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Preventive Effects of Traditional Chinese (Kampo) Medicines on Experimental Osteoporosis Induced by Ovariectomy in Rats

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Abstract. Preventive effects by traditional Chinese (Kampo) medicines, Unkei-to, Hachimi-jio-gan, and Juzen-taiho-to, on the progress of bone loss induced by ovariectomy in rats were investigated for a period of 49 days. The bone mineral density (BMD) of tibia in ovariectomized (OVX) rats decreased by 20% from those in sham-operated (Sham) rats, with the decrease completely inhibited by the administration of any one of these Kampo medicines or 17β-estradiol. From scanning electron microscopic (SEM) analyses, the surface of a trabecular bone of tibia in OVX rats had a porous or erosive appearance, whereas that of the same bone in Sham rats was composed of fine particles. The administration of three Kampo medicines and 17β-estradiol to OVX rats preserved the fine particle surface of the trabecular bone. These results strongly suggest that any of these three gynecological Kampo medicines is as effective as 17β-estradiol in preventing the development of bone loss induced by ovariectomy in rats.

Key words: Ovariectomy — Chinese traditional (Kampo) medicines — Tibiae — Bone mineral density — Electron micrographs — Rat.

Since the peak bone mass of women is less than that of men, and since a large decrease in bone mass occurs in the postmenopause state, women are vulnerable to the osteoporosis known as postmenopausal osteoporosis [1, 2]. The primary cause of postmenopausal osteoporosis is an estrogen deficiency resulting in the decrease in bone mass. An ovariectomized rat model, which artificially produces the depleted state of estrogen, has been used for the study of postmenopausal osteoporosis: Both aged rats and mature rats have been used as animal models to study experimentally-induced osteoporosis and the mature rat model has characteristics that are comparable with those of early postmenopausal trabecular bone loss [3].

Several medications have been reported to be effective for curing osteoporosis based upon the results obtained using these animal models. Estrogen [2, 3], bisphosphonates [3, 4], calcitonin [3, 5], calcium products [3, 6], ipriflavone [7], and anabolic steroids [8] are clinically employed as effective medications.

Traditional Chinese (Kampo) [9] medicines have been reevaluated by clinicians [10], because these medicines have fewer side effects and because they are more suitable for long-term use as compared to chemically synthesized medicines. About forty kinds of Kampo medicines are claimed to be effective for gynecological diseases such as climacteric psychosis, feeling of cold, menstrual disorders, dysmenorrhea, and low back pain. It has been suggested that the effectiveness of Kampo medicines on low back pain seems to correspond to their efficacy in curing osteoporosis [11]. From ancient times in China, Korea, and Japan, women who have had low back pain in climacteric and senescent periods have been treated with Kampo medicines. For example, Unkei-to, Hachimi-jio-gan, and Juzen-taiho-to have been used in treating ovary function failure, used in treating low back pain during the climacteric period, and also used after oophorectomies because of malignant tumors [11, 12]. However, no data are available as to the recovery of bone mass by any of these Kampo medicines.

In order to evaluate the effectiveness of Kampo medicines on osteoporosis, we examined whether three gynecological Kampo medicines, Unkei-to, Hachimi-jio-gan, and Juzen-taiho-to, could prevent the progression of bone loss induced by ovariectomy in rats.

Materials and Methods

Kampo Medicines and Chemicals

Dried extract powders of traditional Chinese (Kampo) medicines, Unkei-to (Wen-Jing-Tang), Hachimi-jio-gan (Ba-Wei-Di-Huang-Wan), and Juzen-taiho-to (Shi-Quan-Da-Bu-Tang) were supplied by Tsumura & Company (Tokyo, Japan). They have been reported to be effective for gynecological diseases [12]. Their herbal constituents and contents are shown in Table 1. A 17β-estradiol was purchased from Sigma Chemicals (St. Louis, MO). All other reagents were purchased from Katayama Chemicals (Osaka, Japan).

Ovariectomy and Administration of Kampo Medicines

Sixty-four female Sprague-Dawley rats, aged 9 weeks, were purchased from Seac Yoshitomi Ltd. (Fukuoka Prefecture, Japan). Twenty-seven days later (at 90-days-old; a mature rat model [3] was used), eight rats (as baseline control) were killed by exanguination under chloroform anesthesia for the evaluation of bone mineral density (BMD) as described in the text that follows. Eight rats were given a sham operation (control rats; first group), and 48 rats were ovariectomized under nembutal (Pentobarbital sodium; 50 mg/kg body weight: Abott Laboratories, IL) anesthesia. They

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Table 1. Herbal constituents and contents in traditional Chinese (Kampo) medicines

Herbs	Contents (% [w/w])		
	Unkei-to	Hachimi-jio-gan	Juzen-taiho-to
Bakumondo (JP,[a] Ophiopogon Tuber)	14.8	—	—
Hange (JP, Pinellia Tuber)	14.8	—	—
Toki (JP, Angelica Root)	11.1	—	10.5
Kanzo (JP, Glycyrrhiza)	7.4	—	5.3
Keihi (JP, Cinnamon Bark)	7.4	4.5	10.5
Shakuyaku (JP, Peony Root)	7.4	—	10.5
Senkyu (JP, Cnidium Rhizome)	7.4	—	10.5
Ninjin (JP, Ginseng)	7.4	—	10.5
Botanpi (JP, Moutan Bark)	7.4	11.4	—
Goshuyu (JP, Evodia Fruit)	3.7	—	—
Shokyo (JP, Ginger)	3.7	—	—
Akyo (JHMC,[b] Ass Glue)	7.4	—	—
Jio (JP, Rehmanniae Root)	—	27.3	10.5
Sanshuyu (JP, Cornus Fruit)	—	13.6	—
Sanyaku (JP, Dioscorea Rhizome)	—	13.6	—
Takusha (JP, Alisma Rhizome)	—	13.6	—
Bukuryo (JP, Hoelen)	—	13.6	10.5
Bushi (Aconite Tuber)[c]	—	2.3	—
Ogi (JP, Astragalus Root)	—	—	10.5
Sojutsu (JP, Atractylodes Lancea Rhizome)	—	—	10.5

[a] Japanese Pharmacopeia
[b] Japanese Herbal Medicine Cordex
[c] Non-JP materials

Table 2. Fluid and food consumption in rats

	Mean daily consumption			
	Fluid (ml) per rat		Food (g) per rat	
	0–1 week	2–7 weeks	0–1 week	2–7 weeks
Sham	26.5 ± 1.7	45.7 ± 4.0	12.8 ± 1.0	16.3 ± 1.1
OVX	29.4 ± 3.1	36.5 ± 2.5[a]	7.9 ± 0.5[a]	20.6 ± 1.7[a]
OVX + Un	24.3 ± 2.5	38.0 ± 2.5[a]	8.1 ± 0.5[a]	19.4 ± 1.7[a]
OVX + Ha	34.8 ± 3.1[b]	48.0 ± 3.5[b]	8.0 ± 0.5[a]	19.8 ± 1.7[a]
OVX + Ju	29.7 ± 3.5	34.1 ± 2.7[a]	12.3 ± 0.9[b]	20.5 ± 1.5[a]
OVX + Pe	27.3 ± 2.0	37.2 ± 2.7[a]	8.1 ± 0.5[a]	20.5 ± 1.7[a]
OVX + Es	30.9 ± 2.9	33.9 ± 2.9[a]	6.7 ± 0.5[a]	17.2 ± 1.6

Rats ($n = 8$) were sham-operated (Sham), ovariectomized (OVX), OVX given Unkei-to (OVX + Un), OVX given Hachimi-jio-gan (OVX + Ha), OVX given Juzen-taiho-to (OVX + Ju), OVX given polyethylene glycol (OVX + Pe), and OVX given 17β-estradiol (OVX + Es), respectively
[a] Significant difference ($P < 0.05$), when compared to Sham rats
[b] Significant difference ($P < 0.05$), when compared to OVX rats

were divided into six groups, those being the second to seventh groups (8 rats per group) after the first group of Sham rats. The first, second, sixth, and seventh groups received distilled water as drinking water. The third to fifth groups received 0.054% (w/w) Unkei-to, 0.043% (w/w) Hachimi-jio-gan, and 0.049% (w/w) Juzen-taiho-to, respectively. These concentrations of Kampo medicines were calculated from the daily fluid consumption and the body weight of the rats (Table 2). It was assumed that the daily doses in humans of Unkei-to, Hachimi-jio-gan, and Juzen-taiho-to were 5.0, 4.5, and 5.0 g, respectively [12]. Each ovariectomized rat in the seventh group was injected subcutaneously with 17β-estradiol (in 20% polyethylene glycol 3000) 5 days/week at a dose of 10 μg/kg body weight. Rats in the sixth group were injected with 20% polyethylene glycol 3000 in the same manner as were rats in the seventh group.

To develop bone loss in ovariectomized rats, all the animals were maintained for seven weeks under regulated 12 hour/12 hour light–dark illumination cycles at constant temperature (24 ± 0.5°C) and humidity (45%–50%). Food (MF pellets, Oriental Yeast Company, Ltd., Tokyo, Japan) and drinking water were supplied *ad libitum.*

Daily fluid consumption, daily food consumption, and body weight were measured at a specified time during the course of the experiment. After terminating the animal by exanguination under chloroform anesthesia, the right leg was dissected from each animal and was stored in a 7.5% formalin-neutral buffer solution (pH 7.2). Longitudinal sections of the tibiae from the right legs were made as described in the following text. The uterus of each rat was dissected out and was weighed.

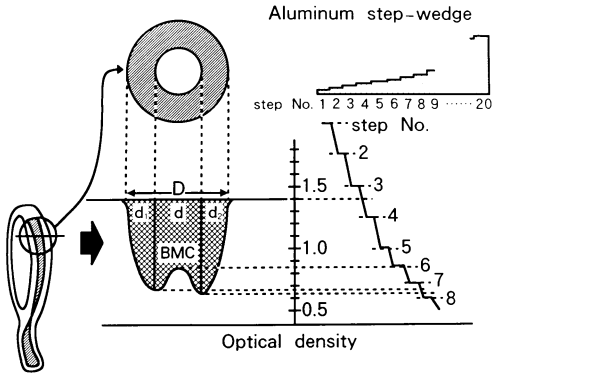
Mesurement of Bone Mineral Density (BMD)

After dissecting the adhering soft tissues, longitudinal sections of tibiae were made by manual grinding with whetstones (≠600). The right tibiae were dehydrated by a step-wise application of 70 and 99.9% (v/v) ethanol solutions. These samples were used for both the measurement of BMD and scanning electron microscopic observation.

The BMD was quantitatively determined by the computed X-ray densitometry (CXD) method (Bonalyzer: Teijin Company, Tokyo, Japan) [13]. The radiographs of the longitudinal sections of tibiae were taken along with an aluminum step-wedge using an X-ray apparatus (Model CMB: Softex Company, Tokyo, Japan) set at 4 mA, 40 cm, 120 sec, and 35 kV. The densitometric pattern of the proximal tibia on an X-ray picture was read by a personal computer (PC-9801; NEC, Tokyo, Japan) using a software program for rat bone density (Version 2. 10A-M: Teijin Company, Tokyo, Japan) (Fig. 1). As an index of BMD and bone mineral content (BMC), ΣGS/D and ΣGS (mm A1) were used, respectively; ΣGS is a value obtained by computer integrating the pattern area, which is obtained opticodensitometrically and is converted into the number of steps on the aluminum step-wedge. As an index of cortical bone width, MCI = $(d_1 + d_2)$/D was used. The D, d_1, and d_2 represent the bone width and both cortical bone widths (see Fig. 1). The area that covers the epiphysis and a part of metaphysis in the proximal tibia was calculated for ΣGS/D (indicated by the circle on a bone at the left side in Fig. 1). For determining the area, one line was placed on the boundary between the epiphyseal cartilage and the epiphysis and another line was made at ⅙ of the length from the tibial mesial end.

For the Precision of CXD Method

Reproducibility of the measurement was assessed in the longitu-



$$\text{Bone mineral density} = \frac{\text{Bone mineral content}}{D}$$

Fig. 1. Densitometric pattern on microdensitometry of computed X-ray densitometry and tibia measurement. The radiograph of the longitudinal section of the tibia was taken along with an aluminum step-wedge using an X-ray apparatus. The densitometric pattern of the proximal tibia on an X-ray picture was read by a personal computer. As an index of bone mineral density (BMD), ΣGS/D (mm A1) was used. The circle on the bone approximately represents the area used for BMD measurement.

dinal sections of removed tibiae from 10 female Sprague-Dawley rats aged 9 and 20 weeks. Bone mineral in the proximal tibia was scanned five times with repositioning of the bone sample within one measurement and coefficients of variation (CVs) were calculated from BMD, BMC, and MCI.

Electron Microscopic Observation

Longitudinal sections of tibiae were next shadowed with carbon before examination in a scanning electron microscope (SEM) (ERA-8000S, Elionix, Tokyo, Japan) at 70 kV and 20 mA specimen current. Three trabecular bones in the metaphysis of one tibia were randomly chosen for the analysis of their surface architecture at a magnification of ×20,000.

Statistics

Data were obtained from 3–5 measurements and were expressed as the means ± standard deviations. Statistical comparisons were made by ANOVA and Scheffé's tests using a statistic software program. The difference was considered significant when $P < 0.05$.

Results

Body Weight and Consumption of Fluid and Food

Increases in the body weight in ovariectomized (OVX) rats were significantly higher than those in sham control (Sham) rats (Fig. 2). After seven weeks, the mean body weights ± SD in Sham and OVX rats were 267 ± 27 ($n = 8$) and 355 ± 25 ($n = 8$), respectively. Increases in the body weight of the groups, which were given Unkei-to (OVX + Un) or Juzen-taiho-to (OVX + Ju) were almost the same as those in OVX rats. However, the increase in body weight of the group, which was given Hachimi-jio-gan (OVX + Ha), was smaller than those in OVX rats (no significant difference) except for at the first one week (significant difference $P <$ 0.05). With the administration of 17β-estradiol to OVX rats (OVX + Es), the increase was almost the same as those in Sham rats. The administration of the vehicle alone had no effect (data not shown).

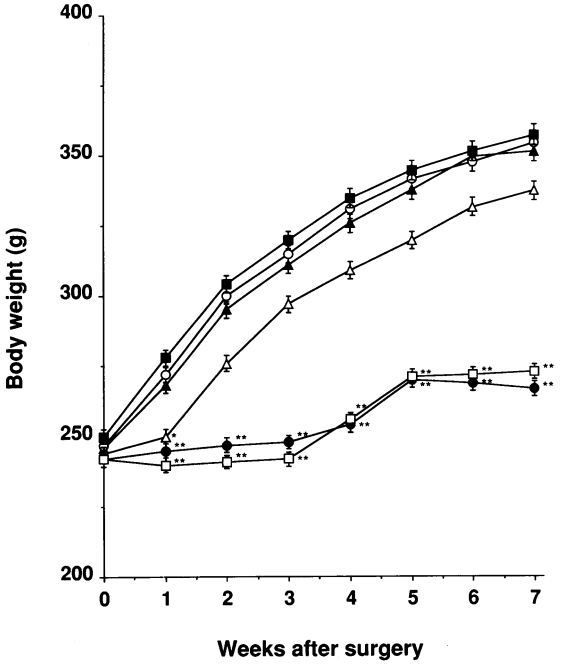


Fig. 2. Changes in body weight in rats. Rats were sham-operated (●), ovariectomized (○), and administered Unkei-to (▲), Hachimi-jio-gan (△), Juzen-taiho-to (■), and 17β-estradiol (□). Since the changes of OVX, which were given a vehicle, were the same as those of ovariectomized, its curve was not shown. Each point represents the mean value ± SD ($n = 8$). *$P < 0.05$ and **$P < 0.01$, significant difference from the ovariectomized group at corresponding times.

Since both fluid and food consumptions in the first one week were smaller than those in the following weeks, two groups (0–1 week and 2–7 weeks) were compared. As shown in Table 2, fluid consumption of OVX + Ha rats in week 0–1 was higher than that in OVX rats. Both consumptions of Sham and OVX + Ha rats in weeks 2–7 were higher than those in others. Food consumption of Sham and OVX + Ju rats in week 0–1 was higher than that in others. Food consumption of OVX rats in weeks 2–7 was higher than that of Sham rats by 26%. Food consumption of OVX + Es rats in weeks 2–7 was comparable to that of Sham rats.

Uterine weight

The uterine weights of Sham (491 ± 45 mg) and OVX rats (112 ± 9 mg) differed significantly ($P < 0.01$). Those of the groups that were given Unkei-to, Hachimi-jio-gan, and Juzen-taiho-to were 126 ± 10, 139 ± 14, and 120 ± 10 mg, respectively. Those of the groups that were injected 17 β-estradiol and its vehicle were 479 ± 42 and 115 ± 10 mg, respectively. The differences between OVX rats and OVX + Un, OVX + Ha, and OVX + Ju rats were not statistically significant.

The Precision of CXD

The coefficients of variation (CVs) of bone mineral mea-

Table 3. Bone mineral density (BMD) in sham-operated and in ovariectomized rats treated with various compounds for seven weeks

	Bone mineral density (ΣGS/D)
Sham	1.00 ± 0.02
OVX	0.80 ± 0.02[a]
OVX + Un	0.95 ± 0.02
OVX + Ha	0.98 ± 0.02
OVX + Ju	1.00 ± 0.02
OVX + Pe	0.79 ± 0.01[a]
OVX + Es	0.97 ± 0.02

Rats ($n = 8$) were sham-operated (Sham), ovariectomized (OVX), OVX given Unkei-to (OVX + Un), OVX given Hachimi-jio-gan (OVX + Ha), OVX given Juzen-taiho-to (OVX + Ju), OVX given polyethylene glycol (OVX + Pe), and OVX given 17β-estradiol (OVX + Es), respectively

[a] Significant difference ($P < 0.05$), when compared with Sham rats

surements for bone mineral density (BMD), bone mineral content (BMC), and an index of cortical bone width (MCI), were 1.98%, 3.74% and 2.78%, respectively.

Bone Mineral Density (BMD)

As shown in Table 3, bone mineral density (BMD) of Sham and OVX rats were 1.0 ± 0.02 and 0.80 ± 0.02, respectively. This indicated that the ovariectomy decreased in the BMD by 20%. The administration of Unkei-to, Hachimi-jio-gan, or Juzen-taiho-to to the ovariectomized rats clearly restored the BMD to the level of Sham rats. The injection of 17β-estradiol to OVX rats also restored it to the level of Sham rats, whereas the vehicle had no effect.

The BMD (ΣGS/D) of normal rats (baseline control) was 0.93 ± 0.02. The value of OVX rats after seven weeks (0.80 ± 0.02) was lower than the baseline BMD ($P < 0.05$).

SEM Analyses

The scanning electron micrograph of the proximal tibiae taken at seven weeks after the ovariectomy is shown in Fig. 3C. Compared to that of Sham rats (Fig. 3A), the connectivity of cancellous bone in the epiphysis and that of trabecular bone in the metaphysis exhibited greater loss than those in the Sham rats. The surface of trabecular bones in the metaphysis in OVX rats (which decreased its width to ⅓–⅕ of those from Sham rats) had a fluffy surface (Fig. 3D), whereas the surface of the trabecular bones from Sham rats was smooth (Fig. 3B). At increased magnification (×20,000), the surface of trabecular bones of Sham rats appeared to be composed of fine particles (Fig. 4A), but that of trabecular bones in OVX rats had a porous or an erosive appearance (Fig. 4B). However, the appearance of the surface of the trabecular bones in OVX rats was restored to that of Sham rats by the administration of Unkei-to, Hachimi-jio-gan, or Juzen-taiho-to, although the surface of Unkei-to bones had some porous appearance (Figs. 4C–E). 17β-estradiol similarly preserved the surface appearance (Fig. 4F), whereas the vehicle had no effect (data not shown).

Three trabecular bones were randomly chosen from one tibia and their surface state was observed. The frequency of the occurrence of three types of the surface states, namely fine particle, porous or erosive, and intermediate between these two types, is shown semiquantitatively in Table 4. Both the Sham and 17β-estradiol rats were 100% fine particle type. The OVX rats were 95.8% porous or erosive and 4.2% intermediate type. The frequency of the occurrence of the fine particle type was restored to 83–96% by the administration of the three Kampo medicines.

Discussion

Ovariectomy caused an increase in body weight (Fig. 2). This is one of the prominent features that has been postulated to provide a partial protection against the development of osteoporosis in long bones [14]. Hachimi-jio-gan delayed the body weight increase only in the first week, whereas the other two Kampo medicines did not. Subcutaneous injection of 17β-estradiol characteristically inhibited the increase of body weight (Fig. 2). It is not known whether Hachimi-jio-gan has a weak estrogen-like effect.

Ovariectomy caused a decrease in fluid consumption but an increase in food consumption in the rats in 2–7 weeks (Table 2). However, Hachimi-jio-gan noticeably increased fluid consumption through the entire experimental period. This may be related to the diuretic action of Hachimi-jio-gan in the rat [15]. The increase in the food consumption in ovariectomized rats (26% increase in OVX over Sham rats in 2–7 weeks) may correlate with the increase of body weight [14, 16]. The decreased food consumption of OVX rats in 0–1 week may be caused by surgical insult. The food consumption in week 0–1 of OVX + Ju rats was the same as that of Sham rats. Juzen-taiho-to has been reported to recover strongly the decreased appetite of mice after surgery [17]. Therefore, it seems likely that Juzen-taiho-to increases the appetite of the rats that were traumatized by surgery. The administration of 17β-estradiol preserved the food consumption (Table 2). Because none of the Kampo medicines caused a decrease in food consumption in ovariectomized rats (during weeks 2–7), their effect may be different from that of estrogen.

Ovariectomy resulted in the dramatic decrease in uterine weights (Sham 491 ± 45 vs. OVX 112 ± 9 mg; $P < 0.01$). Since none of the Kampo medicines increased the uterine weight, it is suggested that these agents do not function as estrogen agonists, at least in the brain [16, 18].

The CXD method for measuring human metacarpal bone mass has been well established [13]. Seo et al.[19] have reported that the correlation between CXD-measured radial BMD and dual energy X-ray absorptiometry (DXA)-measured radial BMD is of statistical significance ($r^2 = 0.733$; $P < 0.01$). Previously the CXD method, using a software program for the rat (Ver. 2), has been evaluated for measuring the BMD of uremic rats [20]. The coefficient of variation (CV) for the removed femoral bone is below 2%, whereas the regression coefficient between the CXD method and the DXA method is 0.743 [20]. In this study, the CV for BMD of the proximal tibia was about 2%, which is comparable to that obtained by other investigators [13, 19, 20].

The BMD of OVX rats after seven weeks of the experiment was lower than that of the beginning of the study (baseline control; see Results). Our results indicate that true bone loss occurs in the ovariectomized rats [3]. It has been previously documented that the bone density of Sprague-Dawley rats continues to increase until approximately six months of age [3, 21]. Therefore, 20% bone loss over a

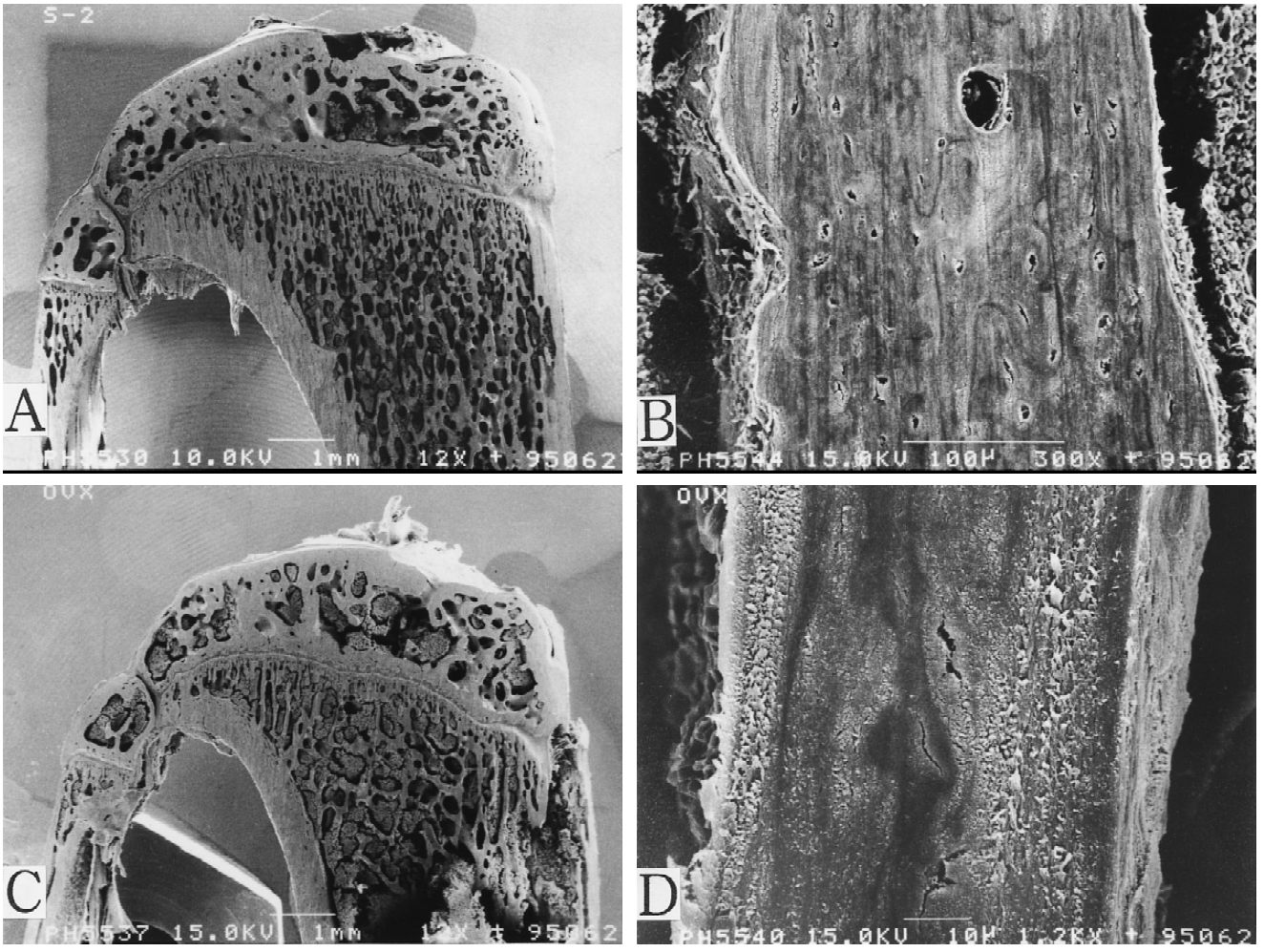


Fig. 3. Scanning electron micrographs of the longitudinal sections of the proximal tibia from sham-operated **(A)** and ovariectomized rats **(C).** Scanning electron micrographs of a trabecular bone in the metaphysis of sham-operated **(B)** and ovariectomized rats **(D).**

seven-week period (Table 3) may precisely express the 20% arrest of bone density increase.

The administration of three Kampo medicines and the injection of 17β-estradiol to OVX rats restored the BMD to the level of Sham rats (Table 3). This strongly suggests that those gynecological medicines are as effective as 17β-estradiol in preventing bone loss, although it is not clear whether the same mechanism as estrogen would be functioning.

Ovariectomy resulted in the decrease of the connectivity of cancellous bones in the epiphysis and that of the trabecular bones in the metaphysis (Figs. 3A, C). Further, the width of the trabecular bones from OVX rats was decreased to ⅓–⅕ that of the trabecular bones from Sham rats (Figs. 3B, D). This is contrary to a previous report indicating that the trabecular connectivity was lost, but that the width of the trabecular bones did not significantly change in the ovariectomized rat [22].

It has been reported that both bone resorption and bone formation are promoted by ovariectomies and the prominent increase of the bone resorption is termed high-turnover osteoporosis [23]. It is possible that the porous or erosive appearance of the tibia surface in OVX rats resulted from this high-turnover osteoporosis. Kampo medicines may be effective in inhibiting the elution of bone calcium, because the porous or erosive appearance in OVX rats was restored by the administration of Kampo medicines to a fine particle composition, which was comparable to the appearance of Sham rats (Figs. 3 and 4). Among the Kampo medicines used, the effect of Unkei-to was weaker than that of the other two, because it still produced some percentages of intermediate-type surface (Table 4 and Fig. 4C). However, the response of these agents is not clear only by SEM analysis at present.

Keihi (Japanese Pharmacopeia [JP] *Cinnamon Bark*) is commonly contained in three Kampo formulas and Toki (JP, *Angelica Root*), Kanzo (JP, *Glycyrrhiza*), Shakuyaku (JP, *Peony Root*), Senkyu (JP, *Cnidium Rhizome*), Ninjin (JP, *Ginseng*), Botanpi (JP, *Moutan Bark*), Jio (JP, *Rehmanniae Root*), and Bukuryo (JP, *Hoelen*) are contained in two of three Kampo medicines (Table 1). There have been no reports on antiresorptive action by these herbs. However, Li et al. [24] have reported an antiresorptive action by the methanolic extract from a herb, Shoma (*Cimicifuga Rhizome*). An ipriflavone is a commercially available, useful drug for osteoporosis with inhibitory activity on bone resorption [7].

Shakuyaku (JP, *Peony Root*), Bukuryo (JP, *Hoelen*), and Toki (JP, *Angelica Root*) have been reported to increase the progesterone content in blood and the ovary [25]. Unkei-to,

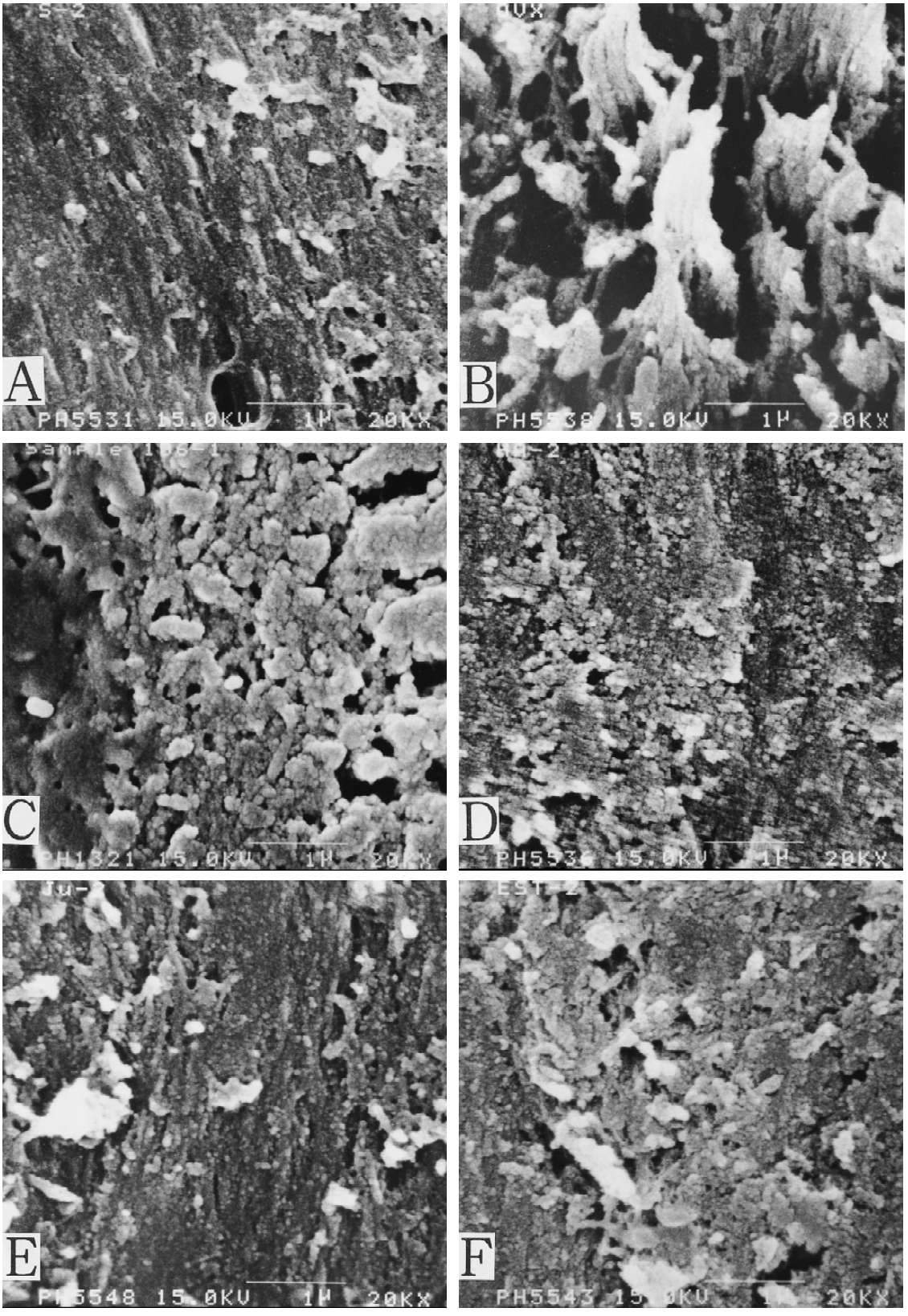


Fig. 4. High magnification of scanning electron micrographs of the surface of the trabecular bone in the metaphysis from sham-operated **(A),** ovariectomized **(B),** and rats administered Unkei-to **(C),** Hachimi-jio-gan **(D),** Juzen-taiho-to **(E),** and 17β-estradiol **(F).**

Table 4. Frequency of occurrence of three types of surfaces of trabecular bones in the metaphysis

	Frequency					
	Fine particles	(%)	Inter-mediate	(%)	Porous or erosive	(%)
Sham	24/24	100	0/24	0	0/24	0
OVX	0/24	0	1/24	4.2	23/24	95.8
OVX + Un	20/24	83.3	4/24	16.7	0/24	0
OVX + Ha	22/24	91.7	2/24	8.3	0/24	0
OVX + Ju	23/24	95.8	1/24	4.2	0/24	0
OVX + Pe	0/24	0	2/24	8.3	22/24	91.7
OVX + Es	24/24	100	0/24	0	0/24	0

Rats ($n = 8$) were sham-operated (Sham), ovariectomized (OVX), OVX given Unkei-to (OVX + Un), OVX given Hachimi-jio-gan (OVX + Ha), OVX given Juzen-taiho-to (OVX + Ju), OVX given polyethylene glycol (OVX + Pe), and OVX given 17β-estradiol (OVX + Es), respectively
Using eight rats, three trabecular bones in the metaphysis per one tibia were analyzed on their surface by SEM at increased magnification

Hachimi-jio-gan, and Juzen-taiho-to contain these herbs at 18.5%, 13.6%, and 31.5% (w/w), respectively (Table 1). Also, Hachimi-jio-gan has been reported to increase the progesterone content in the ovary [26]. However, after ovariectomy, these herbs and Kampo medicines could not increase the synthesis of estrogen in the ovary *in vivo*. The amount of estrogen that has been used in replacement therapy is 0.625–2 mg/day [2]. However, the amount of estrogen contained in the daily dose in Juzen-taiho-to was small when measured by the RIA method using an estradiol antibody (<1.6 ng/day; unpublished data). Therefore, Kampo medicines may enhance some reactions that are triggered by estrogen. It has been reported that the estrogen would influence bone loss either directly by binding to the receptor on the bone [27] or indirectly by influencing calcium regulatory hormones (PTH and vitamin D) [28, 29] and cytokines IL-1 and IL-6 [30]. Therefore, it is probable that Kampo medicines may have an effect through one or both of these indirect actions. Furthermore, because an androgen also associates with an increase of bone mass [31], its *in vivo* content may increase through adrenal synthesis or peripheral conversion. The serum estradiol and androgen levels are under investigation.

Kampo medicines, which have been developed over some 3000 years [9, 10] and are known to have low toxicity, may offer advantages over the longer term over synthetic agent medication. For example, the estrogen replacement therapy needs a conjugate with progesterone to avoid its tumor-inducing effect [32]. Although the preventive mechanism of these agents remains to be explained, this initial study does show that Kampo medicines that have traditionally been effective for the gynecological diseases [9, 11, 12] may also be administered for the prevention of osteoporosis.

In conclusion, three Kampo medicines, as well as 17 β-estradiol, could prevent the development of bone loss induced by ovariectomy in rats. This result strongly suggests that these Kampo medicines are useful for preventing postmenopausal osteoporosis and osteoporosis associated with both the ovary function failure and oophorectomy caused by a malignant tumor.

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