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Feeding with dairy *Propionibacterium acidipropionici* CRL 1198 reduces the incidence of Concanavalin-A induced alterations in mouse small intestinal epithelium

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ABSTRACT

Plant lectins are specific carbohydrate-binding proteins widespread in human and animal diets that cause antinutritional effects. Specific intestinal receptors and microbiota may interact with these dietary components leading to important changes on intestinal physiology. It has been proposed that probiotic microorganisms with suitable surface glycosidic moieties could bind to dietary lectins favoring their elimination from the intestinal lumen or inhibiting their interaction with epithelial cells. In this work, we assessed the effects of Concanavalin A (Con A) on some morphological and physiological parameters related to intestinal functionality such as small bowel architecture, main microflora components and disaccharidase activities of Balb/c mice after long term feeding with this lectin alone (8 mg/kg/day of Con A for 3 weeks) or with the simultaneous consumption of *Propionibacterium acidipropionici* CRL 1198 (5×10^8 CFU/mice/day). Long-term consumption of Con A reduced food efficiency suggesting the alteration of the digestion/absorption function of the intestine in the presence of lectin. This effect could be due to both histological alterations of the intestinal epithelium such as shortening and shedding of microvilli and physiological changes like the decrease of disaccharidase activities. Con A feeding increased enterobacteria and enterococci populations whereas lactobacilli, bifidobacteria and propionibacteria were not affected. Consumption of propionibacteria at the same time than Con A, reduced the incidence of Con A-induced alterations in Balb/c mice and may be an effective tool to avoid undesirable lectin–epithelia interactions in both animals and humans.

1. Introduction

Foods and the metabolites generated during digestion and gastrointestinal transit exert a major role in the consumer's health. Many antinutritional and/or potentially toxic compounds are daily ingested by humans and animals. Lectins are nonimmune proteins or glycoproteins that bind specifically to carbohydrate moieties expressed on the cells surface commonly affecting cellular physiology (Sharon & Lis, 1995). They are present in a wide range of food items such as vegetables, fruits, cereals, beans and mushrooms (Nachbar & Oppenheim, 1980) so their ingestion could be significant. Since lectins are highly resistant to inactivation by cooking and by digestive processes, it is likely that the intestinal epithelium is exposed to many of these proteins that have retained at least some of their biological activity.

Plant lectins from the Leguminosae family, are considered as antinutritive or toxic substances since they lead to some harmful reactions after binding to the membrane glycosyl groups of the intestinal epithelial cells (Vasconcelos & Oliveira, 2004). In this sense, it has been observed that Concanavalin A, the lectin from Jack bean (*Canavalia ensiformis*), causes morphological and physiological changes in the intestinal epithelium that could reduce the absorptive function and nutrient utilization, and increases cellular proliferation and turnover (Fitzgerald et al., 2001; Lorenzsonn & Olsen, 1982). Miyake, Tanaka, and McNeil (2007) have reported that Con A impairs the normal protecting events of intestinal cells by inhibiting the rapidly repairing membrane disruptions by exocytotic reaction. In consequence the failure in the subpopulation of mucus-secreting cells reduces the mucosa lubrication and increases the mechanical stress suffered by the epithelial cells. Con A also exerts antinutritional effects and suppresses food consumption but stimulates intestinal epithelium growth in rats (Baintner, Kiss, Pfuller, Bardocz, & Pusztai, 2003; Fitzgerald et al., 2001; Lorenzsonn & Olsen, 1982). It was also found that Con A affects cell growth of colorectal cancer cell lines in a dose-dependent manner (Kiss et al., 1997). Because of the damage they produce on the epithelial cells and their hypertrophic effects, some lectins such as Con A, would act as tumor promoters (Evans et al., 2002; Kiss et al., 1997; Ryder, Smith, & Rhodes, 1992; Ryder, Smith, Rhodes, Parker, & Rhodes, 1994). On the contrary, Fitzgerald et al. (2001) have reported that Concanavalin A infused into rats' stomachs induces the proliferation of the intestinal epithelium without producing crypt branching. Therefore, short-term exposition

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to lectins that stimulate growth has been proposed as a useful therapy for the hypoplastic intestine. However, effects of long-term consumption of potentially toxic lectins should be evaluated.

Metabolism of ingested food begins with digestion and subsequent absorption of dietary nutrients. The process of digestion is mediated by different hydrolases secreted into the gastrointestinal tract or produced by the enterocytes in the intestinal brush border membrane. In the case of food carbohydrates, disaccharides and some oligosaccharides are hydrolyzed to monosaccharides by a few small intestinal brush border disaccharidases before their transport across the microvillus membrane. Lactase (lactase-phlorizin hydrolase, EC 3.2.1.23-62) is the enzyme essential for hydrolysis of lactose in milk. Sucrase (sucrase–isomaltase, EC 3.2.1.48-10) is an α-glucosidase that hydrolyzes sucrose, maltotriose and about 80% of dietary maltose, whereas maltase (maltase–glucoamylase, EC 3.2.1.20) digests the remaining maltose to glucose. Trehalase (α,α-trehalose glucohydrolase EC 3·2·1·28) hydrolyzes the disaccharide trehalose into its two glucose molecules.

Classical propionibacteria are microorganisms of interest for their role as starters in cheese technology as well as for their functions as probiotics (Cousin, Mater, Foligné, & Jan, 2010; Zárate, Babot, Argañaraz-Martinez, Lorenzo-Pisarello, & Pérez Chaia, 2011). *Propionibacterium acidipropionici* CRL 1198 is a dairy strain with probiotic potential as it modifies in a beneficial manner the composition and metabolism of the intestinal microflora (Perez Chaia, Nader de Macias, & Oliver, 1995; Perez Chaia, Zárate, & Oliver, 1999; Perez Chaia & Zárate, 2005; Lorenzo-Pisarello, Gultemirian, Nieto-Peñalver, & Perez Chaia, 2010). It also resists the manufacture process of a Swiss-type cheese (Perez Chaia & Zárate, 2005) so that this dairy product could be considered as a suitable vehicle for its delivery to the host. Development of cheeses as vehicle for probiotics, like propionibacteria, represents a valuable alternative to yogurt and fermented milks and a research topic of growing interest for industry (Ranadheera, Baines, & Adams, 2010).

In previous studies, we have determined that this strain has the ability to bind and remove some dietary lectins from media, preventing their cytotoxic effects on intestinal epithelial cells (Zárate & Perez-Chaia, 2009). Furthermore, it decreases *in vivo* the incidence of colonic lesions in a murine model fed with Concanavalin A (Zárate et al., unpublished results). In consequence, dairy propionibacteria would be intended to protect the intestinal mucosa from the toxic effects of dietary lectins by avoiding their interaction with epithelial cells. The aim of the present investigation was to assess the effects of long term feeding of mice with Con A on some morphological and physiological parameters related with the small bowel functionality as well as the potential of dairy propionibacteria to prevent any deleterious modification.

2. Materials and methods

2.1. Lectin

The lectin used in this study, Concanavalin A (Con A, from *C. ensiformis*) was provided by Sigma Chemical Co, St Louis, MO, USA.

2.2. Microorganism and culture conditions

The microorganism used in this study, *P. acidipropionici* CRL 1198 was obtained from Laboratorio de Ecofisiología Tecnológica-CERELA (CRL: Centro de Referencia para Lactobacilos, CERELA, Tucumán, Argentina). The strain was stored at −20 °C in 10% (w/v) reconstituted non-fat milk (NFM) containing 0.5% yeast extract. Before using, it was activated by three successive transfers every 24 h at 37 °C, in LAPTg broth (1% tryptone, 1% yeast extract, 1.5% meat peptone; 0.1% Tween 80, 1% glucose, pH:6.5) sterilized at 121 °C for 15 min. For mice feeding trials, an appropriated volume of a 24 h culture was harvested by centrifugation, washed in sterile saline solution (0.9% NaCl) and suspended in the same solution at a concentration of 10^8 CFU mL^{-1} in order to be added to the drinking bottles.

2.3. Animals and experimental procedures

The experimental protocol used in this study was approved by the Ethical Committee of CERELA (Centro de Referencia para Lactobacilos). For Assay 1, sixty male 5-weeks-old BALB/c mice, each weighing 24–26 g, from the inbreed colony of CERELA, were divided into four groups. They were housed in plastic cages with wire-tops in a room under controlled environmental conditions of 25 °C and a 12:12 h light–dark cycle and were allowed free access to a conventional solid balanced diet with the following composition (g/kg): water, 120; proteins, 230; carbohydrates, 538; lipids, 50; vitamins, 22; minerals, 40 (Cargill, Argentina). After 1 week of acclimatization groups were subjected to one of the following treatments: Group 1 was used as Control without treatment; mice of Group 2 were daily gavaged during 3 weeks, with 8 mg/kg of Concanavalin A dissolved in 0.9% NaCl containing 5 mM $CaCl_2$, 5 mM $MnCl_2$ and 5 mM $MgCl_2$, pH 7.2. Animals from Group 3 were allowed to drink *ad libitum* a suspension of *P. acidipropionici* CRL 1198 instead of water, provided in the drinking bottles during 3 weeks. That represented a daily dose of c.a. 5×10^8 bacteria mL^{-1}. Mice of Group 4 were subjected at the same time to the treatments of Groups 2 and 3. Three animals per group were sacrificed by cervical dislocation, on days 7, 14, and 21 during treatments and at days 1 and 7 after cessation of them in order to obtain organs and the small bowel walls.

For Assay 2, fifteen male 6-weeks-old BALB/c mice without any treatment were used.

2.4. Measurement of food intake, organ and body weights and intestinal permeability

The daily food intake of animals of Assay 1 was determined by difference between weights of food supplied every day and the remnant in the feeder 24 h later. Body weights were recorded twice per week since the beginning of treatments. Animals were fasted overnight before sacrifice and organs (stomach, small bowel, liver, spleen and cecum) were carefully removed post mortem and weighed.

On the morning of each day of sacrifice mice were inoculated by gavage with a marker molecule solution containing Na-fluorescein (NaF; Merck, Darmstadt, Germany) with low molecular mass (376 Da) and that diffuses passively through cell membranes. The marker molecule had been dissolved in 0.9% NaCl and was given to mice at 10 mg of NaF/kg of body weight. Blood samples for analyses of marker molecule absorbed were taken by cardiac puncture 1 h after administration. The amount of Na-fluorescein in plasma, in relation to standard dilutions of Na-fluorescein dissolved in PBS buffer, was measured by spectrofluorometry, using a filter set of 485 nm for excitation and 530 nm for emission (Varian-Cary Eclipse).

2.5. Tissue collection and preparation of intestinal mucosa homogenates

The euthanized animals were laid on their backs to the dissecting boards and the abdomen of each animal was opened up after an incision along the mid line. The small bowel was carefully brought out, and a 2-cm-long, whole-thickness segment from the middle of the intestine was taken for histological analyses. The remaining parts were flushed out with cold saline solution, slit opened longitudinally with sterile scissors, and used to prepare the mucosal homogenates. The intestinal mucosa was scrapped off gently with a sterile glass slide and homogenized in 4 parts of cold PBS as previously described (Zárate, Morata de Ambrosini, Pérez Chaia, & González, 2002). Homogenates were used for microbiological analysis or decanted to remove large debris and supernatants stored at −70 °C for further assays of enzyme activities.