Effects of KCA-098 on Bone Metabolism: Comparison with Those of Ipriflavone

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ABSTRACT—We previously found that 3,9-bis(N,N-dimethylcarbamoyloxy)-5H-benzofuro[3,2-c]quinoline-6-one (KCA-098) inhibited bone resorption in organ culture. In this study, to determine if KCA-098 is therapeutically applicable for the treatment of osteoporosis, we compared the effect of KCA-098 on bone tissues with that of ipriflavone, a drug that is clinically used for the treatment of osteoporosis. Both KCA-098 and ipriflavone inhibited parathyroid hormone-, prostaglandin E_2-, 1α,25-dihydroxyvitamin D_3- and interleukin 1β-induced bone resorption of fetal rat bones, but the inhibitory activity of KCA-098 was more potent than that of ipriflavone. In fact, the effective concentrations of KCA-098 were 10 to 100 times lower than those of ipriflavone. Oral administration of KCA-098 (1 and 3 mg/kg) or ipriflavone (100 mg/kg) to ovariectomized rats on a low-calcium diet increased the breaking force and bone density of the femora, indicating that KCA-098 is as effective on the whole animal as ipriflavone. Furthermore, KCA-098 increased the length and calcium content of 9-day chick embryonic femora cultured in vitro, whereas ipriflavone did not, suggesting that KCA-098 had a direct stimulatory effect on bone mineralization. Therefore, KCA-098 seems to be more potent than ipriflavone in stimulating bone tissue formation and may thus be expected to become a useful agent for the treatment of osteoporosis.

Keywords: KCA-098, Ipriflavone, Bone resorption, Bone formation, Ovariectomy

Osteoporosis occurs from the imbalance between bone formation and bone resorption and is the main causal factor of bone fractures in elderly persons. This disorder has been increasing remarkably in frequency along with the increase in life expectancy. Many agents such as calcium and estrogen (the first-generation-anti-osteoporotic drugs) and calcitonin and vitamin D_3 (second-generation ones) have been used clinically in the treatment of the disease (1). Recently, 7-isopropoxy-3-phenyl-4H-1-benzopyran-4-one (ipriflavone, a synthetic compound belonging to the isoflavone group) was shown to have an inhibitory effect on bone resorption (2) and a stimulating effect on osteoblasts (3, 4) and was thus introduced for clinical use as a third-generation drug (5, 6). In order to find other chemically synthesized compounds that affect bone metabolism, we have been studying the effect of derivatives of coumestrol, a weak estrogen structurally similar to ipriflavone, on bone resorption. We found that among such derivatives KCA-098 has an inhibitory effect on bone

resorption of fetal rat femora (7) and a stimulatory effect on the mineralization of chick embryonic bone in culture (8). Therefore, the effect of KCA-098 on bone tissue resembles that of iprilavone. In this present study, we compared the effects of ipriflavone and KCA-098 on bone resorption of cultured fetal rat bone and bone mineralization of cultured chick embryonic femur to determine whether KCA-098 is useful for the treatment of osteoporosis like ipriflavone is. In addition, we compared the effects of these compounds on the physical properties of the femur isolated from ovariectomized rats on a low-calcium diet, an experimental model of osteoporosis.

MATERIALS AND METHODS

Chemicals

Agents used and their sources were as follows: 3,9-bis(N,N-dimethylcarbamoyloxy)-5H-benzofuro[3,2-c]-quinoline-6-one (KCA-098), Kissei Pharmaceutical Co.,

Ltd., Matsumoto; bovine serum albumin (BSA), cell culture grade, Sigma Chemical Co., St. Louis, MO, USA; 7-isopropoxy-3-phenyl-4H-1-benzopyran-4-one (ipriflavone, extracted from Osten™), Takeda Chemical Industries, Ltd., Osaka; synthetic human parathyroid hormone (fragment 1-34, PTH) and synthetic human calcitonin (calcitonin), Peptide Institute, Inc., Minoh; prostaglandin E_2 (PGE_2) and interleukin 1β (IL-1β), Funakoshi Co., Ltd., Tokyo; 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], hydroxypropyl cellulose (HPC), dimethyl sulfoxide (DMSO) and Calcium C-test Wako™, Wako Pure Chemical Co., Ltd., Osaka; phosphate-buffered saline (Ca, Mg free) [PBS($-$)] and BGJb-HW2 (phenol red free), Nissui Pharmaceutical Co., Ltd., Tokyo; and $^{45}CaCl_2$, Du Pont/NEN Research Products, Boston, MA, USA. Medium BGJb-HW2 containing 0.01% BSA (7) was sterilized by passage through a 0.22-μm filter.

Determination of bone-resorbing activity

Bone-resorbing activity was determined by the method of Raisz (9). ^{45}Ca (1.85 MBq/rat) was injected subcutaneously into pregnant Wistar rats (Japan SLC, Inc., Hamamatsu) at 18 days of gestation. The rats were sacrificed by decapitation under ether anesthesia two days after the ^{45}Ca-injection. The labeled long bones were aseptically isolated from fetuses, and the adhering soft connective tissue was completely removed as the bones were rotated on dry paper. The bones were incubated for 24 hr with fresh BGJb-HW2 medium (chase-medium) to remove the physico-chemical contaminant ^{45}Ca. A test sample dissolved in DMSO (1 : 1,000) was then added to fresh chase-medium, and the cultures were incubated for five days. The medium was changed every day. Before the medium was exchanged, an aliquot of the medium was withdrawn for liquid scintillation counting to measure the radioactivity of ^{45}Ca released into the medium. After the incubation, the bones were hydrolyzed in 6 N HCl at 110℃, and the remaining radioactivities in the bones were measured in a liquid scintillation counter.

Bone-resorbing activity was expressed as a percentage of released ^{45}Ca:

^{45}Ca release (%)

$$= \frac{^{45}\text{Ca released into the medium}}{^{45}\text{Ca released into the medium} + ^{45}\text{Ca remaining in the femur}} \times 100$$

Effect of samples was expressed as follows:

$$\% \text{ of control} = \frac{^{45}\text{Ca release (\%) in the treated group}}{^{45}\text{Ca release (\%) in the control group}} \times 100$$

Determination of bone-mineralizing activity

Femora from 9-day-old chick embryos were cultured by the roller tube method (10, 11) for a week with a change of medium every other day. Next the femora were rinsed with PBS($-$) and dried at 60℃ in an electric oven

for 4 days. They were then hydrolyzed in 1 N HCl at 110℃ for 24 hr, and the calcium content in the hydrolysate was determined with a commercial kit, Calcium C-test Wako™. For control of individual variations, pairmate cultures were employed, with one bone taken for the control and the contralateral bone from the same fetus used for the test sample. The culture was carried out by the roller tube method (10).
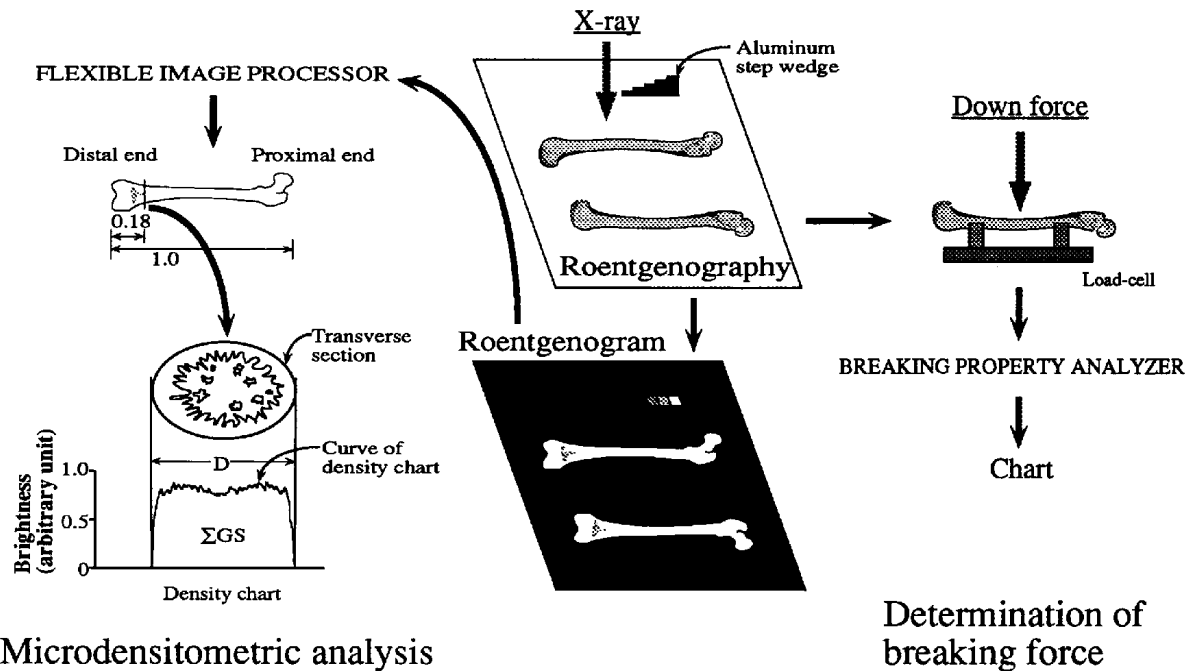
Experimental osteoporosis

Female Wistar rats (Japan SLC, Inc.), 5-week-old, were housed in a room with a 12hr/12hr light-dark illumination cycle at constant temperature (23±1℃). Bilateral ovariectomy or sham operation was carried out under ether anesthesia. During the course of the experiment, the animals were supplied ad libitum with a low-calcium diet chow (Oriental Yeast Co., Ltd., Tokyo; containing 0.03% calcium and 0.8% phosphorus) and distilled water for four weeks. Test compounds suspended in 1% HPC solution were administered orally once a day for four weeks starting the day after the operation. The rats were sacrificed under ether anesthesia at 24 hr after the last administration. Their femora were then removed and separated from surrounding soft connective tissues.

Method for bone roentgenography and microdensitometric analysis

As soon as the femoral dissection had been completed, roentgenograms were taken on a graphic art film (type CS100E, 118 mm × 163 mm, 0.1 mm; Konica Co., Ltd., Tokyo) with a soft X-ray machine (Type SRO-505; Sofron Co., Ltd., Tokyo). An aluminum step wedge of 0.5-mm thickness was placed on the film beside each bone as a density standard, and microdensitometric analysis was then performed. Following the method of Yamazaki and Yamaguchi (12), the density of transverse sections of the femurs was scanned at a point 0.18 from the distal end of the bone (total length of bone was taken as 1.0) on the roentgenograph by a computerized Flexible Image Processor (model PIP-400; ADS Co., Ltd., Nara). Bone density (ΣGS/D) was also calculated by this machine, where D is the outer diameter of the femora, and ΣGS is the area under the curve of the density chart prepared from the X-ray film (Fig. 1).

Determination of breaking force of femora from ovariectomized rats

Immediately after the roentgenography, the breaking force, the amount of force required to break a bone, was measured with a Breaking Property Analyzer (Rheodynacorder, model RDR-1500; Iio Electric Co., Ltd., Tokyo) (13). The femur, placed on the support noses standing 16 mm apart, was broken by pressing down on

Fig. 1. Diagrammatic representation of determination of breaking force and microdensitometric analysis on the roentgenograph of rat femur. Bone density (ΣGS/D) was measured at a point 0.18 (distal metaphysis) from the distal end. D, outer diameter of transverse section; ΣGS, area under the curve of the density chart.

the center of the bone. The load-deflection curve was recorded on an X-Y recorder (Fig. 1). From these results, the breaking force was calculated.

Statistical analyses

Data were expressed as the means±S.E.M. Statistical significance was determined by one-way analysis of variance followed by Dunnett's test for multiple group comparison or Student's t-test for the comparison between two groups for the in vitro experiments and by Student's t-test for the in vivo experiments.

RESULTS

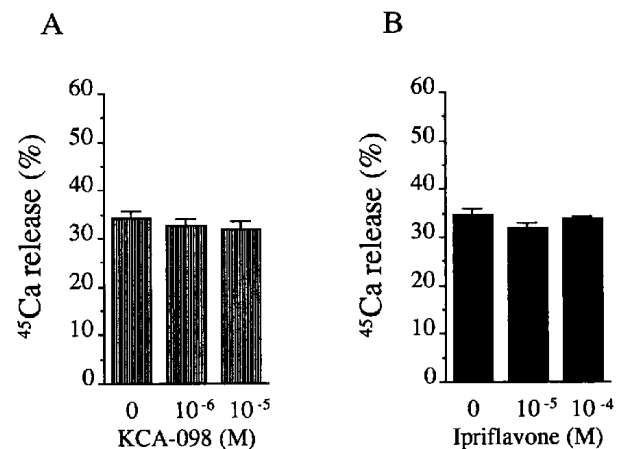*Effects of KCA-098 and ipriflavone on bone-resorbing activity of fetal rat bones*

KCA-098 (Fig. 2A) and ipriflavone (Fig. 2B) had no effect on the basal bone resorption of fetal rat bones cultured in vitro.
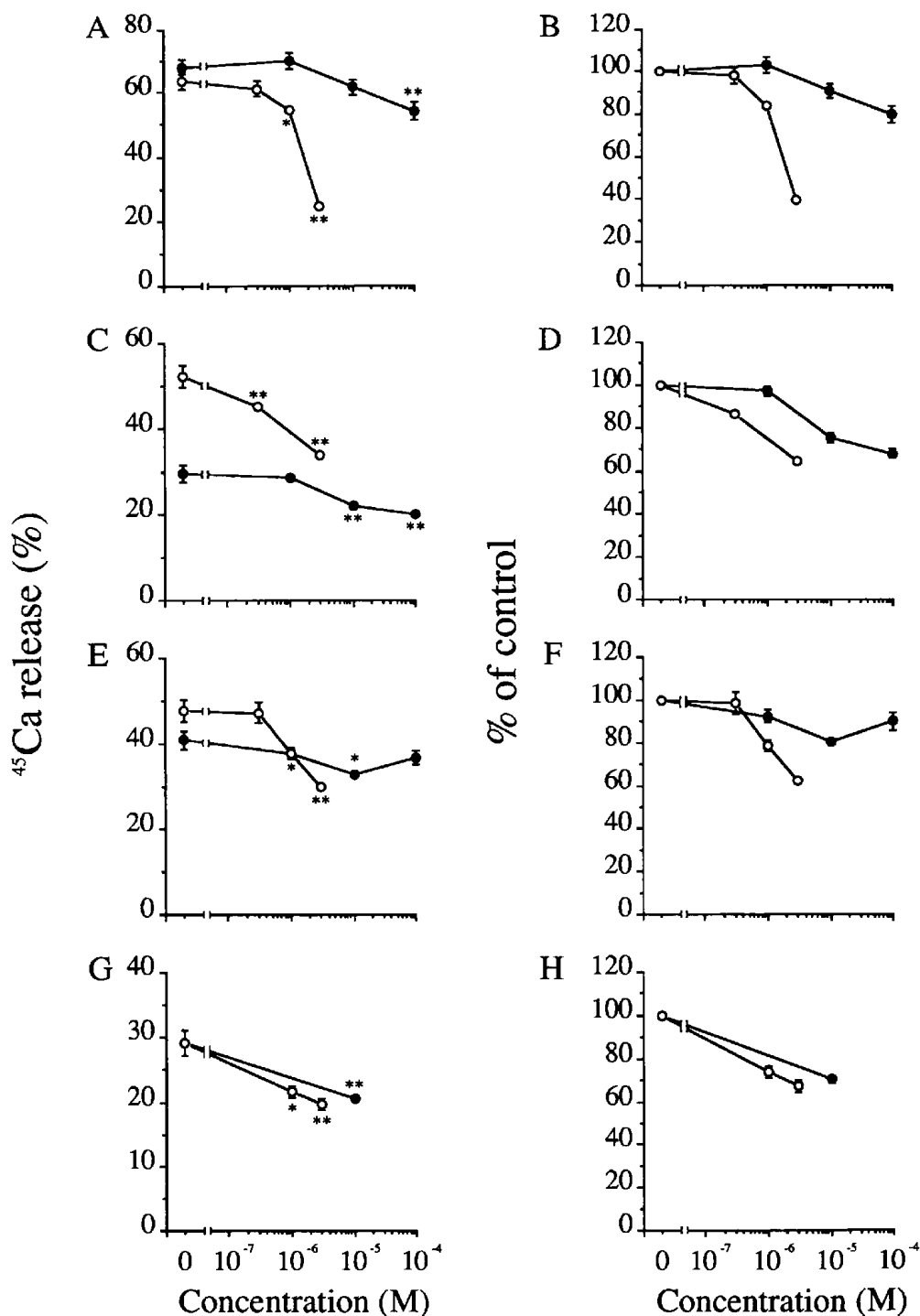
Effects of KCA-098 and ipriflavone on PTH-, PGE₂-, 1α,25(OH)₂D₃- and IL-1β-induced bone resorption

The maximum effective dose obtained from our preliminary experiments was used for each bone resorption-stimulating agent. Both KCA-098 and ipriflavone significantly inhibited PTH- (1.8×10^{-8} M; Fig. 3, A and B), PGE₂ (10^{-6} M; Fig. 3, C and D)-, $1\alpha,25(OH)_2D_3$ (10^{-9} M;
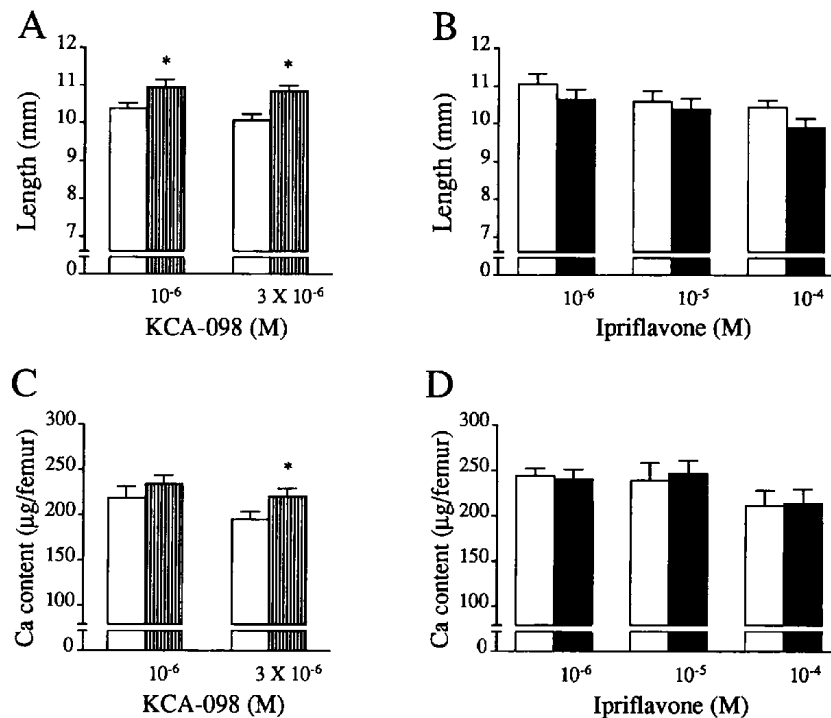
Fig. 3, E and F)- and IL-1β (1 U/ml; Fig. 3, G and H)-induced bone resorption, and the effective concentrations of KCA-098 for inhibition were 10 to 100 times lower than those of ipriflavone.

Fig. 2. Effects of KCA-098 and ipriflavone on basal bone resorption of cultured fetal rat bones. Fetal rat femora (A) and humerii (B) labeled in vivo with ^{45}Ca by injection of the isotope into pregnant rats were chase-cultured for 5 days with KCA-098 (A) or ipriflavone (B). After the culture, the release % of ^{45}Ca was determined. Values represent the means±S.E.M. (n=5).

Fig. 3. Effects of KCA-098 and ipriflavone on PTH-, PGE$_2$-, 1α,25(OH)$_2$D$_3$- and IL-1β-induced bone resorption of cultured fetal rat bones. Fetal rat long bones labeled with ^{45}Ca in vivo were chase-cultured for 5 days with various concentrations of KCA-098 (open circle) or ipriflavone (closed circle) in the presence of PTH (1.8 × 10^{-8} M, A and B), PGE$_2$ (10^{-6} M, C and D), 1α,25(OH)$_2$D$_3$ (10^{-9} M, E and F) or IL-1β (1 U/ml, G and H). After the culture, the % release of ^{45}Ca was determined (A, C, E, G). The % release of ^{45}Ca obtained from the cultures in the absence of inhibitors of bone resorption was slightly different from culture to culture because the bones used were different in the two inhibitor groups. Therefore, the effects of agents on bone resorption were also expressed as % of the control (B, D, F, H; % release of ^{45}Ca obtained from the culture in the absence of KCA-098 or ipriflavone was taken as 100%). The long bones used in this experiment were as follows: A and B, KCA-098 (femur), ipriflavone (humerus); C and D, KCA-098 (humerus), ipriflavone (femur); E and F, KCA-098 (humerus), ipriflavone (radius); G and H, KCA-098 (femur), ipriflavone (femur). Values represent the means±S.E.M. (n=4−5). Some S.E.M.s are smaller than the symbols. *P<0.05 and **P<0.01, compared with the control.

Fig. 4. Effects of KCA-098 and ipriflavone on the elongation and calcium contents of 9-day chick embryonic femora cultured in vitro. Femora from 9-day chick embryos were cultured for 7 days in the absence (control, open column) or presence of various concentrations of KCA-098 (striped column) or ipriflavone (closed column). At the end of the culture period, the length (A, B) and calcium contents (C, D) were determined. Values represent the means ± S.E.M. (n = 5). *P < 0.05 vs. paired control.

Effects of KCA-098 and ipriflavone on bone-mineralizing activity of 9-day chick embryonic femora

KCA-098 increased both the length (Fig. 4A) and calcium content (Fig. 4C) of 9-day-old chick embryonic femora in culture. However, ipriflavone had no effects on either of these parameters (Fig. 4, B and D).

Effects of KCA-098 and ipriflavone on physical properties of the femora from ovariectomized rats on a low-calcium diet
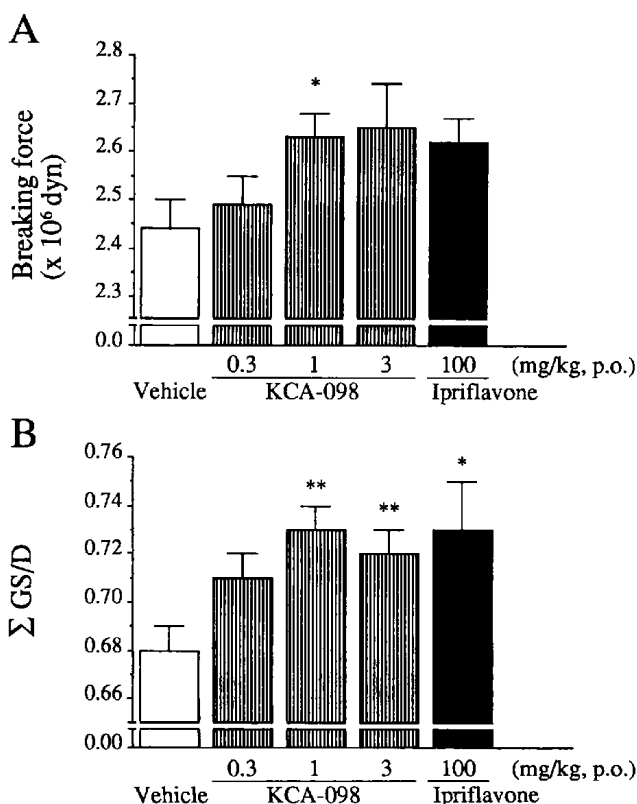
To compare the effect of KCA-098 on bone metabolism in vivo with that of ipriflavone, we administered KCA-098 or ipriflavone orally to ovariectomized rats on a low-calcium diet for four weeks, and changes in the physical properties of their femora were then determined.

Ovariectomy caused a significant decrease in femoral breaking force [$(3.17 \pm 0.08) \times 10^6$ dynes in sham-operated rats vs. $(2.44 \pm 0.05) \times 10^6$ dynes in ovariectomized rats, P < 0.01] and in bone density [$\Sigma GS/D$, 0.75 ± 0.01 in sham-operated vs. 0.68 ± 0.01 in ovariectomized rat, P < 0.01]. KCA-098 (1 mg/kg/day) partially but significantly increased the breaking force (Fig. 5A), whereas the increase by ipriflavone (100 mg/kg/day) was not significant (Fig. 5A). Both KCA-098 (1 and 3 mg/kg/day) and ipriflavone (100 mg/kg/day) significantly increased

the bone density (Fig. 5B).

DISCUSSION

We compared the inhibitory effects of KCA-098 and ipriflavone on bone resorption stimulated by various agents to assess possible differences in action mechanism between these two compounds. Both KCA-098 and ipriflavone inhibited PTH-, PGE_2-, $1\alpha,25(OH)_2D_3$- and IL-1β-induced bone resorption (Fig. 3), whereas they had no effect on the basal bone resorption of fetal bones (Fig. 2). However, KCA-098 may have a stronger effect on the inhibition of stimulator-induced bone resorption because the effective concentrations of KCA-098 were 10 to 100 times lower than those of ipriflavone (Fig. 3). Ipriflavone inhibited the formation of tartrate-resistant acid phosphatase-positive multinuclear cells (TRAP-positive MNC) from murine spleen cells (14) and also reduced the pit formation by mature osteoclasts (15), indicating that ipriflavone inhibits the formation and activation of osteoclasts. Also we found that KCA-098 inhibited TRAP-positive MNC formation in mouse bone marrow culture (manuscript in preparation). Judging from the similar inhibitory patterns of these compounds against bone resorption in organ culture and the inhibitory effects on

A

B

Fig. 5. Effects of KCA-098 and ipriflavone on breaking force and bone density (ΣGS/D) of femora removed from ovariectomized rats on a low-calcium diet. KCA-098 and ipriflavone were orally administered to ovariectomized rats once a day for four weeks. The breaking force (A) and bone density (ΣGS/D, B) of the femora were determined after sacrifice. Values represent the means$\pm$S.E.M. (n=10). *P<0.05 and **P<0.01, compared with the control.

the formation of osteoclasts, the action mechanisms of KCA-098 and ipriflavone appear to be the same.

Usually bone tissue has both bone-forming and -resorbing activity occurring simultaneously. Thus, quantitative separation of the two activities is difficult in organ culture. To determine the bone-forming activity, we used the culture of 9-day chick embryonic femur, because at this early stage, membranous bone formation has just started, and bone-forming activity largely exceeds the bone-resorbing activity. In fact, we previously showed the direct stimulative effects of $1\alpha,25(OH)_2D_3$, $24,25(OH)_2D_3$ and PTH on bone formation in cultures of 9-day chick embryonic femora (11).

KCA-098 increased the length and calcium content of 9-day chick embryonic femur (Fig. 4, A and C) cultured for 6 days, whereas ipriflavone did not (Fig. 4, B and D). We observed that KCA-098 increased the alkaline phosphatase activity and the synthesis of collagenase-digestible protein of osteoblast-like cells, ROS 17/2.8 (manuscript in preparation), suggesting that the increase in cal-

cium content seen in organ culture resulted from the direct activation of osteoblastic activity. KCA-098 also increased the length of the femora, indicating that it may stimulate the proliferation of chondrocytes because at this stage of development, the elongation largely depends on their proliferation. Therefore, KCA-098 may stimulate the total bone formation by influencing both bone and cartilaginous tissues.

Ipriflavone had no effect on calcium accumulation in organ cultures of 9-day chick embryonic femurs (Fig. 4D). However, ipriflavone was shown to increase the alkaline phosphatase activity of osteoblast-like cells (4), suggesting that it stimulates the differentiation of osteoblasts. The discrepant results obtained from the organ and cell culture experiments can not be clearly explained at present. In organ culture, ipriflavone may affect other cells such as chondrocytes or fibroblasts to induce the synthesis or secretion of an inhibitor(s) of bone formation, resulting in no increase in calcium content of the femur.

To determine whether KCA-098 is effective on whole animals and thus applicable for the treatment of osteoporosis, we administered KCA-098 and ipriflavone separately to ovariectomized rats on a low-calcium diet, an experimental model of osteoporosis, and looked for a change in the physical properties of their femora. Ovariectomy brought about a significant decrease in uterine weight (234.0 ± 24.1 mg in sham-operated to 46.6 ± 1.4 mg in ovariectomized rat, P<0.01). The administration of KCA-098 or ipriflavone did not lead to recovery of the uterine weight, indicating that KCA-098 and ipriflavone had no estrogenic activity, even though KCA-098 is a derivative of coumestrol, a weak estrogen.

Ovariectomy significantly decreased the breaking force, i.e., the amount of force necessary to break the femora, as well as the bone density, expressed as ΣGS/D. Oral administration of KCA-098 (1 mg/kg/day) partially but significantly reversed the decrease in the breaking force, and ipriflavone (100 mg/kg/day) also increased it but not significantly (Fig. 5A). Both KCA-098 (1 and 3 mg/kg/day) and ipriflavone (100 mg/kg/day) significantly restored the bone density (Fig. 5B). These results show that KCA-098 and ipriflavone are effective on whole animals in improving bone loss. Yamazaki et al. (16) showed that administration of ipriflavone (25 mg/kg) had no effects on the restoration of the decrease in breaking force and breaking energy of the femur of prednisolone-induced osteoporotic rats, and only higher doses (100 and 400 mg/kg) of ipriflavone significantly reversed the decrease. In the ovariectomized rats examined in this study, administration of 100 mg/kg of ipriflavone was necessary to improve the decrease in bone density. Therefore, KCA-098 may be more potent than ipriflavone in improving the decrease in value of physical properties induced by

ovariectomy, because the administration of very low doses of KCA-098 (1 and 3 mg/kg) restored the decreased breaking force and bone density to their normal levels. This coincides with the findings that the effective concentrations of KCA-098 were lower than those of ipriflavone in inhibiting bone resorption of cultured fetal rat bones.

Both agents had direct action in inhibiting bone resorption as shown by the in vitro experiments. Ipriflavone was shown to increase the synthesis and secretion of calcitonin (17), and our preliminary experiment showed that KCA-098 also stimulated calcitonin secretion in rats (manuscript in preparation). Therefore, in addition to the direct action on bone resorption, in whole animals, KCA-098 and ipriflavone might have an indirect inhibitory effect on the bone resorption through the secretion of hormones. KCA-098 had a direct stimulatory effect on bone mineralization (Fig. 4, A and C) besides the inhibition of bone resorption. Such action would contribute to the improvement of bone loss. Although ipriflavone has a direct stimulatory effect on osteoblasts (4), it had no effect on bone length and calcium content in organ cultures (Fig. 4, B and D), suggesting that in an organ or the whole body, ipriflavone affected other cells as well as osteoblasts, and the direct activation of osteoblasts by ipriflavone may have been hidden by the commitment of other cells sensitized by ipriflavone. Therefore, the bone-strengthening effect of ipriflavone may be mainly dependent on the inhibition of bone resorption. On the other hand, KCA-098 may strengthen the bone by inhibiting bone resorption and also by stimulating bone formation.

In conclusion, KCA-098 and ipriflavone act in almost the same fashion on bone resorption, although the effective concentration of KCA-098 is lower than that of ipriflavone. Judging from the results obtained from the culture of 9-day chick embryonic femora, the action mechanism on bone formation might be different. Thus, KCA-098 may be a more potent stimulator of bone formation than ipriflavone and may be clinically useful for the treatment of osteoporosis.

REFERENCES

1 Fujita T: Introduction. Calcif Tissue Int 51, Supp 1, S1, (1992)
2 Tsuda M, Kitagaki T, Ito T and Fujita T: The effect of ipriflavone (TC-80) on bone resorption in tissue culture. J Bone Miner Res 1, 207–211 (1986)
3 Benvenuti S, Tanini A, Frediani U, Bianchi S, Masi L, Casano R, Bufalino L, Serio M and Brandi ML: Effects of ipriflavone and its metabolites on a clonal osteoblastic cell line. J Bone Miner Res 6, 987–996 (1991)
4 Kakai Y, Kawase T, Nakano T, Mikuni-Takagaki Y and Saito S: Effect of ipriflavone and estrogen on the differentiation and proliferation of osteogenic cells. Calcif Tissue Int 51, Supp 1, S11–S15 (1992)
5 Agnusdei D, Zacchei F, Bigazzi S, Cepollaro C, Nardi P, Montagnani M and Gennari C: Metabolic and clinical effects of ipriflavone in established post-menoposal osteoporosis. Drugs Exp Clin Res 15, 97–104 (1989)
6 Nakamura S, Morimoto S, Takamoto T, Onishi T, Fukuo K, Koh E, Kitano S, Miyashita Y, Yasuda O, Tamatani T, Nakahashi T and Ogihara T: Effect of ipriflavone on bone mineral density and calcium-related factors in elderly females. Calcif Tissue Int 51, Supp 1, S30–S34 (1992)
7 Tsutsumi N, Kawashima K, Arai N, Nagata H, Kojima M, Ujiie A and Endo H: In vitro effect of KCA-098, a derivative of coumestrol, on bone resorption of fetal rat femurs. Bone Miner 24, 201–209 (1994)
8 Kojima M, Tsutsumi N, Nagata H, Itoh H, Ujiie A, Kawashima K, Endo H and Okazaki M: Effect of KCA-098, a new benzofuroquinoline derivative, on bone metabolism. Biol Pharm Bull 17, 504–508 (1994)
9 Raisz LG: Bone resorption in tissue culture: factors influencing the response to parathyroid hormone. J Clin Invest 44, 103–116 (1965)
10 Endo H: Ossification in tissue culture. I. Histological development of the femur of chick embryo in various liquid media. Exp Cell Res 21, 151–163 (1959)
11 Endo H, Kiyoki M, Kawashima K, Naruchi T and Hashimoto Y: Vitamine D_3 metabolites and PTH synergistically stimulate bone formation of chick embryonic femur in vitro. Nature 286, 262–264 (1980)
12 Yamazaki I and Yamaguchi H: Characterization of an ovariectomized osteopenic rat model. J Bone Miner Res 4, 13–22 (1989)
13 Igarashi C and Ezawa I: Effects of whey calcium and lactulose on the strength of bone in ovariectomized osteoporosis model rats. Pharmacometrics 42, 245–253 (1991) (Abstr in English)
14 Morita I, Sakaguchi K, Kurachi T and Murota S-I: Ipriflavone inhibits murine osteoclast formation in vitro. Calcif Tissue Int 51, Supp 1, S7–S10 (1992)
15 Notoya K, Yoshida S, Taketomi I, Yamazaki I and Kumegawa M: Inhibitory effect of ipriflavone on pit formation in mouse unfractionated bone cells. Calcif Tissue Int 51, Supp 1, S3–S6 (1992)
16 Yamazaki I, Shino A, Shimizu Y, Tsukuda R, Shirakawa Y and Kinoshita M: Effect of ipriflavone on glucocorticoid-induced osteoporosis in rats. Life Sci 38, 951–958 (1986)
17 Watanabe K, Takekoshi S and Kakudo K: Effects of ipriflavone on calcitonin synthesis in C cells of the rat thyroid. Calcif Tissue Int 51, Supp 1, S27–S29 (1992)