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Basic nutritional investigation

Evaluation of immune response, microbiota, and blood markers after probiotic bacteria administration in obese mice induced by a high-fat diet

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ABSTRACT

Objective: Obesity is associated with alterations in intestinal microbiota and immunity. The aim of this study was to determine the effect of probiotic *Lactobacillus casei* CRL 431 administration on intestinal and humoral immune response, clinical parameters, and gut microbiota was evaluated using a high-fat diet to induce obesity in a mouse model.
Methods: Adult mice received a conventional balanced diet or a high-fat diet supplemented with milk, milk fermented by *Lactobacillus casei* (FM), *L. casei* as suspension, or water over 60 d. Histology of liver and small intestine (SI), immunoglobulin A-positive cells and macrophages in SI, phagocytic activity of spleen and peritoneal macrophages, and humoral immune response to ovalbumin were studied. Clinical parameters in serum and gut microbiota were also analyzed.
Results: FM was the most effective supplement for decreasing body weight and clinical parameters in serum. The histology of liver and SI was also improved in obese mice given FM. These animals had increased numbers of immunoglobulin A-positive cells and macrophages in SI. The gut microbiota showed that obese mice given probiotics had increased Bacteroides and bifidobacteria. Administration of FM or *L. casei* as suspension enhanced the phagocytic activity of macrophages. The anti-ovalbumin specific immune response was not increased by any supplement assayed.
Conclusion: Administration of probiotics to obese hosts improved the gut microbiota and the mucosal immunity altered by obesity, down-regulated some biochemical parameters in blood associated with metabolic syndrome, and decreased liver steatosis. These results demonstrate the potential use of probiotics in obese individuals to decrease the body weight and to improve the biochemical and immunologic parameters altered by obesity.

Introduction

Obesity has become a serious public health problem and has reached epidemic proportions worldwide, not only in industrialized countries but also in developing countries. Each year, as a result of obesity or overweight, at least 2.6 million individuals are more likely to suffer a heart attack or to die from pathologies related to obesity [1].

Obesity has a multifactorial origin and is strongly associated with: (1) an inflammatory process in which adipose tissue plays an important role [2], (2) liver steatosis, and (3) insulin resistance [3].

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Intestinal microbiota is also modified in the obese host [4]. Studies in animal models and humans showed that the composition of the gut microbiota differs in lean versus obese hosts [5]. These studies suggested the potential role of gut microbiota in the development of obesity and the possible beneficial effect of modifying the gut microbiota as a tool for future treatments. In light of this, probiotic supplementation of the diet appears to be beneficial in combating obesity and its related disorders, due especially to the anti-inflammatory effects of these microorganisms [6].

Probiotics are defined as live microorganisms that, when administered in adequate amounts, provide a health benefit to the host [7]. The beneficial effect of probiotic microorganisms on the obese host has been reported [8,9], and different mechanisms of action were suggested. Probiotics can modulate the lipid profile associated with obesity [10]. A recent study showed that *Bifidobacterium pseudocatenulatum* CECT 7765 modulated metabolic parameters in mice fed a high-fat diet by modifying the expression of key regulators of fatty acid and cholesterol metabolism and transport, lipid levels, and glucose levels in the liver [11]. It has been demonstrated that the combination of two probiotic lactobacillus altered hepatic lipid metabolism in a mouse diet-induced obesity model [12]. It was also described that probiotics can beneficially affect immunologic disorders associated with obesity. In light of these findings, the gene expression of different cytokines and transcription factors in immune cells was analyzed using obese models or obese individuals. Probiotic *Lactobacillus gasseri* SBT2055 prevented body weight gain, and proinflammatory gene expression in the adipose tissue of mice fed a high-fat diet [13]. Another recent study showed that the administration of probiotic yogurt modulated gene expression in the peripheral blood mononuclear cells of overweight and obese individuals [14]. The immunomodulatory effect of different probiotic strains was also demonstrated [15–19].

L. casei CRL 431 is a probiotic bacterium. The mechanisms involved in the immune stimulation mediated by *L. casei* CRL 431 have been extensively studied using experimental models [20–23].

This probiotic strain was also evaluated in clinical studies performed in humans, documenting its effects in different conditions [15–19]. *L. casei* CRL 431 is contained in probiotic products that are consumed in Argentina, and also in other countries such as Chile and Costa Rica. Considering these previous results, specifically the capacity of *L. casei* CRL 431 to activate the gut immune response [20–23], our hypothesis was that supplementation with this probiotic strain would exert a beneficial effect in the intestinal ecosystem on the alterations of the microbiota and the immune response observed in obese hosts, and thereby decreasing other alterations associated with obesity (such as metabolic syndrome, gut immunity, and liver damage). The aim of this study was to evaluate the effect of continuous administration of probiotic bacterium *L. casei* CRL 431 or milk fermented by *L casei* (FM) on different parameters in the obese host by analyzing some parameters of the gut and systemic immune response, microbiota, and blood markers in an obesity mouse model induced by a high-fat diet.

Methods and materials

Bacterial strain and fermented milk

L. casei CRL 431 was obtained from the CERELA Culture Collection (San Miguel de Tucumán, Argentina). Cultures were grown overnight at 37°C in 5 mL sterile Mann-Rogosa-Sharp (MRS) broth (Britania, Buenos Aires, Argentina). The cells were harvested by centrifugation at 5000g for 10 min, washed three times with fresh phosphate-buffered saline (PBS) and then resuspended in 5 mL of sterile 10% (wt/vol) reconstituted non-fat milk. The *L. casei* CRL 431 suspension was diluted 1:30 in water and administered ad libitum to the mice. The final concentration of probiotic bacteria in the drinking water was $2 \pm 1 \times 10^8$ CFU/mL. This count was periodically controlled at the beginning of the administration and each 24 h to avoid modifications of more than one logarithmic unit. The content of the bottles was replaced daily.

To obtain the FM, non-fat dried milk was rehydrated (10% wt/vol) and autoclaved (115°C for 15 min). Overnight cultures of *L. casei* CRL 431 were grown in MRS broth as previously explained. The milk was inoculated with 2% vol/vol of the *L. casei* culture, and incubated at 37°C for 24 h. The final concentration of the probiotic bacteria in the fermented milk was $8 \pm 2 \times 10^8$ CFU/mL. This fermented product was prepared every 2 d and the microbial concentration was monitored. The bottles with FM were replaced daily.

Experimental groups

Protocol to induce obesity by a high-fat diet and sampling procedure

Five-wk-old female BALB/c mice (25 ± 2 g), were obtained from the closed random bred colony maintained at CERELA (Centro de Referencia para Lactobacilos, San Miguel de Tucumán, Argentina). Mice were divided into two groups: non-obese (N) and obese (O). The non-obese group was fed ad libitum with conventional balanced diet (45% carbohydrates, 32% fat, 23% proteins, 6% raw fiber, 10% total minerals, 1.3% calcium, 0.8% phosphorus, 12% moisture, and vitamins) provided by ACA Nutrition Animal (San Nicolas, Buenos Aires, Argentina). Considering that the daily intake of a mouse is 3 g of food, the caloric contribution of the conventional balanced diet would be 16.8 kcal/d. The obese group was fed ad libitum with a high-fat diet that was made in the laboratory using the same conventional balanced diet, with bovine lard and sugar added (both for human consumption, purchased in the supermarket), over 60 d. The high-fat diet contained 43.4% of the conventional balanced diet, 43.4% of bovine lard, and 13.2% of sugar; its caloric contribution was 18.6 kcal/d. These caloric values do not include calories provided by the different dietary supplements. No great variation in calorie intake was observed between the two groups; the main difference was in the percentage of fat between the two diets (conventional and high-fat diet).

Each group was divided into four subgroups (one control and three test) according to the addition of the dietary supplement.

Mice in the N group received a conventional balanced diet and water ad libitum during entirety of the experiment.

We performed three test groups to evaluate the effect of different dietary supplements: milk (N + milk), FM (N + FM), or a suspension of *L. casei* CRL 431 (N + Lc), ad libitum for the duration of the experiment (Fig. 1).

Mice in group O received a high-fat diet and water ad libitum for the duration of the experiment.

We performed the following test obese groups: O + milk, O + FM, and O + Lc. Each test group received a high-fat diet supplemented with milk, FM, or suspension of *L. casei*, respectively for the duration of the experiment (Fig. 1).

Each mouse consumed between 3 and 4 mL of water, milk, FM, or Lc daily.

Mice (n = 6/group) were maintained in collective cages (three animals/cage) in an environmentally controlled room (20°C ± 2°C and 70% ± 5% of humidity), with a 12-h light–dark cycle and unlimited free access to water and the respective food. They were weighed every 5 d during the 60-d experimental period. Three mice from each control and test group were sacrificed by cervical dislocation at day 60. The rest of the animals from each group (three mice) were maintained in the same conditions and used for evaluation of the humoral immune response. Liver was removed for histologic studies. Blood was recovered by cardiac puncture to analyze clinical parameters and the small and large intestine for histologic and microbiology studies, respectively. Both spleen and peritoneal macrophages were extracted for phagocytic activity evaluation.

The experiments were repeated three times. All animal protocols were preapproved by the Animal Protection Committee of CERELA (CRL-BIOT-LI-2010/1 A) and all experiments comply with the current laws of Argentina.

Evaluation of the humoral immune response to ovalbumin antigen

To evaluate systemic immunity, the assay was performed at day 60 of the experiment (Fig. 1). Three mice from each group were injected subcutaneously, three times every 48 h, with 15 μg of chicken egg albumin (OVA; Sigma, St. Louis, MO, USA) in PBS solution. The mice from N or O test groups continued receiving the corresponding diet supplemented with milk, FM, or *L. casei* during and after immunization until the end of the experiment. The mice were sacrificed 10 d after the last OVA injection (day 75 of the experiment), and blood was collected to determine specific anti-OVA immunoglobulin (Ig)G in the serum by enzyme-linked immunosorbent assay (ELISA) test, using plates coated with ovalbumin and a goat anti-mouse IgG antibody conjugated with biotin-SP (Jackson Immuno Research Labs Inc, West Grove, PA) to detect the specific anti-OVA IgG, as described previously [24]. Peritoneal and spleen macrophages were also isolated on day 75 to analyze the phagocytic activity according to a previously described technique [24].