High-resolution magnetic resonance imaging can predict osteoarthritic progression after medial meniscus posterior root injury: randomized in vivo experimental study in a rabbit model

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ABSTRACT

Importance: The field of meniscal root preservation has undergone significant advancement over the past decades; however, the challenge remains to fully understand whether meniscal root repair can ultimately arrest or delay osteoarthritic changes.

Objective: To assess longitudinal changes in articular cartilage, subchondral bone, and progression to meniscal extrusion (ME) using high-resolution magnetic resonance imaging (MRI).

Methods: Medial meniscus posterior root tear was surgically induced in 39 New Zealand white rabbits. Animals were randomly assigned into three experimental groups: partial meniscectomy after root tear (PM, n = 13); root tear left in situ (CT, n = 13); and transtibial root repair (RR, n = 13). Contralateral limbs were used as healthy controls. High resolution 4.7 Tesla MRI of the knee joint was performed at baseline, after 2-, and 4-months of post-surgery. Cartilage thickness was calculated in medial and lateral compartments. In addition, the evaluation of ME, subchondral bone edema and healing potential after root repair were assessed too.

Results: Progressive cartilage thinning, ME, and subchondral bone edema were evident in all 3 study groups after 4-months of follow-up. The mean cartilage thickness in the PM group was 0.53 mm (±0.050), 0.57 mm (±0.05) in the CT group, and 0.60 mm (±0.08) in the RR group. The PM group exhibited significantly higher cartilage loss when compared to the CT and RR groups (p < 0.001). Moreover, progressive ME and subchondral bone edema were associated with a more severe cartilage loss at the final follow-up.

Conclusion: Meniscal root repair did not halt but rather reduced the progression of osteoarthritis (OA). Degenerative changes worsened at a rapid rate in the PM group compared to the RR and CT groups. Early cartilage swelling, persistent subchondral edema, and progressive meniscal extrusion predicted a more severe progression to knee OA.

Level of evidence: II.

What are the new findings

- Medial meniscus posterior root repair doesn't halt but rather reduces the progression to knee osteoarthritis.
- Degenerative changes are more severe in knees with partial meniscectomy after medial meniscus posterior root tear compared to the root repair and conservative treatment.
- Early cartilage swelling, persistent subchondral edema, and progressive meniscal extrusion predict more severe progression to knee osteoarthritis.

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INTRODUCTION

Knee osteoarthritis (OA) is a leading cause of long-term disability and a rapidly expanding public health concern, both in terms of health-related quality of life and financial expenditure [1]. OA is considered the 3rd most common cause of disability following diabetes and dementia [1,2].

Over the last decades, there has been an increasing awareness of the importance of meniscal root tears which are estimated to constitute up to 21% of all meniscal tears [3,4]. The field of meniscal root preservation has undergone significant advancement over the past decades; however, the challenge remains to fully understand whether meniscal root repair can ultimately arrest or delay osteoarthritic changes.

OA is a dynamic and complex process involving metabolic and structural changes in the articular cartilage. Magnetic resonance imaging (MRI) is a critical tool to qualitatively and quantitatively assess articular cartilage by virtue of its excellent soft tissue contrast, high spatial resolution, and ability to quantify cartilage thickness under normal and pathologic condition. It has been theorized that an increase in cartilage thickness precedes any other morphologic changes in early OA [5]. However, it is not clearly understood if it represents an initial non-reversible phenomenon or is the expression of a reparative process characterized by tissue hypertrophy. On the other hand, meniscal extrusion (ME) is associated with osteoarthritis severity [6] and many unanswered questions remain whether the postoperative meniscal extrusion exists immediately, after the root repair, or progressed over time. Furthermore, the point of concern includes the predictive value of ME on cartilage loss. This led us to conduct the present study to evaluate the effects of 3 different therapeutic options for medial meniscus posterior root (MMPR) tears. The main purpose of the study was to assess longitudinal changes in articular cartilage, subchondral bone, and progression to ME in an experimental rabbit model after MMPR injury using high-resolution MRI.

We hypothesized that osteoarthritic changes progress gradually after root repair; albeit to a lesser extent compared to the partial meniscectomy and conservative treatment groups. Additionally, the presence of ME and subchondral edema may influence the degree of cartilage loss.

METHODS

Study design

The experiments were approved by the local animal protection service and were conducted in accordance with the National Institutes of Health Guidelines for the treatment of experimental animals (PROEX 297.8/21). Thirty-nine skeletally mature New Zealand white rabbits were used and MMPRT was surgically induced according to the previously described model [7]. Animals were randomly assigned into 3 experimental groups: root tears treated with transosseous repair (RR, n = 13); root tears left in situ (CT, n = 13); and root tears treated with partial meniscectomy (PM, n = 13).

Briefly, a medial parapatellar approach was used and the medial meniscus posterior root was transected inducing a complete, type 2 root tear. Then the torn meniscal root anatomically repositioned. A Kirschner wire (0.9 mm) was used, and an anteromedial tibial tunnel was created through the root attachment. A 2-0 Ethicon suture (Johnson & Johnson) then passed through the posterior horn of the medial meniscus and pulled out through the transtibial tunnel (Fig. 1).

In the partial meniscectomy group, the medial meniscus posterior root was completely transected from its tibial insertion. Iris scissors were used to remove one-third of the posterior horn of the meniscus (Fig. 2). The collateral and cruciate ligaments were not damaged in any approach, and the arthrotomy was closed in a layer fashion. A soft dressing was applied for 1 week following surgery. All animals were monitored postoperatively and permitted free cage activity. Intramuscular metamizole injection was administered for pain relief during the initial 48 h postsurgery.

High resolution magnetic resonance imaging study

Since the main purpose of this experimental study was to identify and describe early osteoarthritic changes the knees were examined with high resolution MRI at baseline, 2 months, and 4 months after surgery. Within-animal and between-knee longitudinal comparisons were conducted to determine changes in cartilage thickness.
MRI was performed on a Bruker BioSpec 47/40 spectrometer (Bruker BioSpin GmBH, Ettlingen, Germany) operating at 4.7 Tesla and equipped with a 12-cm gradient system with a maximum gradient strength of 50 mT/m. Animals were anesthetized with intramuscular injection of 50 mg/kg of ketamine (Ketolar®, Parke-Davis, Barcelona, Spain) combined with 10 mg/kg of xylazine (Rompun®, Bayer, Leverkusen, Germany).

Cartilage thickness was calculated based on a previously published method using a vector perpendicular to the surface of the articular cartilage [8]. Sagittal 3D gradient echo (3DGE) images were acquired with the following parameters: repetition time (TR) = 100 ms, echo time (TE) = 8 ms, flip angle = 30°, number of experiments (NEX) = 2, field of view (FOV) = 5 × 5 × 2.5 cm³. Articular abnormalities were evaluated using 2D fat-suppressed coronal T1-weighted spin-echo images (TR/TE = 800/10 ms) and 2D T2-weighted fast spin-echo images (TR/TE = 2500/60 ms) acquired with coronal, sagittal, and axial orientation. For all these sequences, the FOV acquired was = 5 × 5 cm². For the T1-weighted and T2-weighted sequences, 4 and 2 images were averaged, respectively.

The evaluation of the cartilage thickness was carried out by two independent observers (M.E.F.V. and D.M.M) using the 3DGE parasagittal images. All measurements of cartilage thickness and articular abnormalities were performed using a software developed by our research team Matlab R2022b (The Mathworks Inc., Natick MA, USA). The softwares allows automatic and precise determination of cartilage thickness and repair, including an interaction term between both factors. The potential effect of meniscal extrusion and subchondral edema on the evolution of cartilage damage over time was evaluated using repeated measures ANOVA with an interaction term with time. Statistical significance was set at 0.05 and an adjusted p-value was calculated considering the number of comparisons between the groups and using the Bonferroni correction. All statistical analysis was performed using R (R: A language and environment for statistical computing, R Foundation for Statistical Computing, 2020, Vienna, Austria).

RESULTS

Longitudinal data of 35 rabbits were included as 4 animals (2 in the PM, 1 in the RR, and 1 in the CT group) died after surgery because of self-inflicted wound infections as a result of stress in captivity. The measurements were performed on the two femoral condyles. After localizing the slice of interest, nine different zone of interest on the medial and lateral femoral condyles were defined by the user (Fig. 3). Zones of interest 1 to 6 corresponding to the weight-bearing area as rabbits have a characteristic locomotion pattern, they walk on a flexed hind leg. The weight-bearing area was selected because it has been demonstrated that these zones show the earliest and most severe histological abnormalities [5].

The signal image profile of each radius shows the cartilage as “a peak” in the profile plot, since, in the 3DGE image, the cartilage appears hyperintense in contrast to the subchondral bone, which has a low signal. Cartilage thickness was calculated as the spatial distance between the T1 and T2-weighted images.

The presence or absence of postoperative meniscal extrusion was assessed on the mid–coronal plane at the level of the medial collateral ligament at baseline, 2-, and 4-months of follow-up [9]. The previously described and validated method for ME was used [10]. Meniscal extrusion was defined as extrusion beyond a reference line connecting both the femoral condyle and tibial plateau and was measured between the medial edge of the tibial plateau and the outer edge of the medial meniscus except for osteophytes. There is no previously described known cutoff value for ME in a rabbit model. It has been reported that the average coronal width of human proximal tibial is 79.8 (±5.8) mm and 18.1 mm in rabbits [11,12]. Based on these dimensions and considering that the ME equal or greater than 3 mm in human knee is related to cartilage damage and OA [13], we reported ME as significant any extrusion beyond ≥0.75 mm of the joint margin in a rabbit knee.

Additionally, meniscal healing was assessed as complete using signal intensity continuity in sagittal, coronal, and axial MRI. Healing if any of the views displayed a loss of continuity, and it was deemed not healed if there was a lack of continuity in any MRI view [10]. The presence of subchondral edema was evaluated and defined as a focal bone marrow signal increase on T2-weighted MRI.

Statistical analysis

The required sample size was determined using statgraphics (Statgraphics Technologies, Inc., The Plains VA, USA) and based on the effect size in previously reported preclinical studies [14,15]. Assuming an alpha level of .05, thirteen animals were included in each group to avoid unforeseeable future complications. The random number table method was used to prevent selection bias.

The normality of the data was assessed by the Shapiro–Wilk test. Qualitative variables were summarized by frequencies and percentages, and quantitative values by mean and standard deviation or by median and interquartile range, depending on the skewness of the data distribution. Between-group comparisons in cartilage thickness were performed using Student’s t test when comparing two groups and one-way ANOVA when comparing three or more groups. Changes at each point of time were compared using a two-way repeated measures ANOVA with factors time and repair, including an interaction term between both factors. The potential effect of meniscal extrusion and subchondral edema on the evolution of cartilage damage over time was evaluated using repeated measures ANOVA with an interaction term with time. Statistical significance was set at 0.05 and an adjusted p-value was calculated considering the number of comparisons between the groups and using the Bonferroni correction. All statistical analysis was performed using R (R: A language and environment for statistical computing, R Foundation for Statistical Computing, 2020, Vienna, Austria).

Fig. 2. Macroscopic illustration of partial meniscectomy limits (the red arrows) leaving intact one-third of the posterior horn of the meniscus. Medial meniscus posterior root remnant (the blue arrow).
A large prevalence of subchondral bone edema was observed in knees with more severe cartilage loss. By 4 months, bone edema persisted in knees with ME and subsequent cartilage loss. Difference continued throughout the follow-up and significantly severe cartilage damage was observed in knees with subchondral edema compared to those without persisting bone marrow edema (Table 1).

Assessment of healing potential in the RR group demonstrated that the meniscus had fully healed in 7 knees (58.3%), had partially healed in 4 (33.3%), and had not healed in 1 (8.3%) knee (Fig. 5).

**DISCUSSION**

The main finding of this study was that the root repair did not avoid cartilage loss and ME over time. Osteoarthritic changes worsened at a more rapid rate in knees with PM than with CT and RR. The presence of meniscal extrusion and subchondral edema increased cartilage loss, and early cartilage swelling phenomenon was not a reparative process.

To date, there remains a paucity of data whether cartilage thickening precedes and contributes to the development of severe osteoarthritic...
changes or is the result of a reparative process. It has been suggested that cartilage thickening in rabbit models reflects hypertrophic response in OA [16]. On the other hand, cartilage swelling has been strongly correlated with the loss of degraded collagen molecules [17]. However, previous studies have observed an initial phase of cartilage hypertrophy followed by cartilage degeneration [18,19]. In the anterior cruciate ligament transected (ACL-T) model in dogs, Brandt et al. [20] found cartilage damage was preceded by cartilage swelling. Moreover, focal increases in cartilage thickness were reported in a cross-sectional study as a potential early sign of OA in human knees with ACL rupture and in knees with early radiographic OA [21,22]. The findings of this study support the hypothesis that cartilage swelling can be the earliest measurable and predictive sign of a more severe OA. The increase in cartilage thickness detected at 2 months of follow-up may explain the absence of significant difference between baseline and at 2 months of follow-up. However, significant decreases in cartilage thickness was found from 2 to 4 months and from baseline to 4 months of follow-up (p < 0.001). This observation prompts us to consider that it is not a tissue expression of a reparative phenomenon but rather signifies enduring cartilage damage. Consequently, we could suggest that a focal increase in cartilage thickness is one of the initial measurable signs that precedes any other morphological changes.

There is a substantial body of literature supporting the efficacy of meniscal root repair leading to a favorable mid-term clinical outcome [3,23–25]. However, the presence of ME after root repair has emerged as a noteworthy subject in recent literature [26]. Current evidence is limited to retrospective and small cohort studies where the confounding variables and lack of a control group can severely compromise drawing a solid conclusion. Studies have reported a correlation between increased ME and a higher likelihood of osteoarthritic progression [23]. Our data show that if ME is present at an earlier postoperative stage it progresses over time, and such extrusion is positively correlated with cartilage loss.

There remains considerable surrounding the temporal relationship between meniscal root repair and postoperative ME. It was suggested that conducting root repair soon after the injury is more effective in averting postoperative ME compared to delayed surgery [27]. We observed that root repair did not completely prevent postoperative ME and cartilage damage progressed gradually. Understanding the current evidence is important as the presence of ME in the early postoperative period may predict a more severe cartilage loss and disease progression. These are valuable findings as they offer predictive insights that help bridge the existing gaps between imaging assessments and clinical judgment. While reducing ME is deemed considered biomechanically crucial, postoperative functional scores indicate that achieving such reduction may not be mandatory to see the clinical benefit following root repair [24,25].

Numerous studies have investigated the structural assessment of postoperative meniscal healing potential in patients who underwent meniscal root repair. Furumatsu et al. [28] described an MRI-based suspension bridge sign as a predictor of arthroscopically favorable meniscal healing in a retrospective study including 58 patients after MMPR repair. Postoperative functional outcomes were assessed at second-look arthroscopy. Twenty-three patients (40%) showed good arthroscopic healing scores. The authors did not find any association between the healing status, functional outcomes, and ME. It is worth noting that the findings may have been influenced by potential selection bias, given that the mean age of the included patients was 62.9 years. Krych et al. [26] prospectively evaluated 43 root tears with pre- and postoperative MRI scans assessing healing status after root repair. The authors noted that, on average, 40% of meniscus roots healed completely, 58% partially, and 2.3% did not heal over a mean follow-up period of 6.3 months (range, 5.1–8). There was no significant progression of cartilage degeneration and subchondral bone abnormalities, albeit mean extrusion worsened significantly in the first 6 months. Our findings partially align with existing data as we observed significant increase in ME in all 3 study groups. Moreover, degenerative changes progressed in knees with ME and persistent subchondral bone edema.

The presence of subchondral insufficiency fractures of the knee (SIFK), previously known as spontaneous osteonecrosis of the knee (SONK), [29,30] has been widely related to meniscal root tears with concomitant extrusion [30]. Several studies have found high rates of OA and the need for arthroplasty in patients with SIFK as a result of mechanical overload [30,31]. Other prior studies have suggested that the presence of bone marrow edema was directly associated with an increased risk of subchondral bone attrition [8]. On the other hand,

Table 1: Knees with meniscal extrusion and subchondral bone marrow edema presented more severe cartilage loss.

<table>
<thead>
<tr>
<th>Meniscal extrusion</th>
<th>Baseline (mm)</th>
<th>2 months (mm)</th>
<th>4 months (mm)</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM</td>
<td>0.70 ± 0.07</td>
<td>0.69 ± 0.11</td>
<td>0.51 ± 0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CT</td>
<td>0.71 ± 0.09</td>
<td>0.75 ± 0.13</td>
<td>0.57 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RR</td>
<td>0.71 ± 0.10</td>
<td>0.76 ± 0.14</td>
<td>0.60 ± 0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Subchondral edema</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>0.71 ± 0.07</td>
<td>0.71 ± 0.11</td>
<td>0.53 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CT</td>
<td>0.70 ± 0.11</td>
<td>0.76 ± 0.13</td>
<td>0.58 ± 0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RR</td>
<td>0.71 ± 0.08</td>
<td>0.77 ± 0.14</td>
<td>0.59 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

PM = partial meniscectomy; CT = conservative treatment; RR = root repair.

a Comparison of three times (repeated measures ANOVA).

Fig. 4. Cartilage thickness showed significant decrease over time in all 3 study groups and the between-group differences were significant with the PM group presenting the most severe cartilage loss at final follow-up. PM = partial meniscectomy; CT = conservative treatment, RR = root repair.

Fig. 4A. Cartilage thickness (mean and SD) in each study group at baseline, 2-, and 4 months of follow-up. Progressive cartilage damage was observed across all 3 study groups. The RR group demonstrated significant degenerative changes, albeit to a lesser degree than in the PM and CT. PM = partial meniscectomy; CT = conservative treatment; RR = root repair.
Granta's et al. noted a longitudinal development of subchondral cyst-like lesions, which were strongly associated with the presence of subchondral bone marrow edema within the same subregion as a predictor of full-thickness cartilage loss [32]. We observed 2 cases of SINK in the PM group and 1 in the CT group preceded by an extended subchondral edema. These findings support a scenario in which chronic subchondral edema could serve as an early radiological predictor of SIFK, triggered by intensified remodeling from increased subchondral cyst formation and bone turnover. While further validation in larger dataset is necessary, early recognition of this radiological finding holds clinical significance, enabling more precise diagnosis and prompting timely intervention.

Several limitations should be acknowledged. First, even though high-resolution spin-echo MRI sequences are a highly sensitive and accurate technique to evaluate articular cartilage, the correlation between radiological and histopathological findings was not examined in this study. Second, medial meniscus posterior root was completely transected from its tibial insertion in all three study groups. In the CT group the tear was left in situ and in the PM group one-third of the posterior horn of the meniscus was removed. One could correlate the osteoarthritic changes observed in the PM group with the additional removal of meniscal tissue in the PM group. However, it is now well known that isolated MMPRTs are biomechanically equivalent to a subtotal meniscectomy [33] and thus, this may not be considered as a potential bias for the PM group. Third, only type 2, radial root injuries were induced in the current experimental model; however, this is the most common type of root tear in clinical practice. There might be a possibility that the healing potential could be influenced by the type of tear. However, no evident association between the root tear types (types 2 and 4) and meniscus healing status was found in binary logistic regression analysis [3]. Fourth, a strict validation of quantitative MRI cartilage volume as a precise endpoint for clinical significance is still necessary. Given the inherent limitation of the present study (limited spatial translation of animal model to human), we were unable to determine a significant threshold for ME that could potentially prevent articular cartilage degeneration. In addition, animals were fully weight-bearing for the follow-up period. From an ethical standpoint, we cannot restrict weight-bearing status in any experimental animal model. However, it has been found that these types of models are suitable for longitudinal preclinical studies and are well adapted for the investigation of both the early and late stages of OA [34].

Despite these limitations, the current study is novel in several ways. First, the main strength of the present study lies in its prospective and experimental nature. Study design is an important concept as the validity of any conclusion depends on the minimization of bias. Experimental large animal models play a crucial role in delineating the time path of degenerative changes and evaluating the effectiveness of therapeutic interventions. Second, in-vivo MRI assessments were conducted at any time point as ex-vivo cartilage measurement for the characterization of volumetric changes can largely restrict the validity of in vivo translation. Third, we are not aware of any clinical or experimental in vivo study exploring the relationship between cartilage thickness and the progression of knee OA in isolated MMPRT model. Lastly, translating research into clinical practice is challenging. As such, one may ask how well the rabbit model translates to clinical practice. Rabbits are the most commonly used animal models, especially for knee OA. Over the last decades, multiple surgically induced instability models of OA have been reported and it is now well-known that these models can induce cartilage lesions that are similar to those observed in humans [5,7,35–43]. Furthermore, an in vivo study represents an appropriate methodology to causally address this question, as opposed to biomechanical and retrospective clinical studies. In addition, the MMPR in rabbits provides anatomical and biomechanical similarities to humans increasing clinical translation [44]. Evidence derived from robust translational research methods should drive our clinical practice, decisions, and changes made to improve the way we deliver care to our patients. As our understanding of OA pathophysiology improves, we are convinced that our ability to develop more accurate disease models will also improve in the future.

In summary, the findings of this study enhance the current literature by providing evidence of OA progression after MMPR repair. This information could lead to a more precise prediction of osteoarthritis risk following MMPRT. Future attempts should focus on the identification of pathologic pathways that could be targeted for interventions to delay or prevent diseases progression.

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Fig. 5. Examples of not healed meniscal repair. The coronal view shows a vertical linear defect observed at the root attachment (A). The sagittal view shows the absence of an identifiable meniscal tissue as high signal replacing the normal dark meniscal signal (B).
CONCLUSIONS

Meniscal root repair did not halt but rather reduced the progression of OA. Degenerative changes worsened at a rapid rate in the PM group compared to the RR and CT groups. Early cartilage swelling, persistent subchondral edema, and progressive ME predicted more severe progression to knee OA in the CT and RR groups.

Compliance with ethical standards

The research complies with national legislation and was approved by the animal protection service and institutional review boards of the authors’ affiliated institutions as well as the regional ethics committee (1221606236918420810087).

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Conceptualization: Lika Dzidzishvili; Irene Isabel López-Torres. Material and preparation: Lika Dzidzishvili, Irene Isabel López-Torres, Emilio Calvo. Data collection and analysis: Lika Dzidzishvili, David Moreno Molera, María Encarnación Fernández-Valle, Irene Isabel López-Torres, Emilio Calvo. The first draft of the manuscript was written by Lika Dzidzishvili. All authors commented on the previous version of the manuscript. All authors read and approved the final manuscript.

Declaration of competing interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lika Dzidzishvili reports financial support was provided by The Spanish Society of Orthopedic Surgery and Traumatology (SECAT). Emilio Calvo reports a relationship with DePuy Synthes that includes: consulting or financial support was provided by The Spanish Society of Orthopedic Surgery and Traumatology (SECAT). Emilio Calvo reports relationships which may be considered as potential competing interests: financial support was provided by The Spanish Society of Orthopedic Surgery and Traumatology (SECAT).

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