REVIEW

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The benefits of human milk oligosaccharides in adult nutrition

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ABSTRACT

Human milk oligosaccharides (HMOs) comprise a large family of extremely diverse oligosaccharides present in high concentrations and which generate a multitude of biological responses. They are generally considered to have a beneficial effect upon the establishment of the microbiota in infants, but they also exert this effect in adult humans. They can impede the attachment of pathogens and toxins such as *Campylobacter jejuni, Escherichia coli, Vibrio cholerae, Salmonella fyris, Helicobacter pylori*, bacterial toxins, *Entamoeba histolytica* and viruses, to the epithelial cells of the gastrointestinal tract. This in effect reduces their pathogenicity as binding to receptors on the epithelial cells is a prerequisite for infection by pathogens. If the pathogens or toxins bind to the HMOs they will be removed from the gastrointestinal tract and not cause disease. Therefore, HMOs are putative protective agents against enteric infections in adults as well as in infants. HMOs are also useful as therapeutic or preventive adjuncts in gut motility disorders and gut pain, and possibly also have beneficial effects in reducing food allergies. Hence, dietary manipulation by the use of HMOs represents a strategy to promote a beneficial gut microbiota and provide health benefits to human adults as well as to infants.

Keywords

Human milk oligosaccharides

Microbiota

Pathogens

Adherence

Colon

Allergy

Introduction

Human milk oligosaccharides (HMOs) comprise a large family of extremely diverse oligosaccharides present in high concentrations in human milk. They form the third most abundant solid component of human milk after lactose (70 g/l) and fat (30–60 g/l) with typical concentrations of 10–15 g/l in mature term milk and exceed the protein content of human milk which is usually 6.0–8.0 g/l [1]. HMOs are not digested in the upper gastrointestinal tract, and only 1–2% is absorbed in infants. The majority of ingested HMOs reach the large intestine where they provide selective substrates for specific gut bacteria [2, 3], modulate the immune system [4], and prevent the epithelial adhesion of intestinal pathogens [5].

The HMOs of human milk are composed of various monosaccharides, namely glucose, galactose, fucose, N-acetylglucosamine and sialic acids (N-acetylneuraminic 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.

The structure of HMOs is a lactose core with the reducing-end elongated with fucosylated and/or sialylated N-acetyllactosamine units [6]. Nearly 200 HMOs have been identified from human milk [7]. Fucosylated HMOs were found to be the most prominent component (~77%), while sialylated HMOs accounted for about 16% of the total abundance of HMOs. The fucosylated HMOs are neutral molecules, while the sialylated HMOs are acidic. In human milk, the most abundant HMO is 2′-fucosyllactose, with a concentration of about 2 g/l (Fig. 1).

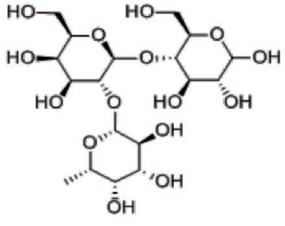

Figure 1 - Structure of 2′-fucosyllactose human milk oligosaccharide

Modification of the microbiota

HMOs are very important in helping to establish an effective microbiota in the human infant. However, when the HMOs

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2′-O-fucosyllactose (2′-FL) and/or lacto-N-neotetraose (LNnT) were fed to healthy adults for 2 weeks, they were also able to modulate the adult gastrointestinal microbiota. There was an increase in the relative abundance of bifidobacteria, of to >25% in some individuals, and a reduction in the relative abundance of two phyla, Firmicutes and Proteobacteria [8]. This is a useful result since bifidobacteria have for long been regarded as beneficial members of the human gastrointestinal microbiota, and low levels have been reported in obese and diabetic individuals, in individuals taking antibiotics, and in patients with irritable bowel syndrome or inflammatory bowel disease.

Also, administration to healthy adults of 2′-FL or LNnT at daily doses up to 20 g was shown to be perfectly safe and well tolerated, and offers the possibility of a strategy to replenish bifidobacteria in adult individuals with low levels of these bacteria.

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 [9]. The safety, tolerance and adverse events of the HMOs were followed. Physical parameters, including pulse rate and blood pressure, remained unchanged during and after HMO uptake. Routine clinical chemistry and haematology analyses also remained stable over the course of the study. Tolerance was good and adverse events were mild. 16S rRNA sequencing analysis revealed that HMOs supplementation specifically modified the adult gut microbiota, with the primary impact being substantial increases in the abundance of *Actinobacteria* and *Bifidobacterium* in particular, and a reduction in Firmicutes and Proteobacteria. The increase in *Bifidobacterium*, reaching more than 25% in some individuals, was dose-dependent but was not dependent on the initial *Bifidobacterium* abundance.

In neither study were there safety or tolerance issues following supplementation with HMOs, but there was a strong impact on the gut microbiota of healthy adult subjects [8, 9]. Collectively, these results show that supplementing the diet with HMOs is a valuable strategy to influence the gut microbiota and specifically promote the growth of beneficial bifidobacteria and improve health in adults as well as in infants.

Interaction with microbial pathogens

Many pathogens recognize the carbohydrate structure on the surface of cells as a receptor. Some HMOs have the same structures as receptors found on the surface of cells, and are therefore potential inhibitors of infection because they are the soluble receptor analogues for the pathogens. Consequently, HMOs can have direct effects on intestinal epithelial structure and function and can interfere with the adhesion of infectious bacteria such as *Campylobacter jejuni, Escherichia coli, Vibrio cholerae, Salmonella fyris, Helicobacter pylori*, bacterial toxins, protozoan parasites such as *Entamoeba histolytica*, and viruses. Enteric infections are a major health challenge for human infants, adults and animals raised for food such as chickens and pigs. Therefore, HMOs are putative protective agents against enteric infection for a wide range of species [5, 10].

Campylobacter jejuni

Campylobacter jejuni is the leading cause of enteric bacterial infection worldwide and poses a substantial challenge to public health. Commercial poultry, particularly broiler chickens, are a reservoir for human infection, with further transmission through contaminated food, water, and direct faecal–oral contact. The bacterial infection causes local acute inflammatory changes in both the small and large intestine leading to abdominal pain, fever, vomiting, headache and diarrhoea. After a *C. jejuni* infection resolves, there is a residual elevated risk of severe complications, including Guillain-Barré syndrome and irritable bowel syndrome.

The first step in campylobacter pathogenesis is adherence to intestinal mucosa [11]. Protein, lipid and carbohydrate fractions prepared from human milk were tested for their ability to inhibit campylobacter adherence to human epithelial cells (HEp-2). Only the oligosaccharide fraction at 3 mg/ml, one-half of the mean concentration in human milk, inhibited infection of HEp-2 cells by the invasive campylobacter strains tested. No inhibition was observed with the lactose, lipid or non-immunoglobulin protein fractions.

Furthermore, the active oligosaccharide fraction caused detectable inhibition at concentrations of only one twentieth of its normal concentration in human milk (0.3 mg/ ml). When the crude oligosaccharide fraction was further separated into neutral and acidic fractions, only the neutral fraction inhibited campylobacter binding and was identified as fucosyl oligosaccharides. The neutral oligosaccharide fraction at one-half its natural concentration in human milk inhibited colonization by the pathogenic campylobacter strain by 93%, and treatment with 2′-FL alone caused 69% inhibition.

A mouse model was also used to determine whether the HMO fraction could inhibit gut colonization in vivo. Mice given neutral HMOs by oral intubation had significantly less campylobacter colonization at both low (10^4 CFU/ml) and high (10^8 CFU/ml) inocula, respectively. Colonization of

mice by pathogenic campylobacter was reduced by several orders of magnitude if the inoculum was accompanied by HMOs.

In another study, the human epithelial cells HEp-2 and HT-29 were infected with a virulent strain of *C. jejuni* [12]. Infected cells were treated with 5 g 2′-FL/l, which attenuated 80% of *C. jejuni* invasion and suppressed the release of mucosal proinflammatory signals of interleukin (IL)-8 by 60–70%, IL-1b by 80–90%, and the neutrophil chemoattractant macrophage inflammatory protein 2 (MIP-2) by 50% [12].

In an in vivo model with 4-week-old male wild-type mice, ingestion of 2′-FL reduced *C. jejuni* colonization by 80%, weight loss by 5% and histological features of intestinal inflammation by 50–70%. Feeding pure 2′-FL may prove useful as a novel clinical prophylactic and therapeutic agent against *C. jejuni* and other enteric pathogens. This offers the possibility that 2′-FL could be a new class of oral agent for the prevention, and potentially treatment, of specific enteric infectious diseases in both adults and infants. *C. jejuni* infection is a recognised public health issue for both adults and infants.

Bacterial toxins

Enterotoxins from *Vibrio cholerae* and enterotoxigenic *Escherichia coli* recognize monosialoganglioside 1 (GMI) on the cell surface as a receptor. This allows the toxins to adhere to the intestinal mucosa and cause diarrhoea.

Human milk contains a large amount of sialic acid bound to oligosaccharides. These sialylated oligosaccharides seem to behave as potential inhibitors against the enterotoxins produced by these bacteria. 3′-Sialyllactose (3′-SL) inhibited the cholera toxin inducing fluid accumulation in a rabbit intestinal loop model [13]. 3′-SL from human milk behaves as a receptor analogue for cholera toxin.

Entamoeba histolytica

Amoebiasis, caused by the protozoan *Entamoeba histolytica*, is the third leading cause of death by parasitic disease, surpassed only by malaria and schistosomiasis. Worldwide, approximately 50 million people are infected with *E. histolytica*, resulting in nearly 100,000 deaths annually. *E. histolytica* is transmitted by the faecal–oral route through contaminated food or water and is often endemic in regions with poor hygiene. Infection is initiated through ingestion of the semi-dormant cyst form of the parasite, which passes through the stomach and small intestine to reach the colon, where it differentiates into motile trophozoites that can colonise and invade the host's mucosa and cause dysentery.

Physiological concentrations of HMOs were able to inhibit *E. histolytica* attachment and cytotoxicity to enteric cell layers in vitro in a dose-dependent manner [14]. The HMOs also rescued epithelial cell layers from *E. histolytica*-induced cytotoxicity when added after the initiation of the co-cultures, indicating that HMOs can halt ongoing cytotoxicity. When the individual HMOs were tested, only lacto-N-tetraose (LNT) (Fig. 2), a neutral tetrasaccharide, significantly protected the intestinal epithelial cells.

Most areas in the world with a high rate of *E. histolytica* infection also have an extreme climate and very limited resources. Therefore, HMOs, which are stable molecules with heat tolerance and low costs, have valuable characteristics that make these oligosaccharides ideal candidates for alternative preventive and therapeutic anti-amoebic agents for treatment of both adults and infants.

Viruses

Avian influenza (AI) infects birds, including poultry, and causes severe economic losses in the commercial poultry sector worldwide. The AI virus subtype H9N2 is highly prevalent with wide distribution around the world. The H9N2 AI subtype is not only infectious to birds, but has also been found to be transmissible to humans and to other animals, such as pigs. In poultry, AI causes acute respiratory tract infection, oedema of the head, and cyanosis of the comb and legs, resulting in reduced egg production, and has a mortality rate of 5–30%. Sialylated HMOs 3′-SL and 6′-sialyllactose (6′-SL) (Fig. 3) have potent inhibitory activity against AI viruses in vitro [15]. 3′-SL exhibited promising antiviral activity against almost all subtypes of viruses tested in the haemagglutination inhibition assay, whereas 6′-SL showed activity against a few selected H1N1, H1N2 and H3N2 subtype strains.

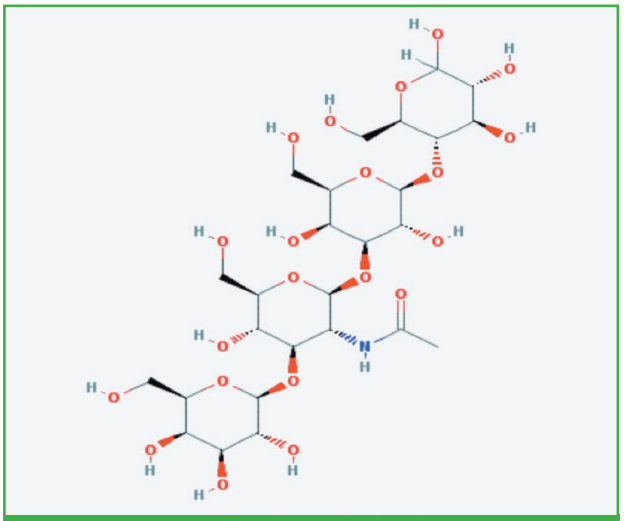

Figure 2 - Structure of lacto-N-tetraose human milk oligosaccharide

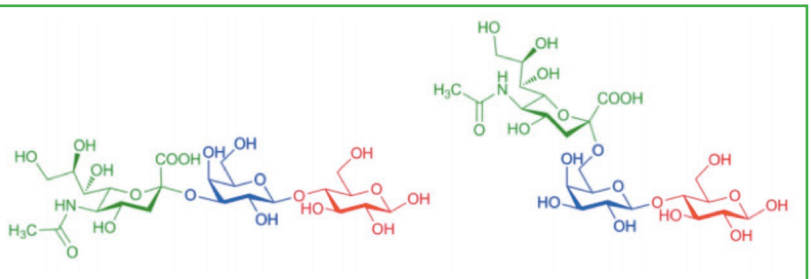

Figure 3 - Structure of sialylated human milk oligosaccharides

The H9N2 virus preferentially binds to α2,3-linked sialyl glycans present in avian mucus cells and 3′-SL was able to mimic the host cell receptors of epithelial cells. Thus, 3′-SL effectively neutralized H9N2 virus by binding at its receptor binding sites and apparently removing them from the colon by simple wash-out.

Moreover, treating chickens with 3′-SL resulted in complete elimination of H9N2 viruses within 24 hours of virus infection. Indirect ELISA assay confirmed complete wash-out of H9N2 viruses from the colon after neutralization by 3′-SL without entering the blood stream. These in vivo results open up possible applications of 3′-SL for the prevention of avian influenza virus infections in birds by a simple cleansing mechanism.

These results are highly significant as they offer the possibility that sialylated HMOs could have beneficial effects in other species as well as in poultry. Viral diseases are a major health challenge for humans and animals. Influenza viruses, particularly influenza A, are the causative agents of seasonal epidemics, causing serious public health problems with 3–5 million cases of severe illness, and about 250,000–500,000 deaths every year worldwide. Unfortunately, many viral vaccines are unable to keep up with the mutation rates of viruses. At the same time, viruses are developing resistance to the currently used drugs, and new drugs need to be developed. The possibility of HMOs contributing to the control of viral diseases is quite novel and interesting.

Colon motor contractions

Various HMOs have a pronounced effect upon gastrointestinal motor contractions as indicated from a mouse colon model of peristalsis [16]. The fucosylated molecules 2′-fucosyllactose (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. Other HMOs such as the acidic sialylactoses 3′-SL and 6′-SL, the neutral lacto-N-neotetraose (LNnT) and galactooligosaccharides (GOS) elicited no effects.

Fucose and fucosylated molecules had the immediate effect of reducing contractility within 5–15 minutes upon colonic neuronally dependent smooth muscle contractions. It is unlikely that these HMO effects occur through stimulation of bifidobacteria, but rather though direct action on neuronally dependent gut migrating motor complexes. These results suggest a specific interaction of fucose and/or fucosylated HMOs with tissue receptors that in turn regulate gut motility, and they could demonstrate anti-nociceptive activity as well.

These observations support the concept that fucosylated HMOs might be useful as therapeutic or preventative adjuncts in gut motility disorders and gut pain, and possibly also have beneficial central nervous system effects.

Allergic diseases

The prevalence of allergic disease has reached epidemic proportions and has been estimated to affect the lives of almost 1 billion people worldwide. Food allergy is considered a ‘second wave’ of the epidemic. Studies suggest food allergy affects nearly 5% of adults and 8% of children. The foods that account for most allergic reactions are cow’s milk, soybeans, wheat, eggs, peanuts, tree nuts, finned fish, shellfish and sesame. As curative treatments are limited, allergic individuals rely largely on allergen avoidance and treatment of acute symptoms. HMOs are recognized as having prebiotic and immunomodulatory effects, which also suggest they may have some therapeutic potential in allergic diseases.

The effect of two HMOs, 2′ FL and 6′-SL, on anaphylactic symptoms induced by oral ovalbumin were studied in an ovalbumin-sensitized mouse model of food allergy [17]. Daily oral treatment with 2′-FL or 6′-SL attenuated food allergy symptoms including diarrhoea and hypothermia. The results suggest that 2′-FL and 6′-SL reduce the symptoms of food allergy through induction of IL-10(+) T regulatory cells and indirect stabilization of mast cells.

In a subsequent study, the HMOs 2′-FL and 6′-SL were shown to modulate human epithelial cell responses related to allergic disease [18]. 6′-SL in particular, may have additional benefits by inhibiting chemokine release induced by

antigen–antibody complex and other inflammatory signals that would in turn inhibit the influx of inflammatory cells to the intestine, potentially attenuating symptoms of food allergy. These findings encourage further investigation of the therapeutic potential of specific HMOs in food allergy.

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