The role of human milk oligosaccharides in preventing necrotising enterocolitis and human immunodeficiency virus transmission

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Abstract

The heavy burden of maternal human immunodeficiency virus (HIV) infection has resulted in a high prevalence of premature births, with an associated increase in the incidence of necrotising enterocolitis (NEC). NEC is a life-threatening inflammatory disease of the gastrointestinal tract that predominantly occurs in preterm infants. Human milk oligosaccharides (HMOs) are carbohydrate compounds which have been shown to have health-promoting effects through their bifidogenic and antiadhesive properties. There is a reduced incidence of NEC in infants who receive human milk, compared to those receiving infant formula. It is suggested that the oligosaccharides found within human milk may act as specific substrates in assisting the growth of selected beneficial bacteria, called probiotics. Probiotics are live microbial food ingredients which have been shown to have health-promoting effects. *Lactobacillus rhamnosus GG* and *Bifidobacterium infantis* have been used as probiotics to reduce the incidence of NEC. Furthermore, HMOs have been associated with protection against postnatal HIV transmission. HMOs may hold key responsibility for the prevention of NEC, and possibly the transmission of HIV, to infants in resource-limited settings and in a developing country, such as South Africa, where HIV plays a major role in the outcomes of preterm neonates.

Introduction

Research, started over a century ago, on human milk oligosaccharides (HMOs) has focused predominantly on their prebiotic effects. Today it is known that the bifidogenic effects of HMOs are one of the many beneficial effects now being discovered.[1] In addition to providing a substrate for the growth of beneficial bacteria, HMOs also directly inhibit pathogens from adhering to the intestinal epithelia through their antiadhesive properties.[1] Recent data suggest that firstly, a relationship exists between the HMOs and the incidence of necrotising enterocolitis (NEC),[2,3] and secondly, they may have an influence on human immunodeficiency virus (HIV) transmission.[4] This article aims to describe the prebiotic and antiadhesive effects of HMOs, and how they could possibly prevent the development of NEC in the presence of HIV infection.

Human milk oligosaccharides

HMOs[5,6] are complex carbohydrates that are highly abundant in breast milk, but not in infant formula.[7] Oligosaccharides are carbohydrates made up of 3-9 monosaccharide units.[5,6] The oligosaccharide concentration of human milk varies with gestational age, lactation duration, and from one mother to another.[6,8] HMOs reach the highest concentration in colostrum (20-25 g/l), and then decrease after roughly two weeks to approximately 5-20 g/l in mature breast milk.[1,6,8-11]

HMOs consists of five different monosaccharide units:
- Glucose.
- Galactose.
- The sialic acid component, known as N-acetylglucosamine.
- Fucose.
- N-acteylneuaminic acid.[1,12]

With a few exceptions, all HMOs contain lactose at their reducing end. They may be elongated (an enzymatic attachment) by the addition of β1-3- or β1-6-linked lacto-N-biose or N-acetyllacetosamine. The latter can be further extended by one or two disaccharides. Chain branching may occur at the β1-6 linkage. Branched lactose molecules may form both linear (para-HMOs) and branched structures (iso-HMOs). Furthermore, oligosaccharide chains can be fucosylated (also known as neutral oligosaccharides) in the α1-2, α1-3 or α1-4 linkage, or sialylated (also known as acidic oligosaccharides) in the α2-3 or α2-6 linkage. The ABH secretor (*Se*) status and Lewis (*Le*) blood group determines the HMO composition of a mother's breast milk.[1] The presence or absence of blood group substances (A, B and H) in the saliva and other body secretions is controlled by a gene on chromosome 19, the fucosyltransferase

locus 2 and the fucosyltransferase locus 3.[13] Both genes are expressed in glandular epithelia and have dominant allele (*Le* and *Se*, respectively) coding for enzymes with fucosyltransferase activity and recessive alleles (*le* and *se*, respectively) that are not functional. Briefly, the fucosyltransferase encoded by the *Se* gene regulates the synthesis of HMOs containing fucose with an α-1,2 linkage. The *Le* gene promotes the synthesis of α-1,3 and α-1,4 linkages.[1] There are up to 200 identified HMOs and their effects appear to be strongly structure dependent.[8,11]

An atypical characteristic of HMOs is that the monosaccharides, of which they are comprised, are bound by specific bonds resistant to the digestive enzymes present in the small intestine.[8,14] Although intact HMOs may be absorbed, the majority of oligosaccharides ingested through breast milk pass through the small intestine undigested, and reach the colon where they act as a fuel source for bifidus-predominant bacteria, thereby gaining the status of prebiotics.[1,15,16] A small percentage of absorbed HMOs are excreted in the urine,[8,11] and may protect the infant from urinary tract infections.[17] High proportions of bifidobacteria are represented in the intestinal tract of breastfed infants, and through the generation of short-chain fatty acids, they protect the enterocyte from pathogens, and may contribute to mucosal integrity.[8,11,17]

HMO functionality is not limited to their prebiotic effects only. The possibility was raised more than a decade ago that HMOs may act as an anti-infective agent by blocking pathogen adhesion to the intestinal epithelial cell walls, thereby possibly preventing enteric infections, such as rotavirus diarrhoea and systemic infections, such as HIV/acquired immune deficiency syndrome.[17] The anti-infective effect that they hold has generated considerable interest. The bifidogenic and anti-adhesive properties of HMOs, as well as their role in NEC and the possible linkage to HIV, are now be described in further detail.

The bifidogenic (prebiotic) effect of human milk oligosaccharides

Factors that influence gastrointestinal tract colonisation can be grouped into two categories: extrinsic and intrinsic. Intrinsic factors include underlying neonatal health, immunological status, gastrointestinal transit time and pH. These factors affect the process of colonisation and the types of organisms established in the neonatal intestinal tract. Extrinsic factors include the geographical area, maternal and surrounding environment bacteria, mode of delivery, feeding practices and drug therapies.[18]

It is well established that the type of delivery has a significant influence on the development of intestinal microflora.[19,20] Infants born by vaginal delivery are more exposed to their mother's microbiota than those born by Caesarean section.[19] Initial exposure to microflora by those born by Caesarean delivery is most likely to be from environmental micro-organisms on the equipment, in the air and on other neonates, with the nursing personnel acting as carriers.[20]

Furthermore, the preterm infant is exposed to bacteria in the neonatal intensive care unit, where the frequent use of broad-spectrum antibiotics affects the colonisation of health-promoting bacteria.[21] The choice of feed plays an important role in the establishment of intestinal flora. *Bifidobacterium* is a predominant organism found in the intestinal tract of breastfed infants. *Lactobacillus* and *Streptococcus* are found in lesser quantities. By contrast, similar amounts of bacteroides and bifidobacterium are found in formula-fed infants, as well as components of the more pathogenic species, such as *Staphylococcus, Escherichia coli* and *Clostridia.[21]* An extraordinary characteristic of the intestinal environment of breastfed infants is the selective nourishment and protective support provided by the intestinal microbiotia.[22]

The term "prebiotic" was introduced and defined by Gibson and Roberfroid as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon".[23,24] Although HMOs fully conform to this definition, it is important to bear in mind that HMOs promote the growth of certain, but not all, health-promoting bacteria.[1] Glycoprofiling results by LoCascio et al[22] provide molecular and mechanistic evidence that specific strains of bifidobacteria have an affinity for selected classes of HMO.[8,11] This suggests that the diversity of specific oligosaccharide structures found within human milk do not provide a direct source of nutrition to the infant, but rather act as specific substrates to assist the growth of selected beneficial bacteria, that in their own right support the development and protection of the neonate.[22] N-acetylglucosamine oligosaccharides have been implicated as important cell wall components for bifidobacteria.[17] In the case of *B. longum by infantis,* this strain has the ability to preferentially consume the most abundant HMOs.[11,22]

Antiadhesive effect of human milk oligosaccharides

There is increasing evidence that oligosaccharides in human milk have a direct inhibitory effect on certain virulence capabilities of pathogenic microorganisms.[11] The destructiveness of most pathogenic microorganisms, e.g. *Campylobacter jejuni, E. coli* and *Salmonella* strains, often depends on their aptitude to adhere to epithelial surfaces.[25] In general, bacterial adhesion is a ligand-receptor interaction between structures on the bacterial surface and complementary structures on the mucosal surface of the host.[11]

HMOs structurally imitate epithelial cell surface carbohydrates, and therefore act as decoys to which infectious agents can bind, instead of to the host, thereby preventing possible infection.[26,27] These molecules can withstand the low pH of the stomach, and resist degradation through enzymes from the pancreas and brush border membrane.[28] With every feed, the gut of breastfed infants is flushed with various oligosaccharides that can effectively reach and block binding sites, thereby reducing the chances of probable pathogen adhesion to these locations.[26]

Human immunodeficiency virus and human milk oligosaccharides

Breastfeeding is the predominant postnatal transmission route for HIV-1 infection in children.[29] However, the majority of breastfed infants do not become HIV-infected, despite continuous exposure to the virus through their mothers' milk over many months. This raises the question: Does human milk have properties that protect the infant against HIV transmission?

Dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN)-bound pathogens are usually transferred into dendritic cell (DC) lysosomes, where they are processed before stimulating T cells to initiate an immune response.[25] However, HIV-1 pathogens are kept hidden within the DC for a number of days without prompting an immune response before they are transferred to CD4+ T lymphocytes, where they multiply and cause disease at a later stage.[11,30] DC-SIGN is a carbohydrate-binding protein. It recognises mannose-containing glycoconjugates, such as HIV-1-gp120, with an even higher binding affinity for *Le* blood group antigens.[25,29,31] Although monomeric *Le* epitopes bind to DC-SIGN, the presence of multivalent *Le* epitopes is required to compete with HIV-1-gp120 for DC-SIGN-binding. The occurrence of multiple *Le* blood group determinants as part of HMOs led scientists to postulate that HMOs compete with HIV-1-gp120 for binding to DC-SIGN, and therefore play a protective role against HIV-1 mother-to-child transmission during breastfeeding.[1,25] Since some HMOs structurally resemble the *Le* blood group antigens that show high-binding affinity for DC-SIGN, and some HMOs carry one or multiple *Le* epitopes, HMOs may compete with gp120 for binding to DC-SIGN and reduce HIV-1 mother-to-child transmission.[31,32]

However, the results by Bode et al suggest otherwise.[4] They found that the concentration of HMOs with secretor or *Le*-active epitopes did not correlate with HIV transmission risk. These results propose that the protection against postnatal HIV transmission is probably independent of the mother's secretor or *Le* blood group status, and that HMOs offer other protective properties, i.e. concentrations of lacto-N-neotetraose.[4] As described, more than 200 different HMOs have been characterised, and investigators have speculated whether or not the amount will rise to 1 000. Hong et al described the blocking of the DC-SIGN as a two-edged sword, indicating that although it may reduce the entrance of certain viruses, such as HIV-1, at the same time it may also reduce the ability of the infant's immune system to detect and fight other pathogens, leading to a potentially increased risk of bacterial or viral gastroenteritis.[29] Hong et al have shown that physiological concentrations of HMOs significantly reduce HIV-1gp120-binding to DC-SIGN.[25,29] Therefore, the question is: Which HMOs are the most potent inhibitors of HIV-1-gp120–DC-SIGN binding?[29] Once individual HMOs have been identified that block HIV-1-gp120 binding to DC-SIGN, it will be important to assess whether or not these HMOs trigger adverse effects.[25]

Necrotising enterocolitis

NEC is the most common serious acquired condition of the gastrointestinal tract in preterm infants.[33] NEC occurs in 4-11% of all premature infants born with a very low birthweight, and an inverse relationship exists between birthweight, gestational age and the incidence of NEC.[33,34] Generally, prematurity, enteral feeding, ischaemia and bacterial colonisation are the most common risk factors for the development of NEC.[35-38] The pathogenesis of NEC in neonates born to HIV-infected mothers has not been clearly elucidated. Some described contributing factors include maternal antiretroviral treatment, maternal sepsis and immunological abnormalities. Although antiretroviral prophylaxis has decreased the perinatal transmission of HIV, the burden of NEC in the HIV-exposed newborn population is significant.[39,40] Infants who receive human milk show a reduced incidence of NEC, compared to those receiving infant formula.[41] Numerous advantages of breast milk have been found over infant formula. These include the earlier achievement of full enteral feeding, the presence of active enzymes which enhance the maturation of the immature intestinal tract, and anti-infective agents which protect the neonate from infections.[42] The effect of prebiotics on NEC has not been studied partly because of the very large sample sizes that are required, and secondly because of ethical concerns with such trials.

Jantscher-Krenn et al found that disialyllacto-N-tetraose (DSLNT) has a protective effect against the development of NEC in neonatal rats.[2] Results by Van Niekerk et al[3] indicate that DSLNT may indeed protect against NEC, not only in neonatal rats as indicated by Jantscher-Krenn et al, but also in human preterm infants. These results suggest that infants diagnosed with NEC received breast milk that contained lower concentrations of DSLNT than infants who did not develop NEC. Specifically designed and well powered cohort studies are required to verify the link between DSLNT and NEC. If confirmed, DSLNT concentrations in mothers' milk could serve as a noninvasive marker to determine whether or not a breastfed infant is at risk of developing NEC.[3]

The role of probiotics in the management of necrotising enterocolitis

When focusing on bacterial colonisation, which plays a pivotal role in the development of NEC,[43,44] it is important to note that no single bacterial species has thus far been associated with NEC. Although *Enterobacteriaceae* spp. is the most frequently pronounced bacteria to be found in association with NEC, *Clostridia* spp. and *Staphylococcus* spp. have also been isolated.[45] Probiotics might be beneficial in preventing the overgrowth of pathogenic organisms, increased feeding tolerance,[46,47] decreasing the number of days until full feeds[48] are reached and preventing nosocomial infections. Furthermore, probiotics potentially compete with other organisms for binding sites and substrate in the bowel.[49,50] Microbiota disturbances early in HIV infection lead to greater dominance of potential pathogens, reduced levels of *Bifidobacteria* and *Lactobacillus* spp. and increasing mucosal inflammation.[51] As HMOs support the growth of these beneficial bacteria, it is therefore proposed that in conjunction with the use of probiotics, HMOs could potentially reduce the risk of NEC. A randomised clinical trial by van Niekerk et al was the first to examine the use of probiotics in the management of NEC in HIV-exposed versus non-exposed, very low-birthweight

infants.[52] The results from this trial concluded that the incidence of death and NEC did not differ significantly between the HIV-exposed and unexposed groups. However, a reduced severity of disease per Bell's staging criteria was found. A significantly lower incidence of NEC was found in the probiotic group than in the control group.[52]

Because the pathogenesis of NEC involves various complex pathways, different probiotic strains may create benefit through different pathways. Evidence suggests that the use of a multistrain or multispecies probiotic should be more effective and more consistent than that of a monostrain probiotic.[53] Randomised controlled trials that used a multistrain probiotic reported a significant decline in NEC, whereas those reporting a lesser decline used a single organism, such as *L. rhamnosus GG*.[49,53,54]

Conclusion

HMOs not only act as a substrate for beneficial bacteria, but also provide antiadhesive effects, thereby reducing the risk of infection.[1] Many unanswered questions remain that surround the mother-to-child transmission of HIV and the role of HMOs. Furthermore, the incidence of NEC in premature infants has increased as the burden of HIV has heightened.[55] Therefore, the question remains: Could HMOs serve as a prophylactic measure against this life-threatening gastrointestinal condition? Further studies are needed to investigate the influence of HMOs on the attachment of pathogens to the intestinal epithelial cell walls. To fully understand the immunological needs of the preterm infant, HMO concentrations in preterm breast milk need to be clearly defined. Future prospects for HMO research are expected to be very useful with regard to the development of management options that are effective in protecting babies from harmful infections.

Breast milk is widely recognised as the optimal feed of choice for both term and preterm infants, as it upholds metabolic, immunological and neurodevelopmental advantages.[56] The World Health Organization[57] recommends exclusive breastfeeding for all infants, and recommends modifications to breastfeeding, such as breast milk expression and pasteurisation, to reduce the risk of HIV transmission, while providing the advantages of breast milk's immune-stimulating properties.[58] The use of breast milk should be strongly advocated in neonatal units. Postnatal breastfeeding practices in resource-limited settings and developing countries such as South Africa, can be supported by practising kangaroo mother care in neonatal units. A 2012 Cochrane review concluded that kangaroo mother care improves anthropometric parameters, breastfeeding and mother satisfaction.[59] Mothers of preterm infants should be encouraged to express their breast milk and pasteurise it, if applicable. Breastfeeding must also be enforced when it is physiologically possible for preterm infants.

Acknowledgements

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