Callus formation during healing of the repaired tendon-bone junction

A RAT EXPERIMENTAL MODEL


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This study was undertaken to elucidate the mechanism of biological repair at the tendon-bone junction in a rat model. The stump of the toe flexor tendon was sutured to a drilled hole in the tibia (tendon suture group, n = 23) to investigate healing of the tendon-bone junction both radiologically and histologically. Radiological and histological findings were compared with those observed in a sham control group where the bone alone was drilled (n = 19). The biomechanical strength of the repaired junction was confirmed by pull-out testing six weeks after surgery in four rats in the tendon suture group. Callus formation was observed at the site of repair in the tendon suture group, whereas in the sham group callus formation was minimal. During the pull-out test, the repaired tendon-bone junction did not fail because the musculotendinous junction always disrupted first.

In order to understand the factors that influenced callus formation at the site of repair, four further groups were evaluated. The nature of the sutured tendon itself was investigated by analysing healing of a tendon stump after necrosis had been induced with liquid nitrogen in 16 cases. A proximal suture group (n = 16) and a partial tenotomy group (n = 16) were prepared to investigate the effects of biomechanical loading on the site of repair. Finally, a group where the periosteum had been excised at the site of repair (n = 16) was examined to study the role of the periosteum. These four groups showed less callus formation radiologically and histologically than did the tendon suture group.

In conclusion, the sutured tendon-bone junction healed and achieved mechanical strength at six weeks after suturing, showing good local callus formation. The viability of the tendon stump, mechanical loading and intact periosteum were all found to be important factors for better callus formation at a repaired tendon-bone junction.

Materials and Methods

We have attempted to clarify the mechanism of tendon-bone healing using a suture anchor technique in a rat model and assessing callus formation by radiological and histological examination.

Study I: healing process. Each rat was anaesthetised with sodium pentobarbitral (50 mg/kg body-weight) and a longitudinal skin incision was made on the medial aspect of the tibia. A tendons and ligaments are usually treated surgically when injured at the site of insertion into bone. Various techniques are used to attach tendon to bone, such as an endobutton, a suture anchor and an interference screw. In order to achieve good long-term results, biological integration between the tendon and bone is required.

The normal tendon-bone junction is a unique structure composed of four zones, including a transitional fibrocartilaginous zone characterised by the presence of calcified fibrocartilage next to the bone and non-calcified fibrocartilage next to the tendon. There is controversy about whether these four zones are restored at the repaired tendon-bone junction. In previous studies the healing of tendon-to-bone has been examined in dogs or rabbits both histologically and biomechanically. However, radiological evaluation was not undertaken and this is essential to assess the integration of tendon-to-bone.
hole 1.0 mm in diameter was drilled across the width of the distal third of the tibia perpendicular to the long axis of the bone, distal to the distal tibiofibular junction. The flexor digitorum fibularis tendons (toe flexors) were divided at the medial aspect of the ankle. We introduced a core suture using the Kessler method9 into the stump of the tendon using a 5/0 nylon suture which was passed through the hole and fixed on the anterior aspect of the tibia (tendon suture group: n = 23; Fig. 1a). As a control, a sham-operative group was prepared. A hole 1.0 mm in diameter was drilled in the tibia as before and a 5/0 nylon thread was passed through it and fixed without invading the flexor tendons (control group: n = 19; Fig. 1b).

Radiological analysis. The amount of callus formed was assessed on lateral radiographs at one, three and six weeks after operation. The area of the callus was measured using the National Institutes of Health (NIH) Image Software, version 1.6 (Bethesda, Maryland).

Histological analysis. At one, three and six weeks after the operation 54 rats were killed and the tibia was harvested for histological examination of the tendon-bone interface. The specimen was fixed in 10% buffered formalin before being decalcified in ethylenediaminetic-acetic acid (EDTA) and embedded in paraffin. Sections of 5 µm thickness were obtained in the axial plane from the bone tunnel. The sections were stained with haematoxylin and eosin and examined under a light microscope. The histological evaluation was performed by three different researchers (NH, YH, KY).

Biomechanical analysis. In order to investigate mechanical healing at the tendon-bone junction in the tendon suture group, we concluded a pull-out test with four examples. Immediately after the specimen was harvested, it was stored at -80˚C prior to thawing to room temperature over one day before the testing. With a vice clip, the tibia was secured to the base plate of an AC servo hydraulic materials testing system (AGS-100D, Shimadzu, Kyoto, Japan) and the muscle belly of the sutured flexor digitorum fibularis was held at the actuator. The actuator was moved vertically at a speed of 5 mm/min to distract the muscle-tendon-bone complex until failure. The ultimate strength at failure (N) and the site of failure were confirmed.

Study II: factors influencing callus formation. In order to clarify the role of the tendon stump, mechanical stress and periosteum in callus formation at the repaired tendon-bone junction, animals were allocated to four additional groups. Role of the tendon stump in callus formation. We induced necrosis of the tendon stump by soaking it in liquid nitrogen for one minute10 and then sutured it to the hole in the bone (necrosis group, n = 16; Fig. 1c).

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Fig. 1a Fig. 1b Fig. 1c Fig. 1d Fig. 1e Fig. 1f

The experimental groups: a) tendon suture group (n = 23), b) control group (n = 19), c) necrosis group (n = 16), d) proximal suture group (n = 16), e) partial tenotomy group (n = 16), and f) periosteal excision group (n = 16).
Role of traction force (mechanical loading) in callus formation. In order to reduce mechanical loading through the sutured tendon we sutured its stump at a site proximal to the standard position of the tibiofibular junction (proximal suture group, n = 16; Fig. 1d). For the same purpose we created a partial tenotomy at the muscle-tendon junction prior to reattaching the tendon at the standard position used in the tendon suture group (partial tenotomy group, n = 16; Fig. 1e).

Role of periosteum in callus formation. We removed 1 cm² of periosteum around the centre of the drill-hole in the tibia prior to suturing the tendon stump to the hole in the bone (periosteal excision group, n = 16; Fig. 1f).

Except for the specimens used for the biomechanical assessment the same histological and radiological analyses undertaken in the first stage of the study were conducted to evaluate the subsequent callus formation. In all cases the limbs were not immobilised and the rats were allowed to move freely after the operation.

Statistical analysis. Statistical differences were evaluated using one-way analysis of variance (ANOVA) and a post hoc analysis with the Tukey-Kramer test. A p-value < 0.05 was regarded as statistically significant.

Results
Study I: healing process
Radiographs. In the tendon-bone suture group, the large cartilaginous callus observed at the site of the repair two weeks after surgery gradually remodelled, decreasing in size by six weeks when the bony prominence at the tendon-bone interface was still present (Fig. 2a). There was little callus formation in the control group (Fig. 2b). The area of callus in the control group was significantly smaller than in the tendon suture group (post hoc analysis with Tukey-Kramer test, p < 0.05) (Fig. 2c).

Histological analysis. The histological findings in each group corresponded well with the radiological appearances. In the tendon suture group, serial histological changes revealed callus formation at the tendon-bone interface. During the first week, granulation tissue at the site of repair was initially composed of inflammatory cells. Intramembranous ossification around the whole tibia took
place under the periosteum without the formation of an intermediate cartilaginous layer (Fig. 3a). By three weeks, woven bone was found on the posterior aspect of the tibia at the transitional tendon-to-bone zone with an intermediate cartilaginous layer. Some chondrocytes began to calcify the surrounding matrix, resembling the endochondral ossification found in fracture healing. By six weeks, the woven bone within the intermediate cartilaginous layer was replaced by lamellar bone (Fig. 3a).

There was little callus formation in the hole in the bone in the control group (Fig. 3b). Intramembranous ossification took place under the periosteum.

Biomechanical analysis. At six weeks after the operation, the muscle-tendon-bone complex failed in all four specimens at the muscle-tendon junction at a mean of 28.8 N (26.60 to 29.35). The sutured tendon-bone junction did not break during the pull-out test.

Study II: factors influencing callus formation

Role of the tendon stump in callus formation. The radiographs show that callus formation was reduced in the necrosis group (Fig. 4) compared with that in the tendon suture group (Fig. 2a).

Role of traction force. The formation of callus was reduced in the proximal suture and partial tenotomy groups compared with that in the tendon suture group (Fig. 4).

Role of the periosteum. Periosteal excision markedly compromised callus formation (Fig. 4).

The area of callus in each group at three weeks after the operation is demonstrated in Figure 5. Without either mechanical load or the biological effect from the tendon...
stump, callus formation at the site of tendon-bone repair was significantly reduced (post hoc analysis with Tukey-Kramer test, p < 0.05) compared with that in the tendon suture group; however, callus formation was even less in the control group (post hoc with Tukey-Kramer test, p < 0.05).

Histological analysis in the necrosis group showed that minimal callus formation was induced at the repaired tendon-bone junction (Fig. 6). Similarly when the traction at the tendon reattachment had been reduced by suturing in a proximal position or by partial tenotomy, callus formation was poor three weeks after the operation (Fig. 6).

In the absence of periosteum the formation of an external callus at the repaired tendon-bone junction was also deficient (Fig. 6).

Discussion
In our model, the tendon-bone junction was mechanically healed six weeks after surgery and was confirmed by the point of reattachment being mechanically stronger than that of the muscle-tendon junction. Radiologically, we found a large amount of callus formed at the suture site during normal healing. At the tendon-bone junction, callus was also confirmed histologically, but the typical four-zone structure of the junction was not observed.

Previously, it has been reported that the junction healed showing the normal transitional four-zone structure. However, the importance of these four zones for the repair process is still a matter of controversy. Leung et al histologically investigated the repaired tendon-bone junction up to 24 weeks after surgery. They found that the structural integration at the site of repair was poor without a typical intermittent fibrocartilage zone, suggesting that the four-zone structure may not be important to obtain good healing. Our results were similar.

Instead of obtaining the four zones during healing, we found that callus formed at the site of normal healing and presume that callus formation is a key factor for healing. For appropriate callus formation in the second part of our study we showed that the viability of the tendon stump, mechanical loading and the presence of periosteum were important.

It has been widely recognised that cell proliferation and differentiation, the presence of a number of growth factors and an intact scaffold greatly contribute to tissue regeneration. As there was little callus formation in the control group, the presence of the tendon stump seemed to be important for the stimulation of callus at the tendon-bone interface. This was tested by preparing a tendon stump necrosis group. Consistent with our hypothesis, callus formation was minimal in this group compared with the normal tendon suture group, suggesting that the tendon stump may release factors related to osteogenesis.

In general, mechanical stress is a key factor for osteogenesis. This principle has been seen to operate in our study because in both groups where the loading was reduced, the callus formation was poor.

Kojimoto et al found that preservation of the periosteum was important for callus formation in bone lengthening in a rabbit model of callus distraction. In a clinical
setting, Wakisaka et al\textsuperscript{13} investigated the importance of the periosteum in a patient undergoing bone lengthening. Our results showed that callus formation occurred at sites where the periosteum was intact, but was deficient where it had been excised.

We demonstrated that a viable tendon stump, mechanical stress, and intact periosteum were all important for callus formation at the site of tendon reattachment. Further studies are necessary to clarify the role of callus in the integration of tendon and bone.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References


