PERIARTICULAR GENE EXPRESSION OF OSTEOPONTIN AND PROGRESSION IN OSTEOPENIA FOLLOWING JOINT IMMobilIZATION

+*Miyama, T; **Nakamura, N; *Nakase, T; *Myoi, A; *Tomita, T; *Hamada, M; *Toritsuka, Y; *Yoshikawa, H
+*Dept. of Orthopaedic Surgery, Osaka Univ. Medical School, Osaka, Japan. 2-2 Yamadaoka, Suita-city, Osaka, Japan, +81-6-6879-3552, Fax: +81-6-6879-3559, miyama@ort.med.osaka-u.ac.jp

Introduction: Joint immobilization is known to result in preferential reduction of bone mass at periarticular regions. However, the underlying mechanisms remain unclear. Temporal and regional increase in the number of osteoclast and its activation following immobilization may be involved in this phenomenon. Osteopontin is one of the major noncollagenous bone matrix proteins produced by osteoblasts and osteoclasts, and is a ligand of αvβ3 integrin, which is specifically expressed by osteoclast. Binding of the proteins produced by osteoblasts and osteoclasts, and is a ligand of phenomenon. Osteopontin is one of the major noncollagenous bone matrix osteoclast and its activation following immobilization may be involved in this mechanisms remain unclear. Temporal and regional increase in the number of osteoblast-like cells of spongiosa, which were also positive for Col I mRNA, as well as terminally differentiated hypertrophic chondrocytes at 1 week after immobilization (Figure 2-1, 2). At 4 weeks, the expressions of OPN mRNA were decreased.

Discussion: This study demonstrated that joint immobilization rapidly increased the number of osteoclasts in metaphyseal trabecular bone. It was notable that OPN mRNA expression was simultaneously increased at metaphyses. OPN mRNA was mainly detected in Collagen I-positive, osteoblast-like cells. Previous studies on αvβ3 integrin-osteopontin interactions as well as a recent study regarding OPN-deficient mice have strongly suggested that OPN is a key molecule for osteoclast-mediated bone resorption. While, our preliminary data shows OPN protein expression is also detected at osteoblast-like (collagen I-positive) cells at metaphyseal spongiosa (data not shown). These findings suggest that regional osteoblast-osteoclast coupling, namely, the increase in OPN expression by osteoblasts and the forming of osteoclasts to periarticular bone with their activation might play an important role in rapid progression in periarticular osteopenia.

Method:

The experimental model
Disuse osteopenia was induced in Wistar male rats (n=50) with an age of 15weeks. The knee joints of the right hindlimb were positioned in full flexion and immobilized by a soft wire (2). After recovery from anesthesia, the rats were allowed to free cage activity. The rats (n=5 per group) were sacrificed at 7, 14, 21 and 28 days after immobilization.

Measurement of BMD of Tibia
To examine the time course of the change in BMD at regions of the tibia, BMD was measured at the proximal metaphyses of the tibia. The bones were scanned with a x-p system (μFX1000, FUJIFILM, Japan). The standard was composed of Phantom UHA-type bone mineral containing 20-400 mg hydroxy apatite/cm³ (Kyoto Science, Kyoto, Japan). The optical density of each sample was analyzed using Bioimage Analyzer System (FUJIFILM, Japan) and the mineral density was quantified.

Histology
The harvested tibias of immobilized and control limbs were dissected free of soft tissues, fixed in phosphate-buffered 4% paraformaldehyde for 24 h, decalcified, dehydrated, embedded and sectioned. Specimens were cut longitudinally in the sagittal plane and analyzed histologically with Tartrate resistant acid phosphatase (TRAP) staining.

Preparation of probes
The following cDNA clones were used as hybridization probes: mouse OPN cDNA and mouse cDNA specific for the alpha chain of procollagen type I (Col I). The specificity of these probes (OPN and Col I) has been confirmed (3) (4). Col I mRNA was used as a marker of bone forming cells (osteoblasts).

In situ hybridization
In situ hybridization techniques was carried out as described (5). To semi-quantitate OPN mRNA expression, the number of OPN-positive cells was counted. The area of measurement was routinely performed from 0.1mm to 1.1 mm distal to the growth plate.

Results:

BMD of the Tibia
Development of regional osteopenia was confirmed by measurement of BMD in proximal tibia metaphyses at 1, 2, 3 and 4 weeks after immobilization. At 2 weeks, a highly significant decrease in total BMD was observed in the immobilized limbs as compared with control limbs.

Histology
In immobilized knee joints, loss of spongiosa was already observed at metaphyses compared with the control joints at 1 week. At 3 weeks, metaphyseal spongiosa was almost disappeared in the immobilized joints. In the immobilized knees, the multinucleated cells with positive staining with TRAP were significantly increased at metaphyses compared with control at 1, 2, 3 and 4 weeks after immobilization (Figure 1). In situ hybridization demonstrated that the expressions of OPN mRNA were mainly detected in osteoblast-like cells of spongiosa, which were also positive for Col I mRNA, as well as terminally differentiated hypertrophic chondrocytes at 1 week after immobilization (Figure 2-1, 2). At 4 weeks, the expressions of OPN mRNA were decreased.

Discussion: This study demonstrated that joint immobilization rapidly increased the number of osteoclasts in metaphyseal trabecular bone. It was notable that OPN mRNA expression was simultaneously increased at metaphyses. OPN mRNA was mainly detected in Collagen I-positive, osteoblast-like cells. Previous studies on αvβ3 integrin-osteopontin interactions as well as a recent study regarding OPN-deficient mice have strongly suggested that OPN is a key molecule for osteoclast-mediated bone resorption. While, our preliminary data shows OPN protein expression is also detected at osteoblast-like (collagen I-positive) cells at metaphyseal spongiosa (data not shown). These findings suggest that regional osteoblast-osteoclast coupling, namely, the increase in OPN expression by osteoblasts and the forming of osteoclasts to periarticular bone with their activation might play an important role in rapid progression in periarticular osteopenia.

Figure 1. TRAP staining of proximal tibia. A:control. B:1 week of immobilization.

Figure 2-1. OPN in situ hybridization. A:control. B:1 week of immobilization.

Figure 2-2. Number of OPN positive cell (n=5) * p<0.05 vs. control (unpaired t-test)


**Dept. of Orthopaedic Surgery, Osaka Rosai Hospital, Osaka, Japan.