Histological Observation of Regions around Bone Tunnels after Compression of the Bone Tunnel Wall in Ligament Reconstruction

Shintaro Maeda1, Hiroki Ishikawa1, Naoaki Tanigawa1, Kyosuke Miyazaki2 and Seiji Shioda1

1Department of Anatomy, Showa University School of Medicine, 1–5–8 Hatanodai, Shinagawa-ku, Tokyo 142–8555, Japan and 2Seikagaku Corporation, 1–6–1 Marunouchi, Chiyoda-ku, Tokyo 100-0005, Japan

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The objectives of this study were to investigate the time-course of influence of compression of bone tunnel wall in ligament reconstruction on tissue around the bone tunnel and to histologically examine the mechanism of preventing the complication of bone tunnel dilation, using rabbit tibia. A model in which the femoral origin of the extensor digitorum longus tendon was cut and inserted into a bone tunnel made proximal to the tibia was prepared in the bilateral hind legs of 20 Japanese white rabbits. In each animal, a tunnel was made using a drill only in the right leg, while an undersized bone tunnel was made by drilling and then dilated by compression using a dilator to the same tunnel size as that in the right leg. Animals were sacrificed at 0, 2, 4, 8 and 12 weeks after surgery (4 animals at each time point). Observation of bone tunnels by X-ray radiography showed osteosclerosis in the 2- and 4-week dilation groups. Osteosclerosis appeared as white lines around the bone tunnel on X-ray radiography. This suggests that dilation promotes callus formation in the bone tunnel wall and prevents the complication of bone tunnel enlargement after ligament reconstruction.

Key words: dilator, dilation, bone tunnel enlargement, ligament reconstruction, callus formation

I. Introduction

Bone tunnel enlargement is a potential complication after anterior cruciate ligament (ACL) reconstruction. The risk of this event can be significantly reduced using the dilation technique, in which an undersized bone tunnel is prepared by drilling and then dilated to the planned size by compressing the bone tunnel wall using a dilation system [4–6]. Ishikawa et al. [4] first reported this phenomenon in 2002, and many subsequent studies have shown a similar effect [1, 8]. However, the underlying histological mechanism is unclear and remains speculative. In this study, we established an experimental rabbit model to investigate the influence of dilation on bone tunnels histologically.

II. Materials and Methods

The bilateral hind legs of 20 Japanese white rabbits (body weight: 3.0–3.5 kg) (40 legs) were used to prepare the model. Surgery was performed under anesthesia with 30 mg/kg Nembutal injected into the auricular vein. To further reduce distress, Xylocaine 1%E (ASTRA ZENECA, Osaka, Japan) was subcutaneously injected around the bilateral knee joints (5 ml each, 10 ml in total) for local anesthesia. After confirming induction of sufficient anesthetic depth, a longitudinal incision of about 4.5 cm was made in the central skin of the knee joint. Following the method reported by Rodeo et al. [9], the extensor digitorum longus tendon (EDL) was cut at the attachment site on the femoral lateral condyle, and a bone tunnel at 45° to the longitudinal axis of the femur was prepared in the proximal tibial diaphyseal region, using a precision hand piece grinder (HP-200S, Toyo Associates Corp., Tokyo, Japan) with an attached drill. The number of rotations was $3.5 \times 10^4$ rpm.
A taper drill with a diameter of 2.1 mm (Hiroshima Yasuri Seizosho, Hiroshima, Japan) was used in the right leg in all animals. In the left leg, a bone tunnel was made using a drill with a diameter of 1.2 mm (Sunhayato Corp., Tokyo, Japan), and a taper dilator (Fujiwara Sangy Inc., Hyogo, Japan) with the same shape as the drill was inserted into the tunnel. The tunnel was dilated by manually compressing the bone tunnel wall with the dilator to a 2.1-mm diameter to prepare a tunnel with the same size as that in the right leg. The bone tunnel was moderately tapered to simulate the shape of the EDL. To minimize errors in precision associated with laterality and individual variation, the space between the tendon and bone was reduced and as tight a contact as possible was made by inserting the dilator until it reached a self-locking position. The dilator was also placed in the tunnel in the right leg to confirm that the angle and size of the bone tunnels were identical in the bilateral legs. The EDL was inserted into the tunnel and fixed to the opposite opening (proximal tibial medial periosteum) with suture thread, and the wound was closed. No plaster fixation was applied.

Four animals each were sacrificed at 0, 2, 4, 8 and 12 weeks after surgery. The bilateral tubiae were excised and X-ray radiograms were immediately acquired. Using an X-ray radiography system (KXO-50R, DS-PH, Toshiba Medical Systems, Tochigi, Japan), frontal images of the bilateral tubiae of the rabbits were acquired. The conditions used were voltage, 46 kV; current, 200 mA; and acquisition time, 0.020 seconds. After acquiring X-ray images of the excised bilateral specimens on the same film (bilateral frontal tubial views). The excised tubiae were then fixed in formalin, decalcified, and paraffin-embedded. Then the serial sections (3-µm thickness) parallel to the bone tunnel were prepared, stained with Masson-trichrome, and observed under light microscopy. A fixation of 10% neutral buffered formalin containing 0.5% w/v cetylpyrindinium chloride (CPC; Wako Pure Chemical Industries, Osaka, Japan) was used.

The 4 animals sacrificed 24 hr after surgery were regarded as the 0-week group. The bone tunnel diameter was measured and the mean was compared between the bilateral legs. The bone tunnel diameter was measured in digital images on a computer using Fuji Computed Radiography FCR PROFECT CS (FUJIFILM Corp., Tokyo, Japan), and a taper dilator (Fujiwara Sangy Inc., Hyogo, Japan) was used in the right leg. The bone tunnel diameters were 2.29±0.07 and 2.22±0.05 mm in the right and left legs, respectively, with no significant difference between the legs. In the 4-week group (Fig. 1b), osteosclerosis appeared in both legs, but was macroscopically more intense on the dilation side. The bone tunnel diameters were 2.66±0.23 and 2.36±0.33 mm in the right and left legs, respectively, with a tendency for the tunnel to be enlarged in the right leg, but with no significant difference between the legs.

In the 8-week group (Fig. 1c), the macroscopic laterality of osteosclerosis severity disappeared, but the bone tunnel diameters were 2.69±0.25 and 2.16±0.03 mm in the right and left legs, respectively, with significant enlargement of the tunnel in the right leg (p<0.01). In the 12-week group (Fig. 1d), the findings were similar to those in the 8-week group. In both legs, intense osteosclerosis was present over the bone tunnel, and the bone tunnel had enlarged on the non-dilation side. The mean bone tunnel diameters were 2.47 mm on the right side and 2.15 mm on the left side, with a tendency for bone tunnel enlargement on the right side, but without a significant difference between the legs.

Comparison of the bilateral legs over time showed enhanced macroscopic osteosclerosis on the dilation side at a relatively early phase (2- and 4-week groups: 3 legs; 8-week group: 1 leg), but no apparent laterality at 8 and 12 weeks. Bone tunnel enlargement was not observed after 2 weeks, but subsequently developed on the non-dilation side (4-week group: 2 legs; 8-week group: all 4 legs; 12-week group: 3 legs).

**Histology with Masson-trichrome staining**

In the 0-week group, relatively large cortical bone fragments were present around the bone tunnel opening. These may have been produced by damage caused by drilling (Fig. 2a, b). The cortical bone fragment on the right side (non-dilation side) was slightly larger, but no other apparent laterality was noted, showing no variability in the precision of this surgical procedure. In the 2-week group (Fig. 3a), woven bone, a feature of osteogenesis, that was assumed to be intramembranous ossification appeared near both ends of the bone tunnel and had entered the bone tunnel. In the central region of the bone tunnel, only immature fibrous tissue was present between the cancellous bone and tendon, forming a 3-layer structure. Bone tunnel invasion by woven bone was enhanced on the dilation side. In the 4-week group (Fig. 3b), woven bone had advanced to the region between the cancellous bone and fibrous tissue, mostly reaching the central region of the bone tunnel, and a 4-layer structure had formed in many regions. The woven bone increased in density and decreased in thickness, showing a tendency for maturation, which was enhanced on the dilation side, as noted in the 2-week group. In the 8-week group (Fig. 3c), osteogenic corticalization had progressed and matured to lamellar bone, giving a distinct 4-layer structure composed of cancellous bone, lamellar bone, fibrous tissue, and tendon.

**III. Results**

**X-ray radiography findings**

The features of osteosclerosis that appeared around the bone tunnel in the rabbits were similar to those observed in humans. Osteosclerosis started to appear at the bilateral bone tunnel openings and extended and intensified over the bone tunnel with time. In the 0-week group, no osteosclerosis was noted and there was no laterality. In the 2-week group (Fig. 1a), a pale image of osteosclerosis appeared at the opening of the bone tunnel in the left leg (dilation side), whereas there was no clear osteosclerosis in the right leg (non-dilation side). The bone tunnel diameters were 2.29±0.07 and 2.22±0.05 mm in the right and left legs, respectively, with no significant difference between the legs. In the 4-week group (Fig. 1b), osteosclerosis appeared in both legs, but was macroscopically more intense on the dilation side. The bone tunnel diameters were 2.66±0.23 and 2.36±0.33 mm in the right and left legs, respectively, with a tendency for the tunnel to be enlarged in the right leg, but with no significant difference between the legs.
Laterality of the osteogenic features had disappeared. In the 12-week group (Fig. 3d), maturation of the 4-layer structure had further progressed and the boundaries were mostly linear. As in the 8-week group, the osteogenic features showed no laterality.

Time-course observation of each tissue showed that osteogenic features oberved at 2 to 4 weeks included rough (Fig. 4a, b), wide mesh-like woven bone that slowly matured and became dense lamellar bone at about 8 weeks, with development of a Haversian canal (Fig. 4c). Twelve weeks, maturation progressed further, unevenness decreased, and the bone became linear (Fig. 4d). Comparison of the time-course changes showed that the whitish lines around the bone tunnels, which indicate osteosclerosis, on X-ray radiography were consistent with histological callus formation along the bone tunnel wall, and the increase in the
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Fig. 3. Histology with Masson-trichrome staining showing findings for bone tunnel opening in the 2-week (a), 4-week (b), 8-week (c), and 12-week (d) groups. OS, ossification; WB, woven bone; CB, cortical bone; LB, lamellar bone; FT, fibrous tissue; T, tendon.

Fig. 4. Progress of osteogenic corticalization in the central bone tunnel. Histological findings for osteogenic corticalization in the central bone tunnel are shown for the 2-week (a), 4-week (b), 8-week (c), and 12-week (d) groups. Arrowheads indicate the Haversian canal. WB, woven bone; OS, ossification; CB, cortical bone; LB, lamellar bone; T, tendon.
severity of osteosclerosis. These osteogenic changes tended to occur earlier (at 4 weeks) in the dilation group, but the laterality disappeared after 8 weeks. Regarding the fibrous tissue layer present between the tendon and bone, the thickness slightly increased in the 4-week non-dilation group (Fig. 5a, b), and this tendency increased after 8 weeks and laterality became apparent (Fig. 5c, d). This finding was consistent with the bone tunnel enlargement noted on X-ray radiography.

IV. Discussion

Callus formation along the bone tunnel wall reached the central region of the bone tunnel within about 4 weeks after surgery, which was consistent with the findings of Rodeo et al. [9] and Yamakado et al. [11]. In similar experiments using a drill alone. In our experimental system, a tunnel was made by drilling alone in the right leg, but by dilation in the left leg in the same animal. The features of osteogenesis and osteosclerosis on X-ray radiography tended to appear earlier in bone tunnels prepared by dilation, showing that this procedure promoted callus formation in the early phase (2 and 4 weeks) after surgery. Thus, dilation may be clinically useful to prevent bone tunnel enlargement. A further concern is that bone tunnel enlargement may occur during surgery. Therefore, animals were sacrificed on the day after surgery to examine this issue. Histologically, the diameter of the bone tunnel made by drilling alone did not differ from that prepared using a dilator, and there was no bone tunnel enlargement, showing that there was no difference associated with the surgical technique.

There are various theories concerning the mechanism of bone tunnel enlargement. It has been suggested that enlargement may be due to bone tunnel invasion by synovial fluid [3], but this cannot explain the phenomenon observed in our non-intraarticular model. A mechanical stress theory [3, 7], based on the bungee cord motion or windshield wiper motion may be clinically most likely because bone tunnels tend to enlarge in the mechanical stress load direction. However, this mechanism does not explain the laterality of bone tunnel enlargement observed in our experimental system. The theory of necrosis induced by high-speed drilling [2, 10] cannot be examined using our experimental system, but this theory is not contradictory to the experimental findings because the region assumed to be heat-necrotized by drilling may have been compressed outward by dilation and mixed with the non-heat-necrotized region. In tissue sections, the thickness of the layer between the transplanted tendon and bone was thicker on the right side, suggesting that this fibrous layer was the heat-necrotized region. On the left side, the fibrous layer may have been compressed and narrowed by dilation (Fig. 5). Since this fibrous layer cannot be visualized by X-ray radiography, the bone tunnel diameter may have appeared greater on the right.
side, in which the fibrous layer was wider. This mechanism may have prevented bone tunnel enlargement.

The phase of callus formation occurred earlier in the dilation group, suggesting a positive influence of the bone tunnel preparation method on tissue repair around the bone tunnel wall (cancellous bone). However, the mechanism of bone tendon enlargement in the non-dilation group in our experimental model remains speculative and requires further investigation.

Patients who undergo ACL reconstruction require a long time before returning to sports activities. Many patients want an accelerated rehabilitation period, but relatively slow rehabilitation in the early phase after surgery is recommended to prevent bone tunnel enlargement. The histological findings in our model show that a prolonged time is required for union of the bone and tendon, which suggests that caution is required in acceleration of rehabilitation in clinical practice. However, preparation of a bone tunnel using the dilation technique in surgery may be advantageous for prevention of bone tunnel enlargement, particularly when used in addition to careful rehabilitation procedures.

V. References

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