Mechanoreceptors of patellar tendon used for ACL reconstruction

Rabbit experiments

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In 17 Japanese white rabbits (weighing 2.6–3.7kg), the anterior cruciate ligament of the right knee was resected and then reconstructed, using the lateral half of the patellar tendon. The contralateral side served as control. Rabbits were killed 10, 20, or 30 weeks postoperatively. Then the reconstructed ligament was harvested and stained, using a modified gold chloride method. Under the light microscope, sensory nerve endings in serial sections were counted after being morphologically identified.

With respect to the number of Pacinian and Ruffini corpuscles, there were no significant differences between the reconstructed ligament and the control patellar tendon at any time after surgery.

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Submitted 96-07-06. Accepted 97-06-30

It has been shown that the anterior cruciate ligament (ACL) contains mechanoreceptors (Schultz et al. 1984, Zimny et al. 1986, Yahia and Newmann 1991), which provide information concerning joint position and interaction between the joint and muscles (Boyd 1954, Freeman and Wyke 1967, Adams 1977, Kennedy et al. 1982, Krauspe et al. 1992). Skoglund (1956) reported the existence of mechanoreceptors of the patellar ligament in the cat. We confirmed that mechanoreceptors are also present in the rabbit patellar tendon. Recent animal studies have shown that mechanoreceptors can still be detected after ACL reconstruction with autogenous tendon grafts (Denti et al. 1994). However, changes in the nerve endings in autografts after reconstruction of the ACL have not been investigated. In the present study, we reconstructed the ACL, using a bone-patellar tendon-bone graft in rabbits, and assessed changes of the nerve endings in reconstructed ligaments by quantifying the receptors, including Pacinian corpuscles, Ruffini corpuscles, and free nerve endings.

Animals and methods

We performed an ACL reconstruction in the right knee of 17 Japanese white rabbits. Initially, the lateral half of the right patellar tendon was harvested together with its bony insertions. After excision of the ACL, holes were drilled in the tibia and femur at the ACL insertion sites, using a drill measuring 3.5 mm in diameter. Then the patellar bone fragment of the bone-patellar tendon-bone was inserted into the femoral tunnel, while the tibial bone fragment was inserted into the tibial tunnel. While exerting appropriate tension on the graft, it was fixed from outside the joint, using AO cancellous screws measuring 4 mm in diameter and 12 mm or 14 mm in length (Figure 1).

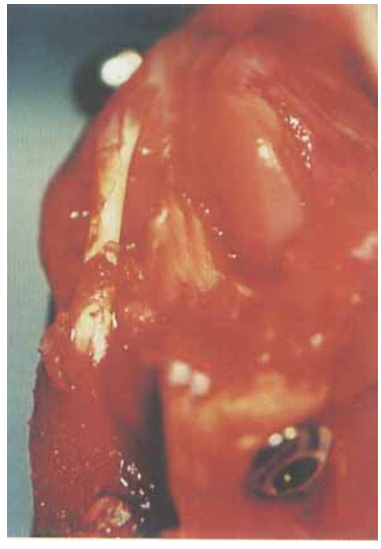


Figure 1. Intraoperative appearance of ACL reconstruction using the lateral half of the patellar tendon.

Postoperatively, the knee was fixed at 45 degrees in a long leg cast for 2 weeks. The rabbits were divided into 3 groups for assessment of mechanoreceptors in the reconstructed ligament at 10, 20, or 30 weeks postoperatively. The lateral half of the bone-patellar tendon-bone complex of the left knee was used as a control.

At 10, 20, and 30 weeks postoperatively, the rabbits of the respective groups were killed for assessment of the stability of the reconstructed ACL. Immediately after death, the knee joint was flexed to 90°, a steel pin measuring 2.0 mm in diameter was inserted through the proximal end of the tibia and connected to a 3 kg load applied by the anterior drawer method, while radiographs were taken. Evaluation was performed by the midpoint method (Murase 1983). Ligament insufficiency was defined as a 20% or greater difference from measurements in the control knee.

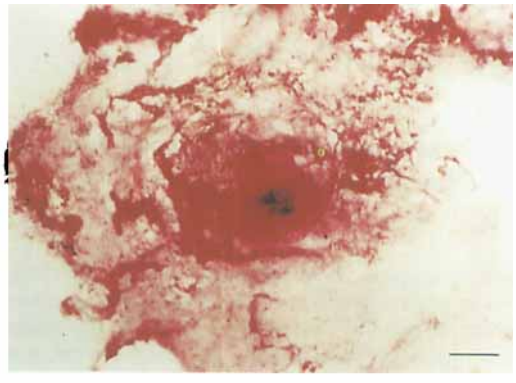
Subsequently, the graft used for ACL reconstruction and the corresponding bone-patellar tendon-bone complex from the control knee were harvested and stained by a modified gold chloride method (Gairns 1930, Zimny et al. 1985). Specimens were frozen and sectioned on a sliding microtome at 100 µm. Serial sections were studied under the light microscope. Pacinian corpuscles, Ruffini corpuscles and free nerve endings were counted after being identified morphologically (Figure 2).

We investigated the total number of each type of sensory nerve ending per ligament for reconstructed ligaments and control patellar tendons. The number is not per unit volume of the ligament or the number per slice, but the total number of each type of sensory nerve ending in all slices for each ligament.

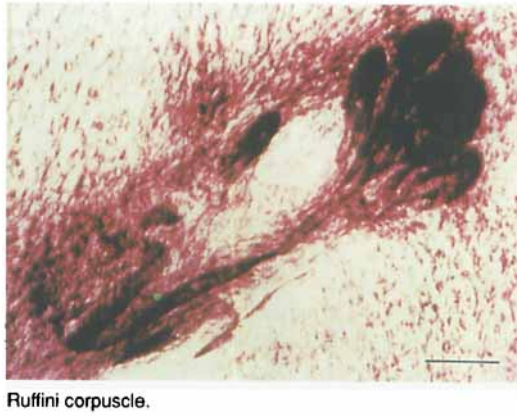
The Wilcoxon signed-ranks test was used to analyze differences between the reconstructed ligament and the control patellar tendon. P-values < 0.05 were considered significant.

Histological investigation of mechanoreceptors was performed in 7 rabbits after 10 weeks, 5 after 20 weeks, and 5 after 30 weeks.

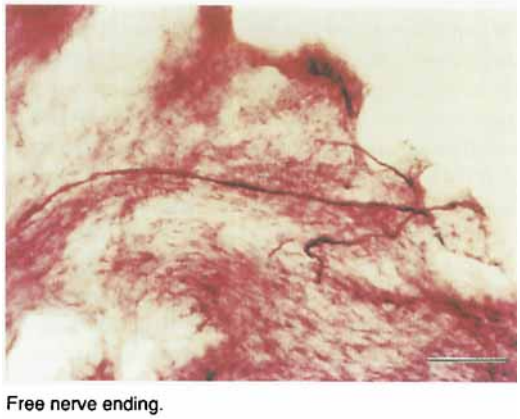
Figure 2. Two types of mechanoreceptors and free nerve endings in the reconstructed ligament and the control tendon. Bar 100 µm. (Modified gold chloride, x100).



Pacinian corpuscle.



Ruffini corpuscle.



Free nerve ending.

Results

Ligament insufficiency was noted in 2 of 7 rabbits at 10 weeks after surgery. In rabbits with ligament insufficiency, the knee joint was unstable and the grafted ACL was reduced in volume and elongated. Very little fibrous tissue was observed around the grafted ligament. Bone union was not complete at the sites of graft insertion and there was a space between the bone and the ligament. In these 2 rabbits, there was none and 2 Pacinian corpuscles in the graft, respectively, while 2 and 5 were found in the control patellar tendon. There were 1 and 3 Ruffini corpuscles in the graft, while there were 5 and 5 in the control patellar tendon. The number of each type of mechanoreceptor in the graft was lower than that in

Comparison of the number of sensory nerve endings per ligament between the reconstruction group (R) and the control group (C). Mean SD

Sensory nerve ending		Postop. 10 w n 5	Postop. 20 w n 5	Postop. 30 w n 5
Pacinian corpuscle	R	3.4 1.5	3.0 1.0	4.0 1.0
	C	7.2 1.6	4.8 1.8	4.8 1.3
		(p = 0.04)	(p = 0.10)	(p = 0.36)
Ruffini corpuscle	R	6.2 2.6	5.2 1.5	4.8 0.4
	C	2.6 1.5	1.6 1.7	2.8 2.0
		(p = 0.08)	(p = 0.04)	(p = 0.10)
Pacinian and Ruffini corp.	R	9.6 2.1	8.2 0.8	8.8 1.3
	C	9.8 2.5	6.4 0.9	7.6 3.1
		(p = 0.79)	(p = 0.07)	(p = 0.47)
Free nerve ending	R	19 2.5	26 12.5	26 10.6
	C	23 6.8	19 4.8	26 3.7
		(p = 0.42)	(p = 0.22)	(p = 0.79)

the control patellar tendon.

In rabbits without ligament insufficiency, the reconstructed ligament had sufficient volume and was covered with reddish fibrous tissue at 10 weeks postoperatively and with whitish fibrous tissue at 20 and 30 weeks postoperatively. Bone union was achieved between the bone fragments attached to the ligament and the femur or the tibia, and the insertion of the ligament was surrounded with cancellous bone. The average number of Pacinian corpuscles at 10 weeks postoperatively was 3.4 in the reconstruction group and 7.2 in the control group, which was significantly lower in the reconstruction group ($p = 0.04$). The two groups showed no significant differences at 20 and 30 weeks postoperatively. The average number of Ruffini corpuscles at 20 weeks postoperatively was 5.2 in the reconstruction group and 1.6 in the control group, which was significantly higher in the reconstruction group ($p = 0.04$). The two groups showed no significant difference at 10 and 30 weeks postoperatively. With respect to the total number of Pacinian and Ruffini corpuscles, there were no significant differences between the reconstruction group and the control group at 10, 20, and 30 weeks postoperatively. The average numbers of free nerve endings were 19 at 10 weeks, 26 at 20 weeks, and 26 at 30 weeks postoperatively. There were no significant differences between the control group and the reconstruction group (Table).

There were 142 (SD 8) slices for the reconstructed ligaments per ligament, and 192 (SD 5) slices for the control patellar tendon per ligament. The total number of slices for the reconstructed ligaments was therefore 2,388, while the total number of control patellar tendons was 3,315.

Discussion

Barrack et al. (1989) reported that patients with ACL injury show reduced proprioceptive functions in the knee. Barrett (1991) assessed 45 patients undergoing ACL reconstruction, using one third of the patellar tendon as a free graft, and reported that proprioception, rather than the clinical excellence of the repair, was the major factor in the outcome of ACL reconstruction. Based on these reports, the presence of mechanoreceptors in the reconstructed ligament appears to be essential to the retention of adequate knee function.

Histologic investigation showed mechanoreceptors in the reconstructed ligaments from 10–30 weeks after surgery. With respect to the total number of the two types of corpuscles and the number of free nerve endings, there were no significant differences between the reconstructed ACL and the control patellar tendon.

10 weeks postoperatively, the number of Pacinian corpuscles in the reconstructed ligament was significantly decreased. However, at 20 weeks postoperatively, the number of Ruffini corpuscles in the reconstructed ligament was increased ($p = 0.04$). Given that Pacinian corpuscles respond to acceleration, we considered that this was a transient reduction caused by lack of stimulation of the Pacinian corpuscles as a result of having the leg in a cast for 2 weeks after surgery and reduced activity postoperatively. Since Ruffini corpuscles are responsible for sensing position, we consider this a transient increase caused by the leg being kept stationary. Nonetheless, further investigations are needed on the relation between the reconstructed anterior cruciate ligaments and control patellar tendons in a postoperative group over 30 weeks.

The number of free nerve endings remained almost unchanged in the ligament, showing no significant differences. Unlike mechanoreceptors, such as Pacinian and Ruffini corpuscles, free nerve endings are pain receptors that do not seem to be influenced by postoperative immobilization.

In 2 rabbits with ligament insufficiency, histological investigation revealed an obvious decrease in the number of mechanoreceptors. This is believed to be because remodeling of the ligament itself was poorer than that in the group with satisfactory stability as a result of postoperative joint instability, and the failure of the mechanoreceptor population to reestablish itself. These results suggest that ACL reconstruction, using a bone-patellar tendon-bone complex, can retain the neurological mechanism of the knee and thus be useful for maintaining proprioception.

Although the present study confirmed that the num-

ber of mechanoreceptors did not change between 10 and 30 weeks after surgery, it remains unknown whether these two types of mechanoreceptors functioned normally in the reconstructed ACL. In addition, quantitative changes in the nerve endings were not examined during the acute postoperative stage before 10 weeks. Therefore, it is not known whether the mechanoreceptors were present immediately after surgery or underwent regeneration. This should be investigated in a future study.

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