Introduction:

Joint contracture is defined as a decrease in both active and passive range of motion (ROM). In our previous study, elasticity of capsule increased after prolonged immobilization [1]. Determination of expression levels of structural collagens is a key to understand these elastic changes. The major structural collagens of the capsule are collagen types I and III [2]. Though some researchers have reported high levels of collagen types I by immunohistochemistry, expression patterns of collagen types I and III in the contracture capsule remain controversial. The purpose of this study was to elucidate the precise expression patterns of collagen types I and III in the capsule after immobilization by multidirectional approach.

Method:

Animals and Tissue Preparation: The protocols for the experiments were approved by the Animal Research Committee of Tohoku University. The unilateral knee joints of S-D rats aged 12-week old 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). Sham operated rats had holes drilled in the femur and the tibia with screws, but the plate was not inserted [3]. Three healthy rats without any surgery were made up the normal group for western blotting.

In Situ Hybridization (ISH): Digoxigenin-labeled single strand RNA probes were prepared. Fragments encoding rat pro-alpha1(I)-collagen (2833-4329bp: GenBank Z78279) and rat pro-alpha1(III) collagen (1462-2097bp: GenBank X70369) were obtained.

Quantitative Real-Time PCR (qPCR): The anterior and posterior capsules were separately obtained and homogenized. The total RNA of the homogenate was purified and cDNA was synthesized. PCR efficiencies and relative expression levels of collagen types I and III as a function of EF1α1 were calculated [4].

Immunohistochemistry (IHC): The sections were deparaffinized and endogenous immunoglobulins were blocked. The slides were washed and incubated with a polyclonal rabbit anti-rat collagen type I (LBb1102, LSL, dilution 1:800) or collagen type III antibody (LBb1393, LSL, dilution 1:800) [4]. The slides were incubated with HRP conjugated goat anti-rabbit antibody. The final detection step was carried out using DAB, 0.1 M imidazole, 0.03% hydrogen peroxidase as the chromogen.

Western blotting: The anterior and posterior capsules were separately resected and homogenized. The supernatants were collected and 100 µg of each protein was separated by SDS-PAGE. The membrane was incubated with rabbit polyclonal anti-human collagen type I antibody (ab292, Abcam, dilution 1:25000), mouse monoclonal anti-human collagen type III antibody (sc-8781, Santa Cruz Biotechnology, dilution 1:1000), or rabbit anti-human β-actin (64967, Cell Signaling Technology, dilution 1:1000). The bound antibodies were detected with HRP-conjugated secondary antibodies.

Statistical Analysis: Differences between the immobilized and control groups were compared at each time point by Mann-Whitney’s U test. A value of P < 0.05 was accepted as statistically significant.

Results:

In Situ Hybridization: Both in the anterior and posterior capsule, much weaker signals of collagen types I and III were detected at 3 days and 1 week in the immobilized group compared to the control group, respectively (Figure 1A-D).

Quantitative RT-PCR: The expression of collagen type I in the immobilized group did not change in the anterior capsule throughout the experimental periods, but significantly decreased at 3 days and 2 weeks in the posterior capsule compared to the control group (Figure 1E, F).

Immunohistochemistry: Both in the anterior and posterior capsule, the staining intensity of collagen types I and III did not apparently change in the immobilized group throughout the experimental periods compared to the control group (Figure 2A-F).

Western blotting: The immunoblots of collagen types I and III in the anterior and posterior capsule were identified. No significant differences were observed among the immobilized group, the control group and the normal group (Figure 3). The intensity of collagen type I was significantly lower in the anterior capsule compared to that in the posterior one.

Discussion:

We revealed no increase in collagen types I and III by ISH, qPCR, IHC and Western blotting. Our result indicated that changes of capsule after immobilization were neither proliferative nor prevalent fibrosis. Synovial or capsular adhesions and cross-links of collagens seemed to be a key to a cause of a joint contracture.

References: