Camel milk Polar Lipids ameliorate dextran sulfate sodium (DSS)-induced colitis in mice by modulating the gut microbiota

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ABSTRACT

Milk contains abundant polar lipids, which are vital constituents of biological membranes. These polar lipids are present in the human diet as phospholipids (PL) and sphingolipids (SL). Nevertheless, the limited focus has been on the attributes and role of camel milk polar lipids (MPLs). In this study, camel MPLs were isolated, and the composition of their lipidome was determined using Ultra Performance Liquid Chromatography-tandem mass spectrometry. This study characterized a total of 333 polar lipids, which encompassed glycerophospholipids and sphingolipids. Camel milk is rich in polar lipids, mainly phosphatidylethanolamine (PE), sphingomyelin (SM), and phosphatidylcholine (PC). The results indicated that MPLs intervention relieved the clinical symptoms and colon tissue damage in mice with DSS-induced colitis, while also suppressing the expression of pro-inflammatory cytokines. Moreover, administration of MPLs partially alleviated mouse gut microbiota dysbiosis by increasing the abundance of probiotics (such as Lachnospiraceae_NK4A136_group, Muribaculaceae) and decreasing the number of harmful bacteria (such as Bacteroides, Parabacteroides). This study was conducted to investigate the potent protective effects of MPLs in camel milk treatments on a mouse model of colitis and provided new ideas for the application of camel milk.

Keywords: Camel milk polar Lipids, DSS, Anti-inflammation, Gut microbiota

INTRODUCTION

Milk is an essential source of nutrients in the human diet. Lipids are one of the main components of milk solids, accounting for 3–5% of the total milk composition. Milk fat is a important source of fat-soluble nutrients and bioactive lipids for mammals, which have anticancer, antibacterial, anti-inflammatory, and immunosuppressive properties (German et al., 2006). A previous study detected 13 lipid subclasses in bovine, caprine, and soy milk (Li et al., 2017). Milk polar lipids (MPLs) exist primarily on the membrane of milk fat globules, comprising mainly phospholipids, glycolipids, and their derivatives (Li et al., 2020; Sun et al., 2022). Polar lipids are minor lipids in milk, accounting for less than 2% of total lipids (Jiang et al., 2022). Human health is significantly affected by fat globule membrane polar lipids (Venkat et al., 2022). Polar lipids have been shown in recent research to serve a variety of physiological purposes, including the treatment of dyslipidemia, the reduction of inflammation, the improvement of cardiovascular disease, and the promotion of intestine and neural development (Yamashita et al., 2021; Venkat et al., 2022).

Inflammatory bowel disease, or IBD, has been more commonplace globally in recent years. The symptoms of IBD include diarrhea, bleeding in the colon, unresolved pain, and decreased appetite (Ng et al., 2017; Wirtz et al., 2017). The incidence of IBD is affected by genetic variation, diet, environmental factors, and the intestinal microbiota (Piovani et al., 2019; Wu et al., 2021). Antibodies, immunomodulators, and steroids are typically used to treat IBD. However, these drugs are ineffective and cause side effects such as fever and hepatorenal damage (Celiberto et al., 2018). Therefore, to prevent or even slow the progression of IBD, safer and more potent naturally occurring bioactive compounds from various food sources are needed, such as polyphenols, peptides, and polysaccharides (Gou et al., 2019). The milk fat globule membrane (MFGM) is primarily composed of sphingolipids, phospholipids, and MFGM proteins. (Anto et al., 2020). Recent studies have confirmed that MFGM attenuated colitis and hepatic injury by improving the colon's mucosal barrier and bacterial community and further inhibiting oxidative stress in the liver (Wu et al., 2022). SM, a component of milk polar lipids, is protective against colitis disease activity (Mazzei et al., 2011). Additionally, it has been demonstrated that when added to HFD-fed animals,
dietary MPLs have anti-inflammatory qualities and can enhance fecal microbial diversity (Garcia et al., 2022).

Camel milk has antioxidant, anti-inflammatory, and immunomodulatory effects, responsible for its nutritional and medicinal benefits (He et al., 2022). The standard anti-inflammatory bioactive component of camel milk was lactoferrin, and the antioxidant effects were attributed to α-lactalbumin, β-caseins, and vitamin C of camel milk (Behrouz et al., 2022). Previous studies also showed that camel milk contains small globules of milk fat and is easy to digest and absorb. (Meena et al., 2014). In contrast to the composition of short-chain fatty acids found in cow's milk and human milk fat, camel's milk fat is predominantly composed of long-chain saturated fatty acids (Konupsayeva et al., 2008). Camel milk fat contains higher phospholipids (PLs) levels than human or cow's milk, and the SM levels are similar in camel and human milk (Garcia et al., 2012). Dietary hemolysin is absorbed by the intestine, which is beneficial to the health of infants (Nishimukai et al., 2003). For infants and adults, camel milk is a promising source of dietary phosphatidylinositol (PI), phosphatidylyserine (PS), PE, and SM (Bakry et al., 2021). Nevertheless, the role and composition of polar lipids in camel milk have not been investigated to the same degree as in human and cow milk. The objective of this study was to analyze the properties of camel MPLs and assess their biological effects on symptoms associated with IBD.

**MATERIALS**

**Polar Lipids Extracted from Camel milk**

The camel milk was supplied from a farm in Alxa, Inner Mongolia, China. Before transportation to the laboratory, the milk samples were refrigerated at 4°C. The milk lipid samples were obtained following the method of Huang et al. (2023). The milk samples were centrifuged at 30 min at 4000 r/min at 4°C to obtain the milk lipid from the upper layer. The milk samples were centrifuged at 30 min at 4000 r/min to obtain the milk lipid from the upper layer. The milk lipid was carried out on samples according to a Folch method with some modification. Briefly, The upper layer of fat was mixed with chloroform/methanol (2:1). The combined chloroform layer was washed twice with 0.9% NaCl and then centrifuged to obtain the organic phase. The collected organic phase was evaporated in a vacuum. Finally, the extraction was added with equal acetone volume, and the precipitate was collected as the MPLs (Yang et al., 2020; Jiang et al., 2022). The MPLs were kept at −20°C until required.

**Untargeted Lipidomics**

The samples were separated using the ultra-high-performance liquid chromatography (UHPLC) Nexera LC-30A system (Shimadzu, Kyoto, Japan) for untargeted lipidomics analysis. The chromatographic conditions comprised a C18 column, 45°C column temperature, and 300 µL/min flow rate. Solvent A comprised of acetonitrile aqueous solution (a 6:4, vol/vol ratio of acetonitrile to water), and Solvent B contained acetonitrile isopropanol solution (a 1:9, vol/vol ratio of acetonitrile to isopropanol). The gradient started with 30% solvent B and was held for 2 min, and then the mobile phase was increased linearly to 100% solvent B over 23 min. Finally, the column was equilibrated for 10 min in 5% solvent B.

Detection used electrospray ionization (ESI) in positive and negative ion modes. After UHPLC separation, the samples were subjected to mass spectrometry (MS) using a Q Active mass spectrometer (Thermo Scientific, Waltham, MA, USA). We collected the MS scans at a resolution of 70,000 at m/z 200 and acquired the MS/MS spectra at 17,500 at m/z 200. The heater temperature was 300°C, the sheath Gas Flow rate was 45 arb, the Auxiliary Gas Flow Rate was 15 arb, the spray voltage was 3.0 KV, the capillary temperature was 350°C, the S-Lens RF Level was 50%, and the MS1 scan ranges were 200–1800.

**Animal model**

Beijing Weitonglihua Laboratory Animal Technology Co., Ltd. (Beijing, China) provided the 7–8 week old male C57BL/6 mice. All mice received a standard chow diet and had ad libitum access to bottled sterile water under constant conditions comprising 25 ± 2°C in a 12 h light/dark cycle. The ethics committee of Inner Mongolia Agricultural University approved the experimental procedures, with animal permit number SYXK 2020–0002. The mice were divided randomly into 4 groups (standard control (CK), dextran sulfate sodium (DSS) treated, MPLs treated, and the MPLs+DSS treated group, 6 mice per group). After 1 week of acclimation, MPLs (50 mg/kg/day MPLs emulsion) were administered to the mice using oral gavage. The polar fats emulsion was obtained using distilled water as a solvent and emulsifying for 3 min in an emulsifier (OuHorA30, Shanghai Ouhe Machinery Equipment Co., China). The CK and DSS groups were given equal doses of distilled water. Then, the mice were allowed free access to the DSS solution (2.5 g in 100 mL of drinking water) as their only water source from d 21 of the study. On the 28th day, all the mice were sacrificed after anesthesia on postnatal by cervical dislocation.
**RESULTS AND DISCUSSION**

**UPLC-QTOF-MS Analysis of MPLs**

The polar lipid composition of camel milk samples (camel MPLs), including glycerophospholipids and sphingolipids, was analyzed using UPLC-Q-Exactive Orbitrap/MS in positive and negative ionization modes. Camel MPLs may positively impact intestinal barrier activities, dyslipidemia, and inflammation (Norris et al., 2017; Anto et al., 2020). Table S1 presents the species and lipid contents of the 5 samples as determined by the various ionization processes. Three hundred 33 species of lipid were detected, including 77 PE species, 60 PC species, 28 PI species, 20 PS species, 4 PG species, 3 PA species, 14 lysophosphatidylethanolamines (LPE) species, 13 lysophosphatidylcholine (LPC) species, 1 lysophosphatidylinositol (LPI) species, 59 SM species, and 54 ceramide (Cer) species (Figure 1B). According to earlier research, 246 lipid molecular structures were found in human milk, animal milk (donkey, cow, goat, and camel milk), infant formula, and formula milk powder. It was discovered that these samples contained a variety of PE species (between 20 and 35 species), PC species (between 27 and 28 species), and SM species (22 species) (Jiang et al., 2022). As shown in Figure 1A, PE, SM, and PC were the most common polar lipid components, consistent with previous reports in mammalian milk (Jiang et al., 2022). The discovery of numerous MPL species has substantially increased our understanding of the composition of camel milk fats. Ethanolamine appears mostly in milk as the head group of PE, and humans cannot synthesize it. As a result, PE produced by milk is an essential source of dietary ethanolamine. Ethanolamine stimulated weaned pigs’ epithelial cell proliferation, intestinal development, and growth (Yang et al., 2016). Dietary SM has been confirmed to function in chemoprevention and chemotherapy for colon cancer (Yamashita et al., 2021). Furthermore, cow milk polar lipids could attenuate LPS-induced intestinal inflammation and reduce intestinal apoptosis (Yang et al., 2020).

The primary glycerophosphatidylcholines in camel milk were PC (16:0/18:1), PC (18:1/18:1), and PC (18:0/18:1). However, human, bovine, and caprine milk contain mostly PC (18:1/16:0) (Sun et al., 2022). The main PEs found in camel milk were PE (16:0/18:1) and PE (18:0/18:2). The main sphingomyelins found in camel milk were sphingomyelin (d16:0/18:1) and sphingomyelin (d22:1/18:0). Sphingomyelin (SM) is the main component of the lipid organized region of the milk fat globule membrane (MFGM). It plays a crucial role in promoting the formation of myelin in the brain and regulating the population of microbes in the gut (Nor-
ris et al., 2019). Additionally, the most abundant Cer in camel milk was Cer (d18:1/16:0), which contrasts with Cer (d18:0/24:1) in human milk and Cer (d18:0/h24:0) in donkey milk (Jiang et al., 2022). Prior research has also demonstrated that camel milk contains higher levels of PE, PC, and SM compared with other types of milk, such as cow, goat, and sheep. Additionally, these components are found in higher concentrations in camel milk than in human milk, potentially enhancing the biological properties of camel milk (Zhao et al., 2022).

**MPLs reduced the symptoms of DSS-induced colitis**

To determine the impact of MPLs on colitis, we induced a colitis model in mice by administering 2.5% DSS (Figure 2A). Unlike the CK group, the DSS group mice had a significantly decreased weight. However, no animal fatalities were observed. MPLs mitigated the extent of weight loss in mice with DSS-induced colitis (Figure 2B). The clinical parameter used to evaluate the severity of IBD was the DAI. Therefore, the level of inflammation in the mice treated with DSS was assessed using the DAI. Figure 2C demonstrates that the addition of MPLs effectively counteracted the DAI score elevation caused by DSS in comparison to the DSS group \((P < 0.05)\). Colon shortening is commonly regarded as a morphological marker of inflammation (Oh et al., 2014). Figures 2D and 2E indicate that the colons in the DSS group were significantly shorter than those in the CK group. Nevertheless, the length of the colons in the MPLs+DSS group was significantly higher than that in the DSS group \((P < 0.05)\). This finding suggests that MPLs have the potential to inhibit colon shortening in mice with colitis. The results indicated that MPL supplementation relieved symptoms of colitis induced by DSS.

The injury to mouse colons was revealed histologically using H&E staining (Figure 3A). The colonic tissue of the CK group appeared normal, with a complete mucosal epithelium, little infiltration of inflammatory cells, and no other significant irregularities. In the DSS group, the mouse colon tissue showed a decrease in goblet cells, crypt collapse, mucosal ulcerations, epithelial dysplasia, and infiltration of mixed inflammatory cells. The histochemical findings demonstrate that MPLs mitigated the damage caused by DSS in the colon tissue, as shown by the improvement in the histopathological score \((P < 0.05, Figure 3B)\). Earlier studies have demonstrated that milk polar lipids' phospholipids and gangliosides regulate systemic inflammation and lipid metabolism (Zhou et al., 2012). Sphingomyelin and gangliosides reduce cholesterol absorption and protect the integrity of the intestinal wall (Zhou et al., 2019). The long-chain fatty acids in the polar lipids of milk have the capacity to enhance the integrity of the intestinal barrier in mice by promoting the proliferation of mucin-producing cells (Lecomte et al., 2016). Hence, we hypothesize that these factors could be the reasons why MPLs reduce symptoms of IBD.
MPLs suppressed DSS-induced inflammatory activation

Oxidative stress enhancement in colon tissue cells can activate inflammatory responses, leading to overexpression of inflammatory cytokine (Bai et al., 2022). In addition, MPO, as an indicator of inflammatory infiltration, can effectively mediate the production of reactive oxygen species (ROS) and reactive nitrogen species in colitis (Wilson et al., 2011). Figure 4E demonstrates that the colon tissue of the DSS group exhibited a substantial increase in MPO activity compared with the CK group ($P < 0.05$). Conversely, the MPO activity was reduced in the MPLs+DSS group ($P < 0.05$). Furthermore, abnormal increases in inflammatory cytokine levels can indicate the severity of colitis (Shao et al., 2020). In particular, in DSS-induced colitis, TNF-α is critical. Increased TNF-α promotes inflammatory IL-1β, IL-6 production, and other cytokines that enhance inflammation (Huang et al., 2019). The levels of

**Figure 2.** Symptoms of colitis in mice after MPL treatment. (A) The protocol of the experiment; (B) Change in mouse body weight; (C) DAI score; (D) Images of colon length analysis; (E) Colon lengths. Data appear as the mean ± SEM (n = 6). Significant differences ($P < 0.05$) are indicated using different letters.
inflammatory cytokines IL-6, IL-10, and IL-1β in the colon tissue of the DSS group mice were significantly increased compared with those in the CK group \( (P < 0.05; \text{Figure 4B-D}) \). MPL treatment decreased the TNF-α, IL-6, and IL-1β levels in the colon tissue treated with DSS \( (P < 0.05) \). Additionally, IL-10 has been reported to inhibit the expression of IL-1β and TNF-α, thereby inhibiting inflammation and infections in the serum (Ouyang et al., 2011). However, high expression or dysregulation of IL-10 may lead to chronic infections (Hofmann et al., 2012). As shown in Figure 4A, we observed an increase in IL-10 in DSS-treated mice colon tissue, consistent with the result of Chen et al. (Chen et al., 2021). This also might reflect a self-defense mechanism in response to the more severe DSS-induced colitis (Pei et al., 2019). Prior research has established that adding milk-derived bioactive components, such as MFGM, oligofructose, and galactooligosaccharide, can mitigate colitis by diminishing the production of pro-inflammatory cytokines (Liu et al., 2021). Sphingolipids in MPLs are metabolized to ceramides associated with intestinal inflammation, which may be responsible for regulating inflammatory factors by MPLs (Zhou et al., 2019). The therapeutic effect of MPLs in treating inflammation may be attributed to their capacity to inhibit intestinal permeability, which is induced by inflammation, through the use of anti-IL-6 and anti-TNF-α agents. Hence, the result suggests that MPLs have the ability to suppress inflammation by decreasing the concentrations of inflammatory cytokines.

**MPLs modulate the gut microbiota of mice with colitis**

In mice, colitis is frequently associated with changes in the composition and diversity of the gut microbiota (Jangid et al., 2020). In this study, we utilized 16S rRNA sequencing to evaluate how MPLs affect the diversity and composition of the gut microbiota. The rarefaction curves used the Sobs index for each sequenced sample, indicating that the sequencing depth was adequate for obtaining a range of the samples (Figure 5A). As shown in Figure 5B, compared with the CK group, the gut microbiota Shannon and Chao indices in the DSS group were markedly decreased in terms of community diversity and richness \( (P < 0.05) \). In comparison, MPL therapy significantly corrected the reduction in the gut microbiota diversity in mice with colitis \( (P < 0.05) \).

The analysis of β-diversity was conducted using the main Bray–Curtis PCoA to uncover the relative differences in species diversity among the groups. Figure 5C demonstrates that, at the level of OTUs, the group treated with MPLs was distinct from the group treated with DSS. This suggests that MPL treatment has the potential to partially reverse the alterations in gut microbiota structure produced by DSS. PLS-DA analysis yielded comparable findings (Figure 5D).

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**Figure 3.** Histopathology of mouse colitis under MPL treatment. (A) Pathological section of the distal colon (100 × magnification) revealed by HE staining. (B) Histological scores were evaluated. Data appear as the mean ± SEM \( (n = 3) \). Significant differences \( (P < 0.05) \) are indicated using different letters.
Consistent intake of milk SM and its metabolites can potentially modify the intestinal flora. The majority of dietary sphingomyelin (SM) and its digestive by-products exert antimicrobial and intestinal regulatory effects after entering the colon (Zhang et al., 2011). The divergent reactions of the gut microbiota observed in different research can be attributed to disparities in animal models, diets, and the duration of milk polar lipid supplementation. Prior research has primarily concentrated on examining the impact of MPLs on the makeup of gut microbiota at the phylum level. These studies have confirmed that MPLs do not modify or decrease the ratio of sequestered bacteria to Bacteroidetes (Anto et al., 2020). The impact of MPLs on the intestinal microbiome of colitis mice was further analyzed by determining the composition of gut bacteria at the family and genus levels. As shown in Figures 6A and 6B, at the family level, the gut microbiota primarily consists of Muribaculaceae, Bacteroidaceae, Lachnospiraceae, and Prevotellaceae. The administration of DSS resulted in a significant increase in the population of Bacteroidaceae and Enterobacteriaceae compared with the control group \( (P < 0.05) \). Conversely, the treatment with MPL effectively suppressed the excessive growth of these microbes (Figure 6D). Prior research has established that the heightened presence of Bacteroidaceae in colitis contributes to the inhibition of the immune system and the development of cancer. Furthermore, Enterobacteriaceae consist of opportunistic infections that can potentially increase the production of inflammatory cytokines and stimulate cells connected to innate immunity (Pickard et al., 2017). However, dietary MPLs reversed the decreases in Lachnospiraceae and Muribaculaceae abundance induced by DSS, which was consistent with the results of previous studies (Chen et al., 2021; He et al., 2022). Additionally, reports indicate a beneficial correlation between Muribaculaceae and the protective function of the intestinal mucus layer (Cao et al., 2021). The changes in microbial structure at the genus level are shown in Figure 6C. Genus level (Linear discriminant analysis Effect Size (LEfSe) analysis demonstrated that DSS resulted in a decreased abundance of Lachnospiraceae\_NK4A136\_group and augmented levels of Bacteroides, Parabacteroides, Escherichia-Shigella, and Klebsiella in the gut \( (P < 0.05) \) (Figure 6E). The results indicated that MPLs could potentially ameliorate intestinal complications in mice with DSS-induced colitis.

CONCLUSION

In this study, we analyzed the camel milk protein levels using Ultra Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry. A total of 333 lipid species were identified, comprising 77 species of PE, 60 species of PC, 28 species of PI, 20 species of PS, 4 species of PG, 3 species of PA, 14 species of LPE (lysophosphatidylethanolamine), 13
species of LPC (lysophosphatidylcholine), 1 species of LPI (lysophosphatidylinositol), 59 species of SM, and 54 species of Cer (ceramide). In vivo, it was revealed that MPL administration prevented losses in body weight, reduced disease activity, and protected colonic tissue on a mouse model of colitis. The MPLs can ameliorate colitis in mice via downregulation of the expression of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β), and modulation of the gut microbiota’s abundance. These findings suggest that camel milk fat/MPLs promise to develop novel health-promoting products.

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Figure 6. Effects of MPLs on the gut microbiota compositions. (A) Histogram of abundance at the family level. (B) Histogram showing genus abundance. (C) Genus level LEfSe analysis. (D-E) Family and genus relative abundances. Data appear as the mean ± SEM (n = 6). Significant differences (P < 0.05) are indicated using different letters (n = 6).


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