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폴리칸이 중년 여성의 골대사에 미치는 영향: 12주간의 무작위배정, 이중눈가림, 플라세보 대조 연구

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Effects of Polycan on bone Metabolism in healthy Perimenopausal Women: a 12-week Randomized, Double-blind, Placebo-controlled study

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배 경: 골다공증은 골대사의 불균형으로 인해 골 흡수가 골 형성보다 많아져 골밀도가 감소함으로서 발생한다. 골다공증의 이상적인 치료목표는 골형성을 증가시키거나 골소실을 방지하여 골량을 현 상태로 유지하는 것이다. 따라서 향후 발생되는 골소실을 예방하는 것이 골다공증의 원칙적이고 효과적인 치료방법이 될 것이다. 본 연구에서는 흑효모 중 *Aureobasidium pullulans*으로부터 유래한 폴리칸(베타-글루칸)이 중년여성의 골대사에 미치는 영향을 규명하고자 하였다. **연구방법**: 골대사에 대한 폴리칸의 효과를 규명하기 위해 12주간의 무작위배정, 이중눈가림, 플라세보 대조 임상연구를 수행하였다. 총 60명(폴리칸 투여군30명, 플라세보 투여군 30명)의 중년 여성 피험자가 등록되어 이 중 총 58명의 피험자가 최종적으로 12주간의 임상연구를 종료하였다. **결 과**: 폴리칸(150 mg/d) 투여 12주 후, 폴리칸 투여군은 요 중 Deoxypyridinoline (DPD) 농도가 유의적인 감소를 보였다(p=0.014). 혈청 중 Osteocalcin(OSC) 농도는 두 군 모두에서 유의적으로 증가하였으며, bone-specific alkaline posphatase (bALP) 와 collagen type 1 cross-linked C-telopeptide (CTx)는 유의적 변화가 보이지 않았다. 폴리칸은 골밀도(BMD)와 혈청 부갑상선 호르몬(iPTH)에 대해 유의적인 변화를 보이지 않았으나, 24시간 요 중 Ca 배설량은 폴리칸 투여군에서 유의하게 감소되었다(p=0.028). 또한 폴리칸 투여군에서 고밀도지단백 콜레스테롤(HDL-cholesterol) 농도의 증가 경향 및 중성지방(triglyceride)의 유의적인 감소가 보였다. 임상연구 기간 중에 발생한 이상반응은 두 군간에 유의적인 차이를 보이지 않았다. **결 론**: 본 연구에서는 폴리칸이 골대사 및 지질에 대해 일부 개선효과가 있음을 보여주었다. 그러나, 골다공증 예방 측면에서 보다 장기적인 임상연구와 피험자 수를 확대하여 골대사 및 지질대사에 대한 폴리칸의 예방적 효과를 규명할 필요가 있을것으로 사료된다.

□ Key words - Polycan, β-glucan, osteoporosis, bone metabolism, osteocalcin, deoxypyridinoline

INTRODUCTION

Osteoporosis is the most common metabolic bone disorder that results from a disturbance in normal bone remodeling, tilting the balance to bone resorption over formation. This imbalance between bone resorption and bone formation results in bone loss and fractures after mineral flux. [1)]

The reduced production of estrogen following menopause leads to accelerated bone loss in perimenopausal women. Therefore, the prevention of bone loss during menopause is the primary strategy used to reduce the

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osteoporotic risk. For years, hormone therapy (HT) was the treatment of choice because it prevents bone loss and osteoporotic fractures in perimenopausal women.[2,3] However, women have sought alternative therapies for preventing osteoporosis because of the increased risks of breast cancer and endometrial cancer associated with HT.[4-6] Finding effective ways to prevent the outbreak of osteoporosis is therefore an important issue. Natural products with properties that preventing the development of bone loss have been studied to prevent the osteoporosis.[7]

β-glucans are polysaccharides consisting of glucose residue jointed by beta linkage.[8] They are found at a high level in the cell wall of fungi, yeast, oat, barley, bacteria, as well as various mushrooms.[9-11] Some evidence reported that the extract of mushroom (*Pleurotus eryngii)* can prevent the bone loss caused by estrogen deficiency.[12,13] Furthermore, polycan (a purified β-glucan from *Aureobasidium pullulans*) has been reported to have osteoporosis preventing effects.[14] However, no investigation has been conducted on the effect of polycan on bone health in perimenopausal women. Therefore, this study was conducted to investigate the effects of polycan on biochemical markers of bone metabolism in Korean perimenopausal women.

METHODS

Subjects

Perimenopausal women were recruited into the study. They were required to have reduced bone density but no evidence of osteoporosis or osteopenia by Dual-emission X-ray absorptiometry (DEXA) scan ($T \geq -1.0$ in the lumbar spine). Inclusion criteria were (1) women 40 years of age or older; (2) established osteopenia as defined by a standard hip BMD (osteopenia T-score of -1 to –2.5 according to WHO classifications). Exclusion criteria were (1) current treatment with vitamin D3, calcium, estrogen, corticosteroids, bisphosphonate, or other medications for bone diseases within six months before the investigation; (2) any diseases affecting the liver or kidney metabolism; (3) any known diseases affecting the absorption from the gastrointestinal tract; (4) any diseases affecting thyroid gland; (5) known cancer; (6) severe dementia; (7) abnormally high serum calcium; (8) body mass index (BMI) >30 kg/m^2. In particular, corticosteroids can cause BMD deterioration and fracture through the drug-disease interactions in elderly.[15]

The study protocol was approved by the Functional Foods Institutional Review Board of Chonbuk National University Hospital and was conducted at the Clinical Trial Center for Functional Foods. All subjects gave written informed consent prior to participation in the study. All study methods and procedures were conducted in accordance with the ethical standards of the declaration of Helsinki and good clinical practice guidelines.

Study Design

This study was a 12-week, randomized, double-blind, placebo-controlled clinical trial followed by a 2-week screening period. Subjects who met the inclusion and exclusion criteria were randomly assigned to one of the two groups, either the polycan treated (n=30) or placebo (n=30) group. Random allocation was generated by a computerized random-number generator through the block-randomization method of a software program (Excel, Microsoft Office 2007) for sequence generation. Randomization sequence and allocation was concealed to all study subjects, research staff, investigators and pharmacists until completion of the study.

The study consisted of 2 periods; screening phase and double-blind study period of 12 weeks. At baseline, informed consent was obtained, following with screening mammogram and physical examinations that included, clinical blood chemistries and DEXA scans were performed. After the screening, subjects were assigned to receive 150 mg/d polycan or placebo. During the intervention period of 12 weeks, subjects were asked to maintain their usual diet and exercise regimens. They were also asked not to take any other functional foods or dietary supplements. Anthropometric and biochemical markers of bone turnover, vital signs were measured before and after the intervention period for both groups. Compliance was assessed by pill counts when the subjects returned to the

study sites to pick up their next supply of tablets.

Measurements

A basic clinical examination including measurement of body weight, systolic and diastolic blood pressure and heart rate was performed at screening, baseline visit and week 12. All subjects were instructed to fast overnight 10 hours prior to their clinic visits. Fasting blood samples and fasting single void, 24h urine samples were collected at screening, baseline and week 12 during morning appointments. The blood samples were measured for osteocalcin (OSC), intact parathyroid hormone (iPTH) and bone-specific alkaline phosphatase (bALP); urine samples were analyzed for deoxypyridinoline (DPD), collagen type 1 cross-linked C-telopeptide (CTx), creatinine, calcium and phophorus. Blood and urine samples were analyzed by the Seoul Clinical Laboratories using commercially available kits.

Statistical analysis

Statistical analysis was performed using SAS version 9.0 for Windows (SAS Institute, Cary, NC, USA). Data are presented as mean±SD values. The Chi-suare test was performed to determine differences at baseline in frequencies of categorized variables between the groups. A linear mixed-effects model was applied to repeated-measures data for each continuous outcome variable. Fixed effects included treatment group, treatment visit, and interaction between treatment group and visit. When the analysis of variance indicated significant differences among groups, *post hoc* tests (Tukey's tests) were used to separate the differences between groups before and after the 12-week intervention period. A value of $P<0.05$ was considered statistically significant.

RESULTS

Among the 107 subjects screened, 47 subjects had laboratory tests and/or physical examination results in the exclusion criteria category and hence were excluded from the study. The remaining 60 subjects fulfilled the study criteria and were distributed equally into two groups: polycan and placebo.

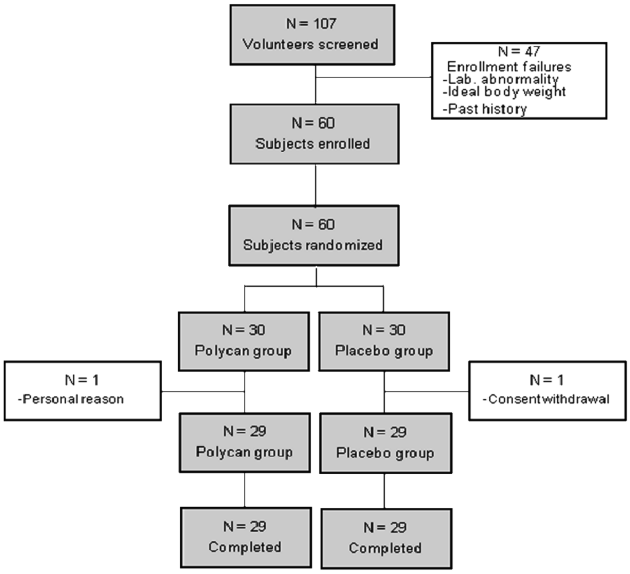

Fig. 1. Flow chart for the study subjects.

One each subjects from both treatment group withdrew from the study because of their personal reasons. At the end of the study, 58 subjects (29 Polycan group, 29 placebo group) were able to finish the study (Fig. 1).

Table 1. Characteristics of the study subjects.

Characteristics	Placebo (n=29)	Polycan (n=29)
Age (y)	52.9±5.8	52.8±6.3
Weight (kg)	60.1±8.1	59.8±9.4
Height (cm)	156.0±5.2	155.5±6.9
BMI (kg/m^2)	24.7±2.9	24.7±2.8
Waist (cm)	88.1±6.0	89.0±7.1
Blood Pressure (mmHg)		
Systolic	118.1±10.9	121.7±8.7
Diastolic	77.7±7.4	81.0±8.1
Pulse rate (bpm)	72.1±9.3	71.5±8.2
T-score		
Lumbar spine	- 0.99±1.38	- 0.84±1.28
DPD (nM/mM crea.)	6.4±1.7	7.1±2.2
CTx (μg/L)	313.6±217.3	343.7±183.2
OSC (ng/mL)	14.2±5.2	16.1±6.8
bALP (U/L)	28.6±9.7	31.8±11.3
iPTH (pg/mL)	31.1±8.7	32.8±10.4
Ca (mg/dL)	201.8±96.7	188.4±92.9

BMI, body mass index; OSC, osteocalcin; DPD, deoxypyridinoline; bALP, bone-specific alkaline phosphatase; CTx, collagen type 1 cross-linked C-telopeptide; iPTH, intact parathyroid hormone; Ca, calcium; IP, phosphorus

The baseline characteristics of all subjects are shown in Table 1. There was no significant difference in age, body mass index, bone metabolic markers or BMD of the lumbar spine between the two groups at baseline. There were tendency toward a higher DPD, OSC, CTx and bALP for subjects randomly assigned to the polycan group, though the difference was not significant.

With polycan treatment, urinary DPD levels decreased significantly on the 4 weeks (p=0.045) and remained low throughout the study (p=0.014). In the polycan treatment group, urinary DPD levels were decreased from 7.1±2.2 at the beginning to 6.2±2.1 nM/mM creatinine (p=0.008) after 12-week of treatment. Placebo decreased the urinary DPD levels from 6.4±1.7 to 5.6±1.5 nM/mM creatinine (p=0.030). Particularly, the change in urinary DPD levels in the polycan group compared with placebo group was greater in subjects with age of fifties (Table 2). Serum OSC levels were increased during the treatment study (p=0.001), but there were not significant difference between both groups. Serum iPTH levels were slightly changed in both groups, but there were not significant difference between both groups (p=0.110). In subanalysis in the subjects with age of fifties, changes of Urinary DPD levels were decreased by 12% in the polycan-treated group and by 10% in the placebo-treated group, respectively (p=0.030) (Fig. 2A). Serum OSC levels were increased in both group but there were not significant difference between both groups (p=0.098) (Fig. 2B).

Urinary CTx levels remained unchanged in both groups throughout the present study. Similarly, the levels of bALP in serum remained unchanged in both treatment groups throughout the present study. Urinary phosphorus levels were significantly decreased in the

Table 2. Changes in biochemical markers of bone metabolism in study subjects

	Age group	Placebo (n=29)		Polycan (n=29)	
		Baseline	After 12 weeks	Baseline	After 12 weeks
DPD (nM/mM Cr.)	Combined	6.4±1.7	5.6±1.5*	7.1±2.2	6.2±2.1*
	41~50 years	5.8±1.9	4.5±0.9	7.2±2.2	6.0±1.8
	51~60 years	6.5±1.7	5.9±1.6*	7.5±2.2	6.7±2.4*†
	61~70 years	7.1±0.6	5.6±0.8	6.2±2.2	5.6±2.0
OSC (ng/mL)	Combined	14.2±5.2	17.0±6.8*	16.1±6.8	19.8±7.7*
	41~50 years	9.6±3.7	10.7±3.4	13.0±7.4	15.6±8.8
	51~60 years	15.4±4.9	18.7±6.5*	18.8±5.3	22.6±5.9*
	61~70 years	19.2±0.8	22.5±1.5	14.6±7.5	19.5±7.7
bALP (U/L)	Combined	28.6±9.7	29.8±9.5	31.8±11.3	31.8±11.7
	41~50 years	24.1±7.8	25.6±8.0	25.8±8.2	25.7±3.1
	51~60 years	28.4±8.6	29.2 ±7.8	35.2±11.5	35.8±12.3
	61~70 years	46.8±6.3	50.1±5.9	32.6±12.9	31.7±11.4
CTx (mg/L)	Combined	314±217	301±157	344±183	341±171
	41~50 years	214±234	148±130	302±250	273±152
	51~60 years	351±217	349±139	396±148	378±156
	61~70 years	292±42	362±86	284±127	359±226
PTH (pg/mL)	Combined	31.1±8.7	33.6±7.5	32.8±10.4	35.0±9.3
	41~50 years	32.3±8.3	36.2±9.2	35.5±12.0	36.9±11.6
	51~60 years	30.8±9.5	32.7±7.2*	30.5±7.5	35.0±6.9*
	61~70 years	29.1±0.2	33.0±2.0	34.0±14.2	32.3±11.5
Ca (mg/dL)	Combined	202±97	184±71	188±93	166±87*
	41~50 years	224±167	186±50	221±109	179±104
	51~60 years	189±60	178±76	184±88	169±86
	61~70 years	259±116	242±90	150±73	137±65

*Significantly different from baseline ($p<0.05$).
†Significantly different from placebo ($p<0.05$).

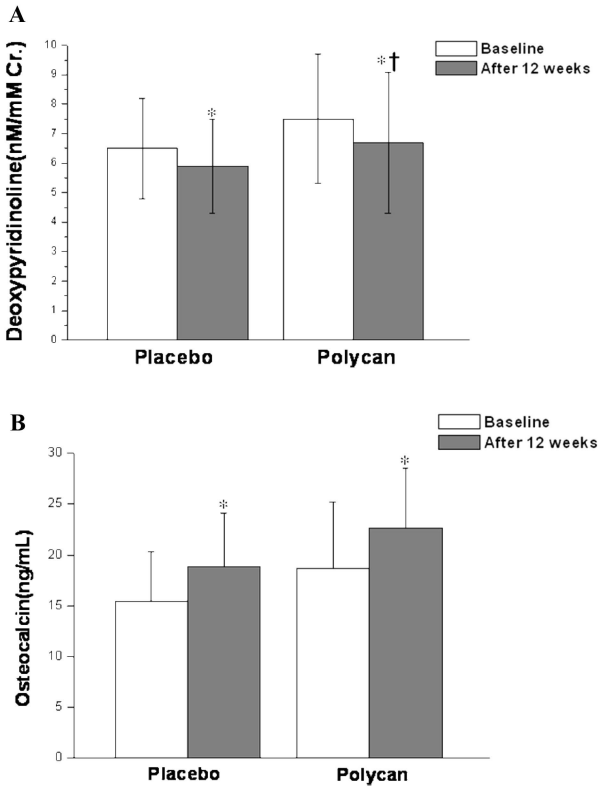

Fig. 2. Effects of polycan treatment on biochemical markers of bone metabolism in subjects with age of fifties. Polycan treatment were associated with a significant decrease in urine levels of DPD (A) and increase in serum levels of OSC(B). *Significantly different from baseline (p<0.05); †Significantly different from placebo (p<0.05)

treatment group. There were significant difference between both groups (p=0.048). In addition, the levels of urinary calcium were significantly decreased in the treatment group. However, there were not significant difference between both groups (p=0.109).

Compared with women taking placebo, polycan treatment group tended to have greater average increases in HDL-cholesterol; however, the comparison between placebo and polycan treatment group did not reach significance (Table 3). Women taking polycan had a significant decrease in triglyceride levels over the course of 12 weeks (p=0.028), while the women in the placebo group had no change in triglyceride levels (p=0.802).

DISCUSSION

This is the first study that documents the efficacy and safety of polycan on bone metabolism in the perimenopausal women. Our results show that the polycan supplementation raises levels of serum OSC, significantly decreases levels of urinary DPD and thereby possibly decreases bone loss. These results are an accordance with the results of Shin *et al.*[14] who have demonstrated that polycan exhibited favorable effects on ovariectomy-induced osteoporosis suggest that polycan may

Table 3. Changes in lipid profiles in study subjects

	Age group	Placebo (n=29)		Polycan (n=29)	
		Baseline	After 12 weeks	Baseline	After 12 weeks
Total cholesterol (mg/dL)	Combined	207.0±41.6	203.7±39.9	198.5±33.0	195.0±33.7
	41~50 years	176.6±28.6	184.7±30.8	185.1±18.6	179.9±23.3
	51~60 years	216.9±39.6	211.8±40.0	195.8±30.6	190.7±27.2
	61~70 years	215.0±72.1	189.5±64.4	224.7±44.0	227.8±42.3
Triglyceride (mg/dL)	Combined	114.7±56.3	112.3±60.1	136.0±68.0	116.7±48.1*
	41~50 years	105.7±80.9	93.0±56.4	138.1±85.2	119.4±52.8
	51~60 years	117.8±50.3	114.3±60.9	116.4±62.1	104.3±48.7
	61~70 years	115.5±27.6	160.5±64.4	178.3±31.3	141.7±33.8*†
HDL- C (mg/dL)	Combined	53.0±12.4	53.9±13.1	47.5±12.2	50.1±12.7
	41~50 years	52.0±17.7	53.3±20.6	47.3±11.2	47.2±10.1
	51~60 years	53.7±11.0	54.8±10.5	50.2±10.9	52.2±8.9
	61~70 years	50.0±11.3	47.5±6.4	42.3±16.1	49.5±22.6
LDL- C (mg/dL)	Combined	131.1±34.9	127.4±35.6	123.4±30.4	121.6±30.1
	41~50 years	103.4±26.1	112.8±26.8	110.2±25.6	108.8±24.5
	51~60 years	139.7±31.2	134.2±34.8	121.9±26.5	117.6±21.7
	61~70 years	141.9±66.3	109.9±70.9	146.7±36.5	150.0±39.9

*Significantly different from baseline (p<0.05).
†Significantly different from placebo (p<0.05).

have a therapeutic use.

In the present study, the levels of two different markers of bone resorption and two markers of bone formation in perimenopausal women were some affected by consumption with polycan for 12 weeks. There is no other published data on the effect of polycan on bone metabolism in human subjects with which to compare our findings. Various biochemical markers are available for the assessment of bone formation and bone resorption. Urinary excretion of DPD have been used as resorption marker for over a decade.[16] Serum OSC and bALP levels have also been used as bone formation markers. In the present study, serum OSC levels were all significantly increased in both groups, while the specific biomarker of bone resorption, urinary DPD levels were all significantly decreased in both groups. In addition, subanalysis showed that the urinary DPD levels were decreased in subjects with age of fifties more than other ages, there were significant difference between both groups (Fig. 2). In terms of the baseline levels of bone formation and resorption markers, such as DPD, CTx, and OSC, the levels were higher in the age of fifties than others, so we thought that bone formation and resorption are generated more in the age of fifties. These results suggest that the polycan is more effective in women in their fifties.

With regard to bone resorption, our results showed decreased urinary DPD levels following polycan treatment. It is suggest that DPD acts as a bridge between collagen fibrils which enter urine with collagen breakdown. As this is a very specific marker for bone resorption, its significant decrease in our study suggests polycan treatment may prevent degradation of collagen the major protein in bone matrix.[17] Moreover, the bone resorption measurements in the present study show a decrease in DPD without any change in CTx. Because CTx is released directly from bone as a result of osteoclastic resorption, whereas DPD is generated in the kidneys as a result of the catabolism of the cross-linking telopeptides,[18] it is possible that the effect of polycan on renal function causes the fall in DPD. Although this study was not designed to investigate the mechanisms of polycan action, a direct effect of polycan on both osteoblasts and osteoclasts is possible. Similarly, changes in cytokine levels may be operative following polycan supplementation.

Our results show that women taking polycan compared to those taking placebo had greater increases in HDL-cholesterol; however, this change was small in magnitude (< 3 mg/dL) and did not reach significance (Table 3). Some clinical reports show that the use of lipids-lowering agents are associated with significant effects on reduction of fracture risk at various sites by increasing bone mineral density (BMD) or affecting other bone metabolic biochemical markers.[19-22] However, it is not yet clear whether these in vitro studies and observed effects on BMD have clinical relevance in preventing bone. In the present study, there was a significant decrease in triglyceride levels in women taking polycan ($p=0.022$). It was reported that barley or oat-derived β-glucan decreased the levels of total cholesterol, LDL-cholesterol and triglyceride.[23-25] To date, most of the human studies investigating the effects of β-glucan have utilized β-glucan derived from oat or barley. However, the present study have been conducted using yeast-derived β-glucan (polycan). Therefore, extended studies are needed to demonstrate the efficacy of polycan on lipid profiles in hyperlipidemic subjects.

There are several limitations to this study. First, although we observed small changes in urinary DPD levels and serum OSC levels in perimenopausal women, it is possible that these changes may be greater at 6 months than what we observed at 3 months. The longer-term trials are needed to evaluate whether polycan supplementation is beneficial for disease prevention in perimenopausal women. Second, despite the random allocation, the baseline levels of OSC, bALP, DPD and CTx were higher in the polycan group; therefore, although we controlled for these baseline differences via the statistical tool, it can still act as a confounding factor. Finally, although it may be partly because of the short-term trial, no group differences in bone mineral density of lumbar spine was observed.

In this study, polycan was generally well tolerated

and appeared to have a good safety profile; i.e., no intergroup differences were noticed with respect to liver function tests or serum creatinine levels.

In conclusion, this is the first study which shows that polycan treatment in perimenopausal women increases serum OSC levels and decreases urinary DPD levels indicating a reduction in bone metabolism. Although long term studies are needed, the treatment with anti-resorptive agents and polycan produced a more favorable bone biomarker profile indicative of healthy bone metabolism in perimenopausal women.

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