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Full Length Research Paper

Reduction of enterotoxigenic *Escherichia coli* colonization by the oral administration of *Lactobacillus casei* as a probiotic in a murine model

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The aim of this study was to examine the reduction of enterotoxigenic *Escherichia coli* (ETEC) colonization by oral administration of *Lactobacillus casei* as probiotic in a murine model. In this study, thirty-six BALB/c mice were divided to two test groups and one control group. One of the test groups was fed probiotic bacteria at six days and other groups at three days; whereas, the control group did not receive any probiotic. 72 h after the last oral administration of probiotic, the all three groups were fed by the ETEC. Than, the numbers of *E. coli* excreted from animal intestinal were determined at various times by colony counting on specific culture media and assessed their rate of colonization. The results of this study showed that *L. casei* is enable to be colonize in the murine gastrointestinal tract and both test groups of murine show significant decrease of ETEC excretion compared to control group (P = 0.001). Also, comparison of test groups indicated that longer time reception of probiotic bacteria resulted in more reduction of colonization of pathogenic bacteria. These findings suggest that *L. casei* can be used as a candidate probiotic to reduce the rate of colonization and establishment of ETEC in the digestive tract and to prevent diarrhea caused by this organism.

Key words: *Lactobacillus casei* sp. strain GG, *Escherichia coli*, probiotic, enterotoxigenic traveler's diarrhea, bacteriotherapy.

INTRODUCTION

Many reasons show that most diseases and infections are associated to lifestyle. In the past few centuries, changing living conditions by reduced physical activity, increased international travel, the modern life stress, artificial human nutrition and basically, Stay away from natural life, susceptibility to infectious diseases in humans has increased (Montalto et al., 2002; Caglar et al., 2005; Senok et al., 2005; Mazmanian et al., 2008). Infectious diseases are still one of the biggest health problems at the 21th century. Among other, the intestinal infection caused by various infectious agents, such as *Salmonella* and *Shigella sp.* and *Escherichia coli* lead to many mortalities in developing and developed countries through consumption of contaminated water and foods. Enterotoxigenic *E. coli* remain as major agent of traveler's diarrhea in humans worldwide and diarrhea in children under five years, especially in developed countries (Bergonzelli et al., 2005; Yates, 2005). According to World Health Organization (WHO) reports, the enterotoxigenic *Escherichia coli* (ETEC) strains lead to 400,000 deaths in children less than five years age, annually (Yates, 2005). Use of antibiotics for prevention and treatment of these diarrhea leads to not only the drug resistance, but also destruction of digestive tract normal flora, and increased colonization of causing bacteria and subsequently, proneness to chronic diarrhea. For this reason, today's WHO advocates the usage of accessory

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strategies to controlling of diseases such as, utilization of preventing and treating power of probiotics. Probiotics, as a live microbial food supplement, have beneficial effects on health of their host by improving of the microbial balance in the digestive tract (Castelli et al., 2006; DuPont, 2007; Bisson et al., 2010). Recently, many studies have reported the antagonistic effects of lactic acid bacteria (such as, Lactobacillus sp., Streptococcus sp. and Bifidobacterium sp.) and other microbial agents including, Clostridium sp., Enterococcus sp. and yeast against various pathogenic bacteria (Hickson et al., 2007; Boirivant and Trober, 2007). Among other, the various species of *Lactobacillus sp.* present in the dairy, there have been more attention. In the one side, these agents by several mechanisms such as, colonization in the gastrointestinal tract, production of antimicrobial substances and organic acids, competition with pathogenic agents for gain of food and the occupation of the host cell surface receptors and the other hand, by improving of innate and acquired immune systems inhibit growth and propagation of pathogenic bacteria and eventually, eradicate them from infection site (Ishida-Fujii et al., 2007; Mc Farland, 2007). The aim of this study was to examine the reduction of ETEC colonization through oral administration of *Lactobacillus casei* as probiotic in BALB/c murine model.

MATERIALS AND METHODS

Animals

Thirty-six 4-weeks-old male BALB/c mice were obtained from the Pasteur Institute of Iran and maintained in Trexler type flexible film plastic isolators with sterile food and water and penicillin (0.3 g/l) for two weeks (Savage and Dubos, 1968; Aiba et al., 1998).

Preparation of *L. casei* to feed the murine

L. casei standard strain was purchased as lyophilized. After transfer to laboratory, bacteria initially were solved in a little PBS buffer and then inoculated in MRS broth medium (Difco). Culture media were incubated in anaerobic conditions for 48 h at 37°C. Then, bacterial pellet was obtain by centrifugation of broth culture media (2000 rpm for 15 to 20 min) and washed by PBS buffer for three times. Finally, pellet was solved in 0.5 ml PBS and its concentration measured by spectrophotometer at 630 nm with OD = 0.5. In this OD, the concentration of bacterial suspension is 1×10^8 CFU/ml. In the last stage, the bacterial suspension was transferred to sterile syringe to feed the mice.

Preparation of enterotoxigenic *E. coli* and identification of biochemical and cultivation characteristics

Enterotoxigenic *E. coli* strain serotype O:114 was obtained from reference laboratory of Tehran and cultured on the MacConkey agar and EMB agar media (Difco). After 24 h incubation at 37°C, growth colonies were studied by macroscopic, microscopic, biochemical characteristics and reaction to specific antiserum and finally, strain was confirmed. After confirmation of strain, *E. coli* was cultured on the trypticase soy broth (TSB) medium (Difco) and incubated at 37°C for 18 - 12 h. Then, such as the method described in the preparation of *L. casei*, concentration of this bacterium also was brought to 1×10^8 CFU/ml and then transferred to sterile syringe to feeding the mice.

The feeding of test group of mice by *L. casei*

Thirty-six BALB/c mice were divided to three groups contained two test groups and one control group; so that, each of test and control group includes eight mice. The first test group during six consecutive days, each time 0.5 ml was fed by *L. casei* suspension (1×10^8 CFU/ml) existed in syringe. Also, the second group was fed by the same amount of bacteria but during three consecutive days (the feeding of *L. casei* to this group carried out after the fourth days of the inoculation to the first group). At this stage, none of the test groups of mice were fed to *L. casei*. After inoculation of *L. casei*, the colonization rate of this bacteria in the gastrointestinal tract of mice was evaluated through inoculation of 5 g of feces them in MRS broth and agar (Difco), at the various times.

Feeding of control and test groups of mice by enterotoxigenic *E. coli* bacteria

After 72 h of the last oral administration of *L. casei* to test group of mice, as the method described at above, all three groups were fed to 0.5 ml ETEC suspension (1×10^8 CFU/ml).

Evaluation of enterotoxigenic *E. coli* excretion in stool

The excretion rate of pathogenic bacteria from animal intestinal were examined by colony count of bacteria grown from 5 g feces on the MacConkey and EMB agar at different times including: 24, 48, 72, 96, 120, 144 and 168 h after feeding of *E. coli*.

Analysis methods of results

Data were analyzed by means of statistical software (SPSS 12.1). To analysis of results, non-parametric statistical methods include: Mann-Whitney U Test (for independent groups) and Wilcoxon (for affiliates) and to evaluate the mean difference among groups, Kruscal-Wallis test were used.

RESULTS

The results of mean count of enterotoxigenic *E. coli* in the control group

The excretion rate of ETEC in control group of mice, which did not receive the probiotic bacteria, compared with two test groups shown in Table 1. As is noted, ETEC has established in mice intestinal after initial reduction, and then the occupancy of bacteria would continue.

Comparison of the results of mean colony count of enterotoxigenic *E. coli* in two groups

The results of this study showed that *L. casei* is colonized in the gastrointestinal tract of test group of mice and whatever the amount and time consuming of this

Table 1. Compression of the results of mean count of ETEC excreted from the two test groups and control, at different times.

Time (h)	24	48	72	96	120	144	168
The first test group	6×10^5	5×10^4	5×10^3	6×10^2	4×10^2	1×10^2	5×10^1
The second test group	6×10^6	1×10^6	2×10^5	2×10^4	1×10^4	2×10^3	1×10^3
The control group	4×10^7	6×10^6	1×10^6	1×10^5	4×10^5	2×10^5	1×10^5

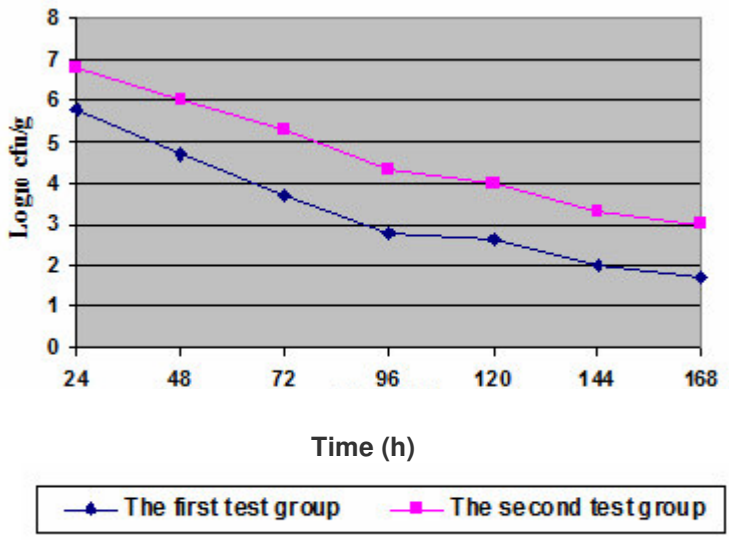

Figure 1. Compression of the results of mean count of ETEC excreted from the two test groups at different times.

probiotic is more, the rate of colonization and the preventive effects is more. Table 1 and Figure 1 show the results of mean colony counts of bacteria excreted from two test groups of mice, in different time according to different times, that the total mean of ETEC excretion in first group is significantly lower than the second group of test (At initial 24 h, P = 0.043 and for later, P = 0.021).

Comparison of the results of colony count mean of enterotoxigenic *E. coli* in two test groups and the control group

Given in Table 1 and Figure 2, compression of the results of mean count of ETEC excreted from the two test groups and control, at different times, indicated that the mean excretion rate of bacteria in the three groups was different significantly and this rate was lower in the first group of test mice than other groups (P = 0.001).

DISCUSSION

Nowadays, because of increased drug resistance and side effects of antibiotics, the using of natural treatment methods such as probiotics is better for treatment and prevention of these infection, that not only are cheap but also are obtained easily and have none problems of antibiotics application(Yates, 2005; Castelli et al., 2006; DuPont, 2007). Generally, if it is possible to establish harmless probiotic bacteria in the digestive system, it is led to inhibition of microbial colonization (Montalto et al., 2002; Caglar et al., 2005; Senok et al., 2005; Mazmanian et al., 2008). The aim of the present study was examine in the reduction of ETEC colonization through oral administration of *L. casei* probiotic in the BALB/c murine.

This study, like other studies, such as studies done by Saxelin, Tuohy, Tsai, Ohashi and colleagues showed that *Lactobacillus sp.* can adhere to epithelial cells of digestive tract of humans and various animals including mice, and the stability proportion of them in the digestive tract dependent to their period and doses amount that are consumed (Asahara et al., 2001; Ohashi et al., 2004; Saxelin et al., 2005; Tuohy et al., 2007; Tsai et al., 2008). This study indicates that if *L. casei* prescribed longer, rate of its colonization and inhibitory effects in the intestine will be higher. The results of this study showed that *L. casei* can be colonized in the intestines of test groups of mice

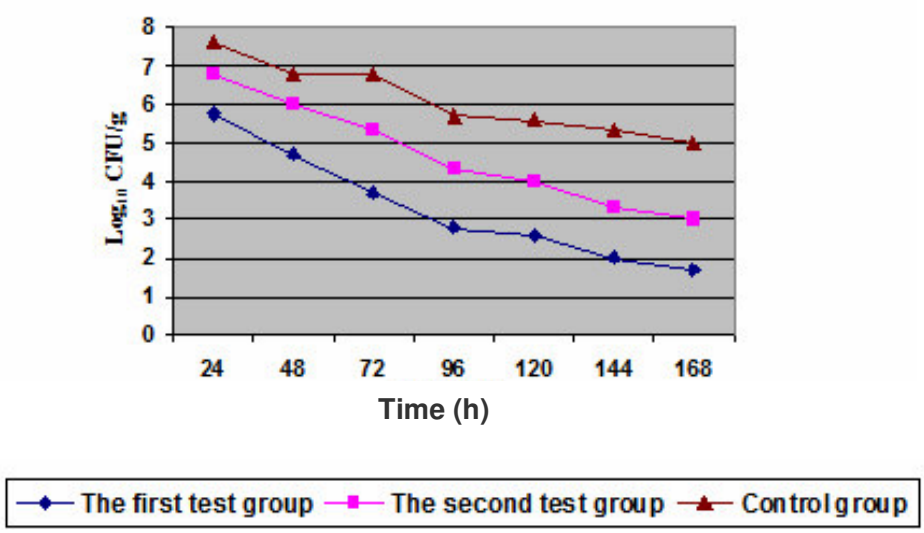

Figure 2. Compression of the results of mean count of ETEC excreted from the two test groups and control, at different times.

and has the ability to reduce the rate of colonization of the ETEC over time; while, the establishment rate of ETEC in intestinal of control group, after initial partial reduction of number, almost would continue constantly at the next times. Also, comparison of the colonization rate of bacteria in first group (that had received *L. casei* for 6 days) and the second group (that had received *L. casei* for three days) indicates that the establishment and exertion of ETEC in first group of test is significantly lower than those in the second group (Table 1). Therefore, it may be concluded that long-term usage of *L. casei* probably will be more effective in preventing of pathogen colonization in the gastrointestinal tract of mouse. The analysis of results of this study, similar to studies done by Sgouras and Kabir and colleagues, showed that colonization of pathogenic bacteria in the digestive system of test groups is significantly lower than the control group (Kabir et al., 1997; Sgouras et al., 2004) (Figure 2). Akalin and his colleagues also showed that feeding of mice with yogurt containing *Lactobacillus acidophilus* would led to the total amount of coliforms in mice stool be less compared with mice that have consumed yogurt without *L. casei*, that is similar to results of our study (Akalin et al., 1997).

Zhao and colleagues concluded from their study that oral usage of probiotics may reduce the carriage state of *E. coli* O157:H7 in cattle and prevents its colonization in digestive tract of cattle (Zhao et al., 1998). Also, results of a research work, done by Kokesova and colleagues, showed that manipulation of intestinal microflora could decrease the severity of Dextrin sodium sulfate-induced colitis in BALB/c mice. They documented by oral administration of probiotic strains that these harmless agents caused amelioration of the clinical signs of intestinal inflammation and improving of clinical appearances of animals (Kokesova et al., 2006). Theses studies support the results of our study implying beneficial effects of probiotics.

In the present study, although, the mechanism of action of *L. casei* in reducing of ETEC colonization and excretion not investigated, but the results of similar studies to our study (Kabir et al., 1997; Sgouras et al., 2004; Kokesova et al., 2006), it seems that the competitive inhibition of pathogenic bacteria from adherence to host cells, production of various metabolic and anti-bacterial substances, such as: different organic acids, bacteriocin, proteinase and hydrogen peroxide and ultimately regulation of immune system activity of host cell, alone or a combination of the above functions, may be inhibitory mechanisms of *L. casei* in deterrence of ETEC colonization (Boirivant and Trober, 2007; Ishida-Fujii et al., 2007).

In summary, the results of our study indicated that *L. casei* can be colonized probably as a probiotic in the gastrointestinal tract of murine model and reduced colonization of ETEC. This study is similar to other studies done by previous researchers reinforces the view that it is better to use probiotic and biotherapy instead of antibiotic for the prevention and treatment of ETEC infections.

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REFERENCES

Aiba Y, Suzuki N, Kabir AM, Takagi A, Koga Y (1998). Lactic Acid-Mediated Suppression of *Helicobacter pylori* by the Oral Administration of *Lactobacillus salivarius* as a Probiotic in a Gnotobiotic Murine Model. Am. J. Gastroenterol., 93: 2097-2101.

Akalin AS, Gonc S, Duzel S (1997). Influence of yogurt and acidophilus yogurt on serum cholesterol levels in mice. J. Dairy. Sci., 80: 2721-2725.

Asahara A, Nomoto K, Watanuki M, Yokokura T (2001). Antimicrobial activity of intraurethrally administered probiotic *Lactobacillus casei* in a murine model of *Escherichia coli* urinary tract infection. Antimicrob. Agents. Chemother., 45: 1751–1760.

Bergonzelli GE, Blum S, Brüssow H, Corthésy-Theulaz I (2005). Probiotics as a Treatment Strategy for Gastrointestinal Diseases? Digestion, 72: 57–68.

Bisson JF, Hidalgo S, Rozan P, Messaoudi M (2010). Preventive Effects of Different Probiotic Formulations on Travelers' Diarrhea Model in Wistar Rats: preventive effects of probiotics on TD. Dig. Dis. Sci., 55: 911–919.

Boirivant M, Trober W (2007). The mechanism of action of probiotics. Curr. Opin. Gastroenterol., 23: 679–692.

Caglar E, Kargul B, Tanboga I (2005). Bacteriotherapy and probiotics' role on oral health. Oral. Dis., 11: 131–137.

Castelli F, Saleri N, Tomasoni LR, Carosi G (2006). Prevention and treatment of traveler's diarrhea. Focus on antimicrobial agents. Digestion, 73: 109–118.

DuPont HL (2007). Therapy for and prevention of traveler's diarrhea. Clin. Infect. Dis., 45: 78–84.

Hickson M, D'Souza AL, Muthu N, Rogers TR, Want S, Rajkumar C, Bulpitt CJ (2007). Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. BMJ., 335: 80–84.

Ishida-Fujii K, Sato R, Goto S, Yang XP, Kuboki H, Hirano S , Sato M (2007). Prevention of pathogenic *Escherichia coli* infection in mice and stimulation of macrophage activation in rats by an oral administration of probiotic Lactobacillus casei I-5. Biosci. Biotechnol. Biochem., 71: 866–873.

Kabir AM, Aiba Y, Takagi A, Kamiya S, Miwa T, Koga Y (1997). Prevention of *Helicobacter pylori* infection by lactobacilli in a gnotobiotic murine model. Gut., 41: 49-55.

Kokesová A, Frolová L, Kverka M, Sokol D, Rossmann P, Bártová J, Tlaskalová-Hogenová H (2006). Oral administration of probiotic bacteria *(E. coli Nissle, E. coli O83* and *Lactobacillus casei*) influences the severity of dextran sodium sulfate-induced colitis in BALB/c mice. *Folia. Microbiol. (Praha)*. 51: 478-484.

Mazmanian SK, Round JL, Kasper Dl (2008). A microbial symbiosis factor prevents inflammatory disease. Nature, 453: 620–625.

McFarland LV (2007). Meta-analysis of probiotics for the prevention of traveler's diarrhea. Travel. Med. Infect. Dis., 5: 97–105.

Montalto M, Arancio F, Izzi D, Cuoco L, Curigliano V, Manna R, Gasbarrini G (2002). Probiotics: history, definition, requirements and possible therapeutic applications. Ann. Ital. Med. Int., 17: 157–165.

Ohashi Y, Umesaki Y, Ushida K (2004). Transition of the probiotic bacteria, *Lactobacillus casei* strain Shirota, in the gastrointestinal tract of a pig. Int. J. Food Microbiol., 96: 61–66.

Savage DC, Dubos R (1968). Alteration in the mouse cecum and its flora produced by antibacterial drug. J. Exp. Med., 128: 97-10.

Saxelin M, Tynkkynen S, Mattila-Sandholm T, de Vos WM (2005). Probiotic and other functional microbes: from markets to mechanisms. Curr. Opin. Biotechnol., 16: 204-211.

Senok AC, Ismaeel AY, Botta GA (2005). Probiotics: facts and myths. Clin. Microbiol. Infect., 11: 958–966.

Sgouras D, Maragkoudakis P, Petraki K, Martinez-Gonzalez B, Eriotou E, Michopoulos S, Kalantzopoulos G, Tsakalidou E, Mentis A (2004). *In vitro* and *in viv*o inhibition of *Helicobacter pylori* by *Lactobacillus casei* strain Shirota. Appl. Environ. Microbiol., 70: 518-526.

Tsai CC, Lin PP, Hsieh YM (2008). Three Lactobacillus strains from healthy infant stool inhibit enterotoxigenic *Escherichia coli* grown *in vitro*. Anaerobe, 14: 61-67.

Tuohy KM, Pinart-Gilberga M, Jones M, Hoyles L, McCartney AL, Gibson GR (2007). Survivability of a probiotic *Lactobacillus casei* in the gastrointestinal tract of healthy human volunteers and its impact on the fecal microflora. J. Appl. Microbiol., 102: 1026–1032.

Yates J (2005). Traveler's diarrhea. Am. Fam. Physician., 71: 2095–2100.

Zhao T, Doyle MP, Harmon BG, Brown CA, Mueller PO, Parks AH (1998). Reduction of carriage of enterohemorrahagic *Escherichia coli* O157:H7 in cattle by inoculation with probiotic bacteria. J. Clin. Microbiol., 36: 641-447.