Research article

Human Milk Oligosaccharides Improve Tight Junction Proteins in Disrupted Intestinal Epithelial Cells

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Abstract

The strength of intestinal barrier through tight junction proteins, such as Zonula Occludens-1 (ZO-1) and Occludin, is an important factor for nutrient digestion and absorption to promote growth of the body. Human milk oligosaccharides (HMOs) can protect the integrity of tight junction on the inflammation of intestinal epithelial cells. However, the distinct biological activity of HMOs in human milk on intestinal barrier and inflammation response remains unclear. This study aimed to investigate the patterns of HMOs in human milk and their preventive effects to the tight junction proteins disrupted by tumor necrosis factor-alpha (TNF-α). Breast milk samples from 23 Thai lactating women were separated using 3kDa cut-off column and then purified using solid phase extraction cartridges. The mass spectra of purified HMOs from individual milk samples were created using MALDI-TOF mass spectrometry. The effect of each pattern of HMOs on TNF-α-induced Caco-2 cell monolayer disruption was examined using transepithelial electrical resistance (TEER) measurement. The expression of ZO-1 and Occludin was assessed using immunofluorescence. Nine patterns of HMOs were found. The two major HMOs were galacto-oligosaccharides and sialyllactose (SL) group, particularly 3'SL and 6'SL. The level of TEER increased in the presence of HMOs, especially 3'SL and 6'SL. ZO-1 and Occludin were increasingly expressed in the presence of HMOs. In conclusion, this study suggests that HMO patterns of SL groups show the most protective effect on disruption of tight junction proteins.

Keywords: Human milk, Human milk oligosaccharides, Tight junction protein, Disruption

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บทความวิจัย

กลุ่มโอลิโกแซ็กคาไรด์ในนมแม่เพิ่มความแข็งแรงของโครงสร้างเซลล์เยื่อบุลำไส้ผ่านโปรตีนที่เกี่ยวข้องกับโครงสร้างของเซลล์ลำไส้

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บทคัดย่อ

ความแข็งแรงของผนังลำไส้โดยการทำงานของโปรตีนโครงสร้างซึ่งได้แก่ Zonula Occludens-1 (ZO-1) และ Occludin มีบทบาทสำคัญต่อการย่อยและดูดซึมสารอาหารเพื่อใช้ในการเจริญเติบโตของร่างกาย โอลิโกแซ็กคาไรด์ในน้ำนมแม่สามารถปกป้องความสมบูรณ์แข็งแรงของโปรตีนโครงสร้างต่อการอักเสบที่เยื่อบุเซลล์ลำไส้ อย่างไรก็ตาม การออกฤทธิ์ทางชีวภาพของโอลิโกแซ็กคาไรด์ในนมแม่ต่อผนังลำไส้ และการตอบสนองต่อภาวะอักเสบยังไม่ชัดเจน วัตถุประสงค์ของการศึกษานี้ เพื่อศึกษารูปแบบของโอลิโกแซ็กคาไรด์ในน้ำนมแม่และฤทธิ์การป้องกันการแตกขาดของโปรตีนโครงสร้างเซลล์ลำไส้ที่เกิดจาก Tumor necrosis factor-alpha (TNF-α) ตัวอย่างนมแม่จากมารดาที่ให้นมบุตร จำนวน 23 คน ถูกแยกด้วยคอลัมน์ขนาด 3 kDa และทำให้บริสุทธิ์มากขึ้นด้วยเครื่องสกัดสารตัวอย่างด้วยตัวดูดซับของแข็ง จากนั้น มีการวิเคราะห์รูปแบบโอลิโกแซ็กคาไรด์ด้วยวิธี MALDI-TOF mass spectrometry ฤทธิ์ของโอลิโกแซ็กคาไรด์แต่ละรูปแบบต่อเซลล์เยื่อบุลำไส้ชนิด Caco-2 ที่ถูกกระตุ้นให้เกิดการแตกขาดด้วย TNF-α ถูกทดสอบด้วยการวัด Transepithelial electrical resistance การแสดงออกของโปรตีนโครงสร้าง ZO-1 และ Occludin ถูกประเมินโดยใช้วิธี Immunofluorescence ในการศึกษานี้พบรูปแบบของโอลิโกแซ็กคาไรด์ในนมแม่ จำนวน 9 รูปแบบ โดยโอลิโกแซ็กคาไรด์หลักในนมแม่ที่พบมี 2 รูปแบบ ได้แก่ Galacto-oligosaccharides และกลุ่ม Sialyllactose (SL) โดยเฉพาะ 3'SL และ 6'SL และพบว่า โอลิโกแซ็กคาไรด์ในนมแม่ โดยเฉพาะอย่างยิ่ง 3'SL และ 6'SL ทำให้ค่า Transepithelial electrical resistance สูงขึ้น อีกทั้ง ZO-1 และ Occludin แสดงออกมากขึ้นเมื่อมีโอลิโกแซ็กคาไรด์ในนมแม่ สรุป ผลการศึกษานี้บ่งชี้ว่า รูปแบบของโอลิโกแซ็กคาไรด์ในนมแม่ กลุ่ม SL มีฤทธิ์สูงสุดในการปกป้องการแตกขาดของโปรตีนโครงสร้างเยื่อบุเซลล์ลำไส้

คำสำคัญ: นมแม่ โอลิโกแซ็กคาไรด์ในนมแม่ โปรตีนที่เกี่ยวข้องกับโครงสร้างของเซลล์ลำไส้ การแตกขาด

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Introduction

The human small intestine is essential for nutrient digestion and absorption to promote growth and maintain normal body functions. Small intestinal epithelial cells (or enterocytes) perform a physical segregation barrier between luminal microbial communities and the mucosal immune system to protect the intestinal cells from external pathogens and toxins[1-3]. Enterocytes have a selectively permeable barrier function for the absorption of nutrients and electrolytes. Two routes of selectively permeable functions include transcellular or trans-epithelial permeability and the paracellular pathway associated with three protein networks, which are the adhesive complex consisting of tight junctions, adherent junctions, and desmosomes[3-5]. Loss of barrier integrity associated with unusual expression and dysfunction of tight junction proteins can lead to various diseases such as ulcerative colitis, Crohn's disease, celiac disease, rheumatoid, obesity, and diabetes[3, 6, 7]. Tumor necrosis factor-α (TNF-α), one of the inflammatory mediators, can cause the alteration of tight junction proteins, such as Zonula-Occludens-1 (ZO-1), Occludin, and Claudin. TNF-α is involved in the pathway of cell death as well as paracellular permeability related to migration and proliferation of the epithelial cell lining and reconstitution of the intestinal mucus[8-10]. Several studies have been shown that TNF-α plays a major role in gut inflammatory conditions and directly diminishes tight junction function in both epithelial and endothelial cells[11-14]. Human milk oligosaccharides (HMOs) are the third most abundant component in human milk. More than 200 unique HMOs have been identified[15]. In general, HMOs consists of 3 major groups: fucosyllactose (FL), non-fucosyllactose, and sialyllactose (SL). The HMOs have several biological functions including modulation of proliferation and differentiation in intestinal epithelial cells via alterations of tight junctions and proinflammatory cytokine signaling[16-20]. There are different types of HMOs among species. Moreover, the secretory status, Lewis blood group, and several environmental factors influence HMOs patterns, resulting in specific effective functions. The distinct biological activity of HMOs on intestinal epithelial cells and several inflammatory responses are likely dependent on the difference in oligosaccharide structure and types of cells or target tissues. The combination of HMOs might have more efficiency effects than individual HMOs. Therefore, this study was conducted to explore patterns of HMOs in human milk and to determine whether or not HMOs improved the integrity of tight junction proteins disrupted in the presence of TNF-α.

Materials and Methods

Human milk collection

Thirty milliliters of human milk were collected from each lactating mother participating in the study described elsewhere[21]. In brief, 23 lactating mother who had unconditional pregnancy and have healthy term newborns were included in this study. The participants with alcohol consumption of more than 7 units per week, antibiotics and immunomodulatory drug within 4 weeks prior to delivery and 6 months after delivery were excluded. This study was approved by the Human Research Ethics Committee, Faculty of Medicine Ramathibodi Hospital, Mahidol University (MURA2020/512). Individual milk samples were centrifuged at 3,000

g, 12,000 g, and then 200,000 g at 4°C for 30 minutes each to discard milk cell debris, lipids, and exosome, respectively. Then, protein fractions (> 3 kDa) were removed by the 3kDa cut-off column; the flow through fraction (< 3 kDa) was collected. Milk peptides were discarded by binding to C18 solid phase extraction (SPE) (Waters, Milford MA, USA), and the unbound C18 SPE column was a highly abundant HMO fraction. The small molecule fraction was concentrated by lyophilization. The HMOs were purified by PGC cartridges (ThermoFisher, Massachusetts, USA), then aliquoted and kept at -80°C until analysis (**Additional file 1: Figure S1**).

Mass spectrometry analysis

Purified oligosaccharides and commercial oligosaccharides standard (Biosynth; UK) were resuspended with 50% ACN/ 0.1% TFA for final concentration of 1 μg/mL. Then, 1 μL of the resuspension was loaded onto the Matrix-Assisted Laser desorption/ ionization (MALDI) target plate. Once dried, 1 μL of the 2,5-dihydroxybenzoic acid (DHB) matrix (Sigma Aldrich; St .Louis, MO), which was solubilized by 50% ACN/ 0.1% TFA to 50 mg/mL final concentration, was dotted on the top of the dried sample dot. The DHB matrix was dotted in 2 layers on the dried sample dot on the MALDI target plate. After completely drying, all mass spectra were acquired on the Matrix-Assisted Laser desorption/ionization Time-of-Flight (MALDI-TOF) mass spectrometer[22] (**Additional file 1**, **Figure S1**). The mass spectra were scanned in the range of m/z 0 to 4,000 with linear positive-ion mode. The spectra corresponded to the ion accumulation of 5,000 laser shots randomly distributed on the spot. All obtained spectra were analyzed with default parameters using FlexAnalysis V.3.4 software (Bruker, Daltonik, Germany). The pattern similarity of oligosaccharides in individual samples was processed with MALDI Biotyper® software (Bruker, Daltonik, Germany) with default parameter.

Cell culture and stimulation experiment

The Caco-2 cells, the human epithelial cell line (passage 31), were grown in Eagle's Minimum Essential Medium supplemented with 20% heat inactivated fetal bovine serum (FBS; GIBCO, Massachusetts, USA), 10% tryptose phosphate broth (TPB), 100 μg/mL penicillin, and 100 μg/mL streptomycin (Sigma Aldrich; St. Louis, MO, USA). Then, cells were incubated with 5% CO_2 at 37°C until 100% confluent. For the purposes of this study, only Caco-2 cell passages between 32 and 41 were used. The Caco-2 cells were seeded on the Transwell polycarbonate filters (Corning-Costar, New York, USA) at a density of 1×10^6 cells per insert (1 cm^2) and monitored regularly by transepithelial electrical resistance (TEER) measurement. Cells were cultured for 18-21 days to present complete tight junction formation by TEER level reached 300-500 Ωcm^2. Thereafter, confluent monolayer epithelial cells were incubated with various concentrations of galacto-oligosaccharide (GOS) (Biosynth; UK) from 2.5 mg/mL to 20 mg/mL with and without TNF-α (at 40 ng/mL concentration) for 24-h incubation; and then epithelial barrier alteration was measured using TEER. In this study, concentrations of TNF-α (20, 40, 60, 80, and 100 ng/mL) and the time course of TNF-α (12, 24, and 48 h) effects on Caco-2 tight junction

permeability were determined by measuring TEER using a Millicell-ERS epithelial volt-ohmmeter (Millipore). The TEER values were calculated by subtracting the background TEER of blank filters and multiplying the filter's surface area. The obtained TEER values were calculated and represented as percentage of relative TEER. HMOs at different concentrations were given to the cells fed 2% FBS culture medium in the presence or absence of human recombinant TNF-α at a concentration of 40 ng/mL. GOS was used as a positive control.

Caco-2 cytotoxicity after TNF-α treatment

The Caco-2 cell line was seeded at 1×10^6 cells in a 24-well plate and allowed to grow until 100% confluence. The monolayer epithelial cells were cultured in DMEM supplemented with 2% FBS with and without TNF-α at various concentrations (20-100 ng/mL) for 24 h in humidity with 5% CO_2 incubator to test cell viability using trypan blue exclusion assay. The concentration of TNF-α at 40 ng/mL, which had a significant decrease in TEER value but no significant cytotoxicity, was used for further experiments. HMOs in the presence or absence of TNF-α were added into the cells and then incubated for 24 h at 37°C. After incubation, cell morphology was observed under a light microscope (Olympus, Tokyo, Japan); then, cell viability was counted by trypan blue exclusion assay using the automated cell counter (Logos Biosystems, Gyeonggi-do, South Korea).

Immunofluorescence assay

To examine the expression and localization of tight junction proteins, Caco-2 cells were seeded at approximately 1×10^6 cells and then incubated at 37°C until fully confluent. Cells were treated with each HMO at 7.5 mg/mL in the presence or absence of TNF-α at 40 ng/mL for 24 h of incubation. Subsequently, cells were washed twice with PBS and fixed with 3.7% paraformaldehyde in PBS for 10 minutes at room temperature. After washing with PBS, the fixed cells were permeabilized with 0.1% Triton-x100 for 15 minutes and then washes with PBS three times. The cells were blocked in 1% BSA/PBS and then incubated with the primary antibody specific to Occludin and ZO-1 proteins (Cell Signaling Technology, Massachusetts, USA) in 1% BSA/PBS (1:30) for 1 h at 37°C. After washing with PBS, the secondary IgG antibody conjugated with Alexa fluor 594 dye in 1% BSA/PBS (1:5,000) was added to the cells and then incubated at room temperature for 1 h. The cells were washed with PBS there times and then mounted in 20% glycerol/PBS. The fluorescence signal on the cells was examined using confocal microscopy (Nikon Instruments, Inc., Melville, New York, USA).

Statistical analysis

The statistical analysis of HMOs' effect on tight junction proteins intensity was performed using a SPSS software package (version 13.0) (SPSS; Chicago, IL, USA) and presented as mean ± standard deviation. The sample distribution or normality test was examined by Shapiro-Wilk test, with the *p*-value less than 0.05, meaning that the data is normal distribution. The mean values were compared using unpaired Student's *t* test or one-way ANOVA analysis of variance with Tukey-Kramer test. The *p*-value less than 0.05 was determined as statistical significance.

Results

Profiling of standard HMOs spectrum using MALDI-TOF mass spectrometry

Spectra of reference HMOs standards consisting of galacto-oligosaccharide (GOS), 2'FL, 3'SL, and 6'SL were detected by MALDI-TOF mass spectrometer to generate specific main spectrum profiles (MSP) database (**Figure 1A**). The most peaks were in the range of 500-1,100 m/z, and all spectra of HMOs standard showed similarities in the range of 500-700 m/z (**Figure 1B**). However, their peaks were small different in intensity and mass. To clearly distinguish the spectrum, multiple main spectrum profiles (MSP) of each HMOs standard were analyzed by MALDI Biotyper ® software (**Figure 1C**). The spectra of HMOs standard were classified into 2 major groups, sialyllactose (or acidic) and neutral oligosaccharide groups.

HMOs patterns based on standard HMOs database generation

Multiple spectra of individual HMOs from MALDI-TOF mass spectrometer were also analyzed using MALDI Biotyper® software. All spectrum profiles of each individual HMOs from human milk samples were compared with MSP HMO standards (**Figure 2A**). To distinctly classify HMOs pattern, log scores of multiple HMOs spectra were collected and analyzed for HMOs pattern classification (**Figure 2B**)**.** There were 9 groups of HMOs patterns considering from high log score of the first 2 oligosaccharides which were found in the samples. Most individual milk samples were classified in HMOs pattern number 1, presenting 2 major oligosaccharides, GOS and 3'SL (**Additional file 1: Figure S2**). The second HMOs pattern, of which the amount in individual milk samples was close to HMOs pattern number 1, consisted of 3'SL and 6'SL. Most of HMOs patterns from this study were mixing between GOS and acid oligosaccharides (3'SL and 6'SL).

Effect of TNF-α on intestinal permeability

The percentage of TEER values significantly decreased, from 106.14 ± 4.36 to 92.67%, with the concentration of TNF-α at 20 ng/mL and dropped to 84.82% at the concentration of TNF-α at 100 ng/mL (**Figure 3A**). The concentration of TNF-α that significantly affected cell morphology were higher than 20 ng/mL (i.e., 20, 40, and 60 ng/mL) compared to the control (**Additional file 1: Figure S4B**). The time course of TNF-α on Caco-2 tight junction permeability is shown in **Figure 3B**. TNF-α concentration at 40 ng/mL significantly affected Caco-2 TEER at 24-h incubation compared to the control (84.67 and 95.37%, respectively). There was a sharp time-dependent drop in Caco-2 TEER when compared between 24 and 48 h of incubation (84.67 and 75.44%, respectively). Those concentrations and incubation time points had no significant difference in cell cytotoxicity compared to the control (**Additional file 1: Figure S4A**). Accordingly, TNF-α at 40 ng/mL concentration in 24 h of incubation time increased paracellular permeability.

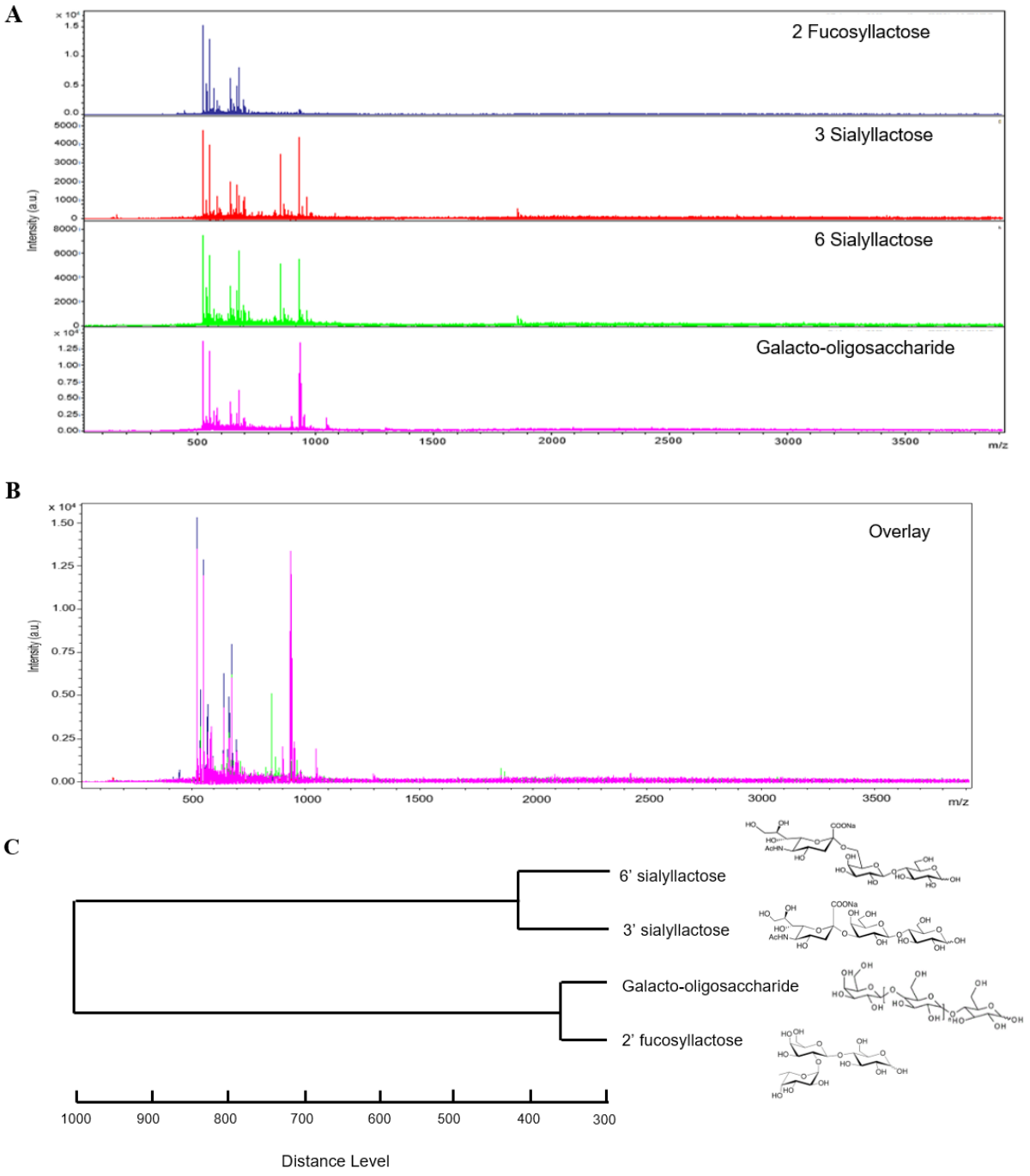

Figure 1. Main Spectra profile (MSP) of standard human milk oligosaccharides (HMOs) using the Matrix-Assisted Laser Desorption/ionization Time-of-Flight (MALDI-TOF) mass spectrometer (MALDI-TOF MS). **(A)** Spectra profile of 4 standards of commercial HMOs performing in mass-to-charge (m/z) and intensity; **(B)** Overlay of MSP of the 4 HMO standards; **(C)** The dendrogram of the 4 MSP HMO standards was clearly separated by using MALDI Biotyper® analysis.

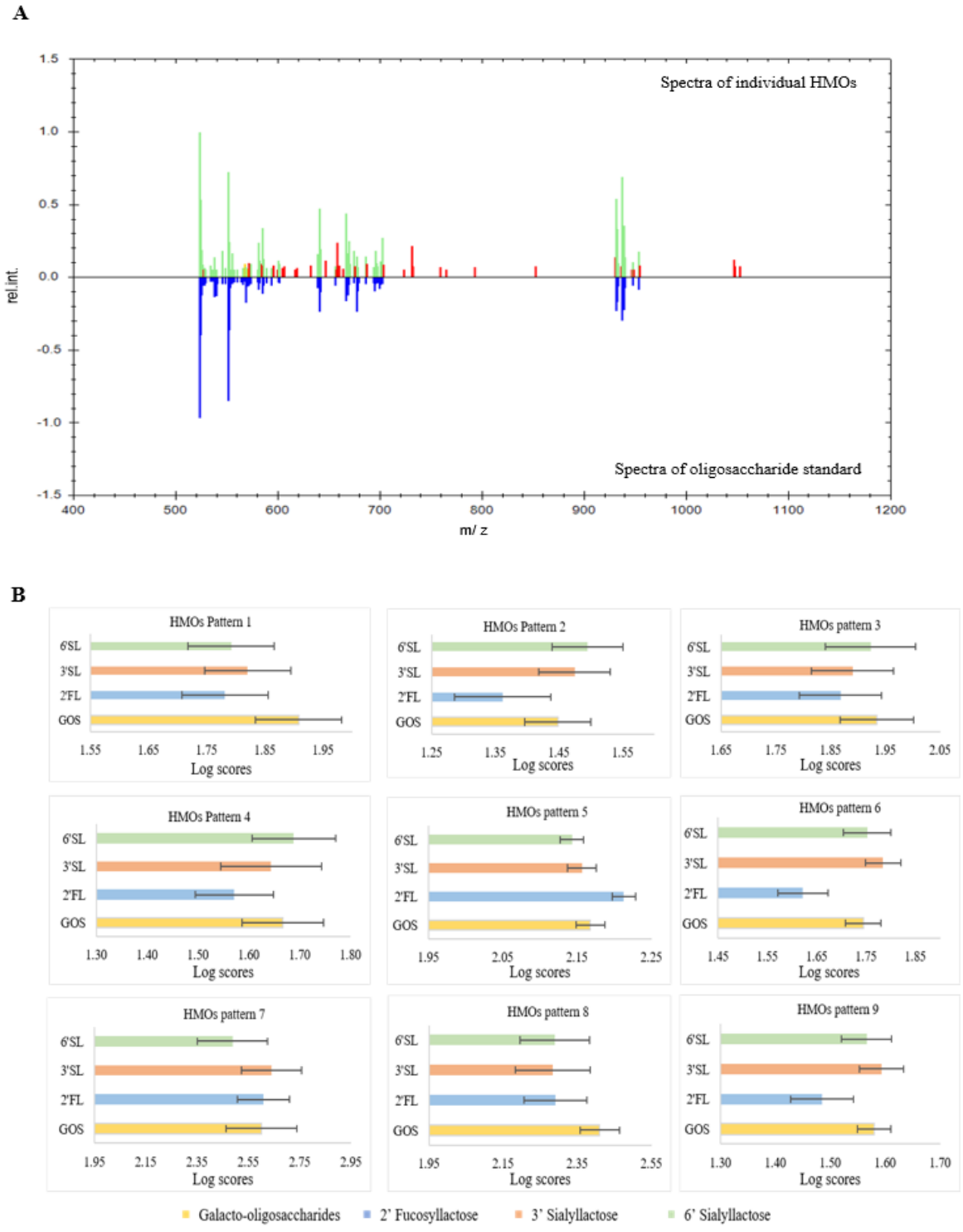

Figure 2. Spectra comparison among the 4 human milk oligosaccharide (HMO) standards and individual HMOs sample. **(A)** Integration of HMOs standard peaks and individual HMOs peaks in m/z and relative intensity. Green and red peaks are similar and different m/z to spectra of oligosaccharide standard, respectively; **(B)** The HMOs patterns were represented by the average log score of oligosaccharides in each pattern.

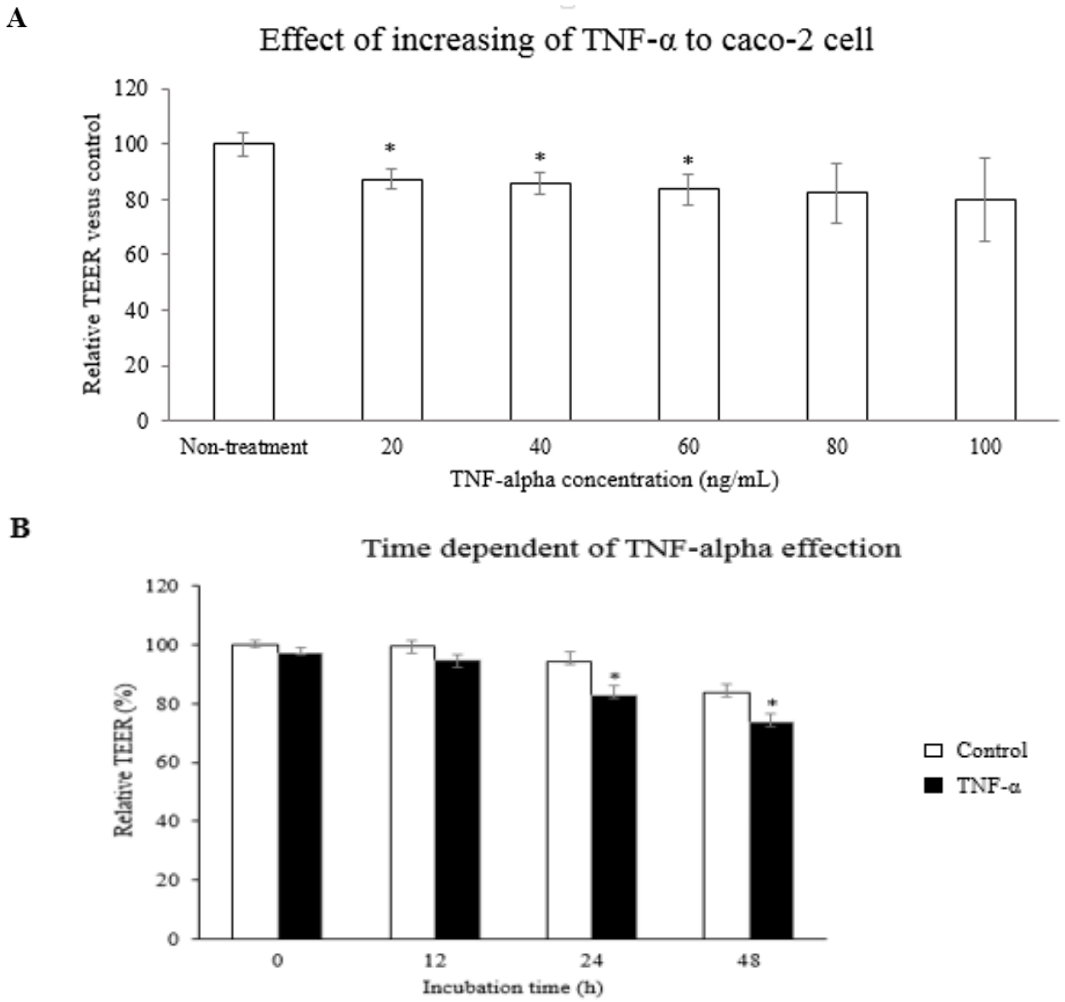

Figure 3. Effect of tumor necrosis factor-alpha (TNF-α) on transepithelial electrical resistance (TEER). **(A)** The concentration of TNF-α affected on intestinal epithelial cell barrier model (Caco-2 cell) at 24 h of incubation time; **(B)** TNF-α (40 ng/mL) produced a time-dependent decrease TEER value on Caco-2 cells at 12 h, 24 h, and 48 h, respectively. *p<0.05 compared to its control at the same time point.

Effect of HMOs patterns on intestinal permeability

In this study, GOS served as a positive control for determination of the efficiency of individual HMOs pattern to Caco-2 cell barrier strength. The permeability measurement of Caco-2 cells incubated with GOS in the presence or absence of TNF-α at a concentration of 40 ng/mL was shown in **Figure 4A**. There was a protective effect of GOS on cell permeability at tight junctions; the significant effect was found when GOS was added at the concentrations of 7.5, 10, and 15 mg/mL. There was no significant difference in cell viability using the cell cytotoxicity assay between control and GOS at those concentrations (**Additional file 1: Figure S3**). The results indicated that the minimum concentration of GOS for significantly preventing epithelial barrier disrupted by TNF-α was 7.5 mg/mL The effect of HMOs pattern on barrier integrity was shown in **Figure 4B**. The Caco-2 cells treated with TNF-α had a significant

decrease in the percentage of TEER (p=0.0087). The GOS treatment showed an increase in the percentage of TEER. The percentage of TEER for some HMOs patterns (pattern No. 3, 5, 6, and 7) in the absence of TNF-α was significantly higher than control (p=0.0025 for pattern No.3 and p<0.001 for pattern No. 5, 6 and 7). The percentage of TEER of HMOs pattern No. 3, 5, 6, and 7 co-incubated with TNF-α was significantly higher than that in the TNF-α treatment alone. The most preventive action belonged to HMOs pattern No. 6 (p<0.001).

Effect of HMOs pattern on tight junction proteins

The alteration of tight junction proteins such as ZO-1 and Occludin expression in parallel with TEER monitoring is usually used for evaluating the permeability of epithelial barrier. ZO-1 and Occludin proteins were markedly decreased in the presence of TNF-α (**Figure 5A** and **5B**) (p<0.001), while the expression of ZO-1 and Occludin was higher when incubated with GOS in the absence or presence of TNF-α than that with TNF-α alone. Likewise, the expression of ZO-1 and Occludin was higher with HMOs in the absence or presence of TNF-α than that with TNF-α alone (**Figure 5C** and **5D**) (p<0.005)

Discussion

In the present study, we found 9 different patterns of HMOs. We had 23 human milk samples; therefore, this is one of the limitations of the present study. However, the normality test of the sample size in this study confirmed that the sample data was normally distributed, which was enough to provide reliable results. The obtained multiple HMOs spectra in the present study were detected using MALDI-TOF mass spectrometry and complied into the standard HMOs database for HMOs pattern classification by MALDI Biotyper® software. The MALDI-TOF mass spectrometer is used for screening the HMOs profiles and individual molecular spectra recognition because it can convert molecules such as protein, lipids, and nucleic acids metabolites into their ionized states, and record them in the format of spectra profiles[25, 26]. This technique can provide a rapid and cost-effective method for analyzing oligosaccharide in their native form in whole oligosaccharide mixtures. The limitation of current technology and computational algorithms results in the inability to reveal particular molecules. Nevertheless, particular spectra profiles of individual metabolites or small molecules can be captured via MALDI-TOF mass spectrometer analysis. Therefore, these spectra profiles can be used for constructing a specific database to recognize and classify individual patterns of biological compounds[22, 26]. The results from the study of Akbari et al. supported the beneficial effects of GOS on the epithelial barrier via tight junction protein function[23]. In the present study, the dominant HMOs patterns were acidic oligosaccharides and GOS, which differs from the results from the study of Donovan et al, showing that fucosyllactose group was more prevalent in human milk[24]. This might be due to maternal factors such as ethnicity, genetic status, nutrition status, and milk microbiota profile contributing to the concentration and composition of HMOs. Xun et al. found that the genetic status of 17 Chinese mothers such as secretor and Lewis gene in the mammary gland influenced HMOs profile

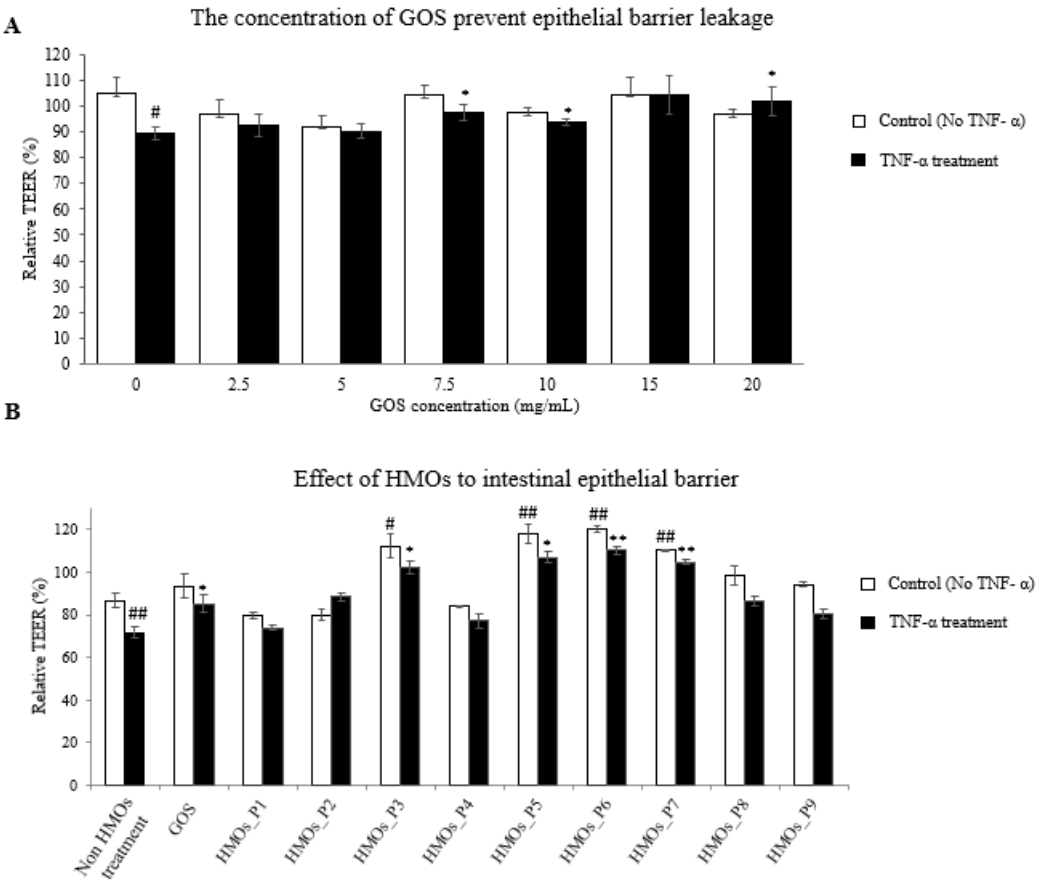

Figure 4. Effect of human milk oligosaccharides (HMOs) on intestinal epithelial permeability. **(A)** The concentrations of galacto-oligosaccharide (GOS) prevent transepithelial electrical resistance (TEER) decreasing on Caco-2 cells; **(B)** Preventive action of HMOs patterns to epithelial barrier integrity.

$^{\#}$ p <0.05 and $^{\#\#}$ p<0.001 versus non-treatment of both tumor necrosis factor-alpha (TNF-α) and HMOs.

* p<0.05 and ** p<0.001 versus TNF-α alone treatment.

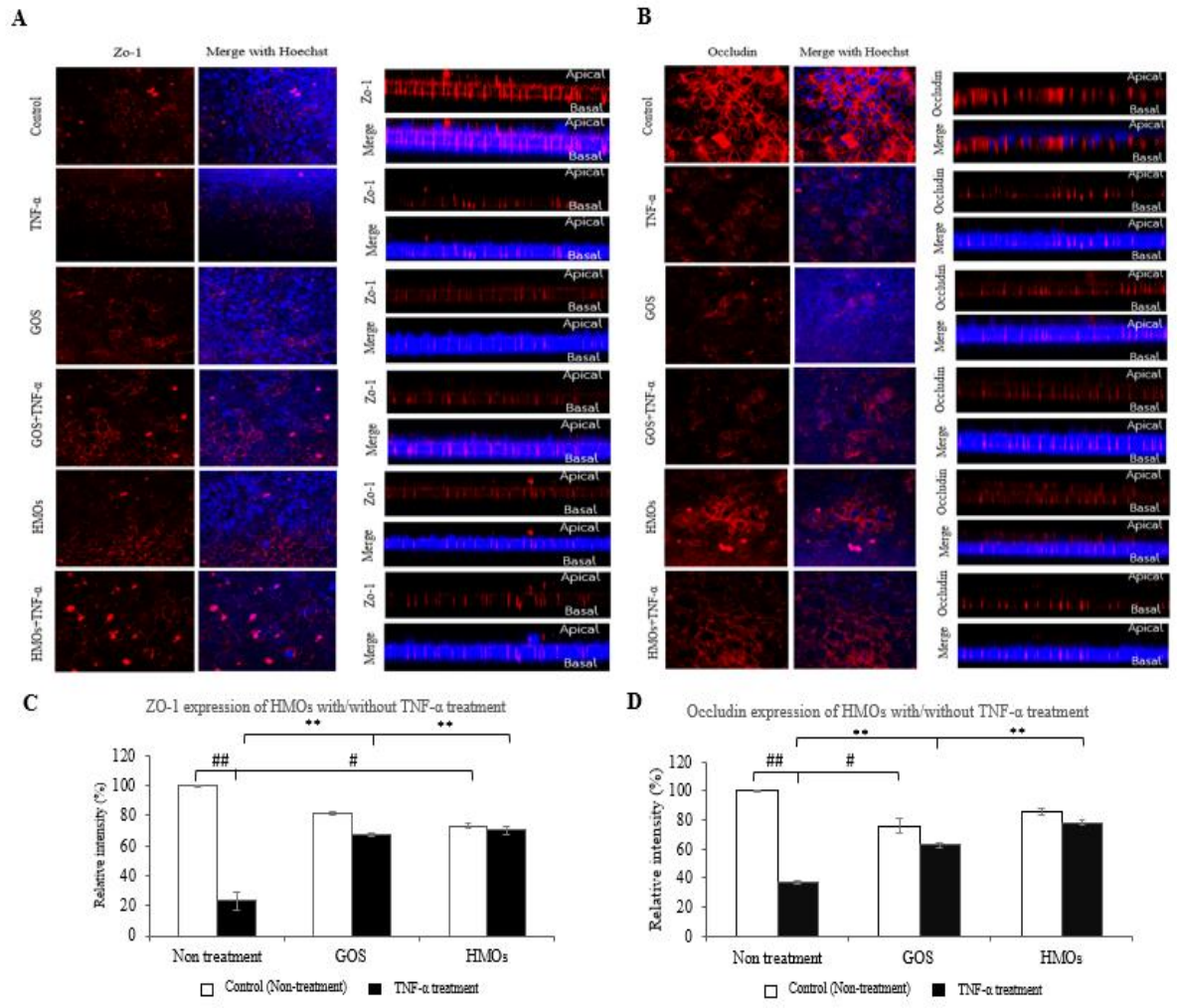

Figure 5. Effect of human milk oligosaccharides (HMOs) on tight junction proteins expression. **(A)** Zonula-Occludens-1 (ZO-1) expression on the epithelial barrier model; **(B)** Occludin expression on the epithelial barrier model. Tight junction proteins are red and nucleus is a blue color; **(C)** The percentage of relative intensity of ZO-1 protein. **(D)** Relative intensity of occluding protein. $^{\#}$ p<0.01 and $^{\#\#}$ p<0.001 versus cell control; * p<0.05 and ** p<0.005 versus TNF-α alone treatment.

which differed from European and American countries[27]. They presented high concentration of 3'SL and low concentration of 2'FL including LNFP I that caused of secretor group and lactation period. However, they suggested that further studies with larger sample sizes are needed. The present study supports the protective effect of acid oligosaccharides and GOS on tight junction proteins. The results of the present study are consistent with those from the study of Zenhom et al., showing that 3'SL decreased mRNA expression of IL-8 and TNF-α mediated via peptidoglycan recognition protein 3 (PGlyRP3)[28]. Huang et al. showed the protective mechanism of 3'SL and 6'SL on intestinal epithelial cells by decreasing of IL-6, IL-8, and IL1β expression that were regulated by TLR4/NF-ĸB pathway[29]. It has been shown that 3'SL and 6'SL promoted cell maturation in the crypt-villus axis model[29, 30]. Apoptosis and necrosis also decrease when exposed to acid oligosaccharide[30]. The differences in substituent-linkage positions of monosaccharide residues and lactose moiety can have significantly different effects on cell biological actions[30, 31]. The different responses on the epithelial barrier treated with several HMOs patterns may be due to the diversity of HMOs structures[31]. The strength of this study was that we utilized the MSP profiling method to analyze and classify HMOs patterns, resulting in finding GOS, 2'FL, 3'SL, and 6'SL. However, according to the findings, it did not represent the entire HMOs in human milk. Notably, the sample population consisted of Asian mothers. The results showed a similar protective effect of sialyllactose on the intestinal barrier although the sample size was small. Oligosaccharides are synthesized from lactose and other monosaccharides by enzymes in the mammary gland. The specific enzymes are influenced by maternal diet and nutrition status. These factors help to support optimal synthesis and level of oligosaccharides secretion in breast milk. Understanding HMOs effects on the barrier of intestinal epithelial cells helps set strategic plans for nutrition intervention and maternal nutrition during lactation. However, we analyzed the patterns of 4 specific oligosaccharides and did not provide quantitative data. Further studies should be explored to determine the optimal ratio between acidic and natural oligosaccharide groups and how maternal diets may modulate the composition to provide advice or recommendation for lactating mothers.

Conclusion

The present study found 9 HMOs patterns according to the oligosaccharides database in MALDI software. All patterns of HMOs, especially GOS, 3'SL, and 6'SL in the present study protected tight junction proteins, which are part of the intestinal epithelial barrier from inflammatory disruption or damage.

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