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Review

Human milk oligosaccharides and the GI microbiota: is there a rationale for the use of HMOs in autism?

Abstract

The gastrointestinal (GI) microbiota plays a key role in health and disease. It also has a longdistance effect influencing the brain through the gut–brain axis. The establishment of an effective GI microbiota is associated with human milk oligosaccharides (HMOs). In animal studies there is evidence that HMOs can influence brain activity and cognitive development. This further suggests that management of the GI microbiota by dietary means could impact upon a wide range of diseases. Autism, which is the familiar name for autism spectrum disorder (ASD), comprises a group of heterogeneous neurodevelopmental disorders characterized by social deficits, repetitive and stereotypical behaviours, insistence on routines and communication impairments. GI abnormalities are a characteristic of a substantial number of children with ASD. These children possess a lower overall abundance of beneficial taxa, such as *Bifidobacteria* and *Akkermansia*, in the GI microbiota. Many children with ASD have higher counts of potentially pathogenic *Clostridia* than normally developing children.

The underlying pathophysiology of ASD remains unknown although sub-optimal breast-feeding practices are associated with ASD. HMOs selectively promote the growth of *Bifidobacteria* in the GI tract, which is associated with numerous beneficial health outcomes.

There are two potential benefits of HMOs in alleviating autism.

Firstly, supplying HMOs to infants though breast-feeding can help establish a functional GI microbiota and thus avoid GI dysbiosis which is commonly related to the onset of autism. Secondly, administration of HMOs may alleviate the symptoms of autism through an effect on the gut–brain axis.

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Introduction

The gastrointestinal (GI) microbiota plays a key role in health and disease.

It contributes to the breakdown of dietary constituents that are non-digestible in the upper GI tract and is intimately involved in various aspects of normal host physiology, such as protection against pathogens, education of the immune system and modulation of GI development.

Furthermore, the GI microbiota plays a pivotal role in, or is the cause of, several diseases.

The composition of the GI microbiota is mainly influenced by genetic factors, age and diet [1].

Numerous diseases in both adults and infants have links to the GI microbiota, including stomach cancer, mucosa-associated lymphoid tissue lymphoma, inflammatory bowel disease, obesity, necrotizing enterocolitis and autism spectrum disorder (ASD).

The GI microbiota not only influences events in the GI tract, but also, has a long-distance effect, exerting an influence remote from the GI tract as far away as the brain. While the ability of the brain to regulate the function of the GI tract has long been recognized, only in the last decade has the bidirectional nature of this relationship been elucidated.

Based on studies using rodents raised in a germ-free environment, the GI microbiota appears to influence the development of emotional behaviour, stress- and pain-modulation systems, and brain neurotransmitter systems [2].

Disruption of the microbiota in male rats leads to long-term changes in visceral pain perception [3].

The GI microbiota influences the function of the brain and central nervous system through metabolic, neuroendocrine and immune systems and impacts upon cognitive development [4, 5].

Human milk oligosaccharides and the gut–brain axis

Human milk oligosaccharides (HMOs) are associated with functional development during early life, mainly related to immunity and GI health through the establishment of an effective GI microbiota. In animal studies, however, there is evidence that HMOs can influence brain activity and cognitive development.

Following chronic oral administration of the HMO, 2'- fucosyllactose (2'-FL), to rats and mice, the animals showed improved performance in various types of learning behavioural tests. In addition, chronic administration of 2'-FL increased the expression of different molecules involved in the storage of newly acquired memories, such as the postsynaptic density protein 95, phosphorylated calcium/calmodulindependent kinase II and brain-derived neurotrophic factor in cortical and subcortical structures. Overall, the data show that dietary 2'-FL affects cognitive domains and improves learning and memory in rodents [6].

A growing body of evidence now indicates a direct effect of HMOs on the gut–brain axis for the appropriate development of many central nervous system functions, such as GI motility. Various HMOs have a pronounced effect upon GI motor contractions, as indicated from a mouse colon model of peristalsis [7].

The fucosylated molecules, 2'-FL and 3'-fucosyllactose (3'-FL), decreased contractility in a concentration-dependent fashion. On a relative concentration basis, 2'-FL is almost three times more active than L-fucose, and 3'-FL is additionally more than twice as effective as 2'-FL. Fucosylated HMOs had an immediate effect in reducing contractility within 5–15 minutes of administration. It is unlikely that these HMO effects occur through stimulation of *Bifidobacteria*, but the results suggest a specific interaction

of fucosylated HMOs with tissue receptors that, in turn, regulate GI motility, by triggering enteric neurons. It appears that, specifically, the vagus nerve represents a key pathway underlying the effect of the HMOs on gut–brain communication. Vagus nerve stimulation potentiates hippocampal long-term potentiation and learning in freely moving rats [8]. Severing the vagus nerve in rats inhibited the beneficial effects of 2'-FL on hippocampal long-term potentiation and learning [9].

The vagus nerve evidently plays an important role in mediating gut–brain axis activity. It is not apparent from these studies precisely how the HMOs affect cognitive development. They could have a direct impact on the gut–brain axis, or possibly, metabolites from digestion of the HMOs by the GI microbiota could influence the enteric nervous system, which in turn influences the vagus nerve that directly signals to the brain. The human GI microbiota has multiple impacts upon human brain health. Excessive stimulation due to bacterial dysbiosis, small intestinal bacterial overgrowth or increased intestinal permeability may produce systemic and/or central nervous system inflammation. Bacterial proteins may cross-react with human antigens to stimulate dysfunctional responses within the adaptive immune system. Bacterial enzymes may produce neurotoxic metabolites such as D-lactic acid and ammonia. Even beneficial metabolites, such as short-chain fatty acids, particularly propionic acid, may exert neurotoxic effects.

The GI microbiota can produce hormones and neurotransmitters that are identical to those produced by humans. Bacterial receptors for these hormones influence microbial growth and virulence [10]. The development of the concept of the gut–brain axis further suggests that management of the GI microbiota by dietary means has the potential to impact upon a wide range of diseases to improve both physical and emotional well-being in humans.

It offers the possibility of dietary interventions for conditions as diverse as irritable bowel syndrome, anxiety, depression, Alzheimer's disease and ASD [11].

The GI microbiota and autism

Autism is the more familiar name for ASD that comprises a group of heterogeneous neurodevelopmental disorders characterized by social deficits, repetitive and stereotypical behaviours, insistence on routines and communication impairments. ASD includes autism, Asperger's syndrome and pervasive developmental disorder not otherwise specified (PDD-NOS). Epidemiology studies show that the incidence of ASD is increasing [12], as evidenced by the number of ASD diagnoses rising at an alarming rate, with the Centers for Disease Control and Prevention estimating the 2014 incidence rate as 1 in 68. Recently, it has been hypothesized that GI bacteria may contribute to the development of autism [13, 14].

GI abnormalities are a characteristic of a substantial number of children with ASD [13].
An estimated 50% or more of these children are affected by a prevalence of symptoms four times greater than in children without ASD.
The most commonly reported symptoms include chronic constipation, diarrhoea, bloating and gastroesophageal reflux and abdominal pain. In addition, GI microbial dysbiosis, an imbalance in the microorganisms that make up the GI microbiota, has been documented in multiple studies of children with ASD [14].

The observation that pain perception and ASD are linked to the GI microbiota is further support for the concept of the gut–brain axis. This concept attributes functions to the GI microbiota in modulating brain plasticity and influencing early development of normal social and cognitive behaviours, such as learning and

memory, which will subsequently influence mood and behaviour [15, 16, 17].

Most studies demonstrate that children with ASD have more bacterial diversity in their GI microbiota and possess a lower overall abundance of potentially beneficial taxa, such as *Bifidobacteria* and *Akkermansia*. Many, but not all, children with ASD have higher counts of potentially pathogenic *Clostridia* than normally developing children. *Clostridia* species synthesize several metabolic products such as phenols, p-cresol and indole derivatives that are potentially toxic for humans.

Furthermore, an abnormal ratio of Firmicutes to Bacteroidetes has been observed, as well as increased levels of certain detrimental taxa, such as Proteobacteria [14].

It appears that the relative balance between inflammatory microorganisms such as *Clostridia* and *Desulfovibrio* and the anti-inflammatory microorganisms *Bifidobacteria* may become destabilized prior to autism development.

The imbalance leads to a leaky GI tract, characterized by a more porous epithelial membrane, resulting in microbial toxin release into the blood, which may contribute to brain inflammation and autism development [18].

ASD and infant nutrition

The underlying pathophysiology of ASD remains unknown, although sub-optimal breast-feeding practices, including non-intake of colostrum and a short duration of breast-feeding, have been shown to be associated with ASD [19].

This relationship follows a dose-response pattern, with the risk for ASD decreasing with more prolonged periods of exclusive breast-feeding for the first six months and continued breast-feeding throughout the first two years of life. Breast-feeding also appears to be less prevalent, and when present, occurs for a much shorter duration, in children with ASD. Many factors contribute to sub-optimal breast-feeding in children with ASD.

One possibility is that mothers of children with ASD terminate or fail to initiate breast-feeding due to temperamental or behavioural issues associated with ASD that may make breast-feeding more challenging [19].

Breast-fed infants develop a GI microbiota that typically contains high levels of *Bifidobacteria*. HMOs, the third most abundant component of human milk, are recognized as selectively promoting the growth of *Bifidobacteria* in the GI tract, although they provide limited direct nutritional support to the infant. Species of *Bifidobacteria* commonly found in the GI tract of breast-fed infants are *Bifidobacterium longum* subsp. *longum* (*B. longum*), *Bifidobacterium longum* subsp. *infantis* (*B. infantis*), *Bifidobacterium breve* and *Bifidobacterium bifidum* [20].

The abundance and prevalence of *Bifidobacteria* in the GI microbiota of breast-fed infants are attributed to their unique ability to catabolize HMOs, which they carry out in various ways. *B. infantis* produces transporters for the uptake of intact oligosaccharides, which are subsequently degraded by intracellular glycosyl hydrolases.

In contrast, *B. bifidum* secretes a number of glycosyl hydrolases and takes up the resulting monosaccharide or disaccharide residues. Another strategy is that of acting as a scavenger, used by *B. breve*, which can only utilize a small fraction of HMOs, and sometimes only by taking advantage of other species such as *B. bifidum* and *B. longum* that are capable of extracellular hydrolysis of larger HMOs [21].

The GI microbiota component *B. infantis*, in particular, is considered to be beneficial to GI health. It has been found to dominate the GI microbiota of healthy breast-fed infants and is associated with numerous beneficial health outcomes. It has also been shown *in vitro* to improve GI barrier integrity and reduce expression of inflammatory genes in intestinal epithelial cells. *B. infantis* grows exceptionally well in

the presence of HMOs, to the exclusion of other potentially harmful bacteria [19].

Is there a rationale for the use of HMOs in autism?

Current treatments for ASD include behavioural therapy, speech and social therapy and nutritional approaches, but no medical treatment has been approved to treat the core symptoms of ASD, such as social communication difficulties and repetitive behaviours. There is considerable consensus that the onset of ASD is related to changes in the GI microbiota. In particular, there appears to be a reduced number of *Bifidobacteria* and increased *Clostridium* spp., *Desulfovibrio* spp., *Sutterella* spp., and possibly, *Veillonellaceae* [22]. Considering the link between the GI microbiota and the brain, modulating the GI microbiota by HMOs may offer potential therapeutic options or a disease avoidance strategy for ASD. Directing an appropriate GI microbiota through HMOs may be a primary role of human milk. The unique oligosaccharides of human milk cannot be digested by the infant, but are utilized by bacteria in the GI microbiota. These oligosaccharides support expansion of a beneficial GI microbiota, particularly species of *Bifidobacteria*, which manifests as differences in the microbiota between breast-fed and formula-fed infants. This is a potential mechanism whereby human milk could confer long-term beneficial effects on infant health well after weaning [23]. HMOs have two potential benefits with respect to alleviating ASD. Firstly, supplying HMOs to infants though breast-feeding can help establish a functional GI microbiota and thus avoid GI dysbiosis, which is commonly related to the onset of autism. Secondly, evidence from animal studies indicates that HMOs affect cognitive domains and improve learning and memory. Therefore, it is possible that administration of HMOs to patients suffering from ASD could help alleviate some of the symptoms through an effect on the gut–brain axis. In addition, administration of HMOs at daily doses of up to 20 g to healthy adults was shown to be perfectly safe and well tolerated [24]. In another study, 100 human adults were randomized into 10 groups, each consuming chemically produced HMOs at various daily doses (5, 10 or 20 g), or 2 g of glucose as a placebo for two weeks [25].

The safety, tolerance and adverse events related to the HMOs were monitored. Tolerance was good and adverse events were mild. Therefore, it would be quite feasible to investigate the effects of HMOs on individuals with ASD.

References

1. Alou MT, Lagier J-C, Raoult D (2016) Diet influence on the gut microbiota and dysbiosis related to nutritional disorders. Hum Microbiome J 1:3–11

2. Mayer EA, Tillisch K, Gupta A (2015) Gut/brain axis and the microbiota. J Clin Invest 125:926–938

3. O'Mahony SM, Felice VD, Nally K, Savignac HM *et al*. (2014) Disturbance of the gut microbiota in early-life selectively affects visceral pain in adulthood without impacting cognitive or anxiety-related behaviors in male rats. Neuroscience 277:885– 901

4. Cryan JF, Dinan TG (2012) Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci 13:701–712

5. Mayer EA, Knight R, Mazmanian SK, Cryan JF *et al*. (2014) Gut microbes and the brain: paradigm shift in neuroscience. J Neurosci 34:15490–15496

6. Vázquez E, Barranco A, Ramírez M, Gruart A *et al*. (2015) Effects of a human milk oligosaccharide, 2'-fucosyllactose, on hippocampal long-term potentiation and learning capabilities in rodents. J Nutr Biochem 26:455–465

7. Bienenstock J, Buck RH, Linke H, Forsythe P *et al*. (2013) Fucosylated but not sialylated milk oligosaccharides diminish colon motor contractions. PLoS ONE 8:e76236

8. Zuo Y, Smith DC, Jensen RA (2007) Vagus nerve stimulation potentiates hippocampal LTP in freely-moving rats. Physiol Behav 90:583–589

9. Vázquez E, Barranco A, Ramirez M, Gruart A *et al*. (2016) Dietary 2'-fucosyllactose enhances operant conditioning and long-term potentiation via gut brain communication through the vagus nerve in rodents. PLoS ONE 11:e0166070

10. Galland L (2014) The gut microbiome and the brain. J Med Food 17:1261–1272

11. Lawrence K, Hyde J (2017) Microbiome restoration diet improves digestion, cognition and physical and emotional wellbeing. PLoS One 12:e0179017

12. De Angelis M, Piccolo M, Vannini L, Siragusa S *et al*. (2013) Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. PLoS ONE 8:e76993

13. Adams JB, Johansen LJ, Powell LD, Quig D *et al*. (2011) Gastrointestinal flora and gastrointestinal status in children with autism–comparisons to typical children and correlation with autism severity. BMC Gastroenterol 11:22

14. Sanctuary MR, Kain JN, Chen SY, Kalanetra K *et al*. (2019) Pilot study of probiotic/colostrum supplementation on gut function in children with autism and gastrointestinal symptoms. PLoS ONE 14:e0210064

15. Cryan JF, O'Mahony SM (2011) The microbiome-gut-brain axis: from bowel to behavior. Neurogastroenterol Motil 23:187–192

16. Foster JA, McVey Neufeld KA (2013) Gut–brain axis: how the microbiome influences anxiety and depression. Trends Neurosci 36:305–312

17. Leung K, Thuret S (2015) Gut microbiota: a modulator of brain plasticity and cognitive function in ageing. Healthcare 3:898–916

18. Weston B, Fogal B, Cook D, Dhurjati P (2015) An agent-based modeling framework for evaluating hypotheses on risks for developing autism: effects of the gut microbial environment. Med Hypotheses 84:395–401

19. Mulle JG, Sharp WG, Cubells JF (2013) The gut microbiome: a new frontier in autism research. Curr Psychiatry Rep 15:337

20. Sakanaka M, Hansen ME, Gotoh A, Katoh T *et al*. (2019) Evolutionary adaptation in fucosyllactose uptake systems supports bifidobacteria-infant symbiosis. Sci Adv 5:eaaw7696

21. Zúñiga M, Monedero V, Yebra MJ (2018) Utilization of host-derived glycans by intestinal Lactobacillus and Bifidobacterium species. Front Microbiol 9:1917

22. Grimaldi R, Gibson GR, Vulevic J, Giallourou N *et al*. (2018) A prebiotic intervention study in children with autism spectrum disorders (ASDs). Microbiome 6: 133

23. Newburg DS, Morelli L (2015) Human milk and infant intestinal mucosal glycans guide succession of the neonatal intestinal microbiota. Pediatric Res 77:115–120

24. Elison E, Vigsnaes LK, Krogsgaard LR, Rasmussen J *et al*. (2016) Oral supplementation of healthy adults with 2'-O-fucosyllactose and lacto-N-neotetraose is well tolerated and shifts the intestinal microbiota. Br J Nutr 116:1356–1368

25. Salomonsson E, Vigsnaes L, Sommer M, Hennet T *et al*. (2016) Human milk oligosaccharides; now as substantial modulators of the adult gut microbiota. Paper presented at the International Scientific Conference on Probiotics and Prebiotics, Budapest, IPC 2016 Proceedings, p 114. Available from: https://azdoc.site/international-scientific-conference-on-probiotics-and-prebio.html