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Review article

Effects of Enteral Administration of Lactobacillus Casei Strain Shirota on Healthy Children, Dysbiosis in Obese Children, and the Elderly

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Introduction

Gut microbiota forms a complex ecological community that influences normal physiology and susceptibility to disease. The development of a stable and diverse gut microbiota is essential for various host physiologic functions such as immunoregulation, pathogen prevention, energy harvest, and metabolism [1]. Simultaneously, dysbiotic gut microbiota associated with disease has altered structure and function and is often characterized by a decrease in species richness and proliferation of pathogenic bacterial taxa. The link between microbes in the human gut and the development of obesity, cardiovascular disease, and metabolic syndromes, such as type 2 diabetes mellitus, is becoming clearer [2,3]. Understanding factors that underlie changes in the composition and function of gut microbiota aids in the design of targeted therapies, one of which is probiotics administration. Probiotics are defined as live microorganisms that confer a health benefit on the host when administered in adequate amounts [4]. The beneficial effects are likely due to improved immunological function of the intestine.

Researchers across the world use various strains of probiotics against immunity-associated diseases. Lactobacillus casei strain Shirota (LcS) is a probiotics strain, which was first successfully cultured by Dr. Minoru Shirota in 1930. Many studies have reported distinct mechanisms of action and clinically validated the disease-prevention effects of LcS.

LcS is a facultative anaerobic strain of Lactobacillus that is tolerant of gastric acid and bile. Fecal recovery of LcS as viable bacteria soon after its ingestion has confirmed that this microorganism survives passage through the intestine. LcS meets the criteria to be considered a probiotic because its efficacy and safety have been established by animal studies and clinical trials. The aim of this article is to review study data concerning the health-promoting effects of LcS.

Effects of LcS Supplementation in Healthy Pre-School and School-Age Children

The intestinal microbiome undergoes dynamic changes during different stages of host growth and development, with the most dramatic compositional changes believed to occur during infancy and childhood [5].

Probiotics are viable bacteria that exhibit a diverse range of beneficial effects on host health, primarily due to the impact of the bacteria on the improvement of gut microbial balance and the intestinal environment. There has been a growing interest in the manipulation of intestinal microbiota with probiotics for the prevention and treatment of certain diseases. However, reports on the analysis of intestinal microbiota in pre-school and school-age children and the effects of probiotic supplementation on the microbiota in these children are sparse. In this context, our study was designed to establish the baseline profile of intestinal microbiota in healthy pre-school and school-age children and investigate the effects of probiotic supplementation on the microbiota. Specifically, we investigated the effects of daily intake of probiotic LcS-fermented milk on the fecal microbiota and intestinal environment of healthy pre-school and school-age Japanese children before, during, and after 6 months of probiotic ingestion, and 6 months following the termination of ingestion (post-ingestion period).

We conducted an open trial and evaluated 23 children [14 boys, 9 girls, mean age (±SD), 7.7±2.4 years; BMI 19.6±4.6]. The composition of intestinal microbiota of healthy pre-school and school-age children resembled that of adults. During probiotic supplementation, Bifidobacterium ($P<0.01$) and total Lactobacillus ($P<0.01$) population levels significantly increased, while those of Enterobacteriaceae ($P<0.01$), Staphylococcus ($P<0.01$), and Clostridium perfringens ($P<0.05$) significantly decreased. A significant increase in fecal concentrations of organic acids and a decrease in fecal pH were observed during the ingestion period. However, the patterns of fecal microbiota and intestinal environment were found to revert to baseline levels (i.e., before ingestion) within 6 months following the cessation of probiotic intake. Our results suggest that consumption of an LcS-containing probiotic product even by healthy pre-school and school-age children favorably modified their intestinal microbiota composition. Notably, however, this effect ceased within 6 months after ingestion of the probiotic was stopped, indicating that LcS does not permanently inhabit the juvenile human intestinal tract and is cleared within months of probiotic discontinuation. Thus, LcS as a probiotic is beneficial for maintaining good intestinal health of children at the time of dosing and perhaps also later in life [6].

Effects of LcS Supplementation on Obese Children

Obese children are at risk of developing non-communicable diseases such as hypertension, type 2 diabetes mellitus, cerebrovascular disorders, and ischemic heart disease in adulthood [7]. As such, obesity should be controlled in children in order to ensure a healthy adulthood. It is currently accepted that the intestinal microbiota plays a role in the pathophysiology of obesity as an important environmental factor for generating energy and regulating inflammation [8,9]. Recent studies have noted a decline in the ratio of Firmicutes and increase in the proportion of Bacteroidetes in the intestinal microbiota of obese adults undergoing treatment with a carbohydrate- and fat-restricted diet [9]. However, these few studies on the relationship between obesity and intestinal microbiota have all been conducted in adults, with little evidence available for children [10-12]. Given that Bifidobacterium is much more dominant than Bacteroidetes in the intestinal microbiota of children [13,14] and reports in animal models have cited the effect of Bifidobacterium on suppressing endotoxinemia and thereby preventing type 2 diabetes mellitus [15] while improving hypercholesterolemia [16], we hypothesized that proliferating Bifidobacterium within the intestine of children could improve such complications.

To confirm this hypothesis, we first compared the baseline intestinal microbiota between obese and non-obese children. Proportions of Bifidobacterium in obese children were lower than those in non-obese children. Next, given that extraneously administered Bifidobacterium is unlikely to reach the large intestine alive, we had obese children drink an LcS-containing beverage, which is the only probiotic proven to effectively proliferate Bifidobacterium within the intestine [17,18]. We then examined potential effects of this drink on weight loss, hyperlipidemia, and blood sugar reduction as primary outcomes and improvement of intestinal dysbiosis and the microbial environment as secondary outcomes.

We conducted an open prospective trial to clarify the effects of LcS-containing beverages in obese children. We compared the intestinal microbiota and organic acid levels between 12 obese (mean age, 10.8 years; BMI Z-score, 2.7±1.7) and 22 control children (mean age, 8.5 years; BMI Z-score, 0.1±0.7), and pre- and post-intervention in obese children. The obese group underwent diet and exercise therapy for 6 months and then consumed LcS-containing beverages daily for another 6 months. Body weight and serological markers were monitored. Significant reductions in fecal concentrations of Bifidobacterium (obese group, 7.9±1.5 vs. non-obese group, 9.8±0.5 log10cells/g; $P<0.01$) along with a significant decline in the Bacteroides fragilis group ($P<0.05$), Atopobium cluster ($P<0.05$), and Lactobacillus gasseri subgroup ($P<0.05$), and acetic acid (obese group, 45.1±16.9 vs. non-obese group, 57.9±17.6 μmol/g; $P<0.05$) were observed in the obese group at baseline. A significant decline in body weight (−2.9±4.6%; $P<0.05$) and elevation in high-density lipoprotein cholesterol level (11.1±17.6%; $P<0.05$) were observed 6 months after ingestion of the LcS-containing beverage compared to baseline. Furthermore, a significant increase in the fecal concentration of Bifidobacterium (before ingestion, 7.0±1.2 vs. after ingestion, 9.1±1.2 log10cells/g; $P<0.01$) and an apparent increase in the acetic acid concentration (before ingestion, 7.0±1.2 vs. after ingestion, 9.1±1.2 log10cells/g; $P<0.01$) were observed 6 months after ingestion.

Our results show that LcS contributes to weight loss and improves lipid metabolism in obese children via a significant increase in fecal Bifidobacterium numbers and acetic acid concentration [19].

Effects of LcS on Risk Management of Infections in Long-Term Inpatients at Health Service Facilities for the Elderly

Physiology, organ function, immunity, and swallowing function decline in the elderly and can cause a myriad of problems in inpatients at health service facilities for the aged. Constipation and diarrhea reduce the quality of life of inpatients, and substantial energy of facility staff is required to care for these issues [20,21]. Additionally, inpatients have increased susceptibility to infections; thus, infections are an intractable problem and easily become severe [22]. Moreover, close contact among inpatients at health service facilities for the aged can readily spread disease [23]. Because there is a possibility of spreading methicillin-resistant Staphylococcus aureus (MRSA) and Clostridium difficile within facilities by contact between staff and bacterial carriers, staff must not become bacterial carriers of harmful bacteria in order to control nosocomial infections [24-26]. Inpatients at health service facilities for the aged are always exposed to infections, thus, infection control among inpatients and staff that is certain, safe, and economical is strongly desired.

We studied the effectiveness of continuous consumption of LcS-fermented milk in an open trial that compared pre- and post-intake for risk management of infections in long-term inpatients at a health service facility for the aged [n=42, mean age (±SD), 82±10 years]. Frequency of fever, constipation, and diarrhea, which are important markers of infection, were evaluated, and intestinal flora, which is considered to affect these symptoms, was analyzed. We also conducted an organic oxidation analysis of feces, which is an indicator of the intestinal environment. Moreover, an analysis of the intestinal flora of facility staff (healthy control group: n=24, mean age (±SD), 40±12 years) was performed, and the contribution of nosocomial infection prevention by LcS-fermented milk intake among staff was investigated.

LcS-fermented milk was continuously consumed for 6 months. Feces were sampled and analyses of fecal microflora, organic acid, and pH were performed. A reduction in the number of days that inpatients experienced a fever,constipation, and diarrhea was observed in the post-intake period compared to the pre-intake period. Furthermore, feces collected in the pre-intake period showed that inpatients had reduced Bifidobacterium and increased Clostridium species abundance compared to those in staff. However, Bifidobacterium proliferated without the detection of MRSA in post-intake samples from inpatients. No bacteria causing nosocomial infections were detected among staff. The acetic acid concentration increased and pH decreased in the feces of inpatients after consuming LcS-fermented milk.

Our results show that LcS-fermented milk is useful for improving clinical conditions and influences the enteral microflora and environment in such inpatients. LcS-fermented

milk may therefore be efficacious for reducing the risk of infection among elderly individuals residing in nursing homes [17].

Effects of LcS on Prevention of a Massive Norovirus Outbreak in a Health Service Facility for the Elderly

To conduct effective risk management in long-stay elderly people at a health service facility, we performed an open case-control study to evaluate the effect of LcS-fermented milk intake on infectious gastroenteritis caused by various Norovirus outbreaks in December 2006. Seventy-seven elderly people (mean age, 84 years) were enrolled in the study. During this period, there was no significant difference in the incidence of infectious gastroenteritis caused by Norovirus between LcS-fermented milk-administered (n=39) and non-administered (n=38) groups. However, the mean duration of fever >37°C after the onset of gastroenteritis was 1–5 (SD 1–7) days in the former group and 2–9 (SD 2–3) days in the latter group, showing a significantly shortened duration of fever in the former group ($P<0.05$). RT-quantitative PCR analysis targeting ribosomal RNA showed both Bifidobacterium and Lactobacillus to be significantly dominant, whereas Enterobacteriaceae was decreased ($P<0.05$), in fecal samples from the LcS-fermented milk-administered group (n=10, mean age, 83 years) compared with the non-administered group (n=10, mean age, 83 years), with a significant increase in fecal acetic acid concentration. Thus, improvement of the intestinal environment associated with an increase in the Bifidobacterium population, such as increased acetic acid concentration and decreased pH, is important in limiting the overgrowth of harmful bacteria in the intestine. Therefore, continuous intake of LcS-fermented milk could positively contribute to the alleviation of fever caused by Norovirus infectious gastroenteritis by correcting the imbalance of the intestinal microflora peculiar to the elderly, although such consumption could not protect them from the disease [18].

Effects of LcS on Control of Proinflammatory Cytokine Responses of Peyer's Patch Cells

Probiotics are viable cell preparations of foods containing viable bacterial cultures or components of bacterial cells that have beneficial effects on the health of the host and include bacteria such as lactobacilli and bifidobacteria [27]. Orally administered probiotics are expected to have resistance against gastric acid and bile, to be delivered live to the intestinal tract, and to normalize the intestinal bacterial flora and thereby contribute to the reduction of various disease risks [28,29]. Moreover, it is becoming clear that probiotics affect the immune system of the host and work effectively against various diseases caused by immune system abnormalities [30,31]. The effects of probiotics on the immune system are classified into two major categories. One effect is the activation of cells in the innate immune system, such as phagocytes and natural killer cells, which are expected to have an inhibitory effect against infections and cancers [32,33]. The other effect is the inhibition of excessive immune responses, which is expected to have an inhibitory effect against inflammatory bowel diseases, allergies, and autoimmune diseases [34-36]. Multiple mechanisms have been proposed to account for the expression of the latter anti-inflammatory activities, including inhibited production of proinflammatory mediators such as interleukin (IL)-8, induced production of anti-inflammatory cytokines such as IL-10, and induction of regulatory T cells.

Clinical application of probiotics is anticipated, thus, it is essential to determine in detail their effects on immune cells in order to utilize probiotics more effectively and safely. Orally administered probiotics are considered to affect the immune system via the following pathways: (i) probiotics are introduced through M cells in Peyer's patch (PP) follicle-associated epithelium to affect macrophages and DCs beneath the epithelium; (ii) DCs in the mucosal lamina propria extend their dendrites to sample intraluminal probiotics; and (iii) intraluminal probiotics stimulate epithelial cells to produce humoral factors, which indirectly affect intestinal immune cells [31,37]. Incidentally, the intestinal immune system exhibits a unique responsiveness that is different from that of the systemic immune system. Therefore, in order to clarify these differences in this study, cells were prepared from the spleen and PP, which are major organs in the systemic immune system and intestinal immune system, respectively.

To clarify the probiotic features of immunomodulation, cytokine production by murine spleen and PP cells was examined in response to probiotic and pathogenic bacteria. In spleen cells, probiotic LcS induced IL-12 production by CD11b+ cells more strongly than pathogenic Gram-positive and Gram-negative bacteria and effectively promoted the development of T helper type 1 (Th1) cells, followed by high levels of secretion of interferon (IFN)-γ. Although the levels of IL-12 secreted by PP cells in response to LcS were lower than those in spleen cells, Th1 cells developed as a result of this low level induction of IL-12. However, IFN-γ secretion by LcS-induced Th1 cells stimulated by a specific antigen was downregulated in PP cells. Development of IL-17-producing Th17 cells was efficiently induced in PP cells by antigen stimulation. LcS slightly, but significantly ($P<0.05$), inhibited antigen-induced secretion of IL-17 without a decrease in the proportion of Th17 cells. No bacteria tested induced the development of IL-10-producing, transforming growth factor-β-producing or Foxp3-expressing regulatory T cells, thus suggesting that certain probiotics might regulate proinflammatory responses through unidentified mechanisms in PP cells.

Taken together, these data show probiotic LcS has considerable potential to induce IL-12 production and promote Th1 cell development. However, secretion of proinflammatory cytokines such as IL-12 and IL-17 may be well controlled in PP cells [38].

Conclusion

LcS has been established as a probiotic because of the long history of confirmed beneficial effects, we performed further investigations to assess its health-promoting effects, such as, beneficial for maintaining good intestinal health of healthy children, contributes to weight loss and improved lipid metabolism in obese children, efficacious for reducing the risk of infection among elderly individuals residing in nursing homes, and the alleviation of fever caused by Norovirus infectious gastroenteritis by correcting the imbalance of the intestinal

microflora peculiar to the elderly. In addition, LcS was found to have the effects on control of proimflammatory cytokine responses of Payer's patch cells.

Based on the results of these studies, LcS is recommended for the treatment of various populations with optimal or suboptimal health.

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