VITAMIN K2, MENAQUINONES, DELAYS THE PROGRESSION OF KNEE OSTEOARTHRITIC CHANGES IN HARTLEY GUINEA PIG.

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Introduction
Vitamin K is a family of structurally similar, fat-soluble, 2-methyl-1,4-naphthoquinones, including phyloquinone (K1), menaquinones (K2), and menadione (K3). Among these, vitamin K2 exerts a powerful influence on bone building, especially in osteoporosis. This effect is demonstrated by its ability of posttranscriptional modification of a number of vitamin-K dependent proteins such as osteocalcin or matrix GLA protein (MGP), a bone protein containing gamma-carboxyglutamic acid. Gamma-carboxylation of the glutamic acid involves the conversion of glutamic acid residues (Glu) to gamma-carboxyglutamic acid residues (Gla). During development, MGP and osteocalcin preferentially accumulate in mineralized bone, and the Gla residues of them promote binding of calcium and phosphate ions. Our previous study revealed that MGP played a critical role in regulating endochondral chondrocyte maturation and ossification processes by inhibiting cartilage mineralization in vitro and vivo assays(5). These findings imply the possibility that MGP function in mineralization might depend on the status of development and the nature of the responding cells. MGP is not only present in the mineralizing zone of the growth cartilage, but also present in articular cartilage. Hence, there might be the possibility that vitamin K influences physiological or pathological course of articular cartilage.

Certain animal species develop spontaneous OA which is thought to be similar to the disease process in humans. Hartley guinea pigs develop reproductively a spontaneous OA in the knee joint, the first lesions appearing in the medial compartment of the tibial plate in about from 6 months of age. Bone spur formation is recognized in the periphery of the medial tibial plateau at 12 months of age. The disease progresses moderate and severe cartilage degeneration by 16 to 18 months of age. The guinea pig therefore represents an excellent animal model for the study of OA disease process. This study demonstrates the effect of oral administration of vitamin K2 on the pathological progress of OA using Hartley guinea pigs.

Materials and Methods
Animals: Hartley strain female guinea pigs (n=50) were obtained at 4 months of age from Charles River Laboratories (Wilmington, MA). Animals were divided into 10 groups (n=5) at random. Food (LRC-4; Oriental Yeast Co., Tokyo, Japan) and water were available ad libitum. One group was harvested at 4 months of age as control subjects. Eight groups were raised for the subsequent 2, 4, 6, and 8 months with or without food containing vitamin K2 (content 30 mg/g). At objective periods, animals were euthanized by administration of lethal doses of pentobarbital (300 mg/kg of body weight, given intraperitoneally), and bilateral knee joints were then dissected for following experiments. The left side of knee joint was subjected for the QCT experiment, and the right side was for the gross appearance and histological experiments.

Gross appearance: The dissected joints were fully exposed by disarticulating the patella and severing the cruciate ligaments. After application of India ink, gross morphologic changes of tibial plateau were evaluated by three blind, independent observers. The tibial plateaus were photographed using a high-resolution digital camera (3.3 million pixels, with a close-up lens). The gross appearance was judged according to the criteria as described by Yoshioka et al. The criteria used were as follows: for grade 1 (intact surface), surface appears normal and does not retain any ink; for grade 2 (minimal fibrillation), surface appears normal before staining but retains the India ink as black patches; for grade 3 (overt fibrillation), surface is velvety in appearance and retains ink as intense black patches; for grade 4 (erosion), loss of cartilage is evident, with exposure of the underlying bone. Furthermore, the area of ink-retained portion and total cartilage surface of tibial plateau were calculated in each individual. The ratio of ink-retained area to total cartilage surface was determined.

Histology: For histological analysis, paraffin-embedded sections were performed using a microtome (LRC-4; Oriental Yeast Co., Tokyo, Japan). Serial sections of 8 µm were obtained by using a computer-assisted staining and digital imaging system (Microarbon, Thomas Jefferson University). Histological scoring of serial sections of each knee (10 to 15 sections/knee) was also performed by three blinded, independent observers. Histopathologic alterations in the knee joints were graded according to the Mankin criteria as described elsewhere. All sections were graded and median scores were determined for statistical analysis. Statistical Analysis: All data are expressed as the mean ± SD. Statistical analysis was performed by using the un-paired t-test. The value P < 0.05 was considered to be significant.

Results
Progressive histopathologic changes characteristic of developing OA were observed concomitantly with aging. This change was initiated by the disruption of the weight-bearing regions of articular cartilage at 6 months of age, and subsequent changes such as cloning of chondrocytes or loss of Safrarin-O staining were recognized from 8 months of age onward. The figure shows the Mankin scores obtained from sections stained with Safrarin-O. As shown in figure, the scores were increased with aging in both groups. Statistical significance was recognized between two groups only in the early phase at 6 months (p < 0.032). This data was consistent with the data of gross appearance.

Discussion
Vitamin K is contained abundantly in a certain kinds of foods such as fermented soybeans or seaweed. The previous epidemiological study has reported that the high consumption of these foods decreased the incidence of fracture, and menaquinones is thus broadly utilized as medicine for osteoporosis in our country. The present study demonstrated that menaquinones could delay the progression of osteoarthritis. To our knowledge, this is the first report to discuss the effect of menaquinones on the pathological feature of OA.

Reference
(1) Yagami K, Suh YJ, Enomoto-Iwamoto M, Iwamoto M. Matrix GLA protein is a developmental regulator of chondrocyte mineralization and, when constitutively expressed, blocks endochondral and intramembranous ossification in the limb. J Cell Biol. 1999; 147: 1097-108

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