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Inhibitory effect of menaquinone-7 (vitamin K_2) on the bone-resorbing factors-induced bone resorption in elderly female rat femoral tissues *in vitro*

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Abstract

The inhibitory effect of menaquinone-7 (MK-7; vitamin K_2) on osteoclast-like cell formation and osteoclastic bone resorption *in vitro* is found (Mol Cell Biochem 228: 39–47, 2001). This study, furthermore, was undertaken to determine the effect of MK-7 on the bone-resorbing factor-induced bone resorption using the femoral-diaphyseal and -metaphyseal tissues obtained from elderly female rats *in vitro*. Femoral-diaphyseal and -metaphyseal tissues were cultured for 48 h in Dulbecco's modified Eagle's medium (high glucose, 4.5%) supplemented with antibiotics and bovine serum albumin. The experimental cultures contained MK-7 (10^{-7}–10^{-5} M). The bone-resorbing factors, parathyroid hormone (1–34) (PTH; 10^{-7} M) and prostaglandin E_2 (PGE_2; 10^{-5} M), caused a significant decrease in calcium content in the diaphyseal and metaphyseal tissues. The PTH or PGE_2-induced decrease in bone calcium content was completely inhibited in the presence of MK-7 (10^{-7}–10^{-5} M). In addition, MK-7 (10^{-7}–10^{-5} M) completely prevented the PTH (10^{-7} M)- or PGE_2 (10^{-5} M)-induced increase in medium glucose consumption and lactic acid production by bone tissues. These results support the view that MK-7 has a direct inhibitory effect on the bone-resorbing factor-induced bone resorption in bone culture using female aged femoral tissues *in vitro*. (Mol Cell Biochem **245**: 115–120, 2003)

Key words: menaquinone-7 (MK-7), vitamin K_2, bone resorption, aging, osteoporosis

Introduction

Bone loss with increasing age induces osteoporosis [1–3]. This loss may be due to induced bone resorption and decreased bone formation. A decrease in bone mass leads to bone fracture. Osteoporosis is widely recognized as a major public health problem [4]. Postmenopausal osteoporosis is resulted from estrogen deficiency. This is partly involved in the deterioration of bone metabolism with increasing age. Pharmacological and nutritional factors are important in preventing age-related bone loss.

There is growing evidence that vitamin K, which is a nutritional factor, may play a role in the regulation of bone metabolism. Vitamin K_2 (menaquinone) is essential for the γ-carboxylation of osteocalcin, a bone matrix protein containing γ-carboxyglutamic acids, which is synthesized in osteoblast of bone tissues [5–7]. MK-7 with seven isoprene units, one of analog of vitamin K_2, is abundant in fermented soybean (*natto*) [8]. It was recently demonstrated that the prolonged dietary intake of MK-7 has a preventive effect on bone loss induced by ovariectomy in rats [9, 10]. Moreover, it has been reported that the dietary intake MK-7 may enhance γ-carboxylation of osteocalcin in the serum of normal individuals [11, 12]. These observations support the view that dietary MK-7 may have a useful role in the prevention of osteoporosis on the basis of the direct promotion of γ-carboxylation of osteocalcin, which is important in the promotion of bone calcification [6, 7].

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Whether MK-7 has a direct anabolic effect on bone metabolism *in vitro* has not been fully clarified, however. It has been recently demonstrated that MK-7 has an anabolic effect on osteoblastic MC3T3-E1 cells and bone tissues *in vitro*, suggesting that the compound can stimulate osteoblastic bone formation [13]. Moreover, MK-7 has an inhibitory effect on osteoclast-like cell formation in bone marrow culture system *in vitro* and a suppressive effect on osteoclasts isolated from rat femoral tissues *in vitro* [14], suggesting that MK-7 can inhibit osteoclastic bone resorption *in vitro*.

The present study, furthermore, was undertaken to determine whether MK-7 can inhibit the bone-resorbing factors-induced bone resorption using the femoral-diaphyseal and -metaphyseal tissues obtained from female aged rats, which bone metabolism was deteriorated. We found that MK-7 could inhibit the PTH or PGE_2-induced bone resorption in bone culture of female aged rat femoral tissues *in vitro*, suggesting that MK-7 can inhibit bone resorption with increasing age.

Materials and methods

Chemicals

Dulbecco's modified Eagle's medium and penicillin-streptomycin solution (5000 units/ml penicillin; 5000 µg/ml streptomycin) were obtained from Gibco laboratories (Grand island, NY, USA). Bovine serum albumin (BSA) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). MK-7 (vitamin K_2; 96.8% purity) was supplied by Honen Corporation (Tokyo, Japan), which was highly purified from the fermented soybean (*natto*). MK-7 was dissolved in ethanol solution (20%). PGE_2 and synthetic human parathyroid hormone were purchased from Sigma. [PTH (1–34)]. Calcium chloride and other chemicals were of reagent grade from Wako Pure Chemical Industries (Osaka, Japan). All water used was glass-distilled.

Animals

Female Wistar rats (conventional), weighing 90–100 g (4 weeks old) or 220–250 g (50 weeks old) were obtained from Japan SLC (Hamamatsu, Japan). The animals were fed commercial laboratory chow (solid) containing 1.1% calcium and 1.1% phosphorus at room temperature of 25°C, with free access to distilled water.

Bone culture

Bone culture was carried out with the procedure as reported previously [15]. Femoral-diaphyseal and -metaphyseal tissues from 4- or 50-week-old female rats were removed aseptically. These tissues were then cultured in a 35-mm dish in 2.0 ml medium consisting of Dulbecco's modified Eagle's medium (high glucose; 4.5%) supplemented with 0.25% bovine serum albumin (fraction V) plus antibiotics, with either bone-resorbing factors (PTH or PGE_2) or vehicle (sterile distilled water) in the absence or presence of MK-7 (10^{-7}–10^{-5} M). Cultures were maintained at 37°C in a water-saturated atmosphere containing 5% CO_2 and 95% air for 48 h.

Bone calcium

The bone tissues were dried for 16 h at 120°C, weighed, and then dissolved in nitric acid solution [15]. Calcium was determined by atomic absorption spectrophotometry [16]. The bone calcium content was expressed as milligrams of calcium per gram of dry bone.

Determination of medium glucose and lactic acid

The concentration of glucose in the medium cultured with bone for 48 h was determined by the colorimetric method using *o*-toludine [17]. Dry weight of the bone tissue was measured after extraction with 5.0% trichloroacetic acid, acetone, and ether. The medium glucose consumed by bone culture in 48 h was expressed as milligrams of glucose per gram of dry bone tissue. Likewise, the medium lactic acid was measured by the enzymatic method previously described [18]. Data were expressed as milligrams of lactic acid per gram of dry bone tissue.

Statistical methods

Data are expressed as means ± S.E.M. Statistical differences were analyzed using Student's paired *t*-test; p values of less than 0.05 were considered to indicate statistically significant difference.

Results

Change in calcium content, glucose consumption and lactic acid production in bone tissues with aging

Femoral-diaphyseal and -metaphyseal tissues obtained from young (4 weeks old) or elderly (50 weeks old) female rats were cultured for 48 h in a serum-free medium without MK-7. Bone calcium content was significantly decreased in the femoral-diaphyseal and -metaphyseal tissues from elderly rats as compared with those of young rats (Fig. 1). Medium