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Skimmed, sterilized, and concentrated bovine late colostrum promotes both prevention and recovery from intestinal tissue damage in mice

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ABSTRACT

Bovine colostrum is a rich source of tissue repair and growth factors, and inhibits gastrointestinal injury induced by the side effects of nonsteroidal anti-inflammatory drugs (NSAID), such as indomethacin. Nonsteroidal antiinflammatory drugs are drugs with analgesic and antipyretic effects, but in higher doses they have inflammatory effects. The pathogenesis of small intestinal damage caused by NSAID is unclear. The present study was performed to investigate the antiinflammatory effects of skimmed, sterilized, and concentrated bovine late colostrum on intestinal injury induced by side effects of NSAID, and then to identify the active ingredient in the colostrum for intestinal tissue. In Japan, the sale of bovine colostrum within 5 d after parturition is prohibited by law. Therefore, we focused on bovine late colostrum obtained from healthy lactating cows 6 to 7 d after parturition. Proliferation of small intestine epithelial cells was stimulated in mice fed the colostrum for 1 wk. With regard to indomethacin-induced enteropathy, both prefeeding and postfeeding with colostrum facilitated growth of the intestinal villi, indicating preventive and healing effects. Furthermore, to identify the active ingredient in the colostrum responsible for this effect, the casein and whey fractions were prepared from the colostrum and fed to normal mice. Only the colostrum casein fraction stimulated intestinal villus elongation, whereas the whey fraction and mature milk casein showed no such effect. Taken together, these observations indicate that the skimmed, sterilized, and concentrated bovine late colostrum, especially the casein fraction, could be used to treat the injurious effects of NSAID in the intestine and could be effective for treatment of other ulcerative conditions in the bowel, suggesting that the colostrum has therapeutic potential for intestinal inflammation.

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INTRODUCTION

Colostrum is the milk produced by female mammals during the first few days after giving birth and is rich in immunoglobulins, growth factors, antimicrobial proteins such as lactoferrin, and a variety of other antimicrobial factors, including interferons, iron-binding proteins, polymorphonuclear leukocytes, macrophages, and lymphocytes (Playford et al., 1999). Compared with mature milk, colostrum contains higher levels of growth-promoting proteins (Lee et al., 2008). Colostrum is useful for treating a wide variety of intestinal disorders, including inflammatory bowel disease (Khan et al., 2002), nonsteroidal antiinflammatory drug (**NSAID**)-induced gut injury (Playford et al., 1999), viral gastroenteritis (Sarker et al., 1998; Huppertz et al., 1999), and chemotherapy-induced mucositis (Howarth et al., 1996). Several clinical studies have suggested that bovine colostrum may have antiinflammatory effects in various intestinal inflammatory disorders (An et al., 2009).

However, the sale of bovine colostrum within 5 d after parturition is prohibited by law in Japan. Therefore, we evaluated the protective and recovery efficacy of skimmed and concentrated bovine late colostrum (**SCBLC**), which can be used as a food in Japan, obtained from healthy lactating cows on d 6 to 7 after parturition. Bovine colostrum inhibits binding of norovirus-like particles to human intestinal Caco-2 cells (Murakami et al., 2010). We also previously reported that bovine late colostrum could prevent the development of diarrhea caused by rotaviruses (Inagaki et al., 2010). However, the active ingredients in the colostrum are still not clear.

Casein accounts for 80% of bovine milk proteins. On the other hand, immunoglobulins make the largest contribution to protein content in bovine colostrum, with α-LA and casein contributing lesser amounts (Kelly,

2003). The contribution of immunoglobulins will decline substantially in any bovine colostrum collected more than 24 h postparturition and the amounts of α-LA and casein will increase proportionately (Kelly, 2003).

The pathogenesis of small intestinal damage caused by NSAID is unclear. Therefore, currently no therapeutic strategy exists for ameliorating such damage (Fukumoto et al., 2011). Mucosal damage of the small intestine is one of the major adverse effects of NSAID, such as indomethacin (**IND**) and aspirin (Fang et al., 1977; Robert and Asano, 1977; Bjarnason et al., 1987). Intestinal injury induced by IND is associated with increased mucosal permeability, microvascular injury, focal intravascular thrombus formation, fibrin deposition, and neutrophil infiltration.

We hypothesized that SCBLC may suppress intestinal inflammation and may be useful as an antiinflammatory agent for treating diseases caused intestinal inflammation. The present study was performed to investigate whether oral administration of SCBLC to mice can ameliorate small intestinal inflammation induced by NSAID such as IND, and then to identify the active ingredient in the colostrum for intestinal cells.

MATERIALS AND METHODS

Bovine Milk Samples

Bovine normal milk was collected from healthy Holstein-Friesian cows held at Gifu University Farm (Gifu, Japan), and then maintained at −20°C until processing. Skimmed and concentrated bovine late colostrum from normal cows was prepared at an industrial level in the facility of Kobayashi Pharmaceutical Co. Ltd. (Osaka, Japan). Briefly, the pooled late colostrum from healthy cows 6 to 7 d after parturition was defatted by centrifugation, pasteurized by heating at 73°C for 15 s, and then concentrated by UF, followed by spray drying.

Isolation of Casein and Whey from Milk Samples

Skimmed and concentrated bovine late colostrum and normal milk were acidified to pH 4.6 using 4 *N* HCl at room temperature, and then centrifuged at 12,000 × *g* for 30 min at 25°C. Skimmed and concentrated bovine late colostrum casein and normal milk casein were obtained as the acid precipitate and SCBLC whey was obtained as the supernatant. Both caseins were dialyzed using a membrane with molecular weight cut-off of 6,000 to 8,000 (Spectrum Laboratories Inc., Rancho Dominguez, CA) against distilled water for 3 d at 4°C, and then finally lyophilized.

Animals

Adult female BALB/c mice (6 to 7 wk old; BW: 15 to 18 g) were purchased from Japan SLC Inc. (Shizuoka, Japan), and were kept in an air-conditioned room with a 12-h light-dark cycle under specific pathogen-free conditions during the experimental period. All animals were bred under these conditions with a solid diet (Clea Japan Inc., Tokyo, Japan) and water ad libitum for 1 wk, and then normal-feeding, healthy animals were used for the experiments. All experimental methods and procedures were conducted according to the Guidelines for Animal Experiments in Gifu University, and all animal experiments were approved by the Animal Experimental Committee of the Faculty of Applied Biological Sciences at Gifu University.

Examination of the Efficacy of SCBLC on the Normal Small Intestine

Nine mice (7 wk old) were divided into 3 groups: control (n = 3), 5 mg of SCBLC/mL (n = 3), and 10 mg of SCBLC/mL (n = 3) groups. For SCBLC groups, animals were allowed to drink 5 or 10 mg of SCBLC/mL ad libitum instead of water for 6 d. Skimmed and concentrated bovine late colostrum was exchanged every day. The control group was given water instead of SCBLC. All groups were allowed to eat a solid diet ad libitum during the experimental period. On d 7, all animals were anesthetized with 30 mL/kg of 3% chloral hydrate (Wako Pure Chemical Industries Ltd., Osaka, Japan), and killed by transcardial perfusion of physiological saline, followed by 4% paraformaldehyde in 0.1 *M* PBS (pH 7.4). A 0.5-cm length of the jejunum was then collected from the middle region of the small intestine in each animal and immersed in the same fixative solution overnight at 4°C. The specimens were dehydrated, embedded in paraffin, sectioned coronally at a thickness of 5 μm, and processed for histological examination as described below (see test 1 in Figure 1).

Examination of the Prefeeding Effect of SCBLC on IND-Induced Enteropathy

Twenty-four mice (7 wk old) were divided into 4 groups: control (n = 6), IND (n = 6), 5 mg of SCBLC/mL (n = 6), and 10 mg of SCBLC/mL (n = 6) groups. For SCBLC groups, animals were allowed to drink 5 or 10 mg of SCBLC/mL ad libitum instead of water for 1 wk, whereas the control and IND groups were given water. All groups were allowed to eat a solid diet ad libitum during the experimental period. On d 7, the IND and both SCBLC groups were subcutaneously