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Effect of KCA-098 on the Function of Osteoblast-Like Cells and the Formation of TRAP-Positive Multinucleated Cells in a Mouse Bone Marrow Cell Population

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ABSTRACT. KCA-098 (3,9-bis(N,N-dimethylcarbamoyloxy)-5H-benzofuro[3,2-c]quinoline-6-one), an analogue of coumestrol (a naturally occurring weak phytoestrogen), dose-dependently increased alkaline phosphatase activity of osteoblastic ROS 17/2.8 cells and freshly-isolated osteoblasts from neonatal mouse calvaria, and reduced cell proliferation. In addition, KCA-098 increased the synthesis of collagenese-digestible protein (CDP) of ROS 17/2.8 cells. On the other hand, KCA-098 had no effect on the basal synthesis of osteocalcin but reduced the 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$)-induced increase in osteocalcin synthesized by ROS 17/2.8 cells. Therefore, KCA-098 had a bidirectional effect on the differentiation of osteoblasts (i.e., stimulating both alkaline phosphatase activity and synthesis of CDP and inhibiting osteocalcin synthesis). However, as KCA-098 stimulated the mineralization of chick embryonic bone in organ culture and recovered the bone density reduced by ovariectomy of rats, it would serve overall to stimulate the differentiation of osteoblasts. On the other hand, KCA-098 inhibited the formation of tartrate-resistant, acid phosphate-positive multinucleated cells (TRAP(+)MNC) induced by 1α,25$(OH)_2D_3$, parathyroid hormone (PTH), and prostaglandin E_2 (PGE_2) in cultures of mouse bone marrow cells, showing that it inhibits the formation of osteoclast-like cells. Coumestrol and 17β-estradiol had no effect on the proliferation and alkaline phosphatase activity of ROS 17/2.8 cells. They did, however, dose-dependently inhibit osteoclast-like cell formation as well as KCA-098 did, indicating that the main action of coumestrol and 17β-estradiol on bone tissue is the inhibition of bone resorption. BIOCHEM PHARMACOL 51;2:133–139, 1996.

KEY WORDS. 3,9-bis(N,N-dimethylcarbamoyloxy)-5H-benzofuro[3,2-c]quinoline-6-one (KCA-098); coumestrol; TRAP(+)MNC; bone resorption; alkaline phosphatase activity; osteoblast-like cell

Loss of estrogen in animals and humans causes a dramatic and precipitous loss of bone that can be prevented by estrogen replacement [1, 2]. However, the continuous administration of estrogen is sometimes accompanied by severe adverse effects such as vaginal bleeding [3] and carcinogenesis [4]. Thus, a compound that has bone-metabolizing activity, but no estrogenic activity, would be a more suitable therapeutic drug than estrogen for treatment of osteoporosis. KCA-098§ (Fig. 1), one of the analogues of coumestrol (a weak phytoestrogen, Fig. 1), was reported to have no estrogenic activity as estimated by the lack of increase in uterine weight of ovariectomized rats [5]. It also inhibited bone resorption of fetal rat femora [5, 6] and increased the calcium and phosphorus contents of fetal rat and chick embryonic femora in organ culture [5, 6]. In addition, oral administration of KCA-098 to ovariectomized rats, an experimental model of postmenopausal osteoporosis, increased both the bone density and the breaking force or amount of force required to break a bone [6, 7]. Therefore, KCA-098 may be a useful compound for treatment of bone diseases such as osteoporosis.

In this paper, to further investigate the mechanism of action of KCA-098 on bone tissues, we conducted cell culture experiments in which KCA-098 was added to cultures of osteoblastic ROS 17/2.8 cells to observe possible changes in alkaline phosphatase, collagenase-digestible protein-synthesizing, and osteocalcin-synthesizing activities. In addition, alkaline phosphatase activity of freshly isolated osteoblasts from neonatal mouse calvaria was also studied. As PTH and 1,25$(OH)_2D_3$ were shown to stimulate osteoclast-like cell formation in cultures of mouse bone marrow cells [8], we employed this system to analyze the inhibitory effect of KCA-098 on bone resorption. Finally, we also studied the effects of coumestrol, the parent compound of KCA-098, and 17β-estradiol, a strong estrogen, on the behavior of osteoblastic cells and on the formation of osteoclast-like cells.

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§ *Abbreviations:* KCA-098, 3,9-bis(N,N-dimethylcarbamoyloxy)-5H-benzofuro[3,2-c]quinoline-6-one; 1α,25$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; CDP, collagenase-digestible protein; NCP, noncollagenous protein; TRAP(+)MNC; tartrate-resistant, acid phosphatase-positive multinucleated cells; PTH, parathyroid hormone; PGE_2, prostaglandin E_2.

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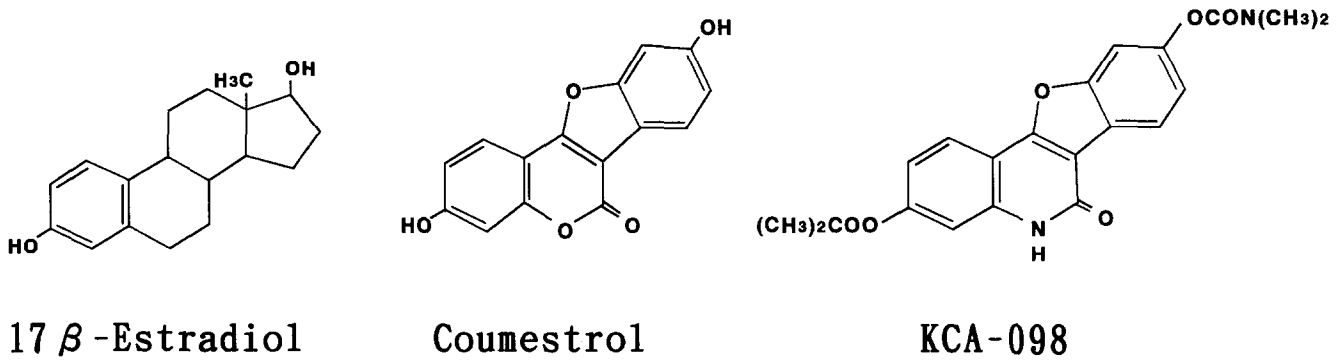

17β-Estradiol Coumestrol KCA-098

FIG. 1. Chemical structures of coumestrol, KCA-098, and 17β-estradiol.

MATERIALS AND METHODS

Reagents

Reagents used and their sources were as follows: KCA-098, Kissei Pharmaceutical Co., Ltd., Matsumoto, Japan); $1\alpha,25(OH)_2D_3$, PGE_2, assay kit for alkaline phosphatase (Wako Pure Chemical Co., Ltd., Osaka, Japan); PTH (fragment 1-34, Peptide Institute Inc., Osaka, Japan); 17β-estradiol, collagenase type VII (Sigma Chemical Co., St Louis, MO); dispase (Godo Shusei Co., Tokyo, Japan); radioimmunoassay kit for rat osteocalcin (Biomedical Technolgies Inc., MA); coumestrol (Eastman Kodak Co., Rochester, NY); Hams F12 and MEM medium (GIBCO BRL, Life Technologies, Inc., NY); DMEM and αMEM (Flow Laboratories, Inc., VA).

Animals

Six-week-old male mice, ddy strain, were obtained from Japan SLC, Shizuoka, Japan.

Cell Cultures

Osteoblastic ROS 17/2.8 cells (purchased from RIKEN Cell Bank, Tsukuba, Japan) were cultured in F-12 supplemented with 10% FCS. All cultures were maintained in a humidified atmosphere of 5% CO_2 in air at 37°C.

Preparation of Cultures of Osteoblasts Freshly Isolated from Neonatal Mouse Calvaria

Freshly-isolated osteoblasts were obtained from neonatal mouse calvaria [9]. Briefly, the calvariae isolated from neonatal mice (2 ~ 3 days after birth) were digested with 0.1% collagenase and 0.1% dispase for 5 min. This procedure was repeated 6 times, and the cell suspensions obtained from the supernatants of the 3rd to 6th digestions were collected and pooled. After the cell suspension had been washed twice with medium by centrifugation (3000 rpm, 5 min), 10^6 cells were inoculated into 60-mm plastic dishes containing MEM + 10% FCS. The cells were subcultured after having reached confluence; after 5×10^6 cells had been incubated at 37°C for 60 min, the adherent cells were used for the experiments. The alkaline phosphatase activity of these cells was at the same level as that of ROS 17/2.8 cells.

Determination of Alkaline Phosphatase Activity

ROS 17/2.8 (10^5) and freshly-isolated osteoblasts (10^5) were inoculated into ϕ 60-mm plastic dishes and incubated overnight. Then, the medium was replaced with fresh medium containing vehicle (0.01% DMSO or 0.1% ethanol) or various concentrations of KCA-098, coumestrol or 17β-estradiol, and the incubation was continued for a further 48 h. The cells, after incubation, were washed twice with PBS(−), scraped into 1 mL of 10 mM Tris-HCl (pH 7.6) buffer containing 0.1% Triton X-100 on ice, and centrifuged. The supernatant was stocked in a deep freezer (−80°C) until use. An aliquot of supernatant was used for the determination of alkaline phosphatase activity by measuring the release of p-nitrophenol from p-nitrophenylphosphate.

Determination of Collagen- and Noncollagenous Protein-Synthesizing Activity

ROS 17/2.8 (1.7×10^4) cells were inoculated into ϕ 35-mm plastic dishes and incubated overnight. Then, the medium was replaced with fresh medium containing vehicle (0.01% DMSO) or various concentrations of KCA-098, and the cells were incubated for an additional 48 h. The cells were labeled with L-[2,3,^{3}H]-proline (185 kBq) for the final 4 hr of this culture period. The cells were washed twice with PBS(−) and then scraped into PBS(−). The protein was precipitated with TCA (final concentration of 5%), washed twice with 5% TCA, ethanol-ether (3:1) twice, absolute ether once and, finally, dried. The dried samples were reconstituted in 0.1 M HEPES buffer (pH 7.4) supplemented with 5 mM $CaCl_2$. Aliquots of reconstituted samples were digested with 50 μg/mL collagenase type VII at 37°C for 120 min in a water bath. The reaction was stopped by the addition of 5% TCA containing 0.25% tannic acid, and the supernatant was counted for collagenase digestible protein (CDP). The precipitates were dissolved in SOLUEN^R-350 (United Technologies Packard, IL),

and were counted for noncollagenous protein (NCP). CDP (%) was calculated by the following formula of Diegelmann and Peterkofsky [10]:

$$\text{CDP (\%)} = \frac{\text{radioactivity of CDP}}{\text{radioactivity of CDP} + 5.4\ x\ (\text{radioactivity of NCP})} \times 100$$

Determination of Osteocalcin Contents

ROS 17/2.8 cells (10^5) were inoculated into ϕ 60-mm plastic dishes and incubated overnight. Then, the medium was replaced with fresh medium containing vehicle (0.01% DMSO and 0.1% ethanol) or various concentrations of KCA-098 with or without $1\alpha,25(OH)_2D_3$ (10^{-8}M); the incubation was continued for a further 48 h. The content of osteocalcin in the medium was determined by radioimmunoassay.

TRAP[+]MNC Formation

TRAP(+)MNC formation from mouse bone marrow cells was performed according to Takahashi *et al.* [8]. Bone marrow cells (10^{-4}) obtained from the tibia of 7 ~ 10-week-old male mice were plated in each well of a multiplate (24-well type) in 0.5 mL of α-MEM containing 10% FCS. Cultures were fed every 2 days by replacing 0.4 mL of the old medium with fresh medium. $1\alpha,25\text{-}(OH)_2D_3$, PTH, or PGE_2 with or without various concentrations of KCA-098, coumestrol, or 17β-estradiol were added at the second day of culture and at each time of medium change. All cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air, and the incubation was continued for 8 days. The cells were then fixed with 10% formaldehyde and stained for tartrate-resistant acid phosphatase activity [11]. TRAP-positive cells containing three or more nuclei were counted as osteoclast-like cells.

Statistical Analysis

Data were expressed as means ± standard errors of means (SEM). Statistical significance was determined by Student's *t*-test.

RESULTS

Effect of KCA-098, Coumestrol, and 17β-Estradiol on the Growth and Alkaline Phosphatase Activity of ROS 17/2.8 Cells

KCA-098 dose-dependently inhibited the growth of ROS 17/2.8 cells; coumestrol did not, and 17β-estradiol did so only slightly but not significantly (Fig. 2). KCA-098 dose-dependently increased the alkaline phosphatase activity of ROS 17/2.8 cells to a value 5 times control activity at 10^{-5} M, whereas coumestrol and 17β-estradiol had no effect (Fig. 2).

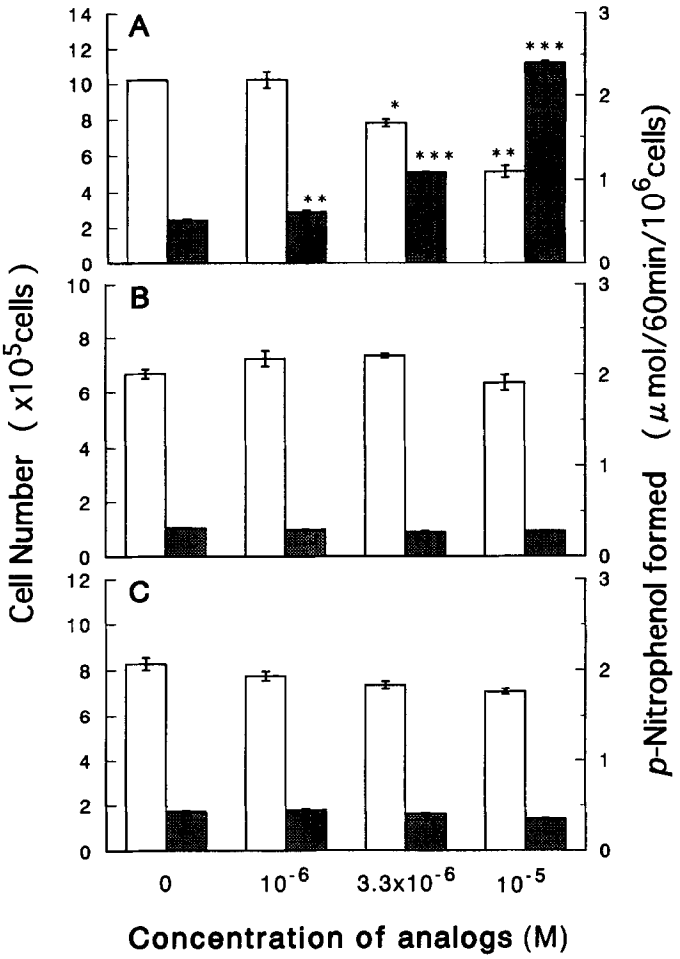

FIG. 2. Effect of KCA-098, coumestrol, and 17β-estradiol on the growth and alkaline phosphatase activity of ROS 17/2.8 cells. ROS 17/2.8 cells (10^5 cells) were inoculated into 60-mm plastic dishes and cultured for 48 hr with various concentrations of KCA-098 (A), coumestrol (B), or 17β-estradiol (C); then, cell number (□) and alkaline phosphatase activity (▨) were determined. Values represent the mean ± SEM (n = 4). *P < 0.05 **P < 0.01 ***P < 0.001 vs control (without analogs).

Effect of KCA-098 on the Growth and Alkaline Phosphatase Activity of Freshly-Isolated Osteoblasts

KCA-098 dose-dependently stimulated alkaline phosphatase activity of osteoblasts freshly isolated from newborn rat calvaria, whereas it inhibited cell proliferation (Fig. 3).

Effect of KCA-098 on the Synthesis of Collagenase-Digestible Protein (CDP) of ROS 17/2.8 Cells

KCA-098 increased the incorporation of L-[2,3,^{3}H]-proline into both CDP and noncollagenous protein (NCP) (data not shown). The incorporation of L-[2,3,^{3}H]-proline into CDP was more prominent than that into NCP. Therefore, it dose-dependently increased the % CDP of ROS 17/2.8 cells (Fig. 4).

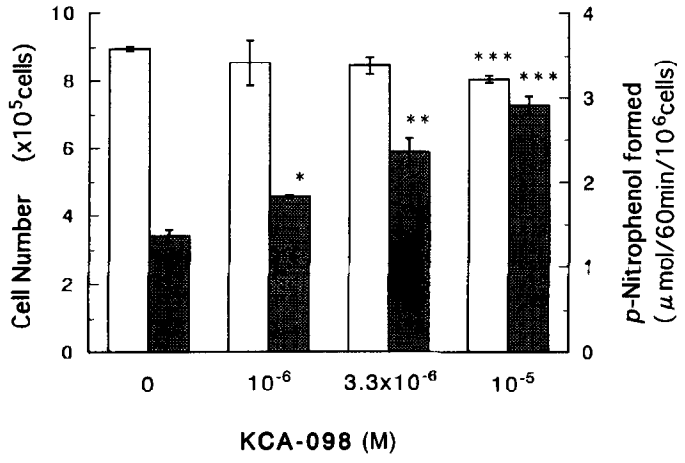

FIG. 3. Effect of KCA-098 on the growth and alkaline phosphatase activity of freshly-isolated osteoblasts. Freshly-isolated osteoblasts (10^5 cells) from neonatal mouse calvaria were inoculated into 60-mm plastic dishes and cultured for 48 hr with various concentrations of KCA-098; then, cell number (□) and alkaline phosphatase activity (▨) were determined. Values represent the mean ± SEM ($n = 4$).
***$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs control (without KCA-098).**

Effect of KCA-098 on the Synthesis of Osteocalcin by ROS 17/2.8 Cells

$1\alpha,25(OH)_2D_3$ increased the content of osteocalcin secreted into the medium (Fig. 5), but KCA-098 alone did not (data not shown). However, KCA-098 dose-dependently inhibited $1\alpha,25(OH)_2D_3$-stimulated osteocalcin synthesis, but stimulated alkaline phosphatase activity (Fig. 5).

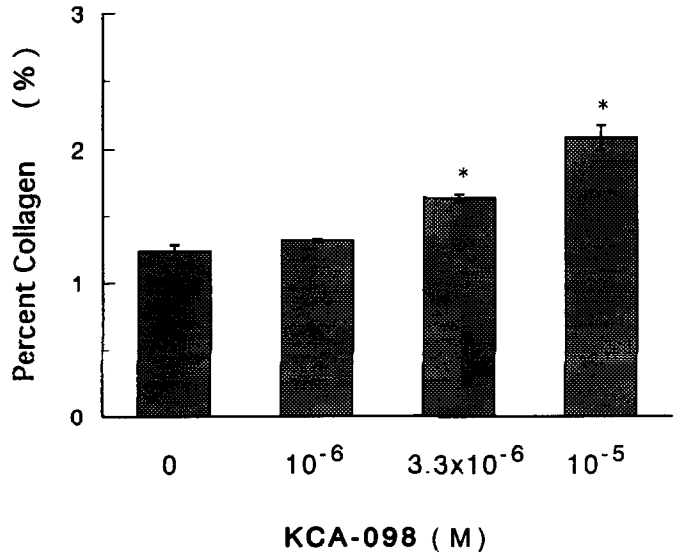

FIG. 4. Effect of KCA-098 on the synthesis of collagenase-digestible protein (% CDP) of ROS 17/2.8 cells. ROS 17/2.8 cells were cultured with various concentrations of KCA-098 for 48 hr. CDP (%) was determined by the incorporation of L-[2,3,^{3}H]-proline into collagenase-digestible protein during the last 4 hr of the culture period. Values represent the means ± SEM ($n = 4$).
***$P < 0.001$ vs control (without KCA-098).**

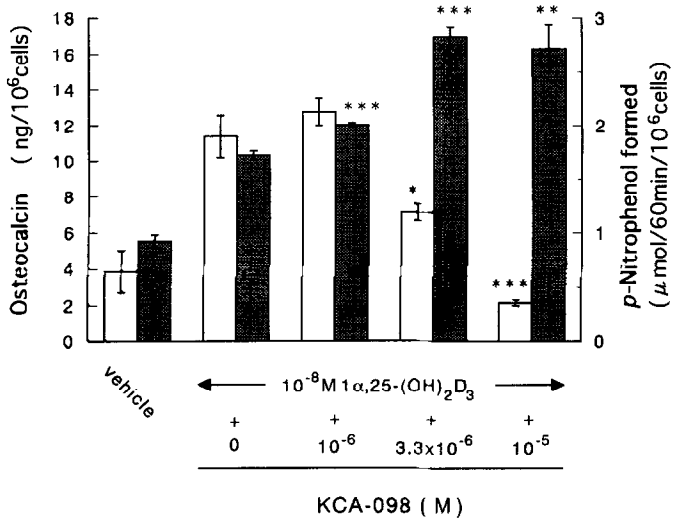

FIG. 5. Effect of KCA-098 on the osteocalcin synthesis of ROS 17/2.8 cells. ROS 17/2.8 cells (10^5 cells) were inoculated into 60-mm plastic dishes and cultured for 48 hr with $1\alpha,25(OH)_2D_3$ (10^{-8} M) with or without various concentrations of KCA-098; then, osteocalcin content in the medium (□) and alkaline phosphatase activity of the cells (▨) were determined. Values represent the mean ± SEM ($n = 4$).
***$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs $1\alpha,25(OH)_2D_3$ (10^{-8} M).**

Effect of KCA-098, Coumestrol, and 17β-Estradiol on Osteoclast-Like Cell Formation

KCA-098, coumestrol, and 17β-estradiol dose-dependently inhibited TRAP-positive MNC formation induced by $1\alpha,25(OH)_2D_3$ (Fig. 6, A–C). KCA-098 also inhibited PGE_2- and PTH-induced TRAP-positive MNC formation (Fig. 7A, B).

DISCUSSION

Hormones, such as calcitonin and vitamin D_3, which regulate the calcium balance in the body, are used for treatment of osteoporosis. Estrogen is also applied for postmenopausal osteoporosis. The chemically-synthesized substance ipriflavone is also used therapeutically [12, 13]. The main mechanism of action of most of these agents, except vitamin D_3, is the inhibition of bone resorption. However, a substance that inhibits bone resorption while stimulating bone formation would be more suitable for treatment of bone diseases. We previously found that KCA-098, a chemically synthesized analogue of coumestrol (a weak estrogen), has such properties of inhibiting bone resorption and stimulating bone mineralization in organ cultures of fetal rat and chick embryonic bones [5, 6]. Oral administration of KCA-098 to ovariectomized rats for 6 weeks led to an increase in both breaking force and bone density [7]. Restriction of exercise by confining a rat to a small cage also induces osteopenia [14]; KCA-098 restored the decrease in breaking force, breaking energy, and bone density of the femora observed in such confined animals [14]. These results show that KCA-098 is effective in whole animals and is a unique and interesting compound that has the possibility of improving bone diseases. However, all these observations were obtained

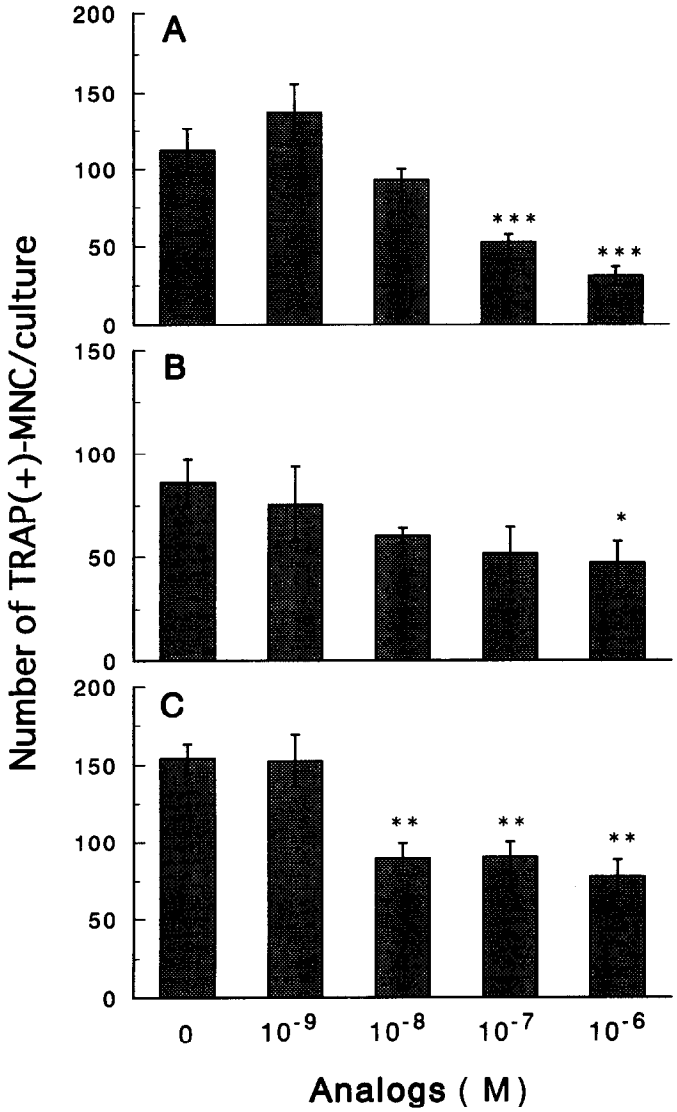

FIG. 6. **Inhibitory effect of KCA-098, coumestrol, and 17β-estradiol on TRAP(+)MNC formation in mouse bone marrow cell cultures. TRAP(+)MNC formation was induced by $1\alpha,25(OH)_2D_3$ (5×10^{-8} M) in cultures of mouse bone marrow cells. $1\alpha,25(OH)_2D_3$ was added on the second day of culture and at the time of medium change. KCA-098 (A), coumestrol (B), or 17β-estradiol (C) was added simultaneously with $1\alpha,25-(OH)_2D_3$. After the 8th day of culture, the cells were stained for TRAP(+)MNC. Values represent the means ± SEM ($n = 4$).**
P < 0.05, **P < 0.01, ***P < 0.001 vs control (without agonists).

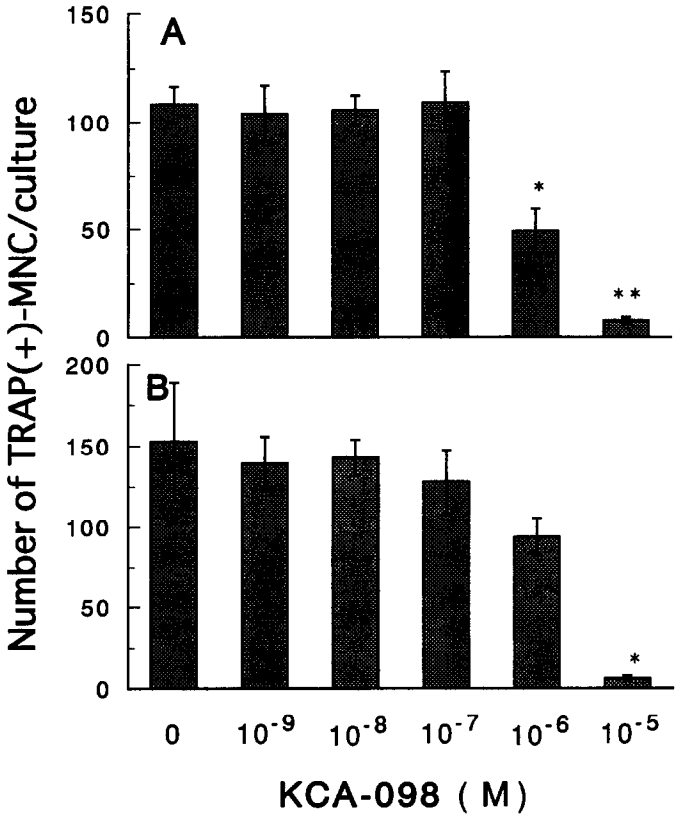

FIG. 7. **Inhibitory effect of KCA-098 on TRAP(+)MNC formation induced by PTH or PGE_2. TRAP(+)MNCs were induced by PTH (A, 2×10^{-8} M) or PGE_2 (B, 2×10^{-7} M). The hormone or prostaglandin was added on the second day of culture and at the time of medium change. KCA-098 was added simultaneously with PTH or PGE_2. At the 8th day of culture, the cells were stained for TRAP(+)MNC. Values represent the means ± SEM ($n = 4$).**
P < 0.05, **P < 0.01 vs control (without KCA-098).

from experiments utilizing organ cultures or whole animals. In this study, to further investigate the effect of KCA-098 on bone metabolism we employed cell culture systems.

Alkaline phosphatase is the most widely recognized biochemical marker for osteoblastic activity. Although its precise mechanism of action is poorly understood, this enzyme is believed to play a role in bone mineralization. Therefore, we examined the effect of KCA-098 on the alkaline phosphatase activity of the rat osteosarcoma cell line ROS 17/2.8 and of osteoblasts freshly isolated from neonatal mouse calvaria. KCA-098 dose-dependently increased alkaline phosphatase activity and concomitantly reduced cell proliferation of both ROS 17/2.8 cells and freshly-isolated osteoblasts. This inhibitory effect on cell proliferation was not a consequence of possible toxicity of KCA-098 because withdrawal of the drug from the medium restored growth to the normal level (data not shown). Such an opposite effect is usually observed between the differentiation and proliferation of cells [15]. Therefore, KCA-098 seems to stimulate the differentiation of osteoblasts. In addition, KCA-098 augmented the % CDP of ROS 17/2.8 cells, indicating that it increased collagen synthesis. This result also supports the stimulatory nature of KCA-098 toward the function of osteoblastic cells.

However, KCA-098 had no effect on the basal level of osteocalcin synthesis. Moreover, it inhibited $1\alpha,25(OH)_2D_3$-stimulated osteocalcin synthesis. The reason why KCA-098 inhibited osteocalcin synthesis is not known. Osteocalcin is the specific product of osteoblasts and is believed to have a role in bone formation. However, its exact role in bone metabolism is not known. Bone formation is accomplished by many processes, including the differentiation and proliferation of osteoblasts, the synthesis of many kinds of matrix proteins,

and mineralization. Therefore, it is unlikely that only one substance can stimulate all of these processes. KCA-098 alone increased alkaline phosphatase activity and the synthesis of CDP, but not the synthesis of osteocalcin. However, it did stimulate the mineralization of chick embryonic bone in organ culture [6] and recovered the density of rat bone decreased by ovariectomy [6]. Therefore, KCA-098 may synergistically act with other substances existing in bone matrix to stimulate the synthesis of osteocalcin and induce mineralization in organ culture. Another possibility is that osteocalcin regulates bone resorption. When the content of osteocalcin in bone was decreased by the administration of warfarin to rats, excess mineralization was observed, indicating that the decrease in osteocalcin brought about the inhibition of bone resorption [16]. In addition, osteocalcin is reported to be a chemoattractant factor of monocytes and macrophages [17], cells having the same lineage as osteoclasts. Osteocalcin is also reported to bind osteopontin [18], known to be an anchor substance for the attachment of osteoclasts to bone surfaces. These results suggest that osteocalcin may be a factor that stimulates bone resorption. KCA-098 inhibited $1\alpha,25(OH)_2D_3$-induced bone resorption of fetal rat bone in culture [5], and inhibited $1\alpha,25(OH)_2D_3$-induced osteocalcin synthesis. Therefore, KCA-098 might inhibit bone resorption by reducing osteocalcin synthesis.

To analyze the inhibitory effect of KCA-098 on bone resorption, we tested the effect of the drug on osteoclast-like cell formation. KCA-098 dose-dependently inhibited TRAP(+)MNC formation induced by $1\alpha,25(OH)_2D_3$, PTH, or PGE_2 in bone marrow cell cultures, suggesting that KCA-098 may block osteoclast formation at a point common to all mechanisms that induce it. However, the mechanism of the inhibitory action of KCA-098 on osteoclast-like cell formation is unknown. Many investigators have shown that various agents modulate the synthesis and secretion by osteoblasts of mediators that stimulate or inhibit osteoclast formation. For example, $1\alpha,25(OH)_2D_3$ and PTH stimulate the secretion of soluble or insoluble factors (bone resorption-stimulating activity = BRSA) from osteoblast-like cells that stimulate osteoclastic activity [19], and estrogen stimulates the secretion of an inhibitory factor of BRSA [20]. TNF and IL-1 stimulate IL-6 production [21], and estrogen inhibits IL-6 secretion in osteoblasts [22]. $1\alpha,25(OH)_2D_3$ also induces PGE_2 production [23]. Thus, one possibility is that KCA-098 may also modulate the synthesis and secretion of such mediators in osteoblasts to regulate bone resorption. Another possibility is that KCA-098 acts directly on preosteoclasts to inhibit their maturation, or directly inhibits the activity of the mature osteoclast.

KCA-098 is a synthetic substance that lacks the weak estrogenic activity of its parent compound, coumestrol. Estrogen replacement therapy is an effective maneuver for the prevention and treatment of bone loss. Therefore, to compare the effect of KCA-098 with that of coumestrol and 17β-estradiol on bone-metabolizing activity, we added coumestrol and 17β-estradiol separately to the cell culture systems. Recent binding studies using radiolabeled estradiol demonstrated that a functional estrogen receptor is present in rat and human osteoblastic cells [24, 25]. However, the effect of estrogen on the proliferation and differentiation of osteoblastic cells is very confusing. Gray *et al.* showed that estrogen inhibits proliferation of rat osteosarcoma cell line UMR 106 and stimulates alkaline phosphatase activity [26]. On the other hand, estrogen had no effect on DNA synthesis, alkaline phosphatase activity, or bone Gla protein level in cultured osteoblastic cells [27]. In the present study, we also found that both 17β-estradiol and coumestrol had no effect on the proliferation and alkaline phosphatase activity of ROS 17/2.8 cells. Ikegami *et al.* recently showed that expression of the estrogen receptor is restricted to the S phase of a synchronized human osteoblast-like osteosarcoma cell line, HOS TE 85 cells [28]. The stimulation of DNA synthesis and proliferation of cells was observed only with synchronized cells. The same events might occur in ROS 17/2.8 cells, which would indicate that both coumestrol and 17β-estradiol had no effects on the nonsynchronized cells.

On the contrary, coumestrol and 17β-estradiol dose-dependently inhibited $1\alpha,25(OH)_2D_3$-induced TRAP(+)MNC formation in the cultures of mouse bone marrow cells. Coumestrol [5] and 17β-estradiol (data not shown) both inhibited the release of ^{45}Ca from cultured fetal rat femora. Therefore, inhibition of bone resorption by coumestrol and 17β-estradiol partly depends on the inhibition of osteoclast formation. The potency of the inhibitory effect was almost the same. 17β-estradiol has the most potent estrogenic activity of the hormones studied, as estimated by the increase in uterine weight of ovariectomized rats. Coumestrol is 4 orders of magnitude less potent than 17β-estradiol, whereas KCA-098 has no activity [5]. There is, thus, no relationship between the potency of estrogenic activity and the degree of inhibition of osteoclast-like cell formation. This result may indicate that KCA-098 has its own receptor rather than utilizing the estrogen receptor. In fact, our preliminary experiment with ROS 17/2.8 cells showed that tamoxifen (10^{-5} M) had no effect on KCA-098-stimulated alkaline phosphatase activity (data not shown). There may be a common structure among coumestrol, 17β-estradiol, and KCA-098 that controls bone resorption but not estrogenic activity. The identification of such a structure should contribute to the development of new drugs for the treatment of bone diseases.

These results show that the main action of coumestrol and 17β-estradiol on bone tissues is the inhibition of bone resorption, whereas the effect of KCA-098, a derivative of coumestrol, covers both the stimulation of bone mineralization and inhibition of bone resorption.

In conclusion, KCA-098 affects osteoblastic cells to stimulate the differentiation of the cells, at the same time inhibitory osteoclast-like cell formation. Our findings, thus, support the results of inhibition of bone resorption and stimulation of bone mineralization obtained by organ culture experiments.

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