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Effects of Polycan on Calcium Bioavailability in Two Different Rat Models of Osteoporosis

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Abstract

This study evaluated the effects of Polycan, a β-glucan produced by *Aureobasidium pullulans* SM-2001, on calcium (Ca) bioavailability in an ovariectomy (OVX) model and a thyroparathyroidectomy (TPTX) model of osteoporosis in the SD rat. Polycan (62.5, 125, and 250 mg/kg body weight) was administered daily with an oral gavage for 4 weeks in both the OVX group (beginning 10 weeks following OVX surgery) and the TPTX group (beginning 4 days following TPTX surgery) while a commercial food product containing 1% milk-borne Ca was available *ad libitum*. After 4 weeks of Polycan administration, all animals were sacrificed and changes in bone mineral density (BMD) in the femur, tibia, and lumbar vertebrae (L_6) were analyzed using dual-energy X-ray absorptiometry. Ca intake was calculated based on the amount of food intake during the 24 h period prior to sacrifice and the Ca balance, absorption, and retention ratios were calculated based on Ca intake, urinary and faecal Ca content, and Ca balance. Polycan treatment resulted in a marked increase in the BMD of the femur, tibia, and L_6 relative to the OVX and TPTX controls with concomitant increases in Ca bioavailability and decreases in secreted Ca. These findings indicate that Polycan may enhance the absorption and bioavailability of Ca and improve Ca balance.

Keywords: β-glucan, Rat, Calcium, Ovariectomy, Thyroparathyroidectomy

Introduction

Osteoporosis is a common metabolic bone disease in modern humans that results from disturbances in normal bone turnover. This disease typically manifests due to an imbalance between bone resorption and bone formation, which can cause bone loss and fracture following mineral flux[1]. Osteoporosis is associated with a significant increase in the frequency of hip fractures, which is a very serious problem that often limits of behavior or threatens the life of a patient[2]. Currently, osteoporosis affects approximately 25 million Americans and it has been estimated that a 50-year-old woman in the United States has about an 11-18% lifetime risk of suffering a hip fracture due to osteoporosis[3]. This number continues to rise in Western countries, and the rate of osteoporosis is predicted to increase by up to 300% in Asia until 2050[4]. The key to postponing or even preventing bone fractures due to osteoporosis later in life may be to not only minimize bone resorption in old age but also maximize peak bone mass during adolescence. A key way to accomplish this would be to increased one's intake of calcium (Ca)[5]. Normally, only about 30% of dietary Ca is absorbed by the body and deposited in bones[6]. Thus, improved Ca absorption would likely have an important mediating influence on the occurrence of osteoporosis and bone fractures.

Various food components, such as β-glucans, may lead to reductions in the digestibility and use of carbohydrates[7]. Feeding rats a diet that includes hard-to-digest or indigestible fermentable carbohydrates such as lactose, oligosaccharides, or resistant starch can accelerate the absorption of both Ca and magnesium (Mg). The primary site of mineral absorption for Ca and Mg was initially believed to be the small intestine but recent reports have indicated that these minerals are also absorbed from the large intestine, particularly the cecum and the colon[8]. The luminal bacteria in the large intestine ferment indigestible carbohydrates, and various studies have suggested that an important correla-

tion exists between an increase in the absorption of minerals and the fermentation of indigestible carbohydrates in the large intestine[4,9].

Several hypotheses regarding the mechanisms underlying these effects have been proposed. One theory suggests that indigestible fibres reach the large intestine in an intact form and are fermented by bacteria in the intestinal lumen, which results in the production of organic acids such as acetate, propionate, and butyrate. Then these acids create a localized drop in pH levels and likely dissolve insoluble Ca salts in the luminal content, accelerating the passive diffusion of minerals via paracellular pathways[4]. Another theory posits that the absorption of short chain fatty acids (SCFAs) is accompanied by the absorption of minerals; SCFAs are products of the colonic bacterial fermentation of dietary fibre, including β-glucans[10,11]. In fact, experimental studies in rodents have demonstrated that fructooligosaccharides, which are a mixture of fermentable and indigestible oligosaccharides, enhance intestinal Ca absorption and increase bone Ca stores via the abovementioned mechanisms[12,13]. Polycan, a β-glucan produced by *Aureobasidium pullulans* SM-2001[14], facilitates the differentiation and activity of osteoblasts in addition to decreasing osteoclast activity *in vitro*[15]. It is suggested here that Polycan may have beneficial effects regarding the absorption and bioavailability of Ca. It has previously been suggested that the synergistic effects of Polycan on osteoblasts and osteoclasts represent a key mechanism by which β-glucans exert anti-osteoporotic activity in mice[15,16].

Thus, the present study evaluated the effects of Polycan on Ca bioavailability in two different rat models of osteoporosis: an oestrogen-deficient ovariectomy (OVX) model and a hypocalcemic and hypoparathyroid thyroparathyroidectomy (TPTX) model. Ca balance, absorption, and retention rates and changes in bone mineral density (BMD) in the femur, tibia, and lumbar vertebrae (L_6) were measured at the end of the Polycan administration period to confirm the influence of this compound on osteoporotic status.

Results

Effects of Polycan in OVX Rats

Changes in Body Weight

There were significant increases ($p<0.01$) for body weight (53.91%) in the OVX group compared to the intact control group. However, no significant changes in body weight were detected in Polycan-treated OVX rats compared to the OVX controls; the weight changes for the Polycan 250, 125, and 62.5 mg/kg groups were −5.65%, −1.69%, and −0.56%, respectively (Table 1).

Changes in BMD

Significant decreases ($p<0.01$) in BMD were detected in the femur, tibia, and L_6 of the OVX rats compared to the intact control rats, but treatment with Polycan significantly and dose-dependently increased ($p<0.01$ or $p<0.05$) the BMD of all three analyzed bones compared to controls (Table 2). The femur BMD of the OVX control group changed by −36.62% compared to the intact control group but the administration of 250, 125, and 62.5 mg/kg Polycan increased BMD by

Table 1. Changes of body weight and gain detected in OVX rats.

Body weight	At initial dosing[1)]	At sacrifice	Gains[2)]
Intact	273.57±15.27	290.00±14.25	16.43±3.41
OVX	352.43±7.85*	377.71±9.71*	25.29±3.59*
Polycan 250	353.43±11.24*	377.29±13.73*	23.86±5.18*
Polycan 125	354.43±16.76*	379.29±17.04*	24.86±2.79*
Polycan 62.5	355.00±11.83*	380.14±13.17*	25.14±6.04*

n=7; (Mean±S.D.), g; [1)]Overnight fasted at 10 weeks after OVX; [2)]Body weight gains during dosing periods (4 weeks); *$p<0.01$ compared to that of intact control by MW test.

Table 2. Changes of bone mineral density detected in OVX rats.

BMD	Right femur	Right tibia	6th lumbar vertebrae
Intact	0.325±0.016	0.287±0.010	0.307±0.014
OVX	0.206±0.019*	0.172±0.010*	0.187±0.014*
Polycan 250	0.277±0.010*,#	0.250±0.008*,#	0.244±0.013*,#
Polycan 125	0.263±0.016*,#	0.242±0.013*,#	0.224±0.012*,#
Polycan 62.5	0.244±0.010*,#	0.216±0.015*,#	0.204±0.011*,##

n=7; (Mean±S.D.), g/cm^2; Bone mineral density was detected using dual-energy x-ray absorptionmetry at sacrifice; *$p<0.01$ compared to that of intact control by MW test; #$p<0.01$ or ##$p<0.05$ compared to that of OVX control by MW test.

34.60%, 27.88%, and 18.52%, respectively, compared to controls. The tibia BMD of the OVX control group changed by −40.03% compared to the intact control group but the administration of 250, 125, and 62.5 mg/kg Polycan increased BMD by 45.11%, 40.30%, and 25.46%, respectively, compared to the OVX controls. The L_6 BMD of the OVX control group changed by −39.02% compared to the intact control group but the administration of 250, 125, and 62.5 mg/kg Polycan increased BMD by 30.21%, 19.68%, and 9.15%, respectively, compared to OVX controls.

Changes in Ca Bioavailability

The sham-operated intact rats and OVX rats ingested similar amounts of food and had similar amounts of Ca intake. However, there was a significant increase ($p < 0.01$) in Ca content in the urine and faeces of the OVX rats compared to the intact control rats such that the Ca balance in the OVX control group significantly decreased ($p < 0.01$). These increased levels were significantly and dose-dependently reversed ($p < 0.01$ or $p < 0.05$) in the Polycan-treated OVX groups, and as a result, the Ca balance in each Polycan group was dramatically enhanced relative to the OVX control group. In addition, the Ca absorption and retention rates were significantly lower ($p < 0.01$) in the OVX control group than in the intact control group. However, these decreases were dose-dependently and significantly reversed ($p < 0.01$ or $p < 0.05$) following treatment with Polycan relative to the OVX control group (Table 3).

The Ca balance of the OVX control group changed by −33.33% compared to the intact control group, and compared to the OVX control group, the administration of 250, 125, and 62.5 mg/kg Polycan altered the Ca balances by 42.65%, 31.58%, and 29.85%, respectively. The Ca absorption rate of the OVX control group changed by −34.83% compared to the intact control group but the administration of 250, 125, and 62.5 mg/kg Polycan increased the Ca absorption rates by 43.29%, 31.35%, and 28.15%, respectively, compared to OVX controls. The Ca retention rate in the OVX control group changed by −35.48% compared to the intact control group but the administration of 250, 125, and 62.5 mg/kg Polycan increased the Ca retention rates by 44.50%, 32.29%, and 28.89%, respectively, compared to OVX controls.

Effects of Polycan in TPTX Rats

Changes in Body Weights

There were significant decreases ($p < 0.01$) in both body weight (−70.83%) in the TPTX group relative to the intact control group. However, no significant changes in these parameters were detected in Polycan-treated TPTX rats compared to the TPTX control group; the weight changes in the Polycan 250, 125, and 62.5 mg/kg groups were 3.36%, 5.88%, and −0.84%, respectively (Table 4).

Changes in BMD

Significant decreases ($p < 0.01$) in BMD were detected in the femur, tibia, and L_6 of the TPTX control group compared to the intact control group but treatment with Polycan significantly and dose-dependently increased ($p < 0.01$) the BMD of all three analyzed bones except

Table 3. Changes of calcium bioavailability in OVX rats.

Calcium bioavailability	Food intake (g/day)	Ca intake (mg/day)	Urinary Ca (mg/day)	Fecal Ca (mg/day)	Ca balance (mg/day)	Ca absorption rate (%)	Ca retention rate (%)
Intact	14.56±0.61	145.57±6.13	0.28±0.03	55.51±3.71	89.78±8.13	61.78±3.51	61.58±3.51
OVX	14.94±0.92	149.43±9.24	0.78±0.13*	88.79±7.89*	59.86±14.48*	40.26±7.71*	39.73±7.78*
Polycan 250	14.81±0.90	148.14±8.97	0.39±0.09**,#	62.36±6.44**,#	85.38±12.91#	57.69±5.80#	57.42±5.84#
Polycan 125	14.87±1.35	148.71±13.51	0.46±0.09*,#	69.49±8.78*,#	78.76±16.64	52.88±7.87**,##	52.57±7.90**,##
Polycan 62.5	15.14±1.29	151.43±12.90	0.57±0.08*,#	73.14±15.44**,##	77.72±17.23	51.59±10.43##	51.21±10.42##

n=7; (Mean±S.D.); *$p < 0.01$ and **$p < 0.05$ compared to that of intact control by MW test; #$p < 0.01$ and ##$p < 0.05$ compared to that of OVX control by MW test.

Table 4. Changes of body weight and gains detected in TPTX rats.

Body weight	At initial dosing[1)]	At sacrifice	Gains[2)]
Intact	182.86±4.17	241.14±8.05	58.29±10.69
TPTX	181.71±7.13	198.71±7.13*	17.00±6.30*
Polycan 250	183.71±4.75	201.29±8.18*	17.57±8.28*
Polycan 125	182.43±3.64	200.43±6.02*	18.00±4.47*
Polycan 62.5	181.43±4.08	198.29±4.54*	16.86±5.37*

n=7; (Mean±S.D.), g; [1)]Overnight fasted at 4 days after TPTX; [2)]Body weight gains during dosing periods (4 weeks); *$p < 0.01$ compared to that of intact control by MW test.

Table 5. Changes of bone mineral density detected in TPTX rats.

BMD	Right femur	Right tibia	6th lumbar vertebrae
Intact	0.302±0.015	0.285±0.013	0.310±0.011
TPTX	0.167±0.023*	0.159±0.017*	0.197±0.007*
Polycan 250	0.215±0.016*,#	0.202±0.009*,#	0.247±0.024*,#
Polycan 125	0.196±0.006*,##	0.194±0.012*,#	0.234±0.010*,#
Polycan 62.5	0.190±0.008*	0.178±0.011*,##	0.220±0.014*,#

n=7; (Mean±S.D.), g/cm^2; Bone mineral density was detected using dual-energy x-ray absorptionmetry at sacrifice; *$p<0.01$ compared to that of intact control by MW test; #$p<0.01$ or ##$p<0.05$ compared to that of TPTX control by MW test.

Table 6. Changes of calcium bioavailability in TPTX rats.

Calcium bioavailability	Food intake (g/day)	Ca intake (mg/day)	Urinary Ca (mg/day)	Fecal Ca (mg/day)	Ca balance (mg/day)	Ca absorption rate (%)	Ca retention rate (%)
Intact	13.71±0.63	137.14±6.26	0.25±0.03	53.92±3.13	82.97±9.19	60.53±4.06	60.35±4.06
TPTX	13.31±0.76	133.14±7.56	0.75±0.10*	95.18±8.28*	37.22±14.48*	28.11±9.41*	27.55±9.44*
Polycan 250	13.59±0.83	135.86±8.25	0.40±0.07*,#	56.29±6.14#	79.17±8.77#	58.50±4.62#	58.21±4.66#
Polycan 125	13.13±0.67	131.29±6.68	0.46±0.06*,#	65.22±7.96*,#	65.60±10.72*,#	50.20±6.65*,#	49.85±6.65*,#
Polycan 62.5	13.23±0.87	132.29±8.69	0.57±0.09*,##	71.33±7.25*,#	60.39±12.62*,##	45.80±7.15*,#	45.38±7.14*,#

n=7; (Mean±S.D.); *$p<0.01$ compared to that of intact control by MW test; #$p<0.01$ and ##$p<0.05$ compared to that of TPTX control by MW test.

for the femur in the Polycan 62.5 mg/kg group, compared to TPTX controls. However, the BMD of the femur in the Polycan 62.5 mg/kg group did exhibit a non-significant increase compared to the controls (Table 5). The femur BMD in the TPTX control group changed by −44.80% compared to the intact control group but the administration of 250, 125, and 62.5 mg/kg Polycan increased BMD by 28.77%, 17.72%, and 13.61%, respectively, compared to TPTX controls. The tibia BMD in the TPTX control group changed by −44.18% compared to the intact control group but the administration of 250, 125, and 62.5 mg/kg Polycan increased BMD by 27.07%, 22.30%, and 12.23%, respectively, compared to TPTX controls. The L_6 BMD in the TPTX control group changed by −36.63% compared to the intact control group but the administration of 250, 125, and 62.5 mg/kg Polycan increased BMD by 25.56%, 18.88%, and 11.62%, respectively, compared to TPTX controls.

Changes in Ca Bioavailability

Sham-operated intact control rats and TPTX rats ingested similar amounts of food and had similar amounts of Ca intake. However, there was a significant increase ($p<0.01$) in the Ca content of the urine and faeces of TPTX rats compared to the sham-operated intact control group such that the Ca balance in TPTX controls significantly decreased ($p<0.01$) compared to the sham-operated intact controls. The increased urinary and faecal Ca content in the TPTX control group were significantly and dose-dependently reversed ($p<0.01$ or $p<0.05$) in the Polycan-treated groups, and as a result, the Ca balance in each Polycan group was significantly enhanced ($p<0.01$) relative to the TPTX control group. In addition, the Ca absorption and retention rates significantly decreased ($p<0.01$) in the TPTX control group compared to the intact control group. However, these decreases were dose-dependently and significantly increased ($p<0.01$ or $p<0.05$) following treatment with Polycan relative to the TPTX control group (Table 6).

The Ca balance of the TPTX control group changed by −55.14% compared to the intact control group but the administration of 250, 125, and 62.5 mg/kg Polycan altered the CA balances by 112.69%, 76.25%, and 62.25%, respectively, compared to TPTX controls. The Ca absorption rate of the TPTX control group changed by −53.56% compared to the intact control group but the administration of 250, 125, and 62.5 mg/kg Polycan increased the Ca absorption rates by 108.11%, 78.58%, and 62.94%, respectively, compared to TPTX controls. The Ca retention rate in the TPTX control group changed by −54.35% compared to the intact control group but the administration of 250, 125, and 62.5 mg/kg Polycan increased the Ca retention rates by 111.30%, 80.95%, and 64.72%, respectively, compared to TPTX controls.

Discussion

Polycan, an ultraviolet light-induced mutant β-glu-

can purified from *Aureobasidium pullulans* SM-2001 (half of the dry material is β-1,3/1,6-glucans), exhibits somewhat different characteristics from other β-glucans derived from various origins[14]. β-glucan is a fibre-type complex sugar (polysaccharide) derived from the cell walls of baker's yeast, oat and barley fibre, and many types of medicinal mushroom that is primarily used to enhance the immune system[17] and lower blood cholesterol levels[18]. A number of studies have demonstrated the favourable effects of various polysaccharides on osteoporosis[4,19]. Moreover, Polycan has been shown to facilitate the differentiation and activity of osteoblasts, decrease osteoclast activities *in vitro*[15], and exert anti-osteoporotic activities *in vivo*[15,16].

In addition, as the synergistic effects on the anti-osteoporotic was observed when administered Polycan with calcium in rat[20] and human[21], respectively, it was estimated that there is a significant promoting effect of the Polycan on calcium bioability.

Actually, the present study found that 4 weeks of continuous orally administered Polycan to OVX and TPTX rats facilitated Ca intake from the intestine, enhanced Ca bioavailability, and increased BMD in the femur, tibia, and L_6. These findings strongly suggest that the anti-osteoporotic effects of Polycan are mediated, at least partially, via improvements in Ca absorption and bioavailability in conjunction with its previously reported synergistic influence on osteoblasts and osteoclasts[15,16].

The oestrogen-deficient OVX model of osteoporosis in Sprague Dawley rats is useful for evaluating osteoporotic drugs because several clinical parameters are clearly deficient within 4-6 weeks of the OVX. The OVX rat model was chosen for the present study because it shares many characteristics with postmenopausal bone loss, which have been summarized in previous studies[22,23], and is recommended by the US Food and Drug Administration as an appropriate test model for the evaluation of skeletal safety and the efficacy of osteoporosis treatments[24]. The hypocalcemic and hypoparathyroid TPTX model of osteoporosis in Sprague Dawley rats is also useful for evaluating osteoporotic drugs, especially the bone-forming effects, because a number of bone formation and mineralization parameters are clearly impaired within 3 days of TPTX surgery due to severe hypocalcaemia and hyperphosphatemia[25]. Hypocalcaemia is primarily responsible for the inhibition of bone formation and mineralization[26] but the decreased bone formation in TPTX rats is likely due to hyperphosphatemia and hypoparathyroidism as well[25].

The increases in body weight that were observed in all OVX groups in the present study are considered to be general signs of oestrogen deficiency[27]. Conversely, decreases in these parameters are considered to be toxicological signs in normal rats but favourable signs in specific disease states, such as obesity. Although β-glucan exerts anti-obesity effects in diet-induced obese rats[28], the present study did not find any significant changes in OVX-induced body weight gain following treatment with Polycan. Previous studies have reported an absence of, or only slightly decreased, changes in body weight within 7 days of TPTX surgery[25] although there is a significant decrease in the body weight of relatively long-term hypocalcemic TPTX rats compared to sham-operated rats[29]. Likewise, the decreased body weight of TPTX animals in the present study were attributed to long-term hypocalcaemia[29]. However, there were no significant and/or meaningful changes in these parameters in any of the TPTX groups treated with Polycan compared to the TPTX control group.

BMD is considered a valuable index for evaluating changes in bone quality. BMD is significantly lower in osteoporotic animals (regardless of the cause of osteoporosis), provides accurate predictable information regarding the efficacy of anti-osteoporotic therapies[30], and contributes to the diagnostic profile of bone quality in human clinical research[31]. The dramatic decrease in the BMD of the femur, tibia, and L_6 in the present study confirmed that both OVX and TPTX induce osteoporosis. Furthermore, the inhibition of this decrease in BMD by Polycan in all three bone types in OVX and TPTX animals supports previous findings demonstrating that Polycan possesses anti-osteoporotic capabilities[15,16].

Treatment with Polycan also increased intestinal Ca absorption and retention rates which were otherwise reduced in OVX rats. Oestrogen is essential for intestinal Ca absorption, and decreases in oestrogen have a direct effect on the skeleton via the perpetuation of Ca deficiency in humans and animals due to diminished Ca absorption and enhanced Ca excretion[32,33]. Recent studies have shown that the oestrogen-induced stimulation of intestinal Ca absorption is oestrogen receptor-dependent and that these receptors are present in rat duodenal cells[34,35]. Because Polycan improves Ca balance in OVX rats, it is of particular interest to further evaluate whether Polycan possesses oestrogen-like capabilities during the stimulation of intestinal Ca absorption in OVX rats.

Treatment with Polycan also increases intestinal Ca absorption and bioavailability which are otherwise reduced in TPTX animals via 1,25 $(OH)_2$ vitamin D synthesis or intestinal resistance to 1,25 $(OH)_2$ vitamin D[36]. The observed effects of Polycan on Ca homeostasis may be mediated by its modulation of Ca-regulating hormones. Parathyroid hormone and 1,25 $(OH)_2$ vitamin D are important hormones that are involved in

the regulation of intestinal Ca absorption[37,38]. These factors are dramatically decreased following TPTX and are the main cause of TPTX-induced osteoporosis[39,40]. The parathyroid glands regulate Ca in the serum and secrete parathyroid hormone if levels become too low, such as when dietary Ca intake is inadequate. The release of parathyroid hormone stimulates the activity of 1-hydroxylase in the kidney, which results in the increased production of calcitriol, the biologically active form of vitamin D3[41]. This increase in calcitriol production restores normal serum levels of Ca in three different ways: 1) by activating the vitamin D-dependent Ca transport system in the small intestine and increasing the absorption of dietary Ca, 2) by increasing the mobilization of Ca from bone into circulation, and 3) by increasing the reabsorption of Ca by the kidneys. Parathyroid hormone is also necessary for increases in bone Ca mobilization and Ca reabsorption by the kidneys[42]. Thus, it is possible that the actions of Polycan may be mediated by changes in parathyroid hormone synthesis or secretion, changes in the biosynthesis of 1,25 $(OH)_2$ vitamin D, and/or increases in the intestinal sensitivity to vitamin D via the modulation of vitamin D receptor expression. Further study is necessary to determine the effects of Polycan on vitamin D metabolism.

β-glucans may lead to reductions in carbohydrate digestibility and use, and feeding rats a diet that includes hard-to-digest or indigestible and fermentable carbohydrates can accelerate the absorption of both Ca and Mg[7]. Luminal bacteria in the large intestine ferment indigestible carbohydrates and several studies have suggested that there is an important correlation between this process and increases in the absorption of minerals[4,9]. Therefore, an increase in the fermentation of indigestible carbohydrates in the large intestine is considered to be a possible mechanism underlying increases in intestinal Ca absorption and bioavailability following treatment with Polycan. It is likely that a combination of these three mechanisms (oestrogen-like capabilities, the modulation of Ca-regulating hormones, and increases in the fermentation of indigestible carbohydrates in the large intestine) is involved with the action of Polycan.

Conclusions

The present findings demonstrate that OVX- and TPTX-induced osteoporosis and decreased Ca bioavailability are effectively inhibited by oral treatment with Polycan. Therefore, it is likely that the anti-osteoporotic effects of Polycan are mediated, at least partially, via improvements in Ca absorption and bioavailability that are supported by complex underlying mechanisms that interact with its synergistic influence on osteoblasts and osteoclasts.

Materials and Methods

Animals

A total of 140 virgin, Sprague-Dawley, specific pathogen-free female rats that were 6 weeks old upon receipt (Charles River Systems, Inc.; Tokyo, Japan) were included in this study. The rats were divided into two primary groups based on different models of osteoporosis, the OVX group or the TPTX group, before being acclimatized for either 7 (OVX group) or 10 (TPTX group) days. All animals were housed in groups of two or three in polycarbonate cages in a room with controlled temperature (20-25°C) and humidity (40-45%) with a 12 : 12 light : dark cycle and ad libitum access to water and commercial feed containing 1% milk-borne Ca (Samyang Foods Co., Ltd.; Wonju, Korea). The OVX and TPTX groups included 56 rats each and 28 sham-operated intact rats were included in a control group. Following recovery from the operation or after a 10-week period of osteoporosis induction, approximately half of the animals exhibited regular changes in body weight and were divided into five groups (n= 7) as follows: non-OVX/TPTX-operated group that received a vehicle dose (sham-operated intact control), OVX/TPTX-operated group that received a vehicle dose (OVX or TPTX control), OVX/TPTX-operated group administered 250 mg/kg oral Polycan (Polycan 250 mg/kg), OVX/TPTX-operated group administered 125 mg/kg oral Polycan (Polycan 125 mg/kg), and OVX/TPTX-operated group administered 62.5 mg/kg oral Polycan (Polycan 62.5 mg/kg).

All animals were cared for according to the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animal Resources, Commission on Life Science, National Research Council, USA.

Test Articles and Dosing

Polycan containing 2.5% β-1,3/1,6-glucan (Glucan Corporation; Busan, Korea) was ordered and stored in a refrigerator at 4°C to protect against light and degeneration. All Polycan doses (250, 125, or 62.5 mg/kg) were diluted in distilled water and administered orally with a gavage once a day for 4 weeks beginning at either 10 weeks (OVX groups) or 4 days (TPTX groups) following surgery. In the sham-operated intact group and the OVX and TPTX control groups, distilled water was orally administered as a control in the identical method and time frame.

OVX and TPTX Surgeries

For the OVX group, rats were anesthetized with ketamine (ICN Biomedicals Inc.; Irvine, California, USA) and xylazine (Wako Pure Chemical Industries Ltd.; Osaka, Japan) before a small midline incision (about 3 cm) was made in the abdomen and the bilateral ovaries were exposed and excised. Rats in the intact control group underwent a sham OVX procedure but not excised. For the TPTX, all rats were anesthetized by same method as the OVX surgery and received a small incision (about 1.5 cm) in the skin and neck muscles and the bilateral thyroparathyroid glands were exposed and excised. Rats in the intact control group underwent a sham TPTX procedure but not excised.

Measurement of Body Weight

In the OVX and TPTX groups, the body weight of each rat was measured at the time of the initial administration of Polycan and at sacrifice. Body weight and weight gains through the 4 weeks of drug administration were calculated.

Measurement of BMD

The right femur, tibia, and L_6 of each rat were dissected out and all soft tissue was eliminated before the BMD (g/cm^2) of the mid-shaft region was measured using dual-energy x-ray absorptiometry (DEXA; Lunar PIXImus; Madison, WI).

Detection of Ca Bioavailability

Prior to sacrifice, each rat was individually housed in a metabolic cage. Samples of urine and faeces were collected throughout the 24 h period before sacrifice using separators; residual food and faeces were weighed. Urine samples were acidified with 2 mL 1 mol/L HCl and stored at -20°C until they were assayed. Ca concentrations in urine were measured using standard colorimetric methods with an automatic analyzer (Hitachi 7080; Hitachi Ltd.; Tokyo, Japan). The collected faeces were dried at 120°C and milled and then the powdered faeces was dissolved in 6 M HCl and warmed to about 80°C for 10 min. Faecal Ca content was measured using a colorimetric method with a kit similar to that used for analyzing urine samples. The Ca intake of each rat was estimated according to its daily food intake and the absorption and retention rates of Ca were calculated using the following equations: Ca absorption rate (%)=(Ca intake−faecal Ca)/Ca intake ×100; Ca balance (mg/day)=Ca intake−(urinary Ca +faecal Ca); Ca retention rate (%)=(Ca balance/Ca intake)×100.

Statistical Analysis

The mean and standard deviations (mean±SD) were calculated with a Mann-Whitney U-Wilcoxon Rank Sum W test (MW test); all statistical analyses were conducted with SPSS for Windows (Release 6.1.3., SPSS Inc.; Chicago, IL, USA). To further analyze any Polycan-induced differences between the intact group and the OVX and TPTX control groups and any differences between the OVX and TPTX control group and the Polycan administration groups, the following equations were used: percent change versus intact control (%)=[(data on OVX or TPTX control−data on intact control)/data on intact control]×100; percentage changes versus OVX or TPTX control (%)=[(data on test groups−data on OVX or TPTX control)/data on OVX or TPTX control]×100.

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References

1. Sakai, A. *et al.* Bone marrow cell development and trabecular bone dynamics after ovariectomy in ddy mice. *Bone*. **23**, 443-451 (1998).
2. Yamaguchi, K. *et al.* Suppressive effect of norzoanthamine hydrochloride on experimental osteoporosis in ovariectomized mice. *Biol. Pharm. Bull.* **22**, 920-924 (1999).
3. Melton, L. J. *et al.* How many women have osteoporosis? *J. Bone. Miner. Res.* **20**, 886-892 (2005).
4. Kruger, M. C. *et al.* The effect of fructooligosaccharide with various degrees of polymerization on calcium bioavailability in growing rat. *Exp. Biol. Med.* **228**, 683-688 (2003).
5. Heaney, R. P. *et al.* Peak bone mass. *Osteoporosis Int.* **11**, 985-1009 (2002).
6. Louie, D. S. in *Calcium and phosphorus in health and disease: Intestinal bioavailability and absorption of calcium* (CRC press, Boca Raton, 1996).
7. Lifschitz, C. H., Grusak, M. A. & Butte, N. F. Carbohydrate digestion in humans from a beta-glucan-enriched barley is reduced. *J. Nutr.* **132**, 2593-2596 (2002).
8. Lopez, H. W. *et al.* Fructooligosaccharides enhance mineral apparent absorption and counteract the deleterious effects of phytic acid on mineral homeostasis in rats. *J. Nutr. Biochem.* **11**, 500-508 (2000).
9. Roberfroid, M. B. Concepts of functional foods: the case of inulin and oligofructose. *J. Nutr. (Suppl.)* **129**, 1398-1401 (1999).
10. Hara, H., Haga, S., Aoyama, Y. & Kiriyama, S. Short-chain fatty acids suppress cholesterol synthesis in rat liver and intestine. *J. Nutr.* **129**, 942-948 (1999).
11. Hara, H., Haga, S., Kasai, T. & Kiriyama, S. Fermen-

tation products of sugar-beet fiber by cecal bacteria lower plasma cholesterol concentration in rats. *J. Nutr.* **128**, 688-693 (1998).
12. Morohashi, T., Ohta, A. & Yamada, S. Dietary fructooligosaccharides prevent a reduction of cortical and trabecular bone following total gastrectomy in rats. *Jpn. J. Pharmacol.* **82**, 54-58 (2000).
13. Scholz-Ahrens, K. E. *et al.* Effects of prebiotics on mineral metabolism. *Am. J. Clin. Nutr. (Suppl.)* **73**, 459-464 (2001).
14. Seo, H. P. *et al.* Production of -1,3/1,6-glucan by *Aureobasidium pullulans* SM-2001. *Korean J. Bitechnol. Bioeng.* **17**, 376-380 (2002).
15. Song, H. B. *et al.* Effect of exopolymers of *Aureobasidium pullulans* on improving osteoporosis induced in ovariectomized mice. *J. Microbiol. Biotechnol.* **16**, 37-45 (2006).
16. Shin, H. D. *et al.* Antiosteoporotic effect of polycan, β-Glucan from *Aureobasidium*, in ovariectomized osteoporotic mice. *Nutrition.* **23**, 853-860 (2007).
17. Czop, J. K. The role of beta-glucan receptors on blood and tissue leukocytes in phagocytosis and metabolic activation. *Pathol. Immunopathol. Res.* **5**, 286-296 (1986).
18. Bell, S. *et al.* Effect of beta-glucan from oats and yeast on serum lipids. *Crit. Rev. Food Sci. Nutr.* **39**, 189-202 (1999).
19. Luegmayr, E. *et al.* Osteoclast formation, survival and morphology are highly dependent on exogenous cholesterol/lipoproteins. *Cell Death Differ. (Suppl.)* **11**, 108-118 (2004).
20. Choi, J.-S. *et al.* Antiosteoporotic effects of Polycan in combination with calcium lactate-gluconate in ovariectomized rats. *Exp. Ther. Med.* **8**, 957-967 (2014).
21. Choi, J.-S. *et al.* Safety and efficacy of polycalcium for improving biomarkers of bone metabolism: a 4-week open-label clinical study. *J. Med. Food.* **16**, 263-267 (2013).
22. Wronski, T. J., Yen, C. F. & Scott, K. S. Estrogen and diphosphonate treatment provide long-term protection against osteopenia in ovariectomized rats. *J. Bone Miner. Res.* **6**, 387-394 (1991).
23. Frost, H. M. & Jee, W. S. On the rat model of human osteopenias and osteoporoses. *Bone Miner.* **18**, 227-236 (1992).
24. US Food and Drug Administration in *FDA Guidelines for preclinical and clinical evaluation of agents for the treatment or prevention of postmenopausal osteoporosis* (Rockville, FDA, USA, 1994).
25. Wergedal, J., Stauffer, M., Baylink, D. & Rich, C. Inhibition of bone matrix formation, mineralization, and resorption in thyroparathyroidectomized rats. *J. Clin. Invest.* **52**, 1052-1058 (1973).
26. Baylink, D., Stauffer, M., Wergedal, J. & Rich, C. Formation, mineralization, and resorption of bone in vitamin D-deficient rats. *J. Clin. Invest.* **49**, 1122-1134 (1970).
27. Lorden, J. F. & Caudle, A. Behavioral and endocrinological effects of single injections of monosodium glutamate in the mouse. *Neurobehav. Toxicol. Teratol.* **8**, 509-519 (1986).
28. Kang, S. A. *et al.* Effect of yeast beta-glucan on serum lipids and leptin levels in the diet-induced obese rats. *Asia Pac. J. Clin. Nutr.* **14**, S114 (2005).
29. Garel, J. M., Gilbert, M. & Besnard, P. Fetal growth and 1,25-dihydroxyvitamin D3 injections into thyroparathyroidectomized pregnant rats. *Reprod. Nutr. Dev.* **21**, 961-968 (1981).
30. Syed, Z. & Khan, A. Bone densitometry: applications and limitations. *J. Obstet. Gynaecol. Can.* **24**, 476-484 (2002).
31. Diez, F. Guidelines for the diagnosis of osteoporosis by densitometric methods. *J. Manipulative. Physiol. Ther.* **25**, 403-415 (2002).
32. Holzherr, M. L. *et al.* Calcium absorption in postmenopausal osteoporosis: benefit of HRT plus calcitriol, but not HRT alone, in both malabsorbers and normal absorbers. *Osteoporos. Int.* **11**, 43-51 (2000).
33. Kalu, D. N. & Orhii, P. B. Calcium absorption and bone loss in ovariectomized rats fed varying levels of dietary calcium. *Calcif. Tissue Int.* **65**, 73-77 (1999).
34. Chen, C., Noland, K. A. & Kalu, D. N. Modulation of intestinal vitamin D receptor by ovariectomy, estrogen and growth hormone. *Mech. Ageing Dev.* **99**, 109-122 (1997).
35. Ten Bolscher, M. *et al.* Estrogen regulation of intestinal calcium absorption in the intact and ovariectomized adult rat. *J. Bone. Miner. Res.* **14**, 1197-1202 (1999).
36. Barrett, E. J. & Barrett, P. in *The parathyroid glands and vitamin D* (eds Boron, W. F. & Boulpaep, E. L.) 1094-1110 (Elsevier Saunders, Philadelphia, PA, USA, 2003).
37. Arden, N. K. *et al.* Size at birth, adult intestinal calcium absorption and 1,25 $(OH)_2$ vitamin D. *QJM.* **95**, 15-21 (2002).
38. Mitamura, R., Hara, H. & Aoyama, Y. Ingestion of raffinose promotes calcium absorption in the large intestine of rats. *Biosci. Biotechnol. Biochem.* **68**, 384-389 (2004).
39. Bonjour, J. P., Fleisch, H. & Trechsel, U. Calcium absorption in diphosphonate-treated rats: effect of parathyroid function, dietary calcium and phosphorus. *J. Physiol.* **264**, 125-139 (1977).
40. Jaeger, P., Jones, W., Clemens, T. L. & Hayslett, J. P. Evidence that calcitonin stimulates 1,25-dihydroxyvitamin D production and intestinal absorption of calcium in vivo. *J. Clin. Invest.* **78**, 456-461 (1986).
41. Murayama, A. *et al.* Positive and negative regulations of the renal 25-hydroxyvitamin D_3 1α-hydroxylase gene by parathyroid hormone, calcitonin, and 1α,25 $(OH)_2D_3$ in intact animals. *Endocrinology* **140**, 2224-2231 (1999).
42. Zhang, Y. *et al.* Effects of *Fructus Ligustri Lucidi* extract on bone turnover and calcium balance in ovariectomized rats. *Biol. Pharm. Bull.* **29**, 291-296 (2006).