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Effect of Exopolymers of *Aureobasidium pullulans* on Improving Osteoporosis Induced in Ovariectomized Mice

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Abstract Treatment with exopolymers of *Aureobasidium pullulans* SM-2001 containing β-1,3/1,6-glucan inhibited osteoclastogenesis of bone marrow stem cells in a co-culture system with calvariae osteoblastic cells. In addition, the treatment increased mineral deposition in osteoblastic cells. These two observations prompted us to evaluate whether the exopolymers could be used as an anti-osteoporotic agent, and efficacy of the exopolymers to prevent bone loss was compared with alendronate, a bisphosphonate, in ovariectomized mice prone to osteoporosis. Administration of the exopolymers to the ovariectomized mice resulted in improved effects on femur weight and histomorphometric changes of femur such as trabecular bone volume (TBV), trabecular bone thickness (TBT), and cortical bone thickness (CBT). In conclusion, the exopolymers treatment inhibited bone loss from osteoporosis induced by ovariectomy, and the effect was comparable to alendronate administration.

Key words: β-Glucans, exopolymers, *Aureobasidium pullulans*, osteoporosis, osteoclasts, osteoblasts

Osteoporosis is a metabolic bone disease that results from a disturbance in the normal bone remodeling, thus tilting the balance to bone resorption over formation, and resulting in bone loss and fractures after mineral flux [10]. The bone remodeling relies on the relative activities of bone formation by osteoblasts and bone resorption by osteoclasts [20]. The frequency of fractures is significantly increased in osteoporosis, and hip fractures in senile patients are a very serious problem because it often limits the patient's life [14]. In particular, many postmenopausal women suffer from osteoporosis caused by estrogen deficiency.

Estrogen-deficient, ovariectomized mice as an osteoporotic animal model have been used for evaluation of osteoporotic drugs, because several parameters of the mice after ovariectomy clearly reflect the osteoporotic symptoms of postmenopausal women. The effect of a drug would be based on observations of bone weight, histomorphometric changes of trabecular bone, and thickness of cortical bone in this model [10]. Alendronate, a nitrogen-containing bisphosphonate, is a potent inhibitor of bone resorption used for the treatment and prevention of osteoporosis, and its anti-osteoporotic effect on ovariectomized animals and postmenopausal women has well been documented [9, 12, 14, 16].

β-glucan has been known to be a powerful immune stimulant, an antagonist to both benign and malignant tumors, to lower cholesterol and triglycerides, normalize blood sugar levels, and heal and rejuvenate the skin [6, 8, 24]. As an immune stimulant, β-glucan has a positive action not only on the macrophage, but also on B lymphocytes, natural killer cells, and suppressor T cells. In addition, β-glucan is an effective antioxidant and free-radical scavenger [21]. β-glucans from various sources are on market and are known to be safe, non-toxic, and orally effective dietary supplements for those who wish to enhance their immune system and lower cholesterol levels. The intake of meals

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containing a modest amount of β-glucan for 4 weeks improved glucose levels and induced low plasma cholesterol levels in men with type 2 diabetes [7]. However, the underlying mechanisms of these benefits have not yet been clearly elucidated.

Exopolymers of *Aureobasidium pullulans* SM-2001, a UV induced mutant, have previously been shown to be mixtures of various polymers, including β-glucans [17]. During our efforts to investigate the cellular effects of the exopolymers, we found a very strong inhibitory activity of the exopolymers on the osteoclast differentiation. In the present study, we tested whether the exopolymers have an anti-osteoporotic effect on the ovariectomized mice by comparing with alendronate, a well-documented anti-osteoporotic drug.

MATERIALS AND METHODS

Materials

Reagents used in cell culture were obtained from Invitrogen (Carlsbad, CA, U.S.A.). Exopolymers of *Aureobasidium pullulans* SM-2001 were supplied by Glucan Co. (S. Korea). Beta-glucans of various sources were obtained from Chi-Hyun Song of Daegu University of S. Korea. Unless otherwise specified, most reagents were purchased from Sigma (St. Louise, MO, U.S.A.).

Osteoblastic Cell Cultures and Assessment of Mineral Deposition with Alizarin Red-S Staining

Primary osteoblasts were isolated from calvariae of newborn ddY mice (2–5 days old) as previously described [13]. Briefly, calvariae were sequentially digested for 30 min in α-MEM (Invitrogen) containing 0.1% collagenase and 0.2% dispase. This procedure was repeated to maximize the yield of osteoblastic cells. The cells isolated were combined, expanded for 2 days in α-MEM with 10% fetal calf serum (FCS) and replated at a density of 10^4 cells/cm^2. The mineral deposition assay was performed as described previously [18]. At confluency, medium was supplemented with 5 mM β-glycerophosphate and 100 μg/ml ascorbic acid, and each material tested was added to the cultures and maintained until the end of the experiment. Alizarin Red-S (AR-S), a dye that binds selectively to calcium salts, was used for the estimation of calcium mineral deposition. Cultures were rinsed with PBS, and followed by fixation (ice-cold 70% ethanol, 1 h). Cultures were rinsed with Milli-Q water (Millipore Corp., Billerica, MA, U.S.A.) and stained for 10 min with 40 mM AR-S (pH 4.2) at room temperature. Cultures were then rinsed five times with Milli-Q water, followed by a 15-min additional wash with PBS to reduce nonspecific AR-S stain. Cultures were washed four times with PBS before the addition of 0.1 ml of 10% (wt/vol) cetylpyridinium chloride (CPC) for 10 min to release the calcium-bound Alizarin Red-S. Aliquots of these AR-S extracts were diluted 10-fold in 10% CPC solution, and the AR-S concentration was determined by absorbance measurement at 562 nm in a microplate reader.

Co-Culture Procedures

Primary mouse osteoblastic cells were obtained from calvariae of newborn mice as described above. Bone marrow cells were isolated from the tibiae and femora of 6 to 8-week-old mice and cultured in a humidified chamber (5% CO_2 in air) at 37°C. After 24 h of culture, the non-adherent cells were collected and centrifuged on a histopaque gradient to obtain the bone marrow macrophage (BMM) cells, and the cells were then washed twice with PBS. This procedure removes stromal cells in the bone marrow cell population. For co-culture experiments, BMM cells (4×10^4 cells) and osteoblastic cells (4×10^3 cells) were mixed and cultured in α-MEM containing 10% fetal calf serum, 10 nM 1-α,25$(OH)_2D_3$, and 1 μM prostaglandin E_2 on a 96-well plate (Corning Inc., Corning, NY, U.S.A.) in the presence or absence of compounds to be tested. Half of the medium was replaced every 2 days [13].

Animals and Husbandry

Twenty-five female ddY mice (6-wk old upon receipt, SLC, Japan) were used after acclimatization for 7 days. Animals were allocated to polycarbonate cages in a temperature (20°C) and humidity (35%) controlled room with a 12-h/12-h light/dark cycle. Food (Samyang, Korea) and deionized water was freely supplied. The 25 mice were randomly allocated to five groups. For therapeutic study, treatment was initiated 4 weeks after ovariectomy, and each sample was then administered for 4 weeks. All procedures were undertaken in accordance with the National Institute of Health and Nutrition Guidelines for the Care and Use of Laboratory Animals.

Operation

One group of mice was subjected to sham operation, and the remaining four groups were ovariectomized as described below. Operations were performed by either bilateral ovariectomy using a dorsal approach or a standard sham operation under general anesthesia induced under ketamine hydrochloride (ICN Biochemicals Inc., U.S.A.) and xylazine hydrochloride (Wako Pure Chemical Industries Ltd., Japan).

Preparations and Administration of Drugs

All test materials were stored in a refrigerator (−10°C) to protect from light and moisture. The test materials were administered at a dosage of 10 ml/kg body weight by oral gavage using corn oil as a vehicle. Exopolymers of *A. pullulans* SM-2001 (hereinafter 'GLU') were orally administered, and alendronate (Merck, Whitestation, NJ, U.S.A.) was orally administered at 10 mg/kg body weight

Table 1. Experimental design of this study.

Group		Dose	Group ID	Vehicle	Route	Schedule
Sham	Sham	10 ml/kg	Sham	D.W.[a]	oral	Once a day for 4 weeks
Ovariectomy	Control	10 ml/kg	Control	D.W.	oral	
	Alendronate	10 mg/kg	Alen	D.W.	oral	
	GLU[b]	100 mg/kg	GLU	corn oil	oral	

*All test articles and vehicle were dosed by gastric gavage for 4 weeks.
[a]D.W., distilled water.
[b]GLU, the exopolymers of *A. pullulans* SM-2001. Operations were conducted under ketamine and xylazine anesthesia.

in distilled water. The dose and schedule of these drugs administered are summarized in Table 1.

Histological Procedures

We evaluated apparent histological features for quantifying pathological changes in femurs. The left side of the femur of each mouse was separated and fixed in 10% neutral buffered formalin (NBF), and then decalcified in decalcifying solution (24.4% formic acid and 0.5 N sodium hydroxide) for 5 days (mixed decalcifying solution was replaced once a day for 3 days). The decalcified bones were cleaved into two sections, embedded in paraffin, sectioned (4 μm) with a microtome, and visualized with hematoxylin-eosin stain.

Criteria Index

Body weight, weight of femur bones, and histomorphometric changes of left-side femur, including trabecular bone volume (TBV) and cortical bore thickness (CBT), were measured. In addition, histological profiles of trochlea epiphyseal regions of the left femur were also examined as previously described [15].

Body Weight. Changes of body weight were measured at operation, 4 weeks after operation, at dosing, and once a week during experimental periods until sacrifice. At dosing and sacrifice day, experimental animals were fasted overnight. In addition, body weight gains during the induction period (Gains I) and dosing (Gains II) were calculated as follow:

Gains I=(Body weight at 4 weeks after operation
−Body weight at operation)
Gains II=(Body weight at sacrifice
−Body weight at dosing)

Bone Weights. At sacrifice, the weight of both sides of femurs was measured and, to reduce deviation due to individual body weight differences, the relative weight (%) was calculated using body weight at sacrifice and absolute weight, as per the following equation:

Relative bone weight=[(Absolute bone weight
/Body weight at sacrifice)×100]

Measurement of Trabecular Bone Volume (TBV). TBV was determined by an automated image analysis (analySIS Image Processing; SIS, Germany) under microscopy (Zeiss, Germany) (×100) in the uniform area of trochlea epiphyseal regions of the right femur (growth plate regions were excluded). TBV was scored as percentage (%) levels.

Measurement of Histomorphometric Index of Trabecular Bone. Trabecular bone thickness (TBT) and trabecular bone length (TBL) were determined by an automated image analysis (analySIS Image Processing; SIS, Germany) under microscopy (Zeiss, Germany) in the trochlea epiphyseal regions of the left femur. TBT and TBL were scored in μm and TBN as number/whole cross-sectional part of epiphyseal region. The most developed of trochlea epiphyseal regions were selected for TBT and TBL scoring.

Cortical Bone Thickness (CBT). CBT was detected in trochlea neck regions of the femur, and they were calculated using an automated image analyzer (analySIS Image Processing; SIS, Germany) under (×100) microscopy (Zeiss, Germany) at prepared histological samples. The thickness was scored in μm.

Osteoclast Cell Number (OCN). Serial 5-mm sections were prepared and stained for tartrate-resistant acid phosphatase (TRAP)-positive cells as described above. OCN was counted as seen under 200 μm^2 of trochlea epiphyseal regions using an automated image analyzer (analySIS Image Processing; SIS, Germany) under microscopy (Zeiss, Germany) at prepared histological samples. Numbers were calculated as number/200 μm^2 of epiphyseal regions.

Statistical Analyses

All data were calculated as mean±S.D. Statistical analyses was conducted using the Mann-Whitney U-Wilcoxon Rank Sum W test (M-W test) with SPSS for Windows (Release 6.1.3, SPSS Inc., U.S.A.).

Results

Effect of the Exopolymers on Mineral Deposition

Our preliminary observation showed that treatment with exopolymers of *A. pullulans* SM-2001 marginally increased mineral deposition in osteoblastic cells of calvariae. To test whether this property is a feature of β-glucans, we treated osteoblastic cells with β-glucans of various sources, and

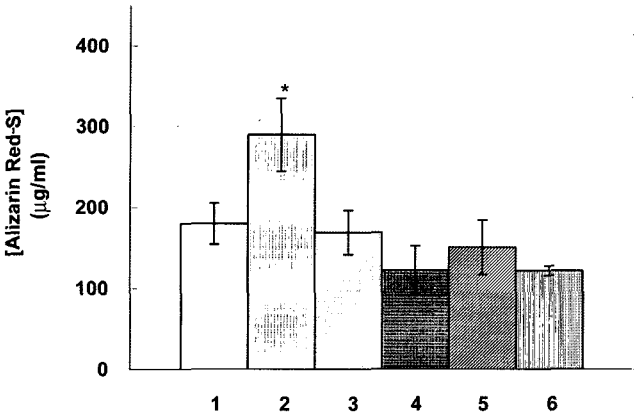

Fig. 1. Effect of β-glucans of various sources on mineral deposition in osteoblastic cells.
The mineral deposition was estimated by AR-S content released from the cells. The detailed procedure is described in Materials and Methods. 1) Control, 2) the exopolymers of *A. pullulans* SM-201, 3) brewer yeast, 4) pustulan, 5) *Ganoderma*, 6) Schizophyllum β-glucans at 10 μg/ml. Data represent mean±S.D. from triplicate experiments.

mineral deposition was evaluated by the release of Ca^{2+}-alizarin Red-S in cetylpyridinium chloride (CPC) solution from the cells. As shown in Fig. 1, besides the exopolymers of *A. pullulans* SM-2001, β-glucans rather decreased mineral deposition in the osteoblastic cells, implicating that the increase of mineral deposition with the exopolymers of *A. pullulans* SM-2001 could be due to other ingredient(s) in the exopolymers rather than β-glucans, or due to differences in structure or composition of β-glucans of *A. pullulans* SM-2001.

Effect of the Exopolymers on Osteoclast Differentiation in Co-Cultures

Osteoclast formation in co-cultures of mouse osteoblastic and bone marrow cells was evaluated in the presence of the exopolymers. The number of TRAP-positive osteoclasts [22] in the presence of 0.5 μg/ml of the exopolymers was scored as 90% of that of vehicle only treatment (Fig. 2). With 5 μg/ml of the exopolymers, a few countable TRAP-positive osteoclasts were observed, and no osteoclast was formed at 10 μg/ml of the exopolymers, (Fig. 2). These observations demonstrated that the exopolymers of *A. pullulans* SM-2001 severely inhibited osteoclast differentiation in the co-cultures of osteoblastic and bone marrow cells.

Changes of Body Weights

Changes of body weight after ovariectomy and drug administration are summarized in Table 2. Body weight gains (Gains I) of all ovariectomized groups were apparently

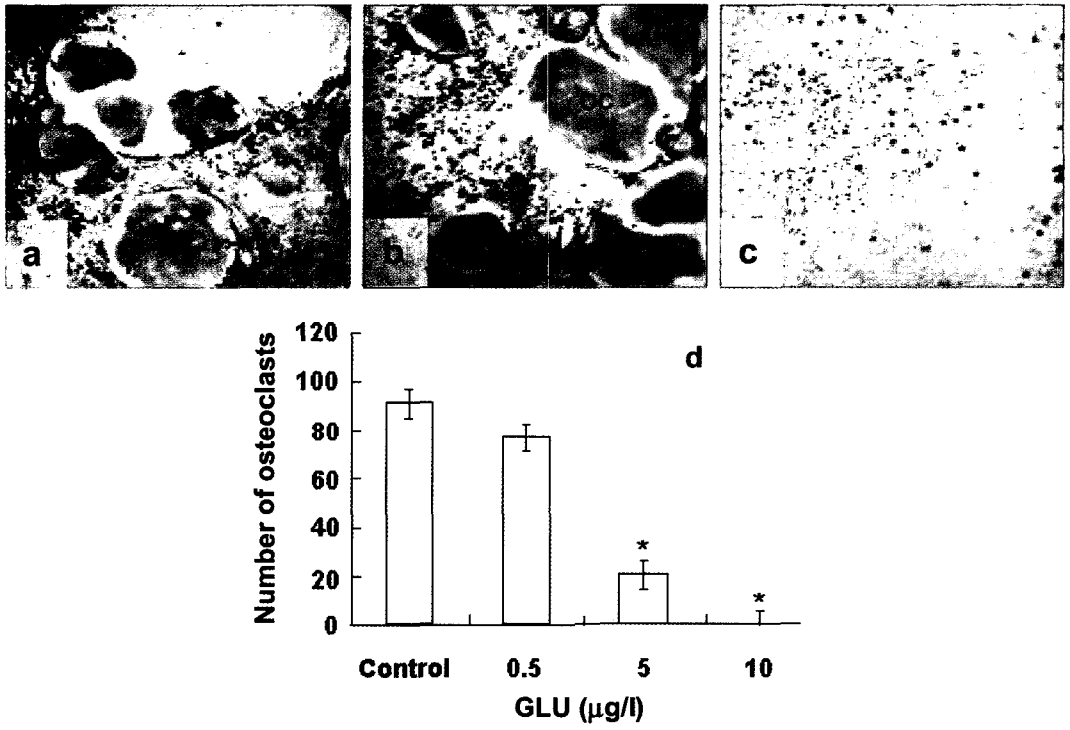

Fig. 2. Effect of the exopolymers of *A. pullulans* SM-2001 on the osteoclasts differentiation.
Osteoclast formation in co-cultures of mouse osteoblastic cells and bone marrow cells was evaluated in the presence of increasing concentrations of the exopolymers. The detailed experimental condition is described in Materials and Methods. (a) Vehicle only, (b) exopolymers 0.5 μg/ml, (c) exopolymers 10 mg/ml, (d) the number of osteoclasts observed under microscope (50×). Tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts were scored as previously described [22]. Data are expressed as mean±S.D. of triplicate cultures and are representative of three similar experiments. *P<0.01.

Table 2. Changes of body weight after ovariectomy and drug administration.

Group	At OVX[1)]	4 weeks after operation	At dosing[a]	Weeks after dosing			At sacrifice[a]
				1 week	2 weeks	4 weeks	
Sham	24.80±1.47	30.90±3.72	30.32±1.99	33.34±2.07	33.86±2.19	34.36±2.19	32.04±1.75
Control	25.08±0.85	36.62±3.33**	34.42±2.88**	36.80±3.70	36.88±3.97	37.44±4.71	35.66±4.69
Alen	25.30±1.17	33.68±2.61	31.56±1.81	34.46±2.76	32.86±4.20	37.02±3.93	36.70±3.73
GLU	25.08±1.12	34.80±1.15**	32.84±0.71**	35.08±1.37	35.30±1.52	36.54±1.37**	34.72±1.20**

Gains	Sham	Control	Alen	GLU
Gains I	6.10±5.00	11.54±3.36**	8.38±2.10	9.72±1.06
Gains II	1.72±1.30	1.24±2.34	3.26±2.73	1.88±1.25

Gains I=(Body weight at 4 weeks after operation−Body weight at operation).
Gains II=(Body weight at sacrifice−Body weight at dosing).
n=5; Mean±S.D., Group ID is listed in Table 1. OVX, ovariectomy.
[a]Body weights were scored at overnight fasted condition.
*$p<0.01$ compared with that of the sham group by M-W test; **$p<0.05$ compared with that of the sham group by M-W test.

larger with the sham group, but the gain of body weight (Gains I) in the control group was statistically significant: Body weight increase is considered a general sign of an estrogen-deficient status of animals [11]. In GLU-dosed animals, the treatment increased body weight, compared with other ovariectomized groups. The oral gavage with either alendronate or GLU did not influence food intake and was not associated with overt signs of toxicity, which might contribute to the bone remodeling process.

Trabecular Bone Volume

TBV changes after ovariectomy and drug administration are summarized in Table 3. A significant ($p<0.01$) decrease of TBV in all experimental groups, compared with that of the sham group, was observed after ovariectomy. TBV in the alendronate and GLU administered groups was significantly ($p<0.01$) increased, compared with that of the control group. The increase of TBV in the alendronate-treated group, compared with control group, was twice as much as those with the GLU-administered groups. Nevertheless, GLU showed a clearly favorable effect on inhibiting the decrease of trabecular bone volume induced by ovariectomy.

Trabecular Bone Thickness

A significant ($p<0.01$) decrease of TBT in the ovariectomized control, compared with that of the sham groups, was detected (Table 3). A significant ($p<0.01$ or $p<0.05$) increase of TBT was observed in all the tested groups including GLU compared with that of the control. GLU administration significantly improved the decrease of trabecular bone thickness induced by ovariectomy, and the efficacy was comparable to that of alendronate.

Trabecular Bone Length

The change of TBL after ovariectomy and drug administration is summarized in Table 3. A significant ($p<0.01$) decrease of TBL in the ovariectomized control was detected, compared with that of the sham group. TBL in all the dosing groups

Table 3. Histomorphometric changes of trabecular and cortical bones at epiphyseal regions of left femur with osteoclast cell numbers.

Volume changes	Sham	Control	Alen	GLU
TBV[a]	47.31±5.95	20.23±1.72*	35.13±4.28*,#	26.26±3.42*,#
Trabecular bone change	Sham	Control	Alen	GLU
TBT[b]	111.00±14.87	66.40±11.70*	93.60±9.56##	90.00±9.19**,##
TBL[c]	1,476.00±233.08	524.40±176.78*	759.40±89.17*,##	985.40±104.08*,#
Cortical bone changes	Sham	Control	Alen	GLU
CBT[d]	271.40±36.98	99.20±20.46*	102.50±22.29*	139.60±12.22*,##
Osteoclast cell changes	Sham	Control	Alen	GLU
OCN[e]	3.60±1.14	13.20±1.48*	15.00±3.00*	11.60±1.95*

n=5; Mean±S.D.; Group ID is listed in Table 1.
[a]TBV, trabecular bone volume (%); [b]TBT, trabecular bone thickness (μm); [c]TBL, trabecular bone length (μm); [d]CBT, cortical bone thickness (μm); [e]OCN, osteoclast cell number (number/200 μm^2); All histomorphometric indices were scored using an automated image analyzer.
*$p<0.01$ compared with that of the sham group by M-W test; **$p<0.05$ compared with that of the sham group by M-W test; [#]$p<0.01$ compared with that of the control by M-W test; [##]$p<0.05$ compared with that of the control by M-W test.

was significantly ($p<0.01$ or $p<0.05$) increased, compared with that of the control. In particular, this histomorphometric index of GLU-administered mice was better than that of the alendronate-treated group.

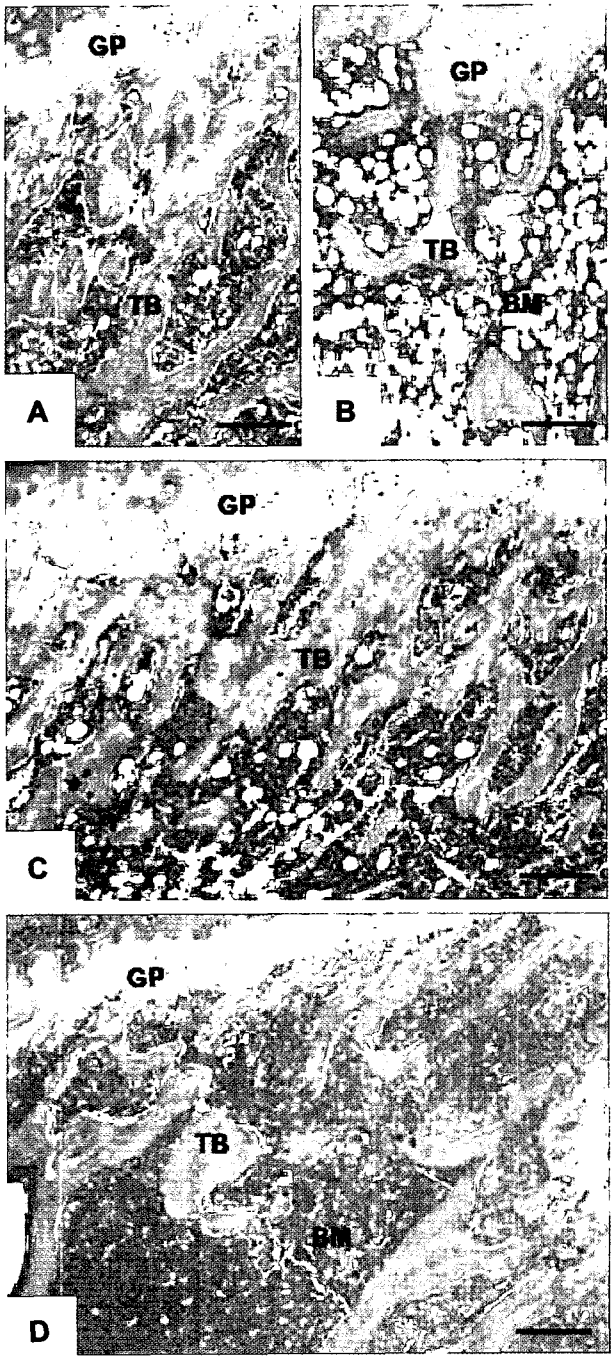

Fig. 3. Histological profiles at trochlear epiphyseal regions of the right femur.
Sample preparations are described in Materials and Methods. A. Sham operated; B. control (vehicle only); C. alendronate administrated; D. GLU adminstered. All micrographs were visualized by staining with hematoxylin-eosin (50×). Trabecular bone (TB), bone marrow (BM), and growth plate (GP) were differentially stained. The bar indicates 200 μm.

Cortical Bone Thickness

A significant ($p<0.01$) decrease of CBT in all the ovariectomized groups was detected compared with that of the sham group. However, CBT in the GLU-dosing groups was significantly ($p<0.01$ or $p<0.05$) increased, compared with that of the control. Interestingly, the CBT of GLU-dosed mice was substantially higher than that of the alendronate-dosed group. In the alendronate-dosed group, values similar to those of the control were obtained.

Osteoclast Cell Numbers

A significant ($p<0.01$) increase of OCN in all the ovariectomized groups was detected, compared with that of the sham-operated animals. The OCN in all the test groups was similar to that of the control. GLU administration did not inhibit the increase of osteoclast cell number induced by ovariectomy and this result was similar to that of the alendronate-treated group.

Histological Profiles

The histological profiles of the epiphyseal region of the right femur are illustrated in Fig. 3. Relatively well-developed trabecular bone was observed in the sham-operated animals (Fig. 3A). However, in the case of the control, many pores in trabecular bone were detected (Fig. 3B). In the alendronate and GLU-dosed groups, more numerous extended trabecular bones were demonstrated than those of the control, but their width and number were somewhat lower than those of the sham group (Figs. 1C and 1D).

The histological profiles of the cortical bone of the right femur are shown in Fig. 4. Well-developed osteomembrane and compact bones were demonstrated in the sham group (Fig. 4A). However, a narrowing of the total width of the cortical bone was detected in the control. In addition, large holes containing cells like bone marrow cells were also demonstrated in this group (Fig. 4B). Similar or slightly increased width of cortical bone was observed in the alendronate-treated group, compared with that of the control. The width and compactness of cortical bones in the GLU group were substantially increased compared with those of control (Fig. 4D). In summary, the exopolymers had favorable effect to ameliorate the histological changes of the trabecular bone at trochlea epiphyseal regions and of the cortical bone at trochlea neck portions of the right femur induced by ovariectomy.

DISCUSSION

When we compared the effect of the exopolymers of *A. pullulans* SM2001 on osteoclast differentiation with equivalent concentrations of β-glucans from other sources, such as yeast, pustulan, or *Ganoderma* β-glucans in a co-culture system, the inhibitory effect of the exopolymers

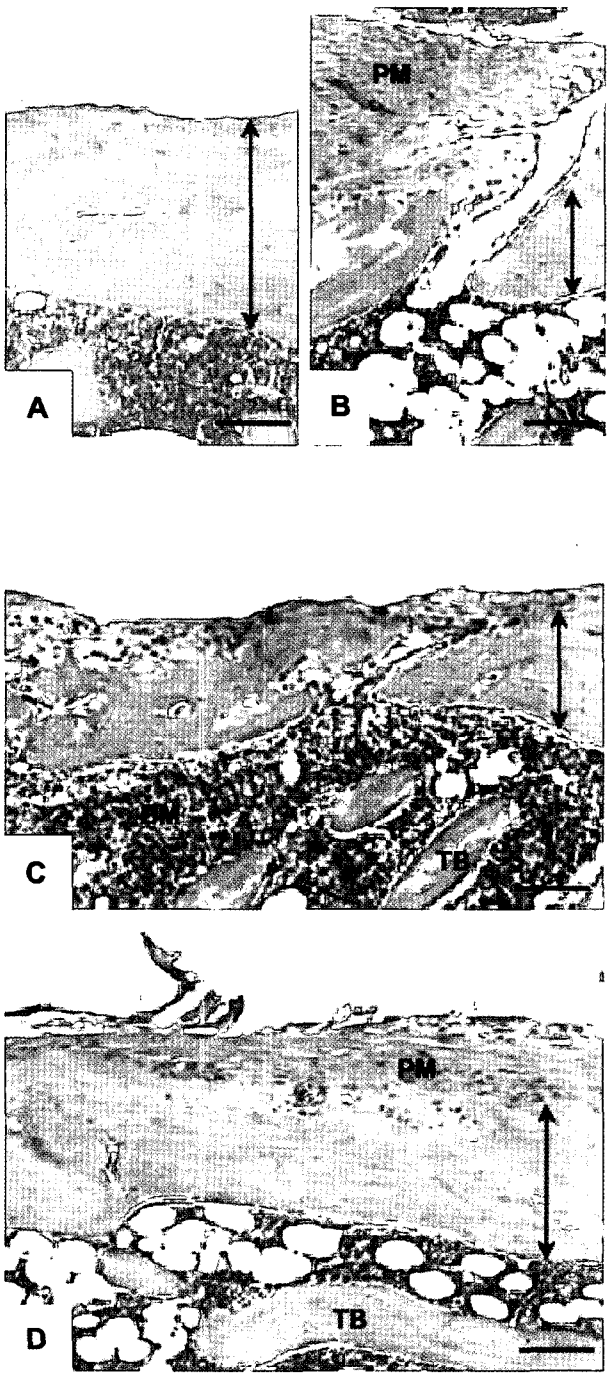

Fig. 4. Histological profiles of cortical regions of the right femur.

The sample preparations are described in Materials and Methods. A. Sham group; B. control group; C. alendronate administered; D. GLU administered. All micrographs were visualized by hematoxylin-eosin staining (100×). Arrows with two heads indicate the width of cortical bone. Trabecular bone (TB) and bone marrow (BM) are differentially stained and periosteum (PM) is noticed as the outmost layer of bone. The bar indicates 100 mm.

was the highest among the materials tested. These data indicate that the inhibitory effect of the exopolymers on the osteoclast differentiation may be due to not only β-glucan, but also other elements in the exopolymers. These observations suggest that β-glucans have an intrinsic property of inhibiting osteoclast formation in co-culture system. It might be of interest to test whether the inhibitory effect of β-glucans could be extended to other cell culture systems, such as mouse RAW 264.7 cells. Elucidation of the underlying mechanism of the exopolymers on the inhibition of osteoclast differentiation might offer a new treatment for osteoporosis.

The efficacy of the exopolymers on trabecular bone was slightly lower that that of alendronate but, in the case of cortical bone, the efficacy was better than that of alendronate. It is expected that the exopolymers would show a beneficial anti-osteoporotic effect on bone fractures that are frequently encountered in postmenopausal osteoporotic patients. Apparently, the osteoclast cell number in the trochlea epiphyseal region of ovariectomized animals was considerably higher than that of the sham-operated group. However, there was no difference between the control and the test article-administered animals. In *in vitro* assay, the exopolymers severely inhibited osteoclastogenesis. This discrepancy has not yet been resolved. It is quite possible that the orally administered exopolymers would not reach the concentration level that eventually inhibits *in vivo* osteoclast maturation. TRAP-positive osteoclast cells of the trochlea epiphyseal region may not entirely reflect bone resorption activity, considering the difference observed in trabecular bone thickness and cortical bone thickness between the control and test article-administered animals.

There are two major therapeutic methods for the treatment of osteoporosis: reducing bone turnover and increasing mineral depositions on bones. As reducing bone turnover compounds, alendronate and other bisphosphonates prevent the formation of these lipid products by inhibiting farnesyl synthase [4, 23, 26], and have been prescribed to millions of patients and proven to be safe with few side effects [3, 19]. On the other hand, parathyroid hormone stimulates osteoblastic bone formation, markedly increases bone mass, prevents vertebral fractures, and has been used to treat osteoporosis [1, 2]. Current study provides evidences that the exopolymers of *A. pullulans* could be an agent embracing both treatment methods. The basis of the beneficial effect depends on two observations: 1) effect of the exopolymers on increasing mineral deposition, and 2) effect of the exopolymers on inhibiting osteoclast differentiation.

In the present study, we showed that yeast-derived exopolymers containing β-glucans considerably inhibited the bone loss of ovariectomized mice. Administration of the exopolymers to the animals increased not only trabecular bone volume, but also cortical bone thickness. These two histomorphometric changes after the administration of the exopolymers encourage us to apply the materials to

humans, since the ovariectomized animals show symptoms very similar to postmenopausal women, and many of them suffer from osteoporosis. In particular, the increase of cortical bone thickness with the exopolymers may contribute to bone strength, whose benefit could not be obtained with a bisphosphonate [5]. The nature of the favorable effect of the exopolymers on cortical bone thickness has not yet been elucidated. Nevertheless, the increasing effect of mineral deposition by the exopolymers treatment may partly explain the effect. Despite the lack of knowledge on the underlying mechanism of how the exopolymer could protect the bone loss of osteoporosis, the anti-osteoporotic activity of the exopolymer could further expand the well-known benefits of β-glucans on health.

Acknowledgments

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