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Various Physiological Functions of Milk Oligosaccharides

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Abstract: ***It has been seen that in all of glyco-compounds i.e. glycosides; glycoconjugates etc. carbohydrate are present as mono, dimer or oligomers, which play a decisive role in the biological function of active constituents. Besides this in some of the cases the glycan is present as free oligomer of monosaccharide known as oligosaccharides. Oligosaccharides are amongst the most biologically diverse and important carbohydrate in biological system. Oligosaccharides are found as natural constituents in fruits, vegetables, milk, blood, bacteria and fungus etc. and have various physiological functions such as improvement of mineral absorption, non oranticariogenecity and improvement of both plasma cholesterol and blood glucose level.[1,2,3] The enormous structural variability possible in oligosaccharide structures is the probable reason for nature using them for the purpose of molecular recognition, transformation of oligosaccharide into glycoconjugates may then yield a specific diagnostic material, a non-toxic and highly specific vaccine or therapeutic product.***

Keywords: Oligosaccharide; antiinflammatory; hypoglycemic

1. Introduction

Milk oligosaccharides are an important source of complex carbohydrates as supplements for the food and the pharmaceutical industries. More than 250 milk oligosaccharides have been isolated from milk of Cow, Buffalo, Donkey, Horse, Sheep, Goat, Bear etc. Human milk oligosaccharides are known to protect breast fed infants from a host of bacterial infection. A broad range of oligosaccharides and their derivatives act as an effective drug against most of acute and chronic diseases. Oligosaccharides play an essential role in many molecular processes impacting eukaryotic biology and diseases and exhibit varied biological activity such as immunostimulant, hypoglycemic, anti-tumor, antiviral, anticancer, anticoagulant, anti-complementary, immunological and anti-inflammatory activities.[4,5,6] Certain cell surface oligosaccharides act as potent antigen and used in tumor vaccines has inspired new approaches to the management of tissue rejection, subsequent to xenotransplantation. Glycosphingolipids is the Globo-, Ganglio and lacto-series have been investigated as components of potential tumor vaccines.[7,8] Milk oligosaccharides inhibit the adhesion of pathogenic microorganisms to the intestinal and urinary tract by acting as receptor analogues, thus preventing gastric and urinary infections. More than 250 oligosaccharides have been isolated from various mammalian[9] milk of different origin e.g.; buffalo, equine, caprine, ezobrown, bear, Japanese black bear, elephant, donkey, rat, dog, echidna, platypus, kangaroo, cow, sheep, goat, mare, camel and human etc. Human milk and colostrums contain more than 80 different oligosaccharides (e.g. fucosyl and sialyl –lactose and lacto-N-tetraose etc.) constituting over 20% of total milk carbohydrate. The oligosaccharides isolated from various milk sources are categorized in two classes i.e. sialylated oligosaccharides and non-sialylated oligosaccharides which have been tested for their varied biological activities. Sialylated oligosaccharides appear to be an essential receptor component for many animal virus families such as Newcastle disease virus (paramyxovirus), cardiovirus (picornavirus) murine and primale polyomavirus (papovirus), rheovirus and enterotoxigenic and uropathogenic E.Coli, influenza A, B and C viruses (orthomyxoviruses), Rotavirus binds in both vivo and vitro.[10-14] Human milk oligosaccharide binds to a wide range of lectins on the surface of epithelial cells living the mouth, oesophagus and stomach and throughout the gastrointestinal system in the new born baby. This in turn prevents opportunistic infection whilst the baby's immune system is developing. Oligosaccharides lectin binding has also been used to target therapeutic agents to diseased cells which express high densities of specific lectins on their surface e.g. GalNAc clusters have been used to target antisensenucleotides to hepatocytes to potentially allow treatment of hepatitis A. Many milk Oligosaccharides contains the basic lactose and lacto-N-tetraose sequence (Gal-GalNAc-GalGlc) or one of its derivatives, which has been shown to be a particularly potent bifidus factor. It has been seen that human milk oligosaccharides structures like LNneoT and LNFPII suppress the production of **IL10** cytokines, which is a potent immunosuppressive cytokine found in breast milk produced by mammary cells.[15] The fucose containing oligosaccharides **Fucα1,2 Gal**[16] also enhance immune system of neonates. Fucose suppresses the skin reaction of allergic contact dermatitis and sialic acid inhibits bronchial allergic reaction in animals.[17] Human milk oligosaccharides are currently used for studying the biosynthesis of I, Lewis blood group related antigens. Infact, some of the oligosaccharides i.e., SialylLe^a [18,19] hexasaccharide have recently been characterized as tumor associated antigen or as differentiation antigens.[20]

The trisaccharide Gal(α1-3)Gal(β1-4)G1c which is found in bovine, ovine, caprine, colostrums, bear, coati and elephant milk is an inhibitor of the binding of clostridium difficile toxin A to the intestinal mucosa in the suckling young of these species. Immostimulant activity of buffalo milk oligosaccharides has been reported by using mouse / SRBC model. These oligosaccharides have ability to stimulate non-specific immunological resistance of the host against parasitic infection.[21] A fraction of oligosaccharides isolated from goat milk reduces intestinal

inflammation in a rat model of dextran sodium sulfate – induced colitis and contribute to the recovery of damaged colonic mucosa. The elephant milk oligosaccharide fraction contained a high ratio of sialyl oligosaccharide, this may be significant with respect to the formation of brain components such as gangliosides of suckling claves.[22,23] Donkey milk oligosaccharides have ability to stimulate non-specific and specific immunological resistance.[24] The oligosaccharide mixture of Donkey's milk has also shown significant stimulation of antibody, delayed type hypersensitivity response to sheep red blood cells in BALB / C mice. The orally treated animals showed a six time increase in HA titre, two times increase in PFC & DTH response. The cow's milk oligosaccharides reduce the adhesion of enterotoxic *E.Coli* strains of the calf.[25] The milk oligosaccharides isolated from camel milk shows anti-tuberculosis activity. N-acetylneuraminlactose sulphate may play an important role in the nutrition of rat pups, which is the dominant oligosaccharide in the dog milk. Goat milk oligosaccharides play an important role in intestinal protection and repair after a damage caused by DSS (dextran sodium sulphate) – induced colitis and their implication in human intestinal inflammation. Goat milk containing galacto-oligosaccharides could be recommended to decrease most of infant allergy and diseases. Goat milk shows therapeutic virtues for individuals with certain dietetic problems.[26] Bovine milk oligosaccharides have several potentially important biological activities including the prevention of pathogen binding to the intestinal epithelial and as nutrients for beneficial bacteria. Although bovine milk oligosaccharides are composed of shorter oligomeric chains than are those in human milk, nearly 40 oligosaccharides are present in bovine milk containing not only lactose core (as in human and other animal milk), but also contains lactose amines as basic core units in it. To date, more than 250 different oligosaccharide structures have been isolated and identified. Most of these carbohydrates possesses a Lactose unit at the reducing end.[27-30] Apart from Lactose, several other core structures like LNT, LNnT, etc have been detected so far.

2. Methods and Materials

Milk oligosaccharides isolated from different sources have been found to show various physiological as well as biological activities.Some of physiological functions studied in milk oligosaccharides are discussed below.

Role of milk oligossacharides as prebiotics

Prebiotics are defined as non-digestible food integredients that beneficially affect the host by stimulating the growth and/or activity of beneficial bacteria in the colon.[31] Galacto-oligosaccharides (GOS), also known as oligogalactosyllactose, oligogalactose, oligolactose or transgalactooligosaccharides (TOS), belong because of their indigestible nature, to the group of prebiotics. Galacto-oligosaccharides are produced through the enzymatic conversion of lactose, a major component of human milk oligosaccharide. Because of the configuration of their glycosidic bonds, galacto-oligosaccharides resist hydrolysis by salivary & intestinal digestive enzyme. Therefore they reach colon virtually intact where GOS shows an excellent source for health promoting bacteria as bifidobacteria & Lactobacilli. For maintaining in a disease-free state the human gut microbiota play a key role in the intestinal immune system. The gut & immune system form a complex structure that provides defense against ingested toxins & pathogenic bacteria. A well-balanced gut micro flora[32] is thought to play a particularly important role in the natural defense of the human body. GOS present in human milk support natural defenses of the human body via the gut microflora indirectly by inhibiting the binding or survival of *E.Coli, Choleratoxin, Salmonella Typhimurium & Clostridia* to the body reducing the chances of getting infected.[33,34] Directly by interaction with immune cells, for example in infants the usage of galacto-oligosaccharides has shown to have a potential role in allergy prevention & reduction of infectious disease.[35,36] Several studies have demonstrated that a fiber-supplemented diet has beneficial effects on rat models of IBD. Thus, lactulose administration ameliorated DSS-induced colitis, improving clinical & inflammatory, bloody faeces & colon myeloperoxidase (MPO) activity.[37] So prebiotics may play a role as modulators of the postnatal development of the immune system.

Role of milk oligosaccharide in Brain Development

Oligosaccharides are abundant components of mammalian milk & have primary roles in immune defense & in brain development. Oligosaccharides in association with lactose play an important role in postnatal brain development. A large amount of glycolipids are required in many newborn mammals undergoing a period of postnatal brain development. These glycolipids are mainly the components of cell membrane of neurons & myelin. Sialylated human milk oligosaccharides (SHMOs) are important components of human milk oligosaccharides. Sialic acids are typically found on the non reducing end & are known binding sites for pathogens & aid in neonatal brain development. Nervous system is not fully developed at the time of birth, the galactose and sialic acid present in milk oligosaccharides may be required for optimal development of the infant brain. A recent comprehensive review of sialic acid research shows that the Neu5Ac in human milk is potentially important for infant brain development & cognition.[38] The tissues of the human Central nervous system (CNS) are higher in Neu5Ac concentration than any other tissues in the body. 65% of the conjugated sialic acids of the CNS reside on cell membrane gangliosides & 32% are attached to glycoproteins.[39] Most of the glycoprotein: neural cell adhesion molecules (NCAM)[40] a polysialylated protein widely expressed on CNS cell membranes & shown to be involved in synaptic plasticity, cell to cell interactions, neuronal outgrowth & memory formation.[41,42,43] Supplementation studies in neonatal pigs & rodents with both free & conjugated radio-labeled Neu5Ac show not only uptake of Neu5Ac into brain glycoproteins & gangliosides but also measurable enhancement of learning & memory.[44,45] Breast milk provides an important source of Neu5Ac for the neonate in context of the low capacity for the neonatal brain. Sialic acids, due to their negative charge & hydrophilic nature, help to modulate the cell-cell

interactions.[46] It is also believed that sialic acid serve as ligands for lectin binding involved in regulating the immune response.[47] In addition, the brain is the organ with the highest level of sialic acids where it plays an important role in facilating neuronal sprouting & plasticity.[48,49] SHMOs are therefore believed to play a key role in postnatal brain development.[50] Elephant milk oligosaccharide fraction contained a high ratio of sialyl oligosaccharide, this may be significant with respect to the formation of brain components, such as gangliosides of the suckling calves.[51]

Role of milk oligosaccharides in mineral absorption

Several studies in animals and humans have shown positive effects of non-digestible oligosaccharides (NDO) on mineral absorption & metabolism, bone composition and architecture. These include inulin, oligo-fructose, fructo-oligosaccharides, soybean oligosaccharide & also resistant starches, sugar alcohols & difructose anhydride. There is evidence for an independent probiotic effect on facilitating mineral absorption. Synbiotics, i.e. combination of probiotics & prebiotics, can include additional effects. Inulin, oligo-fructose & galacto-oligosaccharides are the most intensively investigated prebiotics with regard to mineral absorption & retention. In addition, resistant starches & sugar alcohols have been shown to significantly increase mineral absorption & BMC.[52,53,54] Recently it was shown that difructose anhydride prevented parameters of iron deficiency more effectively than FOS. Most of the studies with respect to bone development have been performed in rats. It was shown that prebiotics stimulated the absorption of iron and of bone-relevant minerals such as calcium, magnesium & zinc in short-term experiments & improved BMC in the long-term perspective. In adult animals absorption of calcium & magnesium was stimulated in groups receiving inulin or resistant starch. However, the effect on calcium absorption was more significant if a combination of the two was given.[55] In growing rats only inulin alone significantly increased BMC & density & decreased urinary excretion of collagen cross links, a marker of bone resorption, but not oligofructose alone or oligo-fructose combined with inulin.[56] Oligo-fructose prevented ovariectomy-induced loss of BMC as indicated by lower bone volume/tissue volume (BV/TV).[57] Magnesium, Boron, Manganese, Copper & Zinc are essential cofactors for enzymes involved in collagen synthesis & other bone matrix[58,59,60] constituents that are required to build up the organic bone matrix, the precondition for mineral accretion. Oligo-fructose not only stimulated calcium absorption but also that of Zinc & Magnesium in rats with & without suppressed magnesium absorption as a result of high calcium supplementation.[61]

Protection by milk oligosaccharides against pathogenic infection

The intestinal mucosa is the largest surface of the human body and it is among the most heavily glycosylated tissue. The mucosa of the intestine is covered with complex glycans, including glycoproteins, glycolipids, mucins and others. The principal function of these glycans is though to be mediation of communication with the extracellular environment, including cell-cell communications, molecular discrimination, barrier functions and diverse signaling actions. To overcome this barrier, the first step of bacterial and viral infections is to recognize and bind specific cell surface glycans of the intestinal mucosa, where sialylated and fucosylated oligosaccharides are the primary targets. Because many milk oligosaccharides contain structural units that they act as soluble receptor analogs that inhibit the adhesion of pathogens, thus preventing infection. Infact, HMO are synthesized by the same glycosyl- and fucosyltransferases enzymes responsible for the formation of glycans present on different cell types. Fucosylated and sialylated milk oligosaccharides inhibit the binding of pathogenic bacteria by blocking bacteria from attaching to target oligosaccharides on the intestinal mucosal surface. Milk oligosaccharides have adhesion-inhibiting activity for both Gram-negative and Gram-positive bacteria[62].

Role of milk oligosaccharide as anti-inflammatory substance

Human milk oligosaccharides may also influence the infants on a systemic level. Obviously, they are partially absorbed in the intestine of babies and can be detected in the urine of breast fed infants.[63] Some evidence exist that milk oligosaccharides may functions as anti-inflammatory factors, contributing to the lower incidence and severity of inflammatory disease in breast-fed infants.[64] Particularly, sialic-acid containing oligosaccharides were found to inhibit the formation of platelet-neutrophil complexes and neutrophil activation.[65] In addition, the acidic oligosaccharide fraction significantly inhibited rolling and adhesion on epithelial cells.[66] Goat milk oligosaccharides have anti-inflammatory effects in rats with trinitrobenzenesulphonic (T) acid-induced colitis and may be useful in the management of inflammatory bowel disease.[67]

Role of Milk Oligosaccharide as Immunomodulator

Some investigations have proposed that breastfeeding enhances development of infants natural and vaccine-induced immunity and that its immunological effects continue after breastfeeding.[68,69] Modulation of cell-mediated immunity has been proposed as a mechanism by which breastfeeding might enhance an infant's natural immune response. Breastfeeding has been reported to increase the production of α-interferron,[70] virus-specific lymphocyte transformation,[71] natural killer cell number and percentage and antibody titers to H. influenza type B (Hib) polysaccharide, poliovirus[72,73] and diphtheria[74] toxoid. In addition, sIgA concentrations have been shown to increase more rapidly during the first six months after birth in infants who are breastfed exclusively than do those of infants who are bottle-fed exclusively. In vitro studies of lymphocytes from breast-fed infants support these observations. The Immunomodulatory effects of human milk are thought to be mediated in part by nucleotides and the T-regulatory cytokines, particularly transforming growth factor-β, which are found in significant concentrations in human milk.

3. Conclusion

The structural variability and functions of different milk oligosaccharides isolated from different animals are responsible for various biological activities which is helpful in drug discovery for different diseases.

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