The effects of meniscal allograft transplantation on articular cartilage
Rijk, P.C.

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Chapter 4

Scintigraphic Assessment of Rabbit Knee Joints after Meniscal Allograft Transplantation

Paul C. Rijk, Berthe L.F. Van Eck-Smit and Cornelis J.F. van Noorden

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Abstract

Purpose: To evaluate scintigraphically changes in rabbit knees after meniscectomy only and after meniscectomy followed by immediate or delayed meniscal allograft transplantation.

Type of Study: Experimental study.

Introduction: It has been demonstrated that meniscal allografts show capsular ingrowth in meniscectomized knees. However, it remains to be established whether a transplanted meniscus can prevent degenerative changes in the long term. In the present study, scintigraphy was used to evaluate degenerative changes in rabbit knees after meniscectomy either or not in combination with immediate or delayed meniscus transplantation.

Methods: Twenty-eight rabbits were divided into 4 groups. Three rabbits developed infective arthritis and were excluded from the study. In group A (6 animals), 2 rabbits were subjected to medial meniscectomy, 3 rabbits underwent transplantation with a freshly harvested medial meniscal allograft immediately after meniscectomy, and 1 animal underwent a sham operation. In group B (6 rabbits), medial meniscectomy was performed. Group C (6 rabbits) was subjected to meniscal transplantation immediately after meniscectomy. Group D (7 rabbits) underwent delayed allograft transplantation at 6 weeks after meniscectomy. The animals in group A underwent scintigraphy at 6 weeks after surgery. In the other groups scintigraphy was performed at 1 year follow-up. Contour changes of the knee joints and uptake of radiolabeled diphosphonate in the subchondral bone were evaluated.

Results: Animals in group A did not show any abnormalities scintigraphically. Medial compartment changes in group B were more pronounced than in group C, but this difference was not statistically significant. A significant increase of contour changes of the femoral condyle was observed in group D.

Conclusions: Immediate meniscal allograft transplantation did not result in a significant protecting effect on articular cartilage against osteoarthritic degeneration on a long-term base. Delayed meniscal transplantation revealed even more degenerative changes of articular cartilage than meniscectomy without transplantation.
Introduction

Over the past decades, progressive degenerative arthritis associated with meniscectomy has been thoroughly documented. Due to recognition of the important role of menisci in the physiology of knee joints, treatment of meniscal tears has evolved from total excision to partial excision, and to meniscal-retaining procedures. Allograft transplantation of menisci is being investigated at present, especially because of the absence of immunological rejection of meniscal tissue. A number of studies showed that meniscal implants can induce capsular healing. However, the ultimate result of transplantation cannot be judged solely on the basis of successful allograft incorporation. It remains to be established whether a transplanted meniscus can prevent or slow down progression of degenerative changes in knees after meniscectomy. Therefore, the present experimental study was performed to establish whether acute and/or delayed allograft transplantation can prevent osteoarthritis in knee joints of rabbits during a long-term follow up of 1 year.

Scintigraphy is the most sensitive diagnostic modality for evaluation of degenerative changes in knee joints. It allows analysis of contour changes of the joint and determination of uptake of bone-seeking agents in subchondral bone, which is increased in osteoarthritis. The purpose of the present scintigraphic study was to test the hypothesis that immediate or delayed meniscal allograft transplantation in rabbit knees can prevent degenerative changes of articular cartilage, which are observed after meniscectomy.

Material and Methods

Twenty-eight mature female New Zealand white rabbits weighing between 3.0 and 3.5 kg were divided into 4 groups. All surgical procedures were performed on the right knee. The nontreated contralateral knees served as paired controls. Three rabbits developed infective arthritis and were excluded from the study.

In group A (6 animals), 2 rabbits were subjected to medial meniscectomy only, 3 rabbits underwent transplantation of a fresh meniscal allograft immediately after medial meniscectomy, and 1 animal was selected for sham operation. These rabbits were scanned at 6 weeks after operation to evaluate scintigraphic abnormalities caused by post-traumatic synovitis. All other animals were subjected to scintigraphy of the knee joints at 1 year after the (first) operation. Group B (6 rabbits) underwent medial meniscectomy only. Group C (6 rabbits) underwent medial meniscectomy followed by immediate transplantation of a freshly harvested meniscal allograft. Group D (7 rabbits) was subjected to medial meniscectomy and delayed transplantation. These animals received a meniscal allograft at 6 weeks after medial meniscectomy. One or 2 days after the bone scan was performed, all rabbits were sacrificed by intravenous injection of sodium pentothal and the knees were removed for histochemical analysis.
Approval of this study was obtained from the local ethical committee for animal experiments.

**Surgical Procedures**
All animals were premedicated with an intramuscular dose of ketamine (50 mg/kg) and xylazine hydrochloride (8 mg/kg). Rabbits were anaesthetized with halothane, oxygen, and nitrous oxide inhalation via a mask. In all animals, the right knee was approached under sterile conditions by a medial parapatellar arthrotomy through the patellar fat pad and gentle lateral displacement of the extensor mechanism. Medial menisci were resected sharply along the periphery, dividing the coronary ligament, and detached from its anterior and posterior tibial bone attachments at the junction of the ligamentous attachment and the meniscal fibrocartilage. Care was taken to avoid injuring collateral and cruciate ligaments. The harvested meniscus grafts were immersed in sterile saline. Acute transplantation was performed immediately after meniscectomy by suturing an appropriately sized fresh allograft in the recipient bed using 6-0 polypropylene. Allografts were selected from removed menisci by the eye on the basis of its match in size and shape with the original meniscus. All allografts were obtained from different animals used in this study and were reimplanted within 2 hours after harvesting. The anterior and posterior horns of the grafts were reattached to the appropriate ligamentous structures; the midportion was sutured to the medial collateral ligament. Allograft position and mobility were controlled during knee flexion and extension, and under valgus and varus stress. The capsule, periarticular tissues, and skin were closed with interrupted 3-0 polyglactin sutures. Delayed transplantation was performed by a 2-step procedure with an interval of 6 weeks between meniscectomy and transplantation using a fresh allograft. The surgical procedure was as described above. In the sham procedure, the joint was surgically opened and exposed as for meniscectomy but the meniscus was not removed. After operation, animals received subcutaneous analgesics (buprenorfine, 0.05 mg/kg) during 24 hours and could move and exercise freely. Antibiotic prophylaxis was given for 72 hours (enrofloxacin 5%, 5 mg/kg).

**Scintigraphic Method**
During scintigraphy, animals were sedated with an intramuscular dose of ketamine (35 mg/kg) and xylazine hydrochloride (5 mg/kg). Anteroposterior images were obtained with a gamma camera (ARC 3000; ADAC, Best, The Netherlands) supplied with a pinhole collimator. Two hours after the intravenous injection of 70 MBq of hydroxymethylene diphosphonate labeled with technetium-99m, anteroposterior images of both knees were captured during 10 minutes.

Four regions of the operated knees (medial condyle, lateral condyle, medial tibia, and lateral tibia) were compared individually with the corresponding regions in the control joints. Differences between control left knee joints and operated right knee joints in uptake of radiolabeled diphosphonate in subchondral bone were defined
as activity and scored as 0 (normal uptake) or 1 (increased uptake). Structural changes of the outline of the bone of the knee joints were defined as contour changes and were scored as 0 (no changes present) or 1 (structural changes present).

Two observers scored all images together to obtain agreement on standards for grading. Two months and 4 months later, 1 observer scored the images twice to establish intraobserver differences. To assess interobserver differences, the second observer performed evaluation again. Finally, both observers together scored the images to obtain a consensus score. All observations were performed blindly.

**Statistical Analysis**
Cohen's kappa test was used to determine interobserver and intraobserver variation. The kappa values were categorized according to Landis and Koch:\(^\text{12}\) 0.00-0.20 slight reliability; 0.21-0.40 fair reliability; 0.41-0.60 moderate reliability; 0.61-0.80 substantial agreement; 0.81-1.00 almost perfect agreement. Comparisons between groups were performed using Fisher's exact test. Statistical significance was set at p ≤ 0.05.

**Results**

**Gross Appearance of Knee Joints**
Capsular ingrowth was observed in all meniscal transplants. None of the transplanted allografts showed extrusion.

**Intraobserver and Interobserver Variation**
The kappa values for intraobserver variation were 0.84 for grading of uptake and 0.54 for grading of contour changes. The kappa values concerning the interobserver agreement of grading of uptake and contour changes were 0.68 for both parameters.

**Scintigraphic Observations**
In all (treated and nontreated) knees, increased activity was observed in the lateral tibia compared to the medial tibia. This finding should be considered as normal. Abnormalities were never observed with scintigraphy in any of the left control knees or in lateral compartments of the right knees. At 6 weeks after the surgical procedure, none of the images of the knees of the rabbits in group A showed increased activity or contour changes.

The consensus scores of groups B, C, and D for activity and contour changes in the medial compartment of the right knee joints are presented in Table 1. One year postoperatively, 5 of 6 rabbits that were subjected to meniscectomy showed scintigraphic abnormalities (Figure 1; Table 1). In the group that underwent meniscectomy and acute transplantation, abnormalities were observed in 2 of 6 rabbits.
Table 1. Consensus score of 2 observers of uptake of radioactive diphosphonate in subchondral bone (activity) and contour changes of the medial compartment in rabbit knee joints at 1 year after meniscectomy only (group B), after meniscectomy and acute meniscal transplantation (group C), and after meniscectomy and 6 weeks delayed meniscal transplantation (group D). Scoring of activity and contour changes in the operated right knee joints is relative to the parameters in the nonoperated left knee joint.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Activity Medial tibial plateau</th>
<th>Activity Medial femoral condyle</th>
<th>Contour Medial tibial plateau</th>
<th>Contour Medial femoral condyle</th>
</tr>
</thead>
</table>
| Group B  
7       | 1                              | 0                               | 1                           | 1                            |
| 8       | 1                              | 1                               | 1                           | 0                            |
| 9       | 0                              | 0                               | 0                           | 0                            |
| 10      | 1                              | 1                               | 1                           | 0                            |
| 11      | 1                              | 0                               | 0                           | 0                            |
| 12      | 1                              | 1                               | 1                           | 1                            |
| Group C  
13      | 0                              | 0                               | 0                           | 0                            |
| 14      | 1                              | 0                               | 0                           | 0                            |
| 15      | 0                              | 0                               | 0                           | 0                            |
| 16      | 1                              | 1                               | 1                           | 1                            |
| 17      | 0                              | 0                               | 0                           | 0                            |
| 18      | 0                              | 0                               | 0                           | 0                            |
| Group D  
19      | 1                              | 0                               | 1                           | 1                            |
| 20      | 1                              | 1                               | 0                           | 1                            |
| 21      | 1                              | 1                               | 1                           | 1                            |
| 22      | 1                              | 1                               | 1                           | 1                            |
| 23      | 1                              | 1                               | 1                           | 1                            |
| 24      | 1                              | 0                               | 0                           | 1                            |
| 25      | 1                              | 0                               | 0                           | 0                            |

1 Activity score: 0 = no increase; 1 = increase. 2 Contour score: 0 = no changes; 1 = changes.

(Table 1). All 7 animals that underwent meniscectomy and delayed transplantation showed increased uptake and/or contour changes in the medial compartment of the right knee joints. No significant differences in uptake of diphosphonate in the medial tibia were observed between meniscectomized knees and knees receiving an immediate or a delayed meniscal transplant (p=0.24 and 0.46, respectively). Animals receiving a delayed transplant showed significantly more uptake in the medial tibia than those receiving an immediate transplant (p=0.021). With respect to the activity of the femur and the contour changes of the tibia, differences were not found between the 3 groups (p=0.38 and p=0.26, respectively). Most contour changes of the medial condyle were observed in group D. This finding was statistically significant (p=0.037).
Discussion

Transplantation of meniscal allografts for treatment of meniscus-deficient knees has progressed to a point that relief of pain is expected shortly after transplantation. However, studies have not yet demonstrated long-term protective effects on articular cartilage after meniscal allograft transplantation. Jackson et al. found an increase in water content and a decrease in uronic acid in meniscal allografts in goat knees, suggesting degeneration at 6 months after implantation. These findings indicated that meniscal allografts may not be able to protect articular cartilage on a long-term basis. Therefore, we have investigated the long-term effects of transplantation of meniscal allografts on osteoarthritic changes in meniscectomized rabbit knees using scintigraphy.

Osteoarthritis in knee joints causes increased uptake of diphosphonate in subchondral bone and contour changes of the bone. Both aspects can be visualized in scintigraphic images. Dieppe et al. found that retention of radioactive diphosphonate in subchondral bone is a good parameter for cartilage destruction. In our study, intraobserver agreement was almost perfect for grading of uptake and moderate for grading of contour changes, whereas interobserver agreement was substantial for both parameters. Therefore, the consensus score as presented in Table 1 can be considered as a reliable measure of osteoarthritic changes in rabbit knees.
The fact that scintigraphic abnormalities were not observed in subchondral bone in the early stages after surgery is consistent with the study of Christensen, who showed that early osteoarthritic development could not be diagnosed by scintigraphy. It can be concluded from these findings that scintigraphic abnormalities observed in later stages do not reflect processes involved in post-traumatic synovitis due to surgery. Although scintigraphic abnormalities in the rabbit knee joints at 1 year after meniscectomy were more pronounced than after immediate meniscal transplantation, this difference was not statistically significant due to small numbers of animals studied. Based on our results, a minimum sample size of 10 animals is required to demonstrate significant differences.

Cummins et al. reported that delayed meniscal allograft implantation at 3 months after meniscectomy arrests degenerative changes and allows healing of the articular cartilage at 3 months afterwards. However, our long-term findings show that contour changes of the femoral condyle at 1 year after delayed transplantation were more prominent than after meniscectomy only. Furthermore, the uptake of radiolabeled diprophosphonate in the medial tibia after delayed transplantation was significantly higher than after immediate transplantation. The pronounced scintigraphic abnormalities after delayed transplantation may be the result of the more extensive twofold surgical procedure in this group, in which articular cartilage was exposed for a longer period to the effects of drying. Aagaard et al. stated that the altered shape of the condyles due to ridge formation after meniscectomy may cause a mechanical conflict between the transplant and the condyles, which could be responsible for the poor outcome in this group.

The rabbits in the present study showed good healing of the grafts within 6 weeks after meniscal allograft transplantation, which is in agreement with the findings of Tachibana. To date, there are no controlled studies available that have compared healing of meniscal allografts transplanted with or without bone plugs. Due to the small size of rabbit knee joints, the horn ligamentous attachments of the transplanted allografts were not retained via transplantation of bone plugs, but the anterior and posterior horn were reattached to the appropriate ligamentous tibial bone attachments in the present study. The effects of this anchoring technique on load distribution and shock absorption could not be determined in our study. Bone plugs increase the antigenic load since bone allografts elicit an immune response. On the other hand, Alhalki et al. reported that implantation of meniscal autografts in human cadaver knees with bone plugs resulted in contact mechanics that were significantly closer to normal as compared with fixation with sutures. However, fixation with bone plugs requires exact size match between donor and recipient, which limits severely the use of this method. Recognition of the importance of appropriate graft selection has led to a number of studies describing radiographic landmarks for the meniscus horn attachment sites which possibly could lead to more reliable meniscus sizing in the future.
Whether acute meniscus transplantation can reduce osteoarthritis in man remains to be proven because animal models cannot completely mimic development of osteoarthritis in the human. The weight-bearing profile in rabbit knees is different from that in human knees. Furthermore, surgical procedures in small animals such as the rabbit are rather difficult and this may introduce artefacts that are hard to interpret when comparing data in small animals with those in larger animals. Nevertheless, rabbit models have proven to be one of the better models for the human situation because of similarities in histological and biochemical aspects of rabbit and human articular cartilage.\textsuperscript{23,24} The histological appearance of the articular cartilage after meniscal transplantation will be reported later in a prospective paper.

It can be concluded from the present study that the protecting effect of acute meniscal allograft transplantation on articular cartilage against osteoarthritic degeneration on a long-term basis is not statistically significant. Delayed transplantation after meniscectomy revealed even more degenerative changes of articular cartilage than meniscectomy.

Acknowledgments

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