Reductions in degree of mineralization and enzymatic collagen cross-links and increases in glycation induced pentosidine in the femoral neck cortex in cases of femoral neck fracture

Department of Orthopaedic Surgery
Jikei University School of Medicine
Tokyo, Japan

Mitsuru Saitou

Introduction: Bone loss and impaired bone quality, which encompass the structural and material properties of bone, have been proposed as major causes of increased bone fragility in osteoporosis. Intramolecular collagen cross-link is candidate for determining the material properties of bone. Collagen cross-links are of two types: lysyl oxidase and lysyl hydroxylase (PLOD1, PLOD2) controlled cross-links (Saito M, JBMR 2003, BONE 2004), and advanced glycation end products (AGEs), pentosidine (Saito M, Anal Biochem 1997). These two types of cross-links play important roles in the expression of bone strength. The cross-link pattern is affected by tissue maturation and senescence. Because the distinctive collagen reactions may be carried to different degrees of mineralization in different bone areas, we should analyze separate fractions from an area, each containing a sample with a different degree of mineralization, which reflects tissue aging. However, the classical whole-bone analysis cannot be used to estimate different degrees of mineralization in certain areas. In the present study, the technique of density gradient fractionation was used to obtain the separation of each osteon in different stages of mineralization. The aim of our study was to understand the distinctive posttranslational modifications of collagen in areas with different degrees of mineralization with and without hip fracture.

Methods: Sixteen female cases of intracapsular hip fracture (78±6 years) and 16 age- and gender-matched postmortem controls (76±6 years) were included in this study. A sample of each femoral neck cortex was fractionated into low-mineralized (1.7 to 2.0 g/ml) and high-mineralized (>2.0 g/ml) portions. The contents of enzymatic cross-links (dihydroxylysinonorleucine, hydroxylysinonorleucine, lysinonorleucine, pyridinoline, and deoxypyridinoline) and nonenzymatic cross-links (pentosidine) and the extent of lysine (Lys) hydroxylation were determined in each fraction by our established HPLC system (Saito M, Anal Biochem 1997).

Results: In the controls, there was no significant difference in the contents of enzymatic cross-links between low- and high-mineralized bone fractions, whereas pentosidine content was significantly higher in high-mineralized bone compared with low-mineralized bone. When comparing enzymatic cross-link contents between controls and fracture cases, a trend toward lower cross-link content in low-mineralized bone and a significant reduction in high-mineralized bone were observed. Pentosidine content of low-mineralized bone was significantly higher in fracture cases than in controls. The extent of Lys hydroxylation was significantly higher in fracture cases than in controls. The higher hydroxylation of Lys in collagen from fracture cases relative to controls was associated with significantly higher values of hydroxylysine-derived cross-link such that the enzymatic cross-link patterns correlated with the extent of Lys hydroxylation in the collagen molecules.

Conclusion: Recently, we reported that mildly hyperhomocysteinemia and vitamin B6 in general population are crucial determinants of detrimental crosslinking of bone collagen in patients with hip fracture (Saito M, Calci Tissue Int 2006) and detrimental cross-link formation reduces bone strength without the reduction in bone mineral density in diabetic rat (Saito M, Osteoporos Int (10) 2006). These results suggest that enzymatic cross-links and excessive formation of AGEs, pentosidine, may play an important role in explaining poor bone quality in osteoporosis.