Hypoxic conditions in the capsule after joint immobilization

Introduction:
Joint immobilization is a useful and commonly performed treatment modality in orthopaedics. However, it also causes unfavorable outcomes such as joint contracture, periarticular osteoporosis, and cartilage degeneration. Once joint contracture is established, it is extremely difficult to regain a full range of motion (ROM) with vigorous and extensive rehabilitation, or even with surgical treatment. In our previous reports, ROM increased after the posterior capsular release in a rat knee flexion contracture model, which indicated the capsule was one of the main causes of joint contracture (1). Further, angiogenesis factors of transforming growth factor-β1 and connective tissue growth factor were increased in the capsule after prolonged immobilization (2). This result might indicate presence of hypoxia in the capsule after immobilization. The purpose of this study was to elucidate changes in the number of blood vessels in the capsule and presence of hypoxia by hypoxyprobe-1 (HP-1) stain in the rat knee contracture model.

Materials and Methods:
Animals: The protocol for the experiments was approved by the Animal Research Committee of Tohoku University. Unilateral knee joints of adult male Sprague-Dawley rats (body weight 380-400g) were immobilized at 150° of flexion with a plastic plate and metal screws for various periods (3 days, 1, 2, 4, 8 and 16 weeks) for blood vessels counts (immobilized group, n=5/each period). Sham operated rats had only screws inserted unilaterally for the same experimental periods (control group, n=5/each period). Sham operated animals made up the immobilized group and the control group, respectively. The other five rats were prepared for HP-1 injection by the same immobilization methods (1, 4, and 8 weeks). However, the contralateral knee was used as a control.

Tissue Preparation: The samples were fixed with 4% paraformaldehyde in 0.1M phosphate-buffered saline and embedded in paraffin. The embedded tissue was cut into 5-μm thick sagittal sections. Standardized serial sections of the medial meniscus of the knee were made. The sections were deparaffinized and endogenous immunoglobulins were blocked. The slides were washed and incubated with a rabbit polyclonal alpha smooth muscle actin (a-SMA) antibody (abcam, dilution 1:100). The slides were incubated with HRP conjugated goat anti-rabbit antibody. The sections after HP-1 injection were incubated with a mouse IgG1 monoclonal antibody (hypoxyprobe inc., dilution 1:400). Biotinylated rabbit anti-mouse IgG (Nichirei, Tokyo, Japan) was applied to the sections for 1 hour at room temperature. HRP conjugated avidin-biotin complex (ABC, Vector Laboratory, Burlingame, CA) was applied for 30 minutes at room temperature. The final detection step in all slides was carried out using DAB, 0.1 M imidazole, 0.03% hydrogen peroxidase as the chromogen.

Numbers of Blood Vessels: Blood vessels were defined as lumen structures with strong a-SMA stain. We divided the capsule into 4 areas (antero-superior, antero-inferior, postero-superior and postero-inferior subdivisions) and counted the number of blood vessels in each subdivision. We also measured each subdivided area in the capsule by image analysis software and calculated average number of blood vessels per unit.

Statistics: Differences between the immobilized group and the control group were compared at each time point by unpaired t-test. Data were expressed as mean SD. A value of p<0.05 was accepted as statistically significant.

Results:
Number of Blood Vessels: The number of blood vessels in the capsule per unit (antero-inferior, postero-inferior) significantly decreased in the immobilized group compared with the control group after 4 weeks. Immunohistochemistry of HP-1: HP-1 was detected around blood vessels in each area of the capsule throughout the experimental periods. In the posterior capsule, the intensity was much stronger in the immobilized group compared to the control group.

Discussion:
The number of blood vessels in the capsule diminished, which might indicate decreased blood flow. HP-1 staining also revealed hypoxic conditions around blood vessels in the capsule. Hypoxia is an important factor of deterioration of joint contracture. However, there was still unclear how immobilization affected the number of blood vessels in the capsule. Further study was needed to clarify the changes of the capsule after immobilization.

References: