Randomized, Double-blind, Placebo-controlled Trial of the Effects of Polycan, β-glucan Originating from Aureobasidium Pullulans, on Bone Biomarkers in Healthy Women

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Polycan originating from *Aureobasidium pullulans* is mostly composed of β-1, 3/1, 6 glucans and possesses an anti-osteoporotic effect. We conducted a randomized, double-blind, placebo-controlled trial to examine the efficacy and safety of the polycan on bone biochemical markers in healthy perimenopausal women. Sixty subjects were randomly allocated to 2 groups—group 1 received 400 mg of polycan and group 2 received placebo—these were administered once daily for 28 days. Fasting blood and urine samples were collected at baseline and 4 weeks after treatment. The primary outcome was change in osteocalcin (OSC) and bone-specific alkaline phosphatase (BALP). Changes in calcium (Ca), phosphorus (P), C-telopeptide of collagen cross-links (CTx), N-telopeptide of collagen cross-links (NTx), and deoxypyridinoline (DPYR) were the secondary outcomes. A safety assessment was performed using adverse event (AE) and laboratory data. After 4 weeks of polycan treatment, OSC, DPYR, and BALP levels changed ($P < 0.05$) significantly from baseline in both groups. However, no significant differences were observed in any markers between the 2 groups, except for P ($P < 0.05$). Interestingly, group 2 showed a significant increase in CTx (65.2%, $P < 0.05$), while CTx in group 1 slightly increased (17.2%). Both groups showed no significant differences in AE. Although 4 weeks of polycan treatment did not have a statistically significant effect on bone metabolism biomarkers, increases in CTx were modestly inhibited by polycan. Further studies in a large population and longer treatment periods are needed to confirm the effect of polycan on bone turnover.

keywords : β-glucans, Polycan, Bone turnover, Osteoporosis, Bone biomarker

Introduction

β-Glucan is a polysaccharide that is found in the cell walls of yeast, oats, barley fiber, and many medicinal mushrooms[1)]. β-Glucan has been reported to be a safe and effective dietary supplement for treating cancer, lowering cholesterol levels, treating diabetes, and enhancing immune function[2,3)]. The polycan, black yeast β-glucan, is a commercial product derived from *Aureobasidium pullulans* SM-2001. The main ingredient of this polycan is β-1, 3/1, 6 glucan and other organic materials such as amino acids, mono- or di-unsaturated fatty acids (linoleic and linolenic acids), and fibrous polysaccharide[4)]. Preclinical studies have suggested that this polycan has favorable effects on bone metabolism. A 4-week treatment with this polycan, as a potent inhibitor of bone turnover, inhibited bone loss from osteoporosis in comparison to treatment with alendronate[2)], decreased osteocalcin (OSC) levels dose-dependently, and inhibited serum calcium (Ca) and phosphorus (P) decrease[5)] in ovariectomized mice. Furthermore, 13 days of polycan

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treatment promoted rib fracture healing[6]. A 12- week of polycan treatment tended to decrease deoxypyridinoline (DPYR) and increase osteocalcin (OSC) and appeared to display a safe and tolerable profile in healthy women[7]. However, at present, insufficient evidence is available from randomized controlled trials to verify the abovementioned effects.

Maintaining balance between bone formation and resorption is a key factor of normal bone metabolism, and this process is regulated by osteoblasts and osteoclasts[4]. When the balance tilts, osteoporosis, the major bone disease in postmenopausal women, can occur[8]. Several treatments for osteoporosis, such as hormone replacement therapy (HRT), bone anabolic agents, and antiresorptive agents, have been widely used to date[9,10]. In postmenopausal osteoporosis, prevention of bone loss by inhibiting bone resorption is a conventional approach[11]. Although the current antiresorptive agents are extensively used, attempts to develop new effective agents or alternative therapies for osteoporosis have been made because of inconveniences such as gastrointestinal irritation and a difficult dosing regimen[10]. Various alternative health supplements such as magnesium[12], potassium[13], vitamin K2(menaquinones)[14], soy isoflavone[15], berberine, vitamin K1, vitamin D3, and hop rho iso-α acid[16] have also been studied for their therapeutic effects on bone metabolism.

There are 2 categories of biochemical markers of bone turnover. The first group comprises bone resorption markers that reflect osteoclast activity and are primarily degradation products of type I collagen, while the other group comprises bone formation markers that reflect osteoblast activity and are by-products of collagen synthesis, matrix proteins, or osteoblastic enzymes. These markers can reflect changes in bone turnover in most circumstances because bone resorption and formation are interconnected[17]. The most sensitive markers include serum OSC and bone-specific alkaline phosphatase (BALP) for bone formation and DPYR, C-telopeptide of collagen cross-links (CTx), and N-telopeptide of collagen cross-links (NTx) for bone resorption[18]. A specific and responsive bone resorption marker can also be used to monitor and establish the short-term effectiveness of antiresorptive therapy in a patient[19].

In the present study, the effects and safety of the polycan on bone turnover markers were evaluated in healthy perimenopausal women. The results of this study are expected to provide basic clinical information from randomized controlled trials about changes in biochemical markers after polycan treatment.

Materials and Methods

1. Study design

This was a 4-week placebo-controlled, double-blind, and equally randomized [1:1] intervention trial to investigate the effects and safety of polycan on bone biochemical markers in healthy women.

2. Subjects

The study protocol was approved by the Institutional Review Board of Daegu Haany University, Daegu Oriental Hospital, and the study was conducted in the clinical trial center of Daegu Haany University, Daegu Oriental Hospital (Daegu, South Korea). All procedures were conducted in accordance with the ethical standards of the Declaration of Helsinki and Good Clinical Practice Guidelines.

Healthy women volunteers aged 40-70 years were recruited in the study. Informed written consent was obtained from all volunteers before the study, and the volunteers were examined for their health status based on clinical history, physical examination, and clinical laboratory testing, including OSC and DPYR.

Exclusion criteria included the following: a history of treatment with HRT, calcitonin, or bisphosphonates within 6 months; a history of the use of β-blockers, any hormone product, soybean-containing dietary supplements; treatment for hyperlipidemia or an endocrine disorder within 2 months; administration of any medication, including vitamin D, K, and Ca supplements within a month; current use of steroids or herbal medicine known to affect bone metabolism; a history of cancer (breast, ovarian, endometrial, cervical, or bone) or a diagnosed disease known to affect bone metabolism (hepatic, renal, or cardiovascular disease); a history of neurological or psychiatric disease; current pregnancy, lactation, or lack of a medically accepted method of contraception in women of reproductive age; being judged by the investigator as inappropriate to participate in this study; and drug addiction. The drugs and foods restricted during the study included the following: all anti-osteoporotic agents; treatment for hyperlipidemia or endocrine disorder; hormone product; anti-thyroid agents; adrenocortical agents; soybean-containing dietary supplements; vitamin D, K, and Ca supplements; and bone and joint health supplements.

3. Interventions

The study comprised 3 site visits (screening, baseline, 4 weeks after baseline) and one telephone contact 2 weeks after baseline. All subjects who were confirmed eligible at screening were enrolled and randomly allocated to the placebo or polycan treatment group in the order of enrollment. Block randomization codes with a 1:1 allocation, with a block size of 2, were used to allocate and identify the subjects (e.g., D001, D002…). At the baseline visit, subjects were given a plastic bottle containing a 35-day supply (including a 7-day surplus tablet supply) of the polycan or placebo. They were instructed to take 2 tablets (placebo or polycan) once a day (total 400 mg per day) 30 minutes after breakfast with a cup of tap water. The polycan dose and duration of treatment period were determined with reference to an earlier clinical trial[20]. The polycan and placebo tablets were manufactured by Silla University Industry-Academic Cooperation Foundation (Busan, South Korea), and each polycan tablet contained polycan (44.4%) and excipients such as microcrystalline cellulose. The placebo tablet was very similar to the polycan tablet with respect to shape, color, weight, and odor, and it contained corn powder (44.4%) and the same excipients as the polycan tablet. To ensure compliance and to verify adverse events (AEs), subjects received a telephone call from research staff after 2 weeks. The subjects were asked to bring their bottle to assess treatment compliance at the end of study and any remaining tablets were counted by the staff.

4. Measurement of Outcomes

The primary outcome was the change in the serum OSC value, and the secondary outcomes were the changes in urinary DPYR, serum BALP, CTx, NTx, Ca, and P after 4 weeks of polycan or placebo treatment as compared to the baseline. For each site visit, all subjects were asked to visit at the same time in the morning. Physical examinations including measurement of vital signs were performed, and fasting blood and single midstream spot urine samples were collected to measure biochemical markers of bone turnover and to assess laboratory abnormalities at baseline and at 4 weeks. All laboratory tests were performed at the Daegu Haany University Oriental Hospital Laboratory (Daegu, South Korea), except for bone biochemical marker tests that were performed at the SungYoon Reference Lab (Daegu, South Korea; The Korean Laboratory Accreditation Program certified).

The polycan safety evaluation was performed throughout the study based on laboratory data, subjects' self-reported symptoms, and abnormal signs observed by a clinical investigator. AE were recorded according to WHO Adverse Reaction Terminology (WHO-ART), and causal relationship to the treatment was evaluated.

5. Sample size

The primary outcome measure was changes in OCS after 4 weeks of treatment. Sample size was calculated using 80% power (α = 0.05) to detect a difference of 13.13 ng/mL in primary outcomes between treatment groups with a standard deviation (SD) of 16.8. Twenty-four subjects were required in each group, and a total of 60 subjects were enrolled assuming a dropout rate of 20%.

6. Randomization and blinding

The randomization code list was computer-generated by a statistician. The statistician recorded each code on paper in an order, placed them in each envelope, and sealed envelopes. The sealed envelopes were given to a clinical trial pharmacist, who was independent from the study, and the principal investigator (PI). During the study period, all the investigators (except the PI), research staff, and subjects were blinded to the randomization codes. The pharmacist received polycan and placebo tablets from the sponsor and enclosed 35-day tablet supplies in consecutively numbered plastic bottles. Whenever a subject was allocated, the pharmacist dispensed a numbered bottle according to the randomization schedule. After completion of all the analyses, the randomization code was disclosed to the investigators.

7. Statistical analysis

Results are expressed as means ± SD. An efficacy analysis was conducted on a per-protocol basis for women with ≥80% compliance, and a safety analysis was conducted on an intent-to-treat basis. All data were confirmed to have followed a normal distribution by using a normality test. Efficacy analysis was performed using the biochemical bone marker values. The mean percent changes in biomarkers from baseline to 4 weeks were calculated for each group, and a paired t-test or Wilcoxon signed rank test was applied to examine the significance of changes in either group. Student's t-test or Wilcoxon rank sum tests were applied to determine significant differences between the changes in the polycan group and those in the placebo group. All tests were 2-sided, and $P < 0.05$ was defined as significant. The effect sizes of each bone biomarker were

reported as Cohen's d and were calculated using mean changes in biomarkers after 4 weeks from baseline according to the group and the pooled SD of mean change of each group. For the safety analysis, Fisher's exact test was used to compare the incidence of AEs between the groups. The SAS statistical software was employed to conduct the analyses (software version 9.2; SAS Institute, Cary, NC).

Results

1. Subjects' characteristics and compliance

The study was performed between December 2010 and March 2011. Among the 127 women who were screened for the study, 60 subjects participated in the study; of these, 2 withdrew from the study after the baseline visit due to AEs. The remaining 58 subjects completed the study, and 2 subjects were excluded from efficacy analyses because of poor compliance (<80%). The data from 56 subjects were included in the efficacy analyses, and all subjects who took a treatment at least once were included in the safety analysis(Fig. 1). No significant differences were observed in baseline characteristics between the placebo and polycan treatment groups(Table 1). Overall compliance was 93.9% for the 58 subjects; for the 56 subjects who were included in the efficacy analyses, compliance was 94.6%.

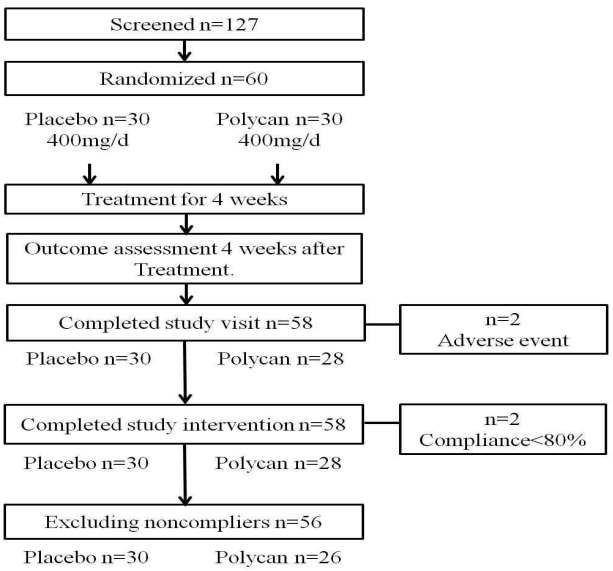

Fig. 1. Flow chart of subjects. Among the 60 women who were allocated to the treatment groups, 58 completed the study visit and intervention. Two women were excluded from the efficacy analysis because of poor compliance (<80%).

2. Changes in bone biomarkers

Table 2 shows the value of bone biomarkers at baseline and after 4 weeks of treatment as well as the mean percent changes in the biomarkers from baseline at 4 weeks in the placebo and polycan groups. As compared to the baseline, OSC and DPYR decreased significantly and BALP increased significantly in both groups ($P < 0.05$). Ca significantly increased only in the placebo group ($P < 0.05$), and NTx and P did not show significant changes in both the groups. CTx showed a significant increase in the placebo group ($P < 0.05$) but a slight, non-significant increase in the polycan group (65.2% vs. 17.2%, respectively). No significant differences in biomarker values were observed between the 2 groups; however, the change of P was statistically significant ($P < 0.05$). The effect size of each biomarker is also presented in Table 2. The effect sizes were generally small to moderate (Cohen's d, <0.20 to <0.50) for DPYR, BALP, and CTx. Serum P showed a moderate effect size. Other outcomes showed small effect sizes (Cohen's d, 0.05–0.19).

Table 1. Baseline characteristics of the subjects

	Placebo n = 30	Polycan n = 30	P-value
Age (y)	48.40±4.55	47.13±6.01	0.13
Height (cm)	159.08±4.90	158.66±4.99	0.59
Weight (kg)	57.23±5.79	56.86±7.54	0.75
BMI (kg/m^2)	22.62±2.12	22.57±2.59	0.85
OSC (ng/ml)	7.82±2.27	7.54±2.93	0.49
DPYR (nM DPYR/mM creatinine)	6.45±2.13	5.62±1.20	0.07

Data represents mean±SD. n: number of subject; BMI: body mass index; OSC: osteocalcin; DPYR: deoxypyridinoline

Table 2. Effects of 4 weeks of polycan treatment on bone biochemical markers

	Placebo n = 30			Polycan n = 26			
	baseline	4 weeks	Change (%)	baseline	4 weeks	Change (%)	Effect size[c]
OSC (ng/ml)[a]	7.26± 2.55	5.99± 2.04	-9.0*	6.67± 2.36	5.66± 2.85	-3.9*	0.08
BALP (ug/L)[a]	12.23± 3.96	13.58± 3.78	15.2*	12.02± 3.63	14.21± 5.02	18.6*	0.31
CTx (ng/ml)[a,#]	0.208± 0.132	0.298± 0.225	65.2*	0.167± 0.082	0.186± 0.118	17.2	0.49
NTx (nM BCE/mM creatinine)[a,##]	58.61± 29.35	57.53± 33.72	0	52.81± 32.36	56.39± 42.47	11.1	0.19
DPYR (nM DPYR/mM creatinine)[b]	6.65± 1.57	6.06± 1.89	-8.6*	6.46± 2.00	5.50± 1.23	-10.0*	0.25
Ca (mg/dl)[a]	10.66± 3.12	11.07± 1.10	8.1*	10.28± 0.66	10.84± 1.54	5.9	0.05
P (mg/dl)[a]	3.97± 0.48	4.63± 0.69	18.4	4.10± 0.56	4.25± 0.78	5.3†	0.57

Data represents mean±SD. n: number of subject. Change (%) represents mean percent change from baseline. a: measured in serum. b: measured in urine. c: Effect sizes are calculated using Cohen's d. OSC: osteocalcin; BALP: bone-specific alkaline phosphatase; CTx: C-telopeptide of collagen cross-links; NTx: N-telopeptide of collagen cross-links; DPYR: deoxypyridinoline; Ca: calcium; P: phosphorus * $P<0.05$ significant differences compared to baseline by paired t-test or Wilcoxon's signed rank test. † $P<0.05$ significant differences between groups by student's t-test or Wilcoxon's rank sum test. # CTx was assessed in 28 subjects in placebo group. 2 of CTx values were excluded as outliers. ## NTx was assessed in 25 subjects in polycan group. 1 of NTx value was excluded as outlier.

3. Safety

Safety assessments were performed on the 60 subjects who received a treatment at least once. A total of 28 subjects experienced 45 AEs during the study, none of which were considered treatment-related. Although there were no significant differences, more patients in the placebo group experienced constipation and diarrhea than in the polycan group (21.4% vs. 5.9% and 14.3% vs. 5.9%, respectively), while nausea occurred more frequently in the polycan group than in the placebo group (11.8% vs. 0%). Other frequent AEs were generally comparable between the 2 groups (Table 3). Two subjects in the polycan group withdrew from the study because of AEs (itching and weakness generalized). There were minor laboratory abnormalities of no clinical significance that were resolved with no specific intervention (data not shown).

Table 3. Summary of most frequent adverse events (AEs)

	Placebo	(%)	Polycan	(%)	P-value[a]
Abdominal discomfort	0	0	1	5.9	1.00
Abdominal pain	1	3.6	0	0.0	1.00
Common cold	3	10.7	3	17.6	1.00
Constipation	6	21.4	1	5.9	0.10
Diarrhea	4	14.3	1	5.9	0.35
Dizziness	0	0	1	5.9	1.00
Drowsiness	1	3.6	0	0.0	1.00
Enteritis	1	3.6	0	0.0	1.00
Fatigue	0	0	1	5.9	1.00
Headache	5	17.9	3	17.6	0.71
Heartburn	2	7.1	0	0.0	0.49
Hot flushes	1	3.6	0	0.0	1.00
Insomnia	0	0	1	5.9	1.00
Itching	1	3.6	1	5.9	1.00
Nausea	0	0	2	11.8	0.49
Nose congestion	0	0	1	5.9	1.00
Skin eruption	1	3.6	0	0.0	1.00
Throat dry	1	3.6	0	0.0	1.00
Tooth ache	1	3.6	0	0.0	1.00
Weakness generalized	0	0	1	5.9	1.00
Total incidence of adverse event	28 (n = 14)		17 (n = 14)		

n: number of subject. % represents incidence rate of each group. a: based on Fisher's exact test

Discussion

In the present study, the effects and safety of polycan were tested in healthy perimenopausal women based on changes in bone biomarkers. Among the outcomes, a marked change in CTx level and a tendency to suppress CTx level increase were seen in the polycan group (Cohen's d, 0.49). NTx and CTx are known to be the most specific and responsive markers among the various bone resorption markers19). These markers start to increase in the perimenopausal period[21]. One study showed that menopause induced increase in bone formation and resorption markers by 37-52% and 79-97%, respectively. NTx and CTx levels remain elevated for 40 years after menopause[22]. In another study, CTx and OSC levels increased steeply to 60% and 35%, respectively, above the premenopausal mean values at menopause[21]. The subjects enrolled in the present study were of perimenopausal age so that their bone biochemical markers probably increased during the study period. Only subjects in the placebo group showed significant increase in CTx levels. These observations tend to support the evidence that polycan led to reduced bone resorption.

In contrast, a significant increase in BALP and decrease in OSC and DPYR were observed in both the groups ($P < 0.05$). However, no significant differences were observed in these markers between the 2 groups. These results are in accordance with an earlier animal study that showed a dose-dependent decrease in OSC level5); however, it is slightly different from an earlier clinical trial that showed decreased DPYR and increased OSC levels after 12 weeks of polycan treatment[7].

Measurement of serum and urinary bone biochemical markers is an easy and non-invasive way to assess quantitative changes in bone turnover[18], and this has proven useful for monitoring the efficacy of both dietary supplements and anti-osteoporotic agents such as bisphosphonates[17]. Bisphosphonates are the representative antiresorptive agents that suppress osteoclast activity and are first-line drugs for postmenopausal osteoporosis. Bone resorption markers decrease rapidly by 50-70% in the telopeptide within the first 12 weeks in response to bisphosphonate treatment[23]. A smaller effect on biochemical markers was observed after treatment with selective estrogen-receptor modulators, such as raloxifene, as compared to that observed with bisphosphonates. Serum CTx, OSC, BALP, and urinary NTx decreased significantly by 31%, 25%, 15%, and 35%, respectively, after the treatment[24]. Both bisphosphonates and estrogen usually have similar reduction effects (40-60%) on the bone resorption and formation markers[17]. The present study showed a reduction in bone biochemical markers such as OSC and DPYR but not BALP and NTx; thus, the polycan seems to suppress bone turnover in a manner similar to bisphosphonates.

Long-term use of bisphosphonates is concerning with respect to the accumulation of microdamage to the organic matrix and possible atypical fracture due to the oversuppression of bone remodeling[25]. Thus, keeping a healthy bone matrix and preserving bone mineral density (BMD) are more important than treatment of already

lowered BMD for the prevention of osteoporosis[16]. Numerous studies have attempted to determine potential agents for the prevention and treatment of osteoporosis. Studies on healthy or osteoporotic postmenopausal women have shown that 30 days of oral magnesium supplementation suppresses bone turnover[12], 2 years of potassium citrate treatment does not have a beneficial effect[13], supplementation with low doses of vitamin K2 may favorably affect bone health[14], and 2 years of soy isoflavone supplementation reduces the loss of whole body BMD without significant changes in serum biochemical markers[15]. As compared to previous supplementation, the polycan can be a helpful agent for preventing osteoporosis. In addition, in our study and in earlier clinical trial, the polycan was generally well tolerated with respect to reported AE or laboratory data despite the minor but uncomfortable side effects such as headache and nausea[7,20].

The present study has some limitations such as a small sample size, short duration of treatment, lack of hard end points such as BMD and bone mineral contents (BMC), and large variability in some bone biomarkers and data from healthy perimenopausal subjects. Further studies with longer periods of treatment and larger sample size are needed to confirm the present observations.

In conclusion, the present study examined the effect and safety of polycan on bone metabolism biomarkers in healthy perimenopausal women. Polycan treatment for 4 weeks resulted in bone turnover suppression as indicated by reduced bone biochemical marker levels. Moreover, the safety profile of polycan was comparable to that of the placebo. These results suggest that polycan has the potential to be a clinically safe and useful supplement for the treatment of osteoporosis.

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