permeability⁽⁴¹⁾. Stress also induces intestinal permeability and allows bacteria and bacterial antigens to cross the epithelial barrier and activate the immune response in the mucosa, which in turn alters the composition of the microbiota⁽⁴⁷⁾.

CONCLUSION

There are various indications that changes in the gut microbiota contribute substantially to the well-being of individuals with ASD. Probiotics may be useful for restoring microbial balance, relieving gastrointestinal problems and minimizing immunological abnormalities. If the consumption of probiotics by children with ASD could

lead to improvements in their behavior, well-controlled studies with significant sample sizes should be conducted.

Nevertheless, it is not enough to focus only on probiotic supplementation as if this in itself would resolve the entire range of symptoms. Parents and healthcare professionals also need to be alert to the profile of the diet of these individuals.

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