Accelerated restriction in range of motion after blood injection in a rat immobilized knee

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Introduction:
Joint immobilization is commonly used as a treatment for joint injuries and diseases. However, it also causes unfavorable outcome such as joint contracture [1]. In our previous reports, restricted range of motion (ROM) was restored after incision of the capsule, which suggested that the capsule was one of the most important causes of joint contracture [2]. Joint hemorrhage is usually occurred by joint trauma and diseases, such as intra-articular fractures, ligament ruptures and haemophilia. Some researchers have reported blood-induced joint damages using animal model (post traumatic model and blood injection model) [3, 4]. Though the influence of bleeding on the synovial membrane and cartilage matrix were reported, precise mechanism of its etiology was still controversial. The purpose of this study was to elucidate the morphological changes of the capsule and measure a joint angle in a rat knee contracture model with single blood injection.

Method:
Animals: 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 (1, 3 days, 1, 2, 4, and 8 weeks). Sham operated rats had holes drilled in the femur and the tibia with screws, but the plate was not inserted [5]. After the operation, the rats were divided into three groups: immobilized-blood injection (Im-B) group, immobilized-normal saline injection (Im-NS) group, and sham-blood injection (Sm-B) group. Fifty μl of autologous blood were administered intra-articularly for the Im-B and Sm-B groups after the surgery. The same amount of normal saline was administered for the Im-NS group.

Histology: Paraffin embedded 5-μm thick sagittal sections in the medial midcondylar region of the knee were made. The sections were stained with Elastica-Masson to observe morphological changes of the capsule and synovial membrane (SM), and with Perls’ Prussian blue to visualize iron deposits in the capsule and SM.

Measurement of joint angle:
Eighty-four rats (1, 2, 4, and 8 weeks: n=7/each group) were prepared for measuring a joint angle. To measure the joint angle correctly with reproducibility, we made a special apparatus for taking lateral x-ray pictures of the knees (Fig. 1A). We measured the angle between the longitudinal axis of the femur and a line passing through the center of the ankle joint and the center of the eminence of the tibia (Fig. 1B). The lateral x-ray pictures were scanned with LP-9200 (EPSON, Tokyo, Japan) and the joint angle was measured with Image J 1.36b (NIH, Bethesda, MD, USA).

We set three torques (450, 900, and 1350 g-cm). To know the influence of the posterior capsule, it was incised at the insertion of the femoral condyle with a surgical knife after measuring the angle at the maximum torque (Fig. 1C). After the release, the joint angle was measured again with the maximum torque. We defined an acquired angle as follow: joint angle after the release – joint angle before the release.

Statistics:
Differences between the experimental and control groups 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:
Histology: Blood clot was observed around the posterior capsule until 3 days in the Im-B group (Fig. 2A). The blood clot was disappeared within 3 days in the Sm-B group (Fig. 2B). Adhesions between the postero-superior synovial fold and SM around the posterior horn of the medial meniscus were observed after 1 week in the Im-B group. The adhesion area extended to the posterior side to diminish the residual joint space after 2 weeks (Fig. 2C). These changes were more severe in the Im-B group than the Im-NS group (Fig. 2D). The area was replaced by fibrous and hypocellular connective tissues after 8 weeks both in the Im-B and Im-NS groups.

The iron deposits in the lining cell and adhesion area were observed after 1 week in the Im-B and Sm-B groups (Fig. 2E). However, Im-NS group showed fewer iron deposits in the adhesion area (Fig. 2F).

Discussion:
We revealed that absorption of the injected blood was delayed in the Im-B group, and the injected blood made severe adhesions and restriction in ROM. Intra-articular hemorrhage is a risk factor of joint contracture, and drainage of the blood or short immobilization periods might be a good strategy to avoid joint contracture.

References: