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Hypocholesterolemic Activity of *Bifidobacteria* Isolated from a Healthy Korean

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This study was undertaken to investigate the hypocholesterolemic activity of *Bifidobacteria* (*B. breve* K-110, *B. breve* K-111, and *B. infantis* K-525) isolated from a healthy Korean. The administration of *B. breve* K-110 and K-111 with a high cholesterol diet significantly protected the increase of serum total cholesterol and LDL cholesterol relative to that of a high cholesterol diet alone. Such a diet supplemented with 0.5% *B. breve* K-111 decreased serum total cholesterol and LDL cholesterol to 57 and 55%, respectively. The administration of *Bifidobacteria* also significantly inhibited the lipid-deposited surface in the aorta. The normalizing activity of serum cholesterol level in cholesterolemic rats was accelerated by *Bifidobacteria*. The normalizing activity of *B. breve* K-111 on serum cholesterol level was superior to that of *B. breve* K-110. These results suggest that *Bifidobacteria* in the human intestine play a role in the prophylactics of arteriosclerosis.

Key words: Hypercholesterolemia, *Bifidobacterium breve* K-110, Cholesterol, Lactic acid bacteria

INTRODUCTION

An elevated level of blood and dietary cholesterol is considered to be a major risk factor for coronary heart diseases, as well as a factor, in addition to high dietary fat and low fiber, in the induction of colon cancer (Reddy *et al.*, 1977). There appears to be a relationship between the consumption of dairy products fermented by lactic acid bacteria and the reduction of serum cholesterol levels in humans (Harrison and Peat, 1975; Mann, 1977; Hepner *et al.*, 1979). Although the hypocholesterolemic effect of lactic acid bacteria has been studied (Gilliland *et al.*, 1985; Ishihara *et al.*, 1989), these studies were not complete in terms of their examination of *Bifidobacteria* isolated from human intestinal microflora.

Therefore, we investigated whether *Bifidobacterium breve* K-110, *B. breve* K-111, and *B. infantis* K-525, which are isolated from Korean intestinal microflora, could decrease the serum cholesterol level in cholesterol-induced hypercholesterolemic mice.

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MATERIALS AND METHODS

Materials

General anaerobic medium (GAM) was purchased from Nissui Pharm. Co, Ltd., (Japan). Tryptic soy broth (TS) and agar were purchased from Difco Co., (USA). The other reagents used were of analytical grades.

Culture of *Bifidobacteria*

Bifidobacterium breve K-110, *B. breve* K-111, and *B. infantis* K-525 isolated according to the method previously described (Park *et al.*, 1998; Han *et al.*, 1999) were used. These *Bifidobacteria* were subcultured in GAM broth for 24 h and then inoculated into TS broth containing 0.1% ascorbic acid and 0.01% sodium thioglycolate and cultured for 24 h at 37°C. The cultured *Bifidobacteria* were collected by centrifugation (5000 × g, 20 min) and supplemented into the normal diet for rats.

Rats and the hypercholesterolemic animal model

Male rats (SD, 180-220 g), purchased from Daehan Animal Center, were fed on tap water and normal diet (lab chow, Samyang Co., Ltd., Korea), and housed at 23°C, 55 ± 10% humidity for 10 days.

To evaluate the hypocholesterolemic effect, rats were

classified into 6 groups (HC, 0.5% K-110, 1% K-110, 0.5% K-111, 1% K-111 and control groups) of 5 rats each. The HC group was fed on a normal diet (95.75~96.5%) supplemented with 1% cholesterol, 0.25% cholic acid and 2.5% olive oil (CCO) for 14 days. The control group received a solid normal diet (rodent chow: 32% protein, 5% fat, 2% fiber, 60% nitrogen free extract and 1% minerals and vitamins), while the 0.5% K-110, 1% K-110, 0.5% K-111, and 1% K-111 groups were fed on a base diet equivalent to the HC group but supplemented with 0.5% *B. breve* K-110, 1% *B. breve* K-110, 0.5% *B. breve* K-111, and 1% *B. breve* K-111, respectively, for 2 weeks. Blood samples were drawn every week by cardiac puncture under ether anesthesia for determination of levels of serum total cholesterol, HDL cholesterol, LDL cholesterol, and serum triglyceride. The cholesterol and triglyceride con-centrations were determined using enzymatic reagent kits from Sigma Co. (U.S.A.).

To evaluate the accelerative effect on cholesterol excretion on the hypercholesterolemic rat model, rats were fed on a normal diet supplemented with CCO for 3 weeks and then classified into 5 groups (0.5% K-110, 1% K-110, 0.5% K-111, 1% K-111, and HC groups) of 5 rats each. The HC group was fed on a normal diet supplemented with CCO for 14 days. The control group was fed on the normal diet described above. The 0.5% K-110, 1% K-110, 0.5% K-111, and 1% K-111 groups were fed on a base diet equivalent to the HC group but supplemented with 0.5% *B. breve* K-110, 1% *B. breve* K-110, 0.5% *B. breve* K-111, and 1% *B. breve* K-111, respectively, for 2 weeks. After final feeding of the *Bifidobacteria* supplemented diet, blood samples were drawn every week by cardiac puncture under ether anesthesia for determination of levels of serum total cholesterol, HDL cholesterol, LDL cholesterol, and serum triglycerides. The cholesterol and triglycerides concentrations were determined enzymatically using enzymatic reagent kits from Sigma Co. (U.S.A.).

Assay of lipid-deposited surface area in the rat descending aorta

To evaluate the effect on lipid-deposited surface area in the descending aorta of hypercholesterolemic rats, rats were fed on a normal diet supplemented with CCO for 3 weeks and then classified into 5 groups (1% K-110, 1% K-111, and HC groups) of 5 rats each. The HC group was fed on a normal diet supplemented with CCO for 2 weeks. The control group was fed on the normal diet described above. The 1% K-110 and 1% K-111 groups were fed on a base diet equivalent to the HC group but supplemented with 1% *B. breve* K-110 and 1% *B. breve* K-111, respectively, for 2 weeks. After final feeding of the *Bifidobacteria* supplemented diet, rats were surgically cut open under ether anesthesia. The descending aorta of the heart was selected, incised, stained with Sudan III and photographed. The lipid-stained lesions of Sudan III-stained aorta were traced and the ratio of lipid-deposited area to total aorta area was calculated. Blood samples were drawn for phospholipid determination before the aorta operation. The phospholipid concentrations were determined using diagnostic kits from Sigma Co. (U.S.A.).

RESULTS AND DISCUSSION

When the hypocholesterolemic activity of *Bifidobacteria* (*B. breve* K-110, *B. breve*, K-111, and *B. infantis* K-525) isolated from a healthy Korean was preliminarily measured, *B. breve* K-110 and *B. breve* K-111 were found to be more effective in decreasing serum total cholesterol and LDL cholesterol than *B. infantis* K-525 (data not shown). Therefore, we investigated the hypocholesterolemic activity of *B. breve* K-110 and K-111 on rats (Table 1). The serum cholesterol level of the control group was increased according to the duration of cholesterol administration. However, the K-110 and K-111 groups, which were treated with a high cholesterol diet containing 0.5 or 1% *Bifidobacteria*, effectively protected the increase of serum total cholesterol and LDL cholesterol, whereas serum triglyceride level was not affected in all groups except the 1% K-111 treated group. Hypocholesterolemic activity of

Table 1. Effects of *Bifidobacteria* on serum triglyceride, total cholesterol, and LDL-cholesterol level of hypercholesterolemic rats.

Group	TG (mg/ml)			Total cholesterol (mg/dl)			LDL cholesterol (mg/dl)		
	Week after treatment			Week after treatment			Week after treatment		
	0	1	2	0	1	2	0	1	2
Control	62.7 ± 14.3	75.5 ± 6.0	72.2 ± 4.2	147.5 ± 9.0	127.3 ± 2.7	78.3 ± 2.9	36.9 ± 5.9	37.1 ± 1.3	37.0 ± 4.1
HC-fed	57.8 ± 9.7	133.5 ± 10.3	81.9 ± 9.5	167.5 ± 16.5	324.9 ± 12.2	431.4 ± 24.6	42.0 ± 5.6	92.3 ± 4.6	224.9 ± 17.8
0.5% K-110	61.3 ± 10.4	91.0 ± 16.9	76.1 ± 11.5	142.0 ± 10.2	308.0 ± 19.1	329.4 ± 52.5*	35.7 ± 4.0	88.3 ± 9.9	176.5 ± 33.1*
1.0% K-110	72.5 ± 9.3	103.3 ± 32.1	84.2 ± 12.1	152.1 ± 4.9	299.2 ± 22.0	236.1 ± 58.7*	38.6 ± 1.8	83.0 ± 8.9	129.0 ± 51.0*
0.5% K-111	59.4 ± 11.2	95.5 ± 20.9	78.7 ± 15.8	160.5 ± 6.1	288.6 ± 9.4	247.1 ± 44.9*	40.9 ± 2.5	74.8 ± 3.0*	122.9 ± 22.3*
1.0% K-111	55.5 ± 6.3	58.0 ± 8.0*	-[a)]	162.0 ± 10.5	253.5 ± 5.2*	-	41.8 ± 4.7	65.0 ± 4.7*	-

[a)]not determined Values are means ± SD

*Significantly different from cholesterol-fed group ($p<0.05$)

Table 2. Effects of *Bifidobacteria* on serum phospholipid and aorta-deposited lipid level of hypercholesterolemic rats

Group	Phospholipid (mg/ml)	lipid-deposited rate in the aorta (%)
Control	71.6 ± 6.2	6.6 ± 0.3
HC-fed	148.6 ± 12.1	22.8 ± 0.5
1% K-110	50.5 ± 7.1*	10.6 ± 1.4*
1% K-111	76.2 ± 2.6*	8.2 ± 2.8*

*significantly different from cholesterol-fed group ($p < 0.05$). Values are means ± SD

serum cholesterol level was *Bifidobacteria* dose-dependent. The decreasing activity of *B. breve* K-111 on serum cholesterol level was superior to that of *B. breve* K-110. A diet supplemented with 0.5% *B. breve* K-110 and 0.5% *B. breve* K-111 decreased serum total cholesterol and LDL cholesterol to 57 and 55%, respectively.

We also measured the inhibitory activity of *Bifidobacteria* on lipid-deposited surface area in the descending aorta of rats fed high cholesterol (Table 2). The lipid-deposited surface area of the control, 6.6%, was increased to 22.8% in the HC group. However, the administration of *Bifidobacteria* significantly inhibited the lipid-deposited surface in the aorta. The inhibitory activity of *B. breve* K-111, which showed inhibition relative to the HC group of up to 46% was superior to that of *B. breve* K-110.

We also investigated the accelerative effect of *Bifidobacteria* on cholesterol excretion in hypercholesterolemic rats (Table 3). When a high cholesterol diet was administered for 3 weeks, the serum cholesterol level of the control group was significantly increased. When the normal diet was replaced 3 weeks after final feeding of the HC diet, the serum cholesterol level was normalized according to the duration of lapse. However, this normalizing activity of serum cholesterol level was accelerated by feeding a diet supplemented with *Bifidobacteria* in a dose dependent manner. The normalizing activity of *B. breve* K-111 on serum cholesterol level was superior to that of *B. breve* K-110. A diet supplemented with 1% *B. breve* K-110 and K-111 decreased serum total cholesterol and LDL cholesterol to 50 and 69% of the control group, respectively.

Lactic acid bacteria are generally recognized as safe organisms, and some probiotic strains have been proposed as an alternative therapy for the treatment of gastrointestinal disorders and cancers (Perdigon *et al.*, 1991; Ishihara *et al.*, 1989). Some researchers have reported that these lactic acid bacteria are effective in decreasing serum lipid concentrations (Rodas *et al.*, 1996; Taranto *et al.*, 1998). Most of the lactic acid bacteria that have been tested originated from fermented foods. However, these bacteria are the dominant organisms in human intestinal microflora. Therefore, we isolated *B. breve* K-110 and K-111, which showed hypocholesterolemic activity, from healthy Korean intestinal microflora. Our results suggest that intestinal microflora of healthy people is capable of lowering serum cholesterol and playing an important role in the prevention of arteriosclerosis. Accordingly, we further suggest that human intestinal *Bifidobacteria* play a role in the prophylactics of cancer and arteriosclerosis.

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Table 3. Normalizing effects of *Bifidobacteria* on the relative values of serum total cholesterol level in rats

Group	TG (mg/ml)			Total cholesterol (mg/dl)			LDL cholesterol (mg/dl)		
	Week after treatment			Week after treatment			Week after treatment		
	0	1	2	0	1	2	0	1	2
Control	61.2 ± 9.0	85.0 ± 16.9	70.3 ± 8.6	92.7 ± 3.8 (100)	61.7 ± 2.0 (67)	78.5 ± 5.6v (85)	25.3 ± 3.7 (100)	21.2 ± 2.6 (84)	19.8 ± 43.5 (78)
HC-fed	77.7 ± 6.9	94.3 ± 3.7	109.1 ± 12.8	408.2 ± 9.1 (100)	152.9 ± 12.9 (37)	140.1 ± 13.0 (34)	107.9 ± 9.5 (100)	39.2 ± 1.8 (36)	35.4 ± 3.2 (33)
0.5% K-110	89.6 ± 9.0	111.5 ± 13.2	115.4 ± 11.8	387.3 ± 3.8 (100)	100.0 ± 19.7 (26)*	78.1 ± 10.5 (20)*	92.0 ± 11.1 (100)	43.1 ± 9.4 (47)	36.0 ± 2.5 (39)
1.0% K-110	79.6 ± 9.6	102.2 ± 15.7	111.2 ± 12.3	444.1 ± 21.9 (100)	96.0 ± 8.9 (22)*	88.4 ± 9.8 (20)*	123.7 ± 2.0 (100)	37.2 ± 9.1 (30)	28.3 ± 2.4 (23)*
0.5% K-111	97.4 ± 6.5	99.6 ± 13.1	105.2 ± 6.3	461.8 ± 10.9 (100)	102.3 ± 28.8 (22)*	75.5 ± 17.0 (16)*	110.0 ± 2.0 (100)	39.2 ± 13.1 (37)	22.4 ± 8.3 (20)*
1.0% K-111	117.5 ± 14.4	107.5 ± 28.1	100.2 ± 6.6	387.0 ± 0.2 (100)	86.9 ± 23.8 (22)*	65.6 ± 5.6 (17)*	85.6 ± 12.2 (100)	28.2 ± 3.7 (33)*	19.5 ± 5.9 (23)*

*Significantly different from cholesterol-fed group ($p < 0.05$) Values are means ± SD
Parentheses indicates relative values compared to cholesterol values of each in non-treated period.

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