Low-Dose Caffeine Enhances Osteoclast Differentiation from Bone Marrow Hematopoietic Stem Cells and Reduces Bone Mineral Density in Growing Rats

INTRODUCTION:
Caffeine-containing beverage consumption has been reported to be associated with reduced bone mass and increased fracture rate in some, but not most, observational studies (1, 2). Heaney suggested that caffeine has no harmful effect on bone status or on the calcium economy in individuals who ingest the currently recommended daily allowances of calcium (1). However, the study of Rapuri and colleagues has indicated that intakes of caffeine in amounts >300 mg/d (approximately 514 g, or 18 oz, brewed coffee) accelerate bone loss at the spine in elderly postmenopausal women (3). Wink and colleagues have also shown that if young, rapidly growing rats are exposed to caffeine, disruption of osteoblasts and retarded bone development occur (4). Therefore, low caffeine consumption has been recommended. However, the effects of caffeine on bone cell differentiation and bone mass need to be further clarified. In the present study, we investigated the effects of low-dose caffeine on the differentiation of bone marrow progenitor cells (HSCs) and bone resorption activity. These in vitro results indicate that caffeine is capable of enhancing the osteoclastogenesis. The results in animal study also showed that caffeine (about 22 mg and 44 mg caffeine/day by diet intake for 20 weeks) significantly reduces BMD and bone calcium content and enhances the osteoclastogenesis of HSCs in growing rats. These findings imply that the enhancement of osteoclastogenesis may cause the reduction in BMD in growing rats fed caffeine-supplemented diets.

METHODS:

Cell culture. Bone marrow cells were prepared by removing femurs from 5-week-old ICR mice and flushing the bone marrow cavity with αMEM containing 10% FBS. Osteoblastogenesis. Osteoblast differentiation was induced by culturing bone marrow cells in osteoblastogenic medium—that was primary culture medium supplemented with 10 μ M dexamethasone, 5 μg/ml ascorbic acid and 10 mM β-glycerophosphate. After 12 days, the osteoblastogenesis of MSCs were confirmed by detecting alkaline phosphatase activity (ALP). After 22 days, to observe calcium deposition, cultures were washed once with PBS, and stained for 5 min with Alizarin Red S stain. Osteoclastogenesis. Cells were seeded in 24-well plates in the presence of mouse recombinant soluble receptor activator of NF-κB ligand (RANKL, 50 ng/ml) and murine macrophage colony-stimulating factor (M-CSF, 20 ng/ml). After 7 days, cells were washed and subjected to a tartrate-resistant acid phosphatase (TRAP) assay, which was used to assay the osteoclast formation. Moreover, pit formation assay was used to detect the bone resorption activity. Animal study. Wister rats (male, 3-week-old) were assigned into 3 groups randomly, 10 each, and fed diets supplemented with 0% (control), 0.1%, and 0.2% caffeine for 20 weeks. The BMD of lumbar vertebra, femur and tibia was determined by Dual-energy X-ray absorptiometry (DEXA, Norland Stratec). The calcium content of femur and tibia were also measured after animals were sacrificed.

RESULTS:
The results of the in vitro study showed that caffeine (0.005-0.1 mM) did not affect the viability of bone marrow stromal cells (data not shown). Caffeine (0.005 and 0.01 mM) in medium could not affect the ALP (Fig. 1A) and osteoblastic mineralization (data not shown). In contrast, osteoblast differentiation, but markedly enhanced the osteoclast differentiation (osteoclastogenesis) (Fig. 1B) and activity of bone resorption by pit formation assay (Fig. 1C). In contrast, genistein (0.01 mM), a phytoestrogen as a positive control, increased the differentiation of osteoblasts, but inhibited the differentiation of osteoclasts and the activity of bone resorption (Fig. 1A-C). In animal study, no significant differences in body weight change and daily diet intake were found between the caffeine-diet groups and the control-diet group. However, the BMD and calcium content in tibia was lower in 0.2% caffeine group than that in control group (Figs. 1D and E). Moreover, osteoclast differentiation of bone marrow cells isolated from caffeine (0.2% in diet)-treated rats was markedly increased as compared with control (Fig. 1F). The osteoblast differentiation (ALP and osteoblastic mineralization) were not affected by caffeine (0.2% in diet) treatment in growing rats (data not shown).

DISCUSSION:
Bone remodeling, an incorporated interaction between the bone resorption and bone formation, plays an important role in the bone homeostasis. The effects of coffee or caffeine on bone metabolism are still controversial. There are about 80-150 and 60-100 mg caffeine per cup for fresh coffee and instant coffee respectively. It has been shown that the intakes of caffeine in amounts >300 mg/day accelerate bone loss at the spine in elderly postmenopausal women (3). Some studies have indicated that the viability of osteoblasts was significantly decreased at concentrations higher than 0.5 mM caffeine (5), and the formations of osteoblast and mineralization were significantly decreased in the presence of 10 μM caffeine (6). In the present study, we found that low-concentration caffeine (0.005-0.01 mM) didn’t affect the cell viability and the differentiation of osteoblast from bone marrow mesenchymal stem cells (MSCs), but could significantly enhance the differentiation of osteoclast from bone marrow hematopoietic stem cells (HSCs) and bone resorption activity. These in vitro results indicate that caffeine is capable of enhancing the osteoclastogenesis. The results in animal study also showed that caffeine (about 22 mg and 44 mg caffeine/day by diet intake for 20 weeks) significantly reduces BMD and bone calcium content and enhances the osteoclastogenesis of HSCs in growing rats. These findings imply that the enhancement of osteoclastogenesis may cause the reduction in BMD in growing rats fed caffeine-supplemented diets.

REFERENCES:

Fig 1. (1) In vitro effects of caffeine on osteoclastogenesis, osteoblastogenesis, and bone resorption activity: (A) Alkaline phosphatase activities during osteoblastogenesis. (B) Osteoclast formation from HSCs (TRAP-positive cells). (C) Activity of bone resorption by pit formation assay. (2) In animal study, growing rats were fed caffeine-supplemented diets for 20 weeks: (D) BMD in tibia. (E) Calcium content in tibia. (F) Osteoclast formation from HSCs. Data are presented as mean±SD (n=10). Columns having different superscripts are significantly different at p<0.05. *: p<0.05 as compared with control.