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Research Paper

Immunomodulation effects of heat-treated *Enterococcus faecalis* FK-23(FK-23) in mice ☆

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Abstract

Objective: The purpose of this study was to evaluate the effects of *Enterococcus faecalis* FK-23(FK-23) on the immune function in mice. **Methods:**Mice were treated with three doses of the FK-23(0.05, 0.1, 0.2, 0.6 g/kg body weight) for 30 days. Delayed type hypersensitivity (DTH), monocyte phagocytic function, serum hemolysis antibody and natural killer cell activity were studied. **Results:** The significant increases in mice treated with 0.6 g/kg bw were found in DTH, phagocytic function of monocyte, serum hemolysis antibody and natural killer cell activity. **Conclusion:**The results from the present study suggest that FK-23 could improve both specific and non-specific immune function.

Keywords: FK-23; immunologic function; probiotics

INTRODUCTION

Colonic microflora are increasingly being shown to be capable of influencing gastrointestinal diseases and disorders[1]. In order to exert their beneficial health effects, it is generally assumed that the microorganisms need to be viable. The use of non-viable instead of viable microorganisms would have economic advantages in terms of longer shelf-life and reduced requirements for refrigerated storage. Non-viable microorganisms expand the potential use of probiotics to areas where the strict handling conditions can not be met, e.g. developing countries.

Among potentially protective foods, growing attention has been dedicated to prebiotics such as fructo-oligosaccharides or fructans, and probiotics[2,3]. Probiotics are live microorganisms that are used as dietary supplements with the aim of benefiting health by influencing the intestinal microbial balance[4-5]. Probiotics are viable microbial food ingredients supposed to be beneficial through their effect in the intestinal tract[6].

A new heat-treated *Enterococcus faecalis* FK-23(FK-23), which was established from serotype 5. *E. faecalis* isolated from the intestine of healthy humans was demonstrated to be a biological response modifier (BRM)[7-9]. Some biological effects of FK-23 have been found in previous studies[10,11]. For example, when given orally it stimulated not only leukocyte reconstituting capacity but also anti-microbial activities in mice treated with cyclophosphamide. Furthermore, it has been shown that FK-23 could inhibit hypertension in spontaneously hypertensive rats[12] and improve Hepatitis type C in humans[13]. The present study was designed to investigate these immunological efficacies of FK-23 administered orally for 30 days to mice.

MATERIALS AND METHODS

Materials

FK-23 was prepared by the method described previously[5-7]. Dinitrofluorobenzene(DNFB), sheep red blood cell(SRBC), guinea pig serum, India ink,

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RPMI1640, concanavalin A(con A), MTT, isopropanol, SA buffer and LDH substrate were purchased from Sigma Chemical Co., Ltd. (St Louis, MO, USA).

Animals and treatment

Male B1/F1 mice were purchased from Shanghai Soppr bk Animal Co. Ltd. They were fed a pellet diet and received filtered tap water. All animals were housed in polycarbonate cages with a 12-h light/dark cycle. Temperature and humidity were controlled at 23.0 ± 0.5℃ and 55.0 ± 5.0%.

FK-23 at doses of 0.05, 0.1, 0.2 and 0.6 g/kg body weight(n=12)was orally administrated to the experimental mice every day for 30 days. The animals for control group were received the same volume of distilled water(0.2 ml) for the same duration.

Delayed-type hypersensitivity(DTH)

After 30 days of oral administration, the hair on the animals' abdomen was removed using barium sulfide and the animals were sensitized to dinitrofluorobenzene (DNFB) by smearing 50 μl 1% DNFB in acetone-gingili oil on the abdominal skin. After seven days, 10 μl 1% DNFB was smeared on the right ear. Forty-eight hours later, the animals were killed and the DTH response to DNFB was evaluated by measuring the weight difference of the right and left ear with an analytical balance.

Phagocytosis of chicken erythrocytes by PEC

Phagocytosis of chicken erythrocytes by peritoneal macrophage was carried out with 12 mice taken from each groups after 30 successive days of FK-23 administration. The spleen and thymus were removed and weighed and the macrophage phagocytosis of chicken erythrocyte calculated by microscopy as follows: Percentage of the phagocytosis(%)=(number of macrophages that phagocytized chicken erythrocytes /total number of macrophages) × 100.

Measurement of humoral immune function

After 30 days of oral FK-23 administration, 12 mice from each groups were injected intraperitoneally with 0.2 ml sheep red blood cells(SRBC: 2% v/v). Blood was collected from the orbital sinus, and the serum was diluted with saline in multiple dilutions. The diluted serum(100 μ l) was then transferred to a hemagglutination microplate, and 100 μl sheep red blood cells(0.5%) was added and mixed. The microplate was incubated for 3 h(37℃, 5% CO_2, 80% humidity) and the degree of agglutination was assessed. A modified Jerne's plaque method was used to count antibody-producing cells, and the number of antibody-producing cells was expressed/10^6 spleen cells.

Measurement of NK cells activity

Spleens were minced under sterile conditions. A single cell suspension of spleen cells was prepared, washed twice, and resuspended in complete medium. YAC-1 mouse lymphoma cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 μg of streptomycin per mL and 100 units of penicillin per mL. The splenocytes were used as effector cells, and YAC-1 cells were target cells.

In vitro killing activity of splenic NK cells against YAC-1 cells was measured using an LDH release assay. Cytotoxicity(%)=(OD experimental release-OD effector spontaneous release-OD target spontaneous release)/(OD target maximum release-OD target spontaneous release) × 100.

Statistical analysis

Data were expressed as mean ± SD and analyzed using one-way analysis of variance(ANOVA). Statistically significant differences were reported as $^{*}P < 0.05$, $^{**}P < 0.01$. Data with values of $P < 0.05$ were generally accepted as statistically significant.

RESULT

Delayed-type hypersensitivity(DTH)

The DTH reaction following DNFB challenge was compared in control mice and in mice that had been orally administered with FK-23 for 30 days. As shown in ***Table 1***, the ear weight of mice treated with 0.1 and 0.6 g g/kg.bw FK-23 was increased significantly compared with control group.

Table 1 **Effect of FK-23 on cellular immunity in mice**

($\bar{x} \pm s$, n=12)

Group	Dosage (g/kg bw • d)	Difference in Weight (mg)
Control	0	14.1 ± 2.2
FK-23	0.05	17.7 ± 2.8
	0.1	19.8 ± 3.9**
	0.2	16.8 ± 4.5
	0.6	19.2 ± 4.3**

**$P < 0.01$ compared to control group, using one-way ANOVA.

Effect of FK-23 on monocytes and macrophages in mice

In ***Table 2***, the percentage of phagocytic index and phagocytosis increased in monocytes obtained from FK-23-treated mice compared to control animals. However this increase was only statistically significant at the highest dose group(0.6 g/kg bw).

Effect of FK-23 on humoral immunity in mice.

As shown in ***Table 3***, the plaque count/10^6 splenocytes was increased in serum obtained from FK-23-treated mice compared to control group. This increase was statistically significant at FK-23 doses of 0.1 and 0.6 g/kg.bw.

Table 2 **Effect of FK-23 on monocyte and macrophage in mice** ($\bar{x}\pm s$, n=12)

Group	Dosage (g/kg bw • d)	Phagocytosis index a	Phagocytosis percent(%)
Control	0	5.02 ± 0.58	18.9 ± 7.5
FK-23	0.05	5.00 ± 0.63	19.4 ± 6.1
	0.1	5.61 ± 0.64	21.8 ± 9.1
	0.2	5.54 ± 0.49	21.4 ± 9.6
	0.6	6.45 ± 0.93[a]	27.7 ± 8.1*

*$P < 0.01$ compared to control group, using one-way ANOVA.

NK cells activity

Among the FK-23 groups, the NK activity in mice treated with 0.6 g/kg.bw was significant increase com–pared to control group(***Table 4***).

Table 3 **Effect of FK-23 on monocyte and macrophage in mice** ($\bar{x}\pm s$, n=12)

Group	Dosage (g/kg bw • d)	Plaque count (count/10^6 spleen cell)
Control	0	70 ± 25
FK-23	0.05	88 ± 31
	0.1	110 ± 33*
	0.2	100 ± 47
	0.6	151 ± 42*

*$P < 0.01$ compared to control group, using one-way ANOVA.

Table 4 **Effect of FK-23 on NK cell activity in mice** ($\bar{x} \pm s$, n=12)

Group	Dosage (g/kg bw • d)	NK activity (%)
Control	0	35.2 ± 14.0
FK-23	0.05	39.0 ± 8.2
	0.1	35.8 ± 8.4
	0.2	47.4 ± 12.3
	0.6	61.1 ± 22.5**

**$P < 0.01$ compared to control group, using one-way ANOVA.

DISCUSSION

Probiotics have been claimed to have a wide range of health benefits, including anti-tumor function and immunostimulation[14,15]. It is known that probiotics have immune enhancement effects, and that each probiotic has a different repertoire of immune effects[16-18]. The term probiotics refers to preparations of microorganisms or components of microbial cells that have a beneficial effect on the health and well-being of the host when ingested[19]. The most commonly used probiotics are *Lactobacillus* and *Bifidobacterium* species, but *Enterococcus* strains and *Escherichia coli* have also been proposed as such in the light of the above definition[20]. FK-23 is a product of *E. faecalis* strain components.

In this study, we also examined the effect of the FK-23 on monocytes since macrophages are known to be one of the target cells of probiotics. A significant increase was observed in the monocyte phagocytic index with the highest probiotic dose used. In order to confirm whether the FK-23 influenced specific immune responses and the humoral immune responses, we evaluated the delayed type hypersensitivity(DTH) reaction to DFNB and serum hemolysis antibody. The results indicate that the FK-23 enhanced the DTH response, and significantly increased the hemolysis antibody level of mice at two probiotic doses. We show here that probiotics FK-23 powder has immune enhancement effects on the cell-mediated immune response, humoral immunity, monocyte/macrophage function, and NK cell activity. Some immune enhancement effects of the FK-23 were not strictly dose-dependent as only two doses (0.1 g/kg bw and 0.6 g/kg bw) had a significant effect while an intermediate dose (0.2 g/kg bw) did not(***Table 1*** and ***3***). In some reports, immune controlling functions of probiotics were not dose dependent. However, Christensen et al reported that probiotics act on macrophage activity and humoral immunity in a dose dependent manner[21-22]. Further investigation on the mechanism of action of these preparations is clearly required. The present study suggested that FK-23 could improve both specific and non-specific immunologic function.

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