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Review Article

Human Milk Oligosacharides: The First Prebiotics

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Abstract

Human milk oligosaccharides (HMOs) are the third largest component of human milk after lactose and lipids and they show a complexity and variety not found in milk of any other species. Although HMOs are significant components in human milk, they are not a source of nutrition for the neonate as they are largely indigestible in the small intestine. Rather, HMOs are now recognised as the first prebiotics and their abundance is seen as a key element in evolution's strategy to establish and guide the development of good health in the human infant and possibly in adults. As prebiotics, HMOs have two major functions. Firstly, they help in establishing a beneficial microbiota in the human infant which is important for health maintenance. In particular they can be metabolized by various species of Bifidobacteria and act as a selective medium since many other bacterial species cannot utilize HMOs. Secondly, HMOs can interact with a wide range of pathogenic micro-organisms and so play an important role in disease avoidance in both infants and adults. The various HMOs which are free in the gastrointestinal tract can bind to pathogens and prevent them from adhering to epithelial cell surfaces which is necessary to initiate disease. Human milk oligosaccharides, as probably the first prebiotics, have a long history of use and shown to be safe when administered to children and adults. Therefore, HMOs have scope for further development as agents for health maintenance and disease avoidance in both children and adults.

Keywords: HMOs; Bifidobacteria; Adults

Introduction

The human neonate is inevitably subjected to the pervasive influence of myriads of bacteria and other micro-organisms which pose a threat to health and well-being. Human milk has emerged from 200 million years of relentless Darwinian pressure as a nourishing food for infants. Milk not only contains important nutrients such as protein, lipids and lactose but also has a large component of complex oligosaccharides which are the third most abundant components in human milk after lactose and lipids. These are known collectively as human milk oligosaccharides (HMOs) and they show a complexity and variety not found in milk of any other species. The HMOs are composed of five monosaccharides: D-glucose, D-galactose, N-acetylglucosamine, L-fucose, and N-acetylneuraminic acid (sialic acid). The sugar fucose is also an unusual molecule in that it has the L-configuration whereas the other sugar molecules in the body have the D-configuration.

Human milk/colostrum contains between 5 and 23 g/L of oligosaccharides, containing a lactose-reducing end elongated with fucosylated and/or sialylated N-acetyllactosamine-galactose units. This translates to over 200 different oligosaccharide structures that differ in their size, charge, and sequence. Despite their presence in human milk in substantial quantities, HMOs were long thought to have no biological significance as they are not directly metabolized by the infant. Now they are recognised as the first prebiotics and their abundance is seen as a key element in evolution's strategy to establish and guide the development of good health in the human infant and possibly in adults [1,2].

Human milk oligosaccharides as prebiotics

The concept of compounds acting as prebiotics was only introduced in 1995 [3]. Prebiotics were described as non-digestible food ingredients that beneficially affects the host by selectively

stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health. This perfectly describes the function which HMOs have been carrying out since time immemorial. Human milk oligosaccharides can justly be described as the first and original prebiotics. They were acting as prebiotics long before prebiotics were recognised.

If HMOs can act as prebiotics then a prerequisite is that they have to resist enzymatic hydrolysis in the gastrointestinal tract, i.e. they should be indigestible. To assess the extent to which selected HMOs are digested they were subjected to *in vitro* digestion studies using enzyme preparations of human and porcine pancreas and intestinal brush border membranes. Maltodextrin used as control substrate was rapidly and completely hydrolyzed, but neutral and acidic HMOs showed a profound resistance against pancreatic juice and brush border membrane hydrolases (Reference). These results strongly suggest that HMOs are not hydrolyzed by enzymes in the upper small intestine. Although some intact HMOs may be absorbed probably the majority of HMOs reach the large intestine, where they serve as substrates for bacterial metabolism and therefore have the characteristics of a prebiotic [4].

Subsequent work on HMOs has established two major functions as prebiotics. Firstly, they help in establishing a beneficial microbiota in the gastrointestinal tract of the human infant which is important for health maintenance. Secondly, HMOs can interact with a wide range of pathogenic micro-organisms and so play an important role in disease avoidance in both infants and adults.

Effect of HMOs on establishing a beneficial microbiota in the gastrointestinal tract

There has long been a recognition that HMOs play a key role in creating and maintaining a healthy infant gastrointestinal microbiota. Infants that are breast-fed have a greater population of Bifidobacteria in their microbiota than formula-fed infants [5]. A large population of Bifidobacteria in the microbiota is associated with positive health benefits. It has now been confirmed that *Bifidobacterium longum* subsp. *infantis, Bacteroides fragilis*, and *Bacteroides vulgatus* strains are able to metabolize HMOs as a sole carbon source and achieve high cell densities. In contrast, *Enterococcus, Streptococcus, Veillonella, Eubacterium, Clostridium*, and *Escherichia coli* strains grew less well or not at all [6]. In another study, none out of 10 *Enterobacteriaceae* strains tested, including several *E. coli* strains and one *Shigella dysenteriae* strain, were able to grow on the HMOs 2′-fucosyllactose, 6′-sialyllactose and lacto-N-neotetraose [7].

The abundance and prevalence of *Bifidobacteria* in the gastrointestinal microbiota of 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 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 [8].

It is possible that HMOs, as prebiotics, can enrich an oligosaccharide-consuming microbial population in the infant gastrointestinal tract. They can orchestrate a shift in the infant microbiota from a non-saccharolytic population dominated by commensals of the birth canal to a population dominated by saccharolytic microorganisms such as Bifidobacteria [5]. This gives the Bifidobacteria a competitive advantage over pathogens which cannot metabolize HMOs. As a result of this selective metabolism, beneficial Bifidobacteria can grow and outcompete harmful pathogens. In addition, *B. infantis* and several other infant-associated bacteria produce short-chain fatty acids and other metabolites (post-biotics) that create an environment further favouring the growth of commensals over potential pathogens [9].

It is evident now that HMOs guide the proper assembly and activity of the gastrointestinal microbiota. Bifidobacteria species can use HMOs as the sole carbon source. Therefore, HMOs have a clear prebiotic effect by selectively stimulating the development of a Bifidobacterium-rich microbiota [10]. In effect HMOs exert a selective pressure on the formation of the gastrointestinal microbiota.

Interactions of HMOs with pathogenic micro-organisms

Many viral, bacterial or protozoan pathogens need to adhere to mucosal surfaces to colonize or invade the host and cause disease. The surfaces of these pathogens have oligosaccharide binding proteins which allow them to recognise receptors on the infant's intestinal epithelial cells and thereby initiate disease.

Human milk oligosaccharides also share common structural motifs with glycans on the infant's epithelial cells. Furthermore, because HMOs are unbound, they can serve as free analogues of pathogen host receptors. Instead of binding to epithelial cell surface glycoproteins or glycolipids, pathogens bind to HMOs which therefore act as decoys and protect infants from infectious diseases. This is a powerful defensive strategy of HMOs [11].

The binding of various HMOs to the cell surface of pathogens, thus inhibiting their ability to bind to oligosaccharides on the surface of epithelial cells, has been described as an antiadhesive strategy. An antiadhesive activity of free HMOs has been described for *Streptococcus pneumoniae*, enterpathogenic *E. coli, Listeria monocytogenes, Vibrio cholerae, Salmonella fyris, Pseudomonas aeruginosa, Noroviruses, Cholera* and *Shiga* toxins, *Rotovirus* and HIV. Fucosylated HMOs inhibit binding of Campylobactor jejuni- to human intestinal mucosa, and also reduce *C. jejuni* colonization of mice [2].

The large diversity of HMO structures suggests multiple functions. Various HMO fractions have been shown to have differing activities. Fucosylated HMOs inhibit the binding of *Campylobactor jejuni* to intestinal cells, whereas sialylated HMOs block the adhesion of E. coli to human erythrocytes [5].

The beneficial effect of α1-2-fucosylated HMO on reducing episodes of *C. jejuni*-associated diarrhoea has been confirmed in a prospective study on almost 100 mother–infant pairs from a transitional neighbourhood of Mexico City [12]. *Campylobacter jejuni* diarrhoea occurred significantly less often in infants whose mother's milk contained high concentrations of 2′FL.

The interaction of HMOs with pathogens is a very important function. This is a system of disease avoidance which does not require antibiotics or any pharmaceutical products. It is highly likely that this effect of HMOs could also operate in adults as part of a health maintenance and disease avoidance strategy.

Healthy infants from 0-14 days old were fed for six months with a cow's milk-based infant formula or the same formula with HMOS (1.0 g/L 2′fucosyllactose and 0.5 g/L lacto-N-neotetraose). The infant formula supplemented with the HMOs was found to be safe, well-tolerated and supported age-appropriate growth [13].

Also, administration to healthy adults of HMOs at daily doses up to 20 g was shown to be perfectly safe and well tolerated [14]. 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 placebo for 2 weeks [15]. The safety, tolerance and adverse events of the HMOs were followed. Tolerance was good and adverse events were mild.

Conclusion

Over a long period of human evolution prebiotics have been selected as significant components of human milk in the form of HMOs. Neonates receive both nutrients and prebiotics from birth in human milk. This strongly suggests that these prebiotics play an important role in growth and development of the human infant.

To date, research on HMOs has demonstrated that they exert a wide range of possible benefits. *In vitro* and *in vivo* work continues to show that HMOs serve as prebiotics that help shape microbiota composition, act as soluble decoy receptors to block pathogens from attaching to host cells and causing disease, and have direct antimicrobial effects to halt pathogen growth and survival.

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