FGF-2 PROMOTES ANCHORING OF THE GRAFTED TENDON IN THE BONE TUNNEL.

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Introduction Although a number of surgical procedures have been reported for anterior cruciate ligament (ACL) injury, reconstruction with an autogenous tendon graft is among the most common method. In this procedure, healing or stable fixation of the grafted tendon in the bone tunnel is crucial for the patients’ safe return to athletic activities. Therefore, early achievement of rigid graft anchorage is of great interest to knee surgeons. Fibroblast growth factor-2 (FGF-2) is a potent mitogenic agent on a variety of cells of mesenchymal and ectodermal origin. With this cytokine, we have already reported that the healing of the fractured bone and the transsected ligament are both significantly promoted 1,2).

These findings allow us to hypothesize that FGF-2 can facilitate the anchoring of the grafted tendon in ACL surgery. The purpose of this study is to examine this hypothesis in vivo experiment.

Methods Forty mature male Japanese Albino rabbits with a mean body weight of 3.4±0.3kg were used. Treatment of each animal was conducted in accordance with the Guide for Animal Experimentation established at our institute. The rabbits were randomly divided into 2 groups of 20 animals (control and FGF-2). According to Rodeo’s procedure 3), the right knee joint of each animal was opened and the long digital extensor tendon was cut at its origin on the lateral femoral condyle. After a 4.0mm-diameter drill hole was made in the proximal tibia at a 45° angle to the long axis of the bone, the tendon was pulled out to the medial side and was fixed with a button. After the fixation of the tendon, 100mg of gelatin gel containing 50micrograms of recombinant human FGF-2 (Kaken pharmaceutical Co., Ltd., Japan) was applied in the bone tunnel around the tendon (FGF-2 group). This gelatin gel is biodegradable, and prepared through the glutaraldehyde crosslinking of acidic gelatin with an isoelectric point of 5.0 as reported previously 4). As for this control, gelatin gel containing no growth factor was applied (control group). The rabbits were housed in individual cages with the operated-on limbs unrestrained. At 1, 3 and 6 weeks after the surgery, 6 animals in each group were sacrificed; four of them were used for biomechanical measurement, and the other two served for histologic and radiologic observation. New bone formation in the tunnel was also assessed using two other animals in each group. For these, calcein was given at 1 and 20 days after surgery 5).

Results Biomechanical testing was performed following Rodeo’s description. All scar tissue, muscle belly adjacent to the tendon and the button were carefully removed. The tibia was mounted on the large drill chuck, the fascia and the tendinous portion of the extensor muscle were grasped in a specially designed jig. Then the whole specimen and devices were fixed on a testing machine (TENSILON UTM-2.5T, Toyo Baldwin Co., Ltd., Japan) so that the testing direction was parallel to the bone tunnel. The load was applied by means of a 1.8N/mm vs. 6.8±1.3mm), the failure load was standardized by dividing it by the length of the bone tunnel (strength-to-length ratio). For radiologic evaluation, 3mm thick section was cut out vertically to the bone tunnel and soft X-ray radiogram was obtained. Following this, the sample was fixed in phosphate-buffered 20% formalin, decalcified, sliced in 3 micrometers sections. Stained with H&E or azan, histological observation was carried out on these sections with light and polarized light microscopy. In the calcine labeled animals, the specimens were fixed in 70% ethanol, treated with Villanueva bone staining. The sections were observed under normal or ultraviolet microscopy.

A Student’s t-test was used to compare the biomechanical data between the FGF-2 group and the control group at each time point.

Discussion There were few previous reports about enhancement of anchoring of the tendon in the bone using growth factors. Low dose BMP-2 was reported to accelerate tendon-to-bone healing at 2 weeks postoperatively in dogs 6), and OP-1 was reported to promote new bone formation within the tendon substance in the bone tunnel in sheep ACL reconstruction model at 6 weeks after surgery 7).

The present study demonstrated that locally administered FGF-2 promotes anchoring of the tendon in the bone tunnel in the early phase of the healing process, probably through enhanced bone formation.

Our preliminary experiments demonstrated that the percentages of the injected 125I-labeled FGF-2 that remained localized were approximately 20%, 3%, and 1% at 1, 2, and 3 weeks, respectively. This is consistent with our results that new bone formation was promoted at the early stage after surgery. A better outcome may be achieved with a more refined method of administration.