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Comparative activities of milk components in reversing chronic colitis

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ABSTRACT

Inflammatory bowel disease (IBD) is a poorly understood chronic immune disorder for which there is no medical cure. Milk and colostrum are rich sources of bioactives with immunomodulatory properties. Here we compared the therapeutic effects of oral delivery of bovine milk-derived iron-saturated lactoferrin (Fe-bLF), angiogenin, osteopontin (OPN), colostrum whey protein, Modulen IBD (Nestle Healthsciences, Rhodes, Australia), and *cis*-9,*trans*-11 conjugated linoleic acid (CLA)-enriched milk fat in a mouse model of dextran sulfate-induced colitis. The CLA-enriched milk fat significantly increased mouse body weights after 24 d of treatment, reduced epithelium damage, and downregulated the expression of proinflammatory cytokines and nitrous oxide. Modulen IBD most effectively decreased the clinical score at d 12, and Modulen IBD and OPN most effectively lowered the inflammatory score. Myeloperoxidase activity that denotes neutrophil infiltration was significantly lower in mice fed Modulen IBD, OPN, angiogenin, and Fe-bLF. A significant decrease in the numbers of T cells, natural killer cells, dendritic cells, and a significant decrease in cytokine expression were observed in mice fed the treatment diets compared with dextran sulfate administered mice. The Fe-bLF, CLA-enriched milk fat, and Modulen IBD inhibited intestinal angiogenesis. In summary, each of the milk components attenuated IBD in mice, but with differing effectiveness against specific disease parameters.

Key words: angiogenin, Osteopontin, conjugated linoleic acid, Modulen IBD, iron-saturated bovine lactoferrin (Fe-blf)

INTRODUCTION

Inflammatory bowel disease (**IBD**) is a chronic immune disorder affecting over 1.4 million people in the United States and 12 to 13/100,000 people worldwide (Podolsky, 1991). In healthy individuals, the intestine becomes inflamed in response to a pathogen then returns to a state of immunological tolerance once the pathogen is eradicated. However, in individuals with IBD, inflammation is not downregulated and the mucosal immune system remains chronically activated, leading to persistent intestinal inflammation (Kaser et al., 2010). Epidemiological and family studies demonstrate that genetic factors play a key role in susceptibility to IBD (Ek et al., 2014). Inflammatory bowel disease manifests as 2 clinical diseases, namely Crohn's disease and ulcerative colitis (**UC**; Podolsky, 1991; Kaser et al., 2010). Crohn's disease affects the entire wall of any region of the gastrointestinal tract, whereas the pathology associated with UC is confined to the mucosa of the colon and rectum. Even though UC and Crohn's disease are generally accepted as being clinically distinct conditions with distinguishing clinical, anatomical, and histological findings, no diagnostic procedure has been established to distinguish between them (Sands, 2004). Nearly 10% of patients suffering with IBD have indeterminate features between UC and Crohn's disease that cannot be clearly classified (Podolsky, 1991). Inflammatory bowel disease often predisposes to other diseases including arthritis, pyoderma gangrenosum, primary sclerosing cholangitis, and nonthyroidal illness syndrome (Liu et al., 2013). Neither Crohn's disease nor UC is medically curable; current treatment involves administration of high-dose steroids, immunomodulators, and surgery (Triantafillidis et al., 2011).

Milk and colostrum are rich sources of bioactives, including proteins, peptides, and lipids that play crucial roles in both neonates and adults, and represent potential health-enhancing nutraceuticals (Krissansen, 2007; Kanwar et al., 2009). Dairy bioactives have demonstrated bioavailability and anti-inflammatory

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and immunomodulatory properties capable of treating various human disorders (Krissansen, 2007; Kanwar et al., 2009). Orally administered bovine lactoferrin (**LF**) has been reported to reduce zymosan-induced ear-skin inflammation in mice (Hartog et al., 2007), adjuvant-induced arthritis in rats (Hayashida et al., 2004) and mice (Samarasinghe et al., 2014), and inflammation in other inflammatory disorders (Conneely, 2001); oral bovine milk-derived iron-saturated lactoferrin (**Fe-bLF**) inhibits cancer in mice (Kanwar et al., 2008a, 2012). Lactoferrin promotes intestinal cell growth (Nemet and Simonovits, 1985), inhibits myelopoesis (Gentile and Broxmeyer, 1983), and has beneficial effects on iron (Ke et al., 2015) and lipid metabolism (Ono et al., 2013). It inhibits the formation of toxic reactive oxygen species by chelation and storage of plasma iron in the liver and spleen, which contributes to the amelioration of inflammatory states (Artym, 2009). For an average adult person, 1.4 to 3.4 g of lactoferrin can be administered per day without inducing any side effects (EFSA, 2012).

Angiogenin is a potent stimulator of the formation of new blood vessels (neoangiogenesis; Gao and Xu, 2008). It promotes the migration, invasion, and proliferation of endothelial and smooth muscle cells, as well as the formation of tubular structures. It activates inositol-specific phospholipase C, which promotes a transient increase in the intracellular levels of 1,2-diacylglycerol and inositol trisphosphate that facilitate angiogenesis (Bicknell and Vallee, 1988). Milk-derived angiogenin (also known as ribonuclease 5) induced myogenic differentiation and promoted muscle weight gain and grip strength when orally administered to mice (Knight et al., 2014).

Osteopontin (**OPN**) is a highly phosphorylated sialoprotein, which forms a prominent component of the mineralized extracellular matrices of bones and teeth (Butler, 1989). It plays a role in wound healing, immunological responses, tumorigenesis, bone resorption, calcification, obesity, and diabetes (Kahles et al., 2014). A short spliced from of OPN, termed OPN-c, upregulates several metabolic pathways to generate energy and acts as inhibitor of apoptosis (Shi et al., 2014). Osteopontin is a potent neuroprotectant that protects cortical neuron cultures against death induced by oxygen and glucose deprivation (Meller et al., 2005). It is regarded as a proinflammatory cytokine that facilitates the recruitment of monocytes or macrophages and stimulates cytokine secretion in leukocytes (Uede, 2011), it has also been associated with various inflammatory diseases (Uede, 2011); however, few studies have examined the effects of oral administration of OPN. Orally administered bovine OPN suppressed the growth of subcutaneous tumors in mice through a process involving inhibition of angiogenesis by blocking peptides that were absorbed into the circulation (Rittling et al., 2014). Bovine milk OPN dissolved in drinking water was orally administered to dextran sulfate (**DSS**)-treated mice in a model of IBD (da Silva et al., 2009). The treated mice experienced less weight loss, reduced colon shortening and disease activity, improved red blood cell counts, and reduced gut neutrophil activity.

The ingestion of colostrum is believed to play an essential role in gut growth and development in the neonate (Van Barneveld and Dunshea, 2011). Supplementing formula-fed piglets with a low-molecular weight fraction of bovine colostrum whey resulted in improved intestinal barrier function (De Vos et al., 2014). It has been reported that the colostrum whey proteins can be administered at 30 g/d for 6 mo without inducing side effects in humans (Kennedy et al., 1995).

Conjugated linoleic acid consists of a series of positional and geometric dienoic isomers of linoleic acid that occur naturally in foods (Sébédio et al., 1999). It was reported that *cis*-9,*trans*-11 CLA, which is the major form of CLA in milk, improved weight gain and feed efficiency in rats (Chin et al., 1994). The ability of *cis*-9,*trans*-11 CLA to reduce the incidence and severity of atherosclerotic lesions is widely studied and hotly debated (Lee et al., 1994). It modified cardiovascular risk biomarkers in spontaneously hypertensive rats (Herrera-Meza et al., 2013). Dietary milk fat naturally enriched with *cis*-9,*trans*-11 CLA and vaccenic acids attenuated allergic dermatitis and airway disease in mice (Kanwar et al., 2008b). Oral *cis*-9,*trans*-11 CLA inhibited allergic sensitization and airway inflammation in mice via a *peroxisome proliferator-activated receptor-*γ (PPARγ)-related mechanism (Jaudszus et al., 2008). The dose rate of CLA has been speculated to be 3.5 g for a 70-kg individual (MacDonald, 2000).

Modulen IBD (Nestle Healthsciences, Rhodes, Australia) is a powdered casein extract naturally enriched in the anti-inflammatory factor transforming growth factor (**TGF**)-β2, intended for use as a sole source of nutrition in the active phase of Crohn's disease and for nutritional support during the remission phase (Fell et al., 2000). It has been shown to reduce inflammation in the gut and promotes gut mucosal healing (Fell, 2005), and was reported to significantly increase the BW of pediatric Crohn's disease patients (Buchanan et al., 2009). Modulen IBD is generally administered at 800 mg 3 times a day for a period of 6 mo (Triantafillidis et al., 2010).

Previous studies have acknowledged that consuming probiotics in milk and acidified milk can provide protection against weight loss and intestinal inflammation in a DSS-induced murine model of UC (Lee et al., 2015),