EFFECT OF VITAMIN K2 AFTER THE TRABECULAR CONNECTIVITY WAS LOST IN OVARIECTOMIZED RATS

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This study suggests that an 8-week administration of MK-4 protects against the loss of trabecular bone volume to some extent in the osteoporotic rat model induced by ovariectomy and a calcium-deficient diet, even though the treatment is started after the trabecular connectivity was lost. When compared with the previously reported apparent effectiveness of the early treatment, the efficacy of medication of the delayed treatment was markedly decreased. These results suggest that the earlier administration of vitamin K2 probably produce better results compared with the delayed therapeutic intervention. Because once connectivity loss occurs, it would be more difficult to re-establish connectivity, and only possible to increase the thickness of the remaining trabeculae.

**Introduction:** Two types of vitamin K occur in nature: vitamin K1 (phylloquinone, 2-methyl-3-phytyl-1,4-naphthoquinone), which is derived from plants, and vitamin K2 (menaquinones), a series of vitamins with multi-isoprene units at the 3-position. Vitamin K2 activates osteoblasts to enhance calcification, acts as a coenzyme of γ-carboxylase for the Gla protein, and is also reported to inhibit bone resorption. We have previously demonstrated that menatetrenone (MK-4), a vitamin K2 with four isoprene units significantly protects against the loss of trabecular bone volume (38%, p<0.01) and its connectivity (46%, p<0.01) in ostepenic rats when treatment is begun just after the ovariectomy. (JMBR 2000, in press) However, it is still unknown whether it is possible to restore trabecular bone mass and connectivity if therapeutic intervention occurs after the trabecular connectivity has been lost. The purpose of this study was to clarify the effect of MK-4 using the 3-D morphometry by micro-CT, when the treatment was initiated after the trabecular connectivity was lost.

**Methods:** Forty-two 12-week-old Fisher 344 breeder female rats weighing 156.1 ± 6.3 g were purchased and maintained at the animal research facility. After a 1-week acclimation period, the rats were randomized by weight into four treatment groups: the ovariectomized (OVX+MK-4) group (n=14), fed a calcium-deficient diet and treated with MK-4 (Eisai Co., Ltd., Tokyo, Japan), the ovariectomized (OVX untreated) group (n=14), fed a calcium-deficient diet and untreated, the sham-operated (Sham+MK-4) group (n=7), treated with MK-4, and the sham-operated and untreated (Sham untreated) group (n=7). Both the ovariectomy and sham operations were performed at an age of 13 weeks, and the animals were kept in pairs at 22 ± 1 temperature/humidity light/dark cycle until they were 29 weeks old. During the 16-week period, the animals were given free access to food and water. Ovariectomized rats were given food containing 0.2% calcium, and the sham-operated rats were given a regular diet containing 0.2% calcium. MK-4 treatment was initiated eight weeks after the ovariectomy. Body weight and food intake were measured once a week. To examine whether the expected dose of MK-4 had been taken, MK-4 intake from the regular diet was calculated from these data. Thus, MK-4 intake was adjusted to the previously decided amount throughout the experimental period. The mean daily doses over the 8-week treatment period were 30 mg/kgBW/day. Blood samples were collected at 0, 4, 8, 12 and 16 weeks after ovariectomy. All procedures performed on the rats were approved by the Institutional Animal Care and Use Committee and complied with the American Journal of Physiology guidelines. After the rats were sacrificed, the right tibias were harvested and the total lengths were measured to assess the influence of longitudinal growth. Proximal metaphyseal area with the exclusion of the area located within 1.0 mm from the epiphysial growth plates in order to exclude the primary spongiosa, was scanned with micro-CT (MCT-1250MF, HITACHI Medical Corporation, Kashiwa, Japan) to nondestructively acquire a 3-D CT stack consisting of 100 consecutive slices at a spatial resolution of 26 μm. All the slice images were then noise-eliminated and segmented using the local thresholding procedure, and the volume of interest composed of the entire secondary spongiosa of the volumetric data was prepared to analyze the 3-D trabecular microarchitecture. A parametric analysis was carried out using bone volume fractions (BV/TV), fractal dimension, and the connectivity density for quantitative analyses. The fractal dimensions of the secondary spongiosa were measured using a 3-D box-counting method implemented on the personal computer. A topological analysis was carried out to calculate the first Betti number, β1, which is defined as the topological connectivity estimated from an enumeration of the number of closed loops in the analyzed volume, and the connectivity density, β1/TV (1/mm3), was referred for a relative comparison. 3-D reconstruction of trabeculae in these groups was performed by surface-rendering methods using the AVS Medical Viewer (KGT, Co., Ltd., Tokyo, Japan) installed on a workstation (ONYX2, Silicon Graphics, Inc., Mountain View, CA). ANOVA (Tukey Kramer multiple comparison procedure) at the p = 0.05 level was used to examine the differences between each group.

**Results:** All animals tolerated the experiment without complications. The body weight at 16 weeks in the OVX groups was significantly higher than that of sham-operated animals. No difference in the total length of the tibia in each group was observed. The BV/TV, fractal dimension, and the connectivity density decreased significantly in OVX untreated animals compared to the sham-operated animals. In the OVX + MK-4 group, this loss of trabecular bone mass and fractal dimension were significantly prevented (p<0.05), and BV/TV was approximately 19% higher than in OVX untreated animals. The connectivity density in OVX + MK-4 animals, however, showed no statistically significant differences. These findings agreed with the 3-D reconstructed images.

**Discussion:** This study suggests that an 8-week administration of MK-4 protects against the loss of trabecular bone volume to some extent in the osteoporotic rat model induced by ovariectomy and a calcium-deficient diet, even though the treatment is started after the trabecular connectivity was lost. When compared with the previously reported apparent effectiveness of the early treatment, the efficacy of medication of the delayed treatment was markedly decreased. These results suggest that the earlier administration of vitamin K2 probably produce better results compared with the delayed therapeutic intervention. Because once connectivity loss occurs, it would be more difficult to re-establish connectivity, and only possible to increase the thickness of the remaining trabeculae.

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