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Randomized control trials

The effects of co-administration of probiotics with herbal medicine on obesity, metabolic endotoxemia and dysbiosis: A randomized double-blind controlled clinical trial

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SUMMARY

Background & aims: Probiotics help maintain balance in composition of the gut microbiota, and have been considered as a potential treatment for obesity. This study was conducted in order to assess the effects of probiotics when combined with herbal medicine in treatment of obesity. Probiotics were tested for the ability to modulate gut microbiota, gut permeability, and endotoxin level, which may have correlation with factors involved in obesity.
Methods: A randomized, double-blind, placebo controlled study was conducted, in which patients with higher BMI (>25 kg/m^2) and waist circumference (>85 cm) were enrolled and randomly assigned to receive *Bofutsushosan* with either probiotics or placebo capsules for a period of eight weeks. Assessment of body composition parameters, metabolic biomarkers, endotoxin level, gut permeability, and fecal bacteria in stool was performed at baseline and at week 8. The study was registered at the Clinical Research Information Service, approved by the Korea National Institute of Health (KCT0000386).
Results: Although both groups showed a significant reduction in weight and waist circumference ($p = 0.000$), no significant differences in body composition and metabolic markers were observed. In correlation analysis, change in body composition showed positive correlation with endotoxin level ($r = 0.441$, $p < 0.05$ for BW; and $r = 0.350$, $p < 0.05$ for fat mass) and the population of gut *Lactobacillus plantarum* ($r = 0.425$, $p < 0.05$ for BW; and $r = 0.407$, $p < 0.05$ for BMI). The Gram negative bacterial population in gut also exhibited positive correlation with changes in body composition (WC) and total cholesterol level ($r = 0.359$, and 0.393, for the former and later parameters, respectively, $p < 0.05$ for both). While, the profile of gut *Bifidobacterium breve* population showed negative correlation with endotoxin level ($r = -0.350$, $p < 0.05$).
Conclusions: Correlations between gut microbiota and change in body composition indicate that probiotics may influence energy metabolism in obesity. Correlation between endotoxin level and weight reduction indicates that probiotics may play an important role in prevention of endotoxin production, which can lead to gut microbiota dysbiosis associated with obesity.

1. Introduction

Gut microbiota composition is regarded as one of the major etiological factors involved in control of body weight and many studies investigating its link to obesity have been conducted in the past several years.[1–3]

Many reports have demonstrated participation of gut microbiota in development of obesity by several mechanisms involving increased gut permeability and metabolic endotoxemia. This can be explained by a fat-enriched diet inducing Gram negative bacteria-derived lipopolysaccharide (LPS), which is closely correlated with abdominal fat deposit and derangement in intestinal microbiota.[4] Endotoxemia involves an inflammatory process, which is triggered by disruption of tight gap junction proteins when exposed to

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LPS originating from the gut, thereby increasing intestinal permeability.[5] Therefore, alteration in gut barrier induces many metabolic disorders preceded by endotoxemia and inflammation, such as increased gut permeability (known as 'leaky gut syndrome')[6] and inflammatory bowel disease (IBD).[7]

Because probiotics help maintain balance in composition of gut microbiota, they have been considered as a potential treatment for obesity. Many studies supporting anti-obesity and lipid-lowering effects of probiotics[8] as well as anti-inflammatory and anti-oxidative activity in pathogenic conditions such as non-alcoholic fatty liver disease (NAFLD)[9] and steatohepatitis (NASH) have been reported.[9] Probiotics also help to maintain integration of gut barrier and reduce intestinal permeability, which subsequently decrease endotoxin level.[10]

Bofutsushosan (BTS), an oriental herbal medicine containing 18 components, is widely used as an anti-obesity medication in East Asia. Its effects on obesity, cardiovascular diseases, and insulin resistance have been researched in various studies and the results showed decreasing effects on triglyceride level and lipid metabolism as well as correlation with obesity associated-genes.[11]

This study was conducted in order to assess the effects of probiotics when combined with herbal medicine in treatment of obesity. In order to investigate correlation of obesity-related factors with alteration of gut microbiota, modulation of gut microbiota composition, gut permeability, and endotoxin level by probiotics was demonstrated.

2. Materials and methods

2.1. Subjects

The study protocol was approved by the Institutional Review Board of Dongguk University Oriental medical hospital (approval number 2011-SR21) and written informed consent was obtained from each patient prior to conduct of the study. A total of 64 female subjects aged 19–65 years meeting the criteria of BMI $>$25 kg/m^2 and waist circumference $>$85 cm were recruited from advertisement in a local newspaper between April and August 2011. Among 64 patients who underwent screening, 50 subjects fulfilled the criterion of the study. All subjects underwent appropriate investigations in order to exclude any condition that might affect weight gain. The exclusion criteria are shown in Table 1. Other than these restrictions, subjects were encouraged to maintain their usual dietary intake and lifestyle during conduct of the study.

2.2. Study design and intervention

This was a randomized, double-blinded, placebo controlled study, which was conducted in order to determine whether there is an additional effect of co-administration of probiotics and BTS, compared to BTS alone. The study was registered at the Clinical Research Information Service, approved by the Korea National Institute of Health (KCT0000386). Subjects were randomly assigned to receive BTS and probiotics ($n = 25$) or BTS and placebo ($n = 25$). The study medications were taken twice per day for a period of eight weeks. Subjects were treated with BTS co-administered with either probiotics or placebo twice per day for a period of eight weeks. BTS was administered in the form of herbal extracts (Tsumura & Co, Japan) 3g per administration. Probiotics (DUOLAC 7 which is a fourth generation dual coated probiotics, Cell Biotech, Gimpo, Korea) or placebo capsules were also given twice per day, co-administered with BTS extracts. One capsule of Duolac 7 included 5 billion viable cells of *Streptococcus thermophiles* (KCTC 11870BP), *Lactobacillus plantarum* (KCTC 10782BP), *Lactobacillus acidophilus* (KCTC 11906BP), *Lactobacillus rhamnosus* (KCTC 12202BP), *Bifidobacterium lactis* (KCTC 11904BP), *Bifidobacterium longum* (KCTC 12200BP), and *Bifidobacterium breve* (KCTC 12201BP). Placebo capsules were identical in appearance, except for the bacterial strains.

2.3. Dietary and exercise intervention

Subjects were informed of dietary guidelines of the study after enrollment. At the beginning of the study, dietary intake was assessed using 24-h dietary recall methods. The guidelines suggest that subjects maintain a daily diet, limiting caloric intake to 20–25 kcal/kg, according to subject's weight. Participants were instructed to record daily food intake in their diet diary provided to them. From the second visit, food intake patterns and caloric values were assessed by clinical research coordinator (CRC) every two weeks until the end of the study. Amount of intake was calculated in kcal unit. Participants were also instructed to maintain their usual exercise program during the entire study. In the exercise diary provided to them, participants recorded the degree of exercise performed on each day (criteria of mild/moderate/severe exercise were supplied to every volunteer) which were checked by CRC every two weeks until the study completed.

2.4. Analytical measurements

The primary outcome measures were the difference in weight and gut permeability between the two groups. The secondary outcomes included change in blood lipid level, waist circumference, blood pressure, BMI, main bacterial strains of intestinal microbiota, endotoxin level, and KOQOL (The Korean version of obesity-related quality of life). Progress of analytical measurements is shown in Table 2.

Table 1
Exclusion criteria of the recruitment of the subjects.

Exclusion criteria
Hypothyroidism
Cushing's syndrome
Heart diseases
Cancer
Lung diseases
Severe renal dysfunction (Cr $>$ 2.0 mg/dl)
Liver dysfunction (ALT, AST $\geq$ 2.5 fold upper limit of normal)
Non-insulin dependent diabetes mellitus with FBS $>$ 140 mg/dL, neuropsychiatric diseases
Eating disorder
Subjects who underwent anatomical change such as incision
Pregnancy, breast feeding, or planning of pregnancy
Subjects who have lost 10% of body weight within six months of the study.

Table 2
Progress of analysis in the course of study.

	Blood analysis	Stool analysis	Gut permeability test	BP	BW	WC	BIA	KOQOL
Visit 1 Week 0	O	O	O	O	O	O	O	O
Visit 2 Week 2				O	O	O		
Visit 3 Week 4				O	O	O	O	
Visit 4 Week 6				O	O	O		
Visit 5 Week 8	O	O	O	O	O	O	O	O

BP: blood pressure, BW: body weight, WC: waist circumference, BIA: bioelectrical impedance analysis, KOQOL: Korean version of the obesity-related quality of life scale.