Devascularization of the anterior cruciate ligament by synovial stripping in rabbits

An experimental mode

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In rabbits, synovial stripping of the anterior cruciate ligament was performed, and histologic and mechanical changes were followed up to 2 months. The operation did not immediately affect the strength of the ligament or its histological structure. However, a gradual deterioration of mechanical properties, associated with collagen necrosis and an ineffectual reparative response, was evident. Thus, synovial stripping of the ligament with the attendant concomitant devascularization leads to ligamentary insufficiency despite the lack of structural damage to the ligament by the contusion itself.

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To evaluate whether devascularization of the anterior cruciate ligament can cause deterioration of knee joint stability, we denuded the ligament of its synovial cover without any structural damage to the ligament itself.

Methods

Animals

New Zealand white rabbits were anesthetized by an intraperitoneal injection of Nembutal (pentobarbitone sodium 60 mg/kg body weight). The knees were shaved and scrubbed. A medial parapatellar incision was made and the retinaculum opened. The meniscofemoral ligament was excised to allow exposure of the anterior cruciate ligament. A 2-0 prolene thread around the anterior ligament was passed up and down to detach the synovium. This maneuver leads to devascularization of the ligament by disruption of the blood vessels which enter the ligament substance through the surrounding synovium.

At the conclusion of the procedure, the ligament was stripped naked of synovium without damaging its fibers. In order to ensure lack of such damage the ligament was examined closely under a x8 magnification lens. The arthrotomy was closed in layers. In the contra-lateral knee a sham operation was carried out in which the ligament was similarly exposed but not stripped. These procedures were performed on 30 animals designated as the experimental animal group.

In addition, 12 animals formed a control group, where a sham operation (arthrotomy only) was performed on the right limb, while the left limb served as an untreated control. Thus, two kinds of control limbs were used, sham-operated limbs, in which the ligament was not stripped and normal untreated limbs. 10 animals in the experimental group were killed immediately after the operation as well as 4 animals in the control group. The same number of animals were killed after 2 weeks and after 9 weeks.

All animals survived the operation and recovered uneventfully. They limped for 2–3 days and later ran without favoring the leg. None of the animals developed septic complications or a knee effusion.

Mechanical evaluation of ligaments

In 5 experimental animals and 2 control animals, at each time interval, both knee joints were dissected immediately after death. They were tested within 5 minutes of dissection in an Instron Universal Testing Apparatus (Instron Table Model 1026) as bone-ligament-bone preparation. Ligament lengths were measured using a caliper. As the anterior cruciate ligament has a broad insertion and a spiral course, it is impossible to measure an accurate length for calculations of strain (Noyes et al. 1974, Butler et al. 1984). Instead, in this study an average value was calculated for all

ligaments (10.2 ± 0.7 millimeters). This value was used as a basis for strain calculations. Deformation was measured using cross-head displacement.

The ligaments were tested at a speed of 100 percent elongation per minute, as this rate is close to the physiologic limit of performance of the ligament under normal loading conditions (Noyes et al. 1974). Load to linear ultimate failure, as well as load to ultimate tensile strength (UTS), were measured (Noyes et al. 1974). These were expressed as ratio to ligament strength in the contralateral (unoperated) limb. This was done in order to check for interanimal variability in the strength of the ligaments, as well as for changes in the ambient conditions in which the test was performed.

Energy absorbed to failure of the ligament was calculated as the area under the curve of force versus elongation of the ligament. In addition, values of stress to 20 percent strain were measured. This measurement was performed both on experimental and control ligaments. Similar measurements were also performed on the contralateral, i.e. unoperated knees.

Histology

5 experimental animals and 2 control animals were killed at each time interval at 0, 2, and 9 weeks following operation. Ligaments from both knees of the animals were processed for histology. The ligaments were dissected and isolated.

In order to measure the cross-sectional area, the ligament was transected with a sharp knife into three equally long pieces, immediately after dissection (to avoid drying). The cross-section area of each of the pieces was measured with an image analyzer under a ×8 magnification. This is an approximation of the ligament cross-section area as it actually changes continuously along the ligament. To calculate stress values the average value of a cross-section of the pieces was used.

The tissue was then fixed for 48 hours in buffered solution containing formalin 4 percent and cetylpyridinium chloride (CPC) 5 percent (the latter component is used to retain the glycosaminoglycans of the sample). It was later transferred to formalin 10 percent – phosphate buffered saline (pH 7.4) for 24 hours and then embedded in methacrylate (Westen et al. 1981). Tissue sections from the three regions of the ligament (the part of the ligament adjacent to the proximal femoral attachment, midsubstance of the ligament and the part of the ligament adjacent to the distal tibial attachment) were stained with alcian blue (pH 1) for sulfated glycosaminoglycans and with alcian blue (pH 2.5) for nonsulfated glycosaminoglycans. Other slides were stained with Masson's trichrome and HE. Quantification of the histologic results was carried out with an image analyzer (JAVA, Jandel Scientific, 65 Koch Road, Corte Madera, CA 94925, U.S.A.). These factors included average nuclei size, cell density, ratio of cell nuclei to extracellular matrix as well as number of synovial layers surrounding the ligament. Results were calculated with CSS: Statistical Program (StatSoft Inc., 2325 East 13th street, Tulsa, OK 74104, U.S.A.) employing the module CSS: QUICK ANOVA/ANCOVA and statistical significance determined by *P*-values.

Results

Macroscopic observations

In the experimental animals, the operation did not alter the appearance of the ligament, neither immediately nor after 2 weeks. On the other hand, at 9 weeks the ligament appeared lax and small. At this stage the ligament was surrounded by hypertrophic synovium (Figure 1). No alterations were noticed macroscopically in the menisci and cartilage during the follow-up period. There were no macroscopic changes in the cruciate ligament in animals of the control group.

Microscopic observations

Ligament cross-section area was not altered by the operation itself, as evidenced by a normal cross-sec-

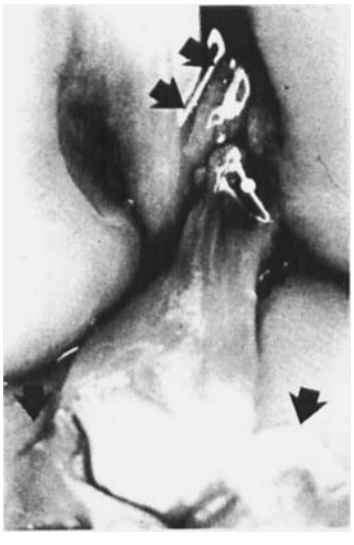

Figure 1. The anterior cruciate ligament two months after devascularization. Note hypertrophic synovium (arrows) which fills the intracondylar notch of the femor, ×3.5.

Figure 2 A. The anterior cruciate ligament two weeks after devascularization. Note areas of collagen necrosis (arrows) as well as mitotic activity of tendon fibroblasts, large in size and unevenly distributed. Masson's trichrome, ×200.

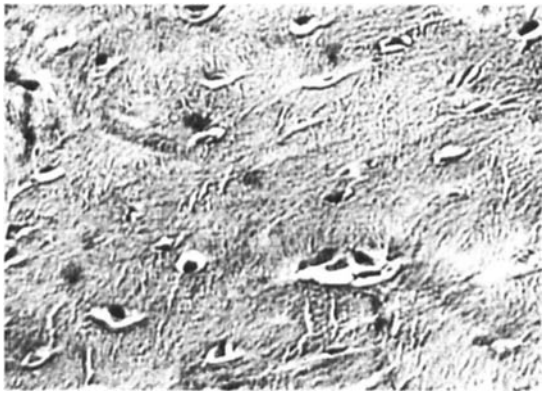

Figure 2 B. Section similar to Figure 2 A, from a sham-operated joint, in which arthrotomy only was performed. Note smaller size of the resting fibroblasts (lack of mitotic figures), and their even distribution. The collagen bundles are uniform in size and shape. Masson's trichrome, ×200.

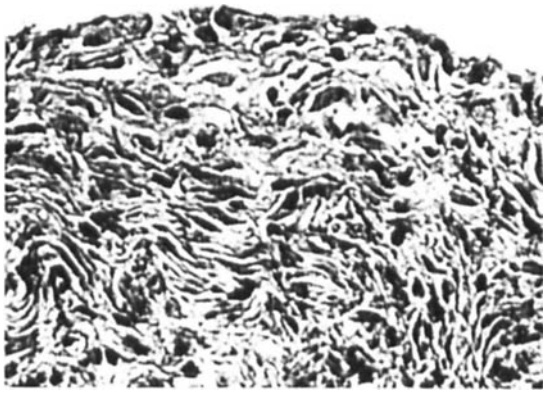

Figure 3. The anterior cruciate ligament two months after devascularization. Note the great increase in cell density and the concurrent decrease in size and organization of the collagen bundles. Masson's trichrome, ×200.

Table 1. Histological properties of devascularized anterior cruciate ligaments . Mean *SD*

Weeks after operation	Nuclei to matrix ratio (percent of ligament occupied by nuclei)	Cell density (nuclei per 1000 microns^2)
0	0.8 *0.1*	1.0 *0.70*
2	1.1* *0.1*	1.8* *0.20*
9	5.5* *1.0*	2.8* *0.15*

*$P < 0.001$ (ANOVA and Student's *t*-test).
The data on animals in the experimental group (sham-operated knee) and in both knees in the control group were at all the time intervals similar to those in the experimental group at zero time (immediately after operation).

tion area immediately after the operation ($2.7 \times 10^6 \pm 3 \times 10^5$ microns^2) and at 2 weeks. However, it diminished markedly two months after the operation ($1.1 \times 10^6 \pm 4 \times 10^5$ microns^2).

The ligament structure was not affected by the operation, as evidenced by a normal histological appearance immediately after the operation. However, 2 weeks later early signs of collagen necrosis appeared (Figure 2). This was followed at the later observation period, by an intense fibroblastic proliferation. The average number of synovial layers surrounding the ligament (Lindblad and Hedfors 1985) increased from 0.7 ± 0.3 to 5.1 ± 2.3 after 2 months, and the ligament structure appeared disorganized (Figure 3). The collagen bundles decreased in size while cell density and nuclei to matrix ratio increased, especially after 2 months (Table 1). Cruciate ligaments in the control group had a histology like that in the untreated contralateral ligament.

Biomechanic results

Strength of the ligament, as assessed by UTS and linear load to failure, was not affected by the operation itself. However, both UTS and linear load to failure decreased slightly after 2 weeks and markedly after 2 months (Table 2). Similar changes were observed in the amount of energy absorbed to failure of the ligament. The stress to strain ratio decreased from 7.47 ± 0.8 mP in the range from 0–20 percent strain in normal animals to 3.5 ± 0.5 mP in operated limbs of animals in the experimental group, 2 months after the operation. Sham operation did not affect any of the mechanical parameters tested. The ligaments in untreated limbs of animals in the control group behaved similarly to those in the sham-operated groups. Thus, we did not observe a detrimental effect of arthrotomy without synovial stripping from the ligament.

Table 2. Mechanical properties of devascularized anterior cruciate ligaments loaded in tension to ultimate failure or ultimate tensile strength. Mean ratio experimental/sham-operated knee, *SD*

Weeks after operation	Energy absorbed by ligament loaded in tension to failure	Ultimate tensile strength (UTS)
0	1.15 *0.15*	1.30 *0.20*
2	0.82* *0.09*	0.55* *0.08*
9	0.28* *0.08*	0.20* *0.03*

*$P < 0.001$ (ANOVA and Student's *t*-test).
The data on both knees of the animals in the control group were at all times tested similar to those in the experimental group at zero time.

Discussion

The sequence of events of partial damage of the anterior cruciate ligament is not clear. Opinions expressed in the literature range from an always benign course, as suggested by Nielsen et al. (1984) and Odensten et al. (1985), to a course similar to that of a complete rupture, at least in a subset of patients in which most of the ligament is damaged (Noyes et al. 1989). While it may be expected that patients with a tear of most of the fibers of the ligament will develop progressive instability, the progression of instability in patients with only sub-synovial bleeding without disruption of the ligament fibers, Sandberg Type 1 (Sandberg and Balkfors 1987), is less explicable. Such partial tears progress to clinical instability in some cases (Noyes et al. 1989, On et al. 1990).

The mechanism causing instability in a visually intact ligament has been explained by the disruption of microscopic collagen fibrils demonstrated by electron microscopy (Kennedy et al. 1976). Such a mechanism cannot explain the gradual deterioration of ligament anatomy and function in our model; if microscopic disruption of the ligament occurred during the operation, then tensile strength would have decreased immediately after the operation. Furthermore, ligament function was unaltered in sham-operated animals. Thus, the progressive functional insufficiency could be related to the initial devascularization. This conclusion appears valid despite the fact that blood flow through the ligament was not directly determined in this study.

The gradual ligament insufficiency in our model is similar to our own clinical observations (On et al. 1990) as well as those of other authors. Arthroscopies performed early after the initial trauma, often demonstrate contusion of the ligament and synovial bleeding (McDaniel 1976, Johnson 1981, Monaco et al. 1982). This injury pattern is faithfully mimicked by this model. Progressive knee instability frequently follows in patients (Noyes et al. 1989), as in the animals examined in this model. The end-result, in humans, appears to be a lax and fibrotic anterior cruciate ligament, with few blood vessels on its surface. Synovitis limited to the notch is a quite constant accompanying finding as well (Johnson 1981). All these phenomena were also observed in our experiment.

The major difference between the clinical situation and our model is the time frame in which the deterioration occurs as well as the variety of end-results observed clinically. In rabbits the deterioration of the ligament occurs much more rapidly. Therefore, evaluation of partial damage to the anterior cruciate ligament in humans may require a long-term follow-up.

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