Meniscectomy leads to an early increase in subchondral bone plate thickness in the rabbit knee

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ABSTRACT We evaluated morphological changes in the tibial bone after meniscectomy in a rabbit model. 15 rabbits subjected to a medial meniscectomy in the right knee and a sham-operation in the left. Histomorphometric parameters were evaluated in the subchondral bone plate and the underlying trabecular bone, 13, 25 and 40 weeks after surgery. 5 rabbits were used as unoperated controls.

Meniscectomized knees had a thicker subchondral bone plate than sham-operated contralateral ones in 13 of the 15 rabbits ($p = 0.01$), but the trabecular bone showed no morphological differences. The meniscectomized knees of these rabbits developed mild osteoarthrosis, described elsewhere, which may have been partly due to a change in the mechanical properties of the thickened subchondral bone plate.

Our findings suggest that the first bony response after meniscectomy occurs in the subchondral bone plate rather than in the trabecular bone.

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The risk of radiographic knee osteoarthrosis (OA) increases after meniscectomy (Roos et al. 1998). Many patients undergoing total knee replacement have had a meniscectomy (Neyret et al. 1994). However, the sequence of intraarticular changes leading to osteoarthrosis after meniscectomy is unclear. Although the entire organ system of the joint is involved in the disease, the focus has been on the articular cartilage. Meniscectomy changes the loading situation in the subchondral bone. It modifies the bony architecture, according to Wolff's law, and possibly even the mechanical properties of the subchondral bone. A change in stiffness has been thought to be involved in the progression of cartilage damage (Radin and Rose 1986). Previous clinical studies have shown an alteration in bone mineral density as well as a sclerotic and thickened subchondral bone plate in OA (Grynpas et al. 1991, Rockborn and Messner 2000). However, the early bony response to meniscectomy is still unclear and can not be evaluated in clinical studies. One factor involved in the mechanical properties of the bone is its architecture. In this study, we assessed the morphological changes in the subchondral bone in a rabbit model with meniscectomy. In the same rabbits, we have already shown a reduction in the mineral content of the subchondral bone 13 to 40 weeks after removal of the meniscus (Messner et al. 2000).

Animals and methods

Animal experiment

20 adult New Zealand White female rabbits were obtained from Lidköping (Sweden) rabbit farm and housed 1 in each cage with an area of 0.5 m^2. At operation, the rabbits weighed between 3.3–5.1 kg. The experiment was approved by the regional ethics committee, and the guidelines for animal use and care were followed. We have already reported various findings concerning these animals, which are not related to the purpose of the present study (Messner et al. 2000, 2001, Fahlgren et al. 2001).

15 rabbits underwent a medial meniscectomy in the right knee and a sham operation in the left knee, using intravenous anesthesia with 15 mg/kg ketamine and 1.5 mg/kg xylazine hydrochloride.

The operation was performed, as described elsewhere (Messner et al. 2000). Briefly, the medial meniscus was removed via two capsular incisions, anterior and posterior to the medial collateral ligament. A similar operation, done as a sham procedure, included incision of the capsule and visualization of the meniscus, but the meniscus was left intact. Before the wound was closed, the joint was irrigated with some drops of saline and oxytetracycline hydrochloride and, in addition, a single dose of the same antibiotic (10 mg/kg) was given intravenously.

Wound healing was assessed daily until full functional recovery. At the predetermined time, the animals were killed with an overdose of pentobarbitural natrium.

Follow-up and sampling

5 rabbits each were killed at 13, 25 or 40 weeks after surgery. The remaining 5 rabbits were used as unoperated controls. At death, all rabbits were skeletally mature. After death, the hind limbs were disarticulated at the hip joint. Before histological processing, the soft tissues around the knee were removed. The tibial shaft was cut below the tibial insertion of the medial collateral ligament.

Histological processing

Tibial specimens were fixated in 10% formalin and decalcified in 22% formic acid. The medial tibial plateau was divided into three frontal sections (anterior, central and posterior), each 3 mm wide. Sections were embedded in paraffin and then cut into 5 μm-thick slices, which were stained with alcian-blue/periodic acid Schiff and safranin-O.

Histomorphometry

Histomorphometry was done using a Jenaval light microscope at a screen magnification of ×120. The technically best section of a minimum of 5 sections from each area measured was taken for further analysis and a single investigator (AF) made all the measurements in a blinded fashion. Each section was divided into a central and a peripheral part of the medial tibial plateau. The central part was the area under the central depression of the joint surface, and the peripheral part the area under the surface normally covered by the meniscus. The findings in both parts were analyzed separately.

Subchondral bone plate thickness and juxtachondral bone density were measured with a digitizing camera (CoolSNAP) and Image analysis software (Image-Pro Plus Program 4.1, Media Cybernetics, USA). The bone plate thickness was the mean distance between a line connecting the most advanced bone tissue islands in the calcified cartilage and a line connecting the most proximal parts of the marrow cavity. The square (2000 μm-wide by 1500 μm-deep) for measuring the juxtachondral bone volume density was placed with its proximal border at the line that connected the most advanced bone tissue islands in the calcified cartilage, and extended down into the cancellous bone. The subchondral bone plate covered about one third of the area measured and because of less bone density in the cancellous part of this area, the density measurement mainly reflects the amount of bone comprising the subchondral bone plate. The bone tissue area was measured relative to the total area measured.

Histomorphometry of the trabecular bone was done by using the Merz eyepiece graticule that extended over an area of 3600 μm by 3600 μm. This square was placed at the proximal part of the marrow cavity, and if it partly covered the marrow cavity, that part was excluded from the total area measured. Bone volume density (BV/TV) and bone surface density (BS/TV) were analyzed directly. Trabecular bone surface density (BS/BV), trabecular thickness (TB.Th), and trabecular number were then calculated from BV/TV and BS/TV, using Parfitt's formulas (Parfitt et al. 1983, Parfitt et al. 1987) (Table 1).

Statistics

We used the Statistica 5.5 program (StatSoft, Inc, Tulsa OK, USA). The Wilcoxon signed rank test was used to calculate differences between the meniscectomized and sham-operated knees. Regression analysis was used for the histomorphometric parameters and time. Confidence intervals were based on t-statistics.

Results

The subchondral bone plate

The subchondral bone plate in the entire medial

Table 1. Histomorphometric measurements

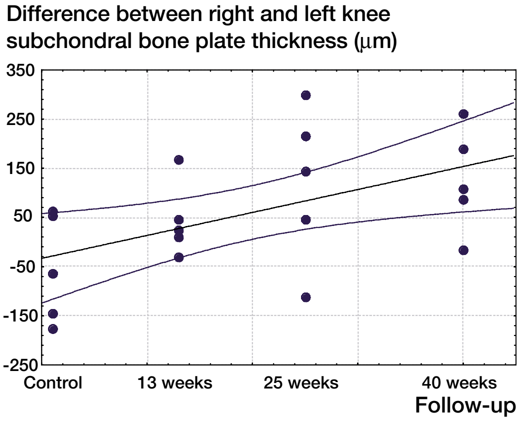
Primary measurements	Abbreviations	Formula
Subchondral bone plate thickness (µm)	B.Pl.Th	
Juxtachondral bone volume density (%)	B.Dn	
Bone volume density (%)	BV/TV	$(P*100)/(F*36)$
Bone surface density (mm^2/cm^3)	BS/TV	$(N/F)*k_{sv}$ $(k_{sv}=(4*103)/(\pi*36*d))$
Derived measurements		
Trabecular bone surface density (mm^2/mm^3)	BS/BV	$(N/P)*k_{sv}$ $(k_{sv}=(4/\pi*d))$
Trabecular thickness (µm)	Tb.Th	2000 / (BS/BV)
Trabecular number (/mm)	Tb.N	(BV/TV*10) / Tb.Th

F number of fields measured, P number of hits on trabecular area, N number of intersections with trabecular perimeter, d grid constant, 36 number of possible hits per field (Merz & Shenk 1970).

Table 2. Histomorphometric characteristics of meniscectomized (ME) and contralateral sham-operated knees (Sham). Difference between ME and Sham (Diff). Mean values concerning the subchondral bone plate of the medial tibial plateau

	Central site			Peripheral site		
	ME	Sham	Diff (95% CI)	ME	Sham	Diff (95% CI)
Subchondral bone plate thickness (µm)	519	456	63 (9–116)	491	394	97 (32–161)
Juxtachondral bone volume density (%)	37	36	1 (-2.6–4.2)	31	36	-5 (-9– -2)

tibial plateau (central and peripheral data combined) was thicker in most of the meniscectomized knees than in the sham-operated ones (13/15, p = 0.01). This was due to an increase in the peripheral (12/15, p = 0.01) and central parts (11/15, p = 0.05). The mean increase in thickness was 25% peripherally and 14% centrally (Table 2). The peripheral subchondral plate thickness increased with the follow-up time ($r^2 = 0.27$, p = 0.02) (Figure 1), an effect that was not significant in the central part of the subchondral bone plate. We found no significant difference in the juxtachondral bone volume density from sham-operated knees or controls.



Note the difference in thickness of the subchondral bone plate in the peripheral part of the medial tibial plateau between the right and left knees, in control and operated animals at three follow-ups (13, 25 and 40 weeks after surgery) ($r^2 = 0.27$, p = 0.02).

The trabecular bone

We also found no effects of meniscectomy on bone volume or any other trabecular parameters, and no difference between the peripheral and central sites. Most confidence intervals were small enough to exclude any relevant effects (Table 3).

Discussion

The early response, with an increase in the thickness of the subchondral bone plate, is probably a reaction to a change in the mechanical situation,

Table 3. Histomorphometric characteristics of meniscectomized (ME) and contralateral sham-operated knees (Sham). Difference between ME and Sham (Diff). Mean values concerning the cancellous bone of the medial tibial plateau

	Central site			Peripheral site		
	ME	Sham	Diff (95% CI)	ME	Sham	Diff (95% CI)
Bone volume density (%)	30.6	30.4	0.07 (-5.10–4.85)	46.2	45.9	0.31 (-2.5–3.14)
Trabecular bone surface density (mm^2/mm^3)	10.3	10.9	-0.58 (-2.34–1.19)	9.4	9.7	-0.30 (-0.99–0.39)
Trabecular thickness (µm)	200	189	9.13 (-23.4 –41.6)	216	208	7.83 (-7.71–23.4)
Trabecular number (/mm)	1.54	1.61	-0.07 (-0.31–0.17)	2.15	2.21	-0.07 (-0.26–0.13)

according to Wolff's law. The meniscus transmits axial loads and increases the weight-bearing area of the tibiofemoral joint. After a meniscectomy, the tibiofemoral contact area is reduced and moved posteromedially, which increases the stress on another area of the tibial plateau (Kurosawa et al. 1980, Baratz et al. 1986, Odgaard et al. 1989). The thickening of the peripheral subchondral bone plate in our rabbits therefore indicates that some of the central load is transferred to the cortex in the periphery via the subchondral bone plate, which would then be subjected to a bending stress, increasing towards the periphery.

The greater stress may explain the moderate time-dependent increase in subchondral bone plate thickness. Moreover, this effect was associated with an increase in cartilage degeneration with time in the same animals, as reported elsewhere (Messner et al. 2000). The relationship between an increase in subchondral bone thickness and in severity of cartilage lesions has been reported in an animal model of spontaneous osteoarthrosis (Carlson et al. 1996). It has also been shown in patients whose changes in cartilage and in subchondral bone increased simultaneously (Shimizu et al. 1993). The association between an increase in cartilage changes and bone remodeling has also been noted by Muehleman et al. (2002) who found that the changes in cartilage could be reduced in an osteoarthrotic model by reducing bone remodeling with the use of a bisphosphonate.

Apart from an increase in stress, other factors may have affected bone remodeling in our model. The joint trauma following an injury or an operation increases blood flow and inflammation, both of which could have increased bone remodeling (Ghosh and Cheras. 2001, Shymkiw et al. 2001). The pain following the operation may have caused immobilization and subsequent local osteoporosis (Kannus et al. 1992). However, these effects would probably have been mild and not lasted a long time, but the effect of an absent meniscus was permanent. Moreover, the surgical trauma of the sham operation was almost the same as that of the meniscectomy.

Our specimens have previously been analyzed by DEXA, which showed 30% less bone mineral density in the medial tibial plateau of the meniscectomized knees than in the sham-operated knees (Messner et al. 2000). The absence of a similar reduction in histological bone density may be due to a reduction in mineral content in the matrix of bone undergoing rapid remodeling (Meunier and Boivin 1997), so that the same cancellous bone volume would consist of younger bone, which has not completed its secondary mineralization. This is supported by the findings of Grynpas et al. (1991) who showed that osteoarthrosis was associated with an abnormally low bone mineral content per volume bone tissue together with thickening of the subchondral bone. However, the difference between DEXA and the histological finding could also be due to examination of different locations with these techniques. With the former method, we measured the entire bone, including the frontal and posterior cortexes, but with the latter, we evaluated only the frontal sections in the bone.

We found an early reaction to meniscectomy with thickening of the subchondral bone plate. This effect of an intra-articular injury suggests that subchondral bone may play an important role in the development of posttraumatic osteoarthrosis.

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