The canine ‘groove’ model of osteoarthritis is more than simply the expression of surgically applied damage

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Summary

Objective: Recently a new canine model of osteoarthritis (OA; the ‘groove’ model) has been described. This model is based on surgically applied mechanical damage of the articular cartilage followed by transient forced loading of the affected joint. Ten weeks after surgery this model shows characteristics of OA, mimicking human OA. To establish whether the observed characteristics of degeneration in this model represent the surgically applied damage, or are the results of progressive features of OA, we evaluated this ‘groove’ model shortly after surgery.

Methods: In 20 female Beagle dogs, articular cartilage of the weight-bearing areas of the femoral condyles in the right knee was damaged without affecting the underlying bone. After surgery dogs were let out on a patio 5 days/week for 4 h/day. The dogs were forced to load the experimental joint by fixing the contralateral control limb to the trunk 3 days/week. The severity of OA was evaluated at 3 (n = 10) or 10 weeks (n = 10) after surgery. Synovial inflammation, cartilage damage and cartilage matrix turnover were determined.

Results: Ten weeks after surgery osteoarthritic features were found, as described previously. Proteoglycan (PG) synthesis, percentage release of newly formed PG, and that of total amount of PG were enhanced, whereas PG content was significantly diminished (all P < 0.05). Importantly, 3 weeks after surgery these characteristics of OA were not yet evident.

Conclusions: The present results clearly show that the characteristics observed 10 weeks after induction of joint degeneration in the groove model are not just the expression of the surgically applied damage but are the result of progressive features of (experimental) OA.

Key words: Osteoarthritis, Animal model, Cartilage, Proteoglycans.

Introduction

Osteoarthritis (OA) is a degenerative joint disease, which is characterized by damage of articular cartilage and changes in the subchondral bone and is frequently accompanied by secondary synovitis. Age has been identified as one of the main risk factors. Most often, OA is diagnosed at a relatively late stage of the disease since, apart from symptoms, no early markers are available. Ideally, therapies to prevent progression of the disease should be applied early in the course of OA. For this reason studies on early changes in OA are important. During further development of markers to detect the early changes in the process of human OA, animal models may be helpful. Several animal models of OA have been developed to study biochemical and histological changes associated with joint degeneration in the early stages of OA in vivo.

Recently a new canine model of OA (the ‘groove’ model) has been described. In this canine model, damage to the articular cartilage of the weight-bearing areas of the femoral condyles in one knee, not damaging the subchondral bone and not causing joint instability, is the trigger for development of OA. To strengthen this trigger for development of OA, loading of the affected joint is forced/intensified, by fixing the contralateral control limb to the trunk of the dog temporarily. Biochemical and histological evaluation showed degenerative changes in the joint, which closely resembles those in the anterior cruciate ligament transection (ACLT) model, which is one of the most frequently used (canine) models of OA, both models mimicking human early OA very well.

In the grove model at 10 weeks, collagen was damaged and proteoglycan (PG) turnover was disturbed: an ineffective synthesis combined with an enhanced release resulted in a diminished PG content. Matrix metalloprotease activity measured in synovial fluid was enhanced. Histologically, moderate cartilage destruction characterized by loss of safranin-O staining, fibrillation of the articular surface and chondrocyte clustering was evident. These characteristics of OA were also clearly visible in the tibial plateau, although this cartilage was not harmed during surgery. All changes were very consistent between animals. Most important, only mild signs of inflammation were present as demonstrated histologically.

In the canine groove model the degenerative changes in the cartilage matrix integrity are slowly progressive over time in the first year after induction, while synovial inflammation decreases. Whether the slowly progressive phase...
in the first year after the initial induction phase will proceed into full blown OA in several years, as demonstrated for the ACLT model \textsuperscript{11}, needs still to be demonstrated. Although the changes in the groove model are slightly progressive over time in the first year of follow-up, it might be disputed whether the observed changes are actual characteristics of OA or merely the result of the surgery-applied cartilage damage. Therefore, in the present study we evaluated cartilage damage in this ‘groove’ model shortly after surgical procedures, and compared it to the changes observed 10 weeks after induction.

Materials and methods

**ANIMALS**

Female Beagle dogs, mean age 2.4 ± 0.3 years, weighing 10–15 kg, were obtained from the animal laboratory of Utrecht University, the Netherlands. They were housed in groups of two to three dogs per pen, and were let out on a patio in large groups for at least 2 h daily. They were fed a standard diet and had water ad libitum. The dogs were divided into four groups of five animals each. The study was approved by the Utrecht University Medical Ethical Committee for animal studies.

**ANAESTHESIA, GENERAL SURGERY, AND POST-SURGICAL TREATMENT**

After induction with Nesdonal, the dogs (in groups of five) were anaesthetized with halothane in a mixture of oxygen and nitrous oxide. Surgery was carried out through a 2–2.5 cm medial incision close to the ligamentum patellae in one knee. Care was taken to prevent bleeding and soft tissue damage as much as possible. After surgery, synovium, fasciae and skin were sutured. The contralateral unoperated knee served as a control. The animals received analgesics (Buprenorphine 0.01 mg/kg) and antibiotics (Amoxicyclin 400 mg/kg) during the first 3 days after surgery. Starting 2 days after surgery, the dogs were let out daily on the patio, again. At the end of the experiment, the dogs were killed with an intravenous injection of euthe-}

**GROOVE MODEL**

In all dogs (n = 20), the cartilage of the right lateral and medial condyles was damaged with a Kirschner-wire (1.5 mm diameter) that was bent 90° at 0.5 mm from the tip. This ensures that the depth of the grooves was restricted to around 0.5 mm. In utmost flexion, 10 longitudinal and diagonal grooves were made on the weight-bearing parts of the femoral condyles without damaging the subchondral bone \textsuperscript{2,3}. The latter was checked by histology at the end of the experiment. There was no absolute visual control over the procedure, but macroscopic evaluation after killing of the animals showed similar patterns in all affected knees. To ensure (intensified) mechanical loading of the affected knee, the dogs were forced to load the experimental joint intermittently by fixing the contralateral limb to the trunk for approximately 4 h per day, 3 days per week, for 3 weeks (two groups of five animals) and 10 weeks (2 × 5 animals). This is less than 10% of the total loading time of the affected joint. Thus, the control limb was not weight bearing for less than 10% of the time. We demonstrated that the control limbs of these animals did not differ from those of animals from which the limbs were not fixed to the trunk \textsuperscript{9}. The severity of OA was evaluated at 3 (n = 10) or 10 weeks (n = 10) after surgery.

**SYNOVIAL TISSUE ANALYSIS**

Three infra-patellar synovial tissue samples per joint (medial middle and lateral) were fixed in 4% phosphate-buffered formalin (pH 7.0) and embedded in paraffin. Deparaffined sections were stained with haematoxylin–eosin. The histological sections were examined separately in random order and independently by two observers who were not aware of the source of the synovium. Each specimen was analysed to determine the degree of inflammation, using the slightly modified \textsuperscript{18} criteria described by Goldenberg and Cohen \textsuperscript{19}. For assessing the overall grade, three specimens from each knee were considered as a unit.

**CARTILAGE ANALYSIS**

Cartilage samples for histological and biochemical analyses were obtained from predetermined locations on the weight-bearing areas of the femoral condyles and the tibial plateau of both experimental and control joints. The locations were identically paired with the same location in the contralateral joint \textsuperscript{9}. Cartilage was cut as thick as possible, while excluding the underlying bone. Samples were cut into full-thickness cubes, kept in 200 µl culture medium (Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 0.085 mM ascorbic acid, 2 mM glutamine, 100 IU/ml penicillin, 100 mg/ml streptomycin, and 10% heat-inactivated Beagle serum), and weighed (3–10 mg; accuracy 0.1 mg).

For histology, four samples from tibial plateau and four from femoral condyles from each knee were fixed in 4% phosphate-buffered formalin containing 2% sucrose (pH 7.0). Cartilage degeneration was evaluated in safranin-O–fast-green stained sections by light microscopy according to the slightly modified \textsuperscript{20} criteria of Mankin \textsuperscript{21}. The tide mark between cartilage and bone was not present in our cartilage samples, since bone was not included. Also as a result of our dissection method, cartilage samples were not covered with pannus. Therefore, the maximum score that could be obtained was 11 instead of the original 14, when all criteria described by Mankin (including pannus, clefts to calcified zone, and tidemark crossed by blood vessels) could have been included. With respect to staining, paired control and experimental samples (of the same animal) were stained in the same safranin-O baths. The samples of the five animals of each of the four groups were stained in the same assay. Specimens were graded in random order by two observers unaware of the source of the cartilage. The average of the four specimens was used for statistical evaluation.

For biochemical analysis, the cartilage samples were cultured individually in 96-well culture plates (NUNCNLABO, Denmark) in 200 µl culture medium. Cartilage explants were cultured according to standard procedures as described previously \textsuperscript{11,20}. For femoral condyles and tibial plateau, cartilage PG content, PG synthesis, PG retention, and PG release were determined and averaged for six explants per parameter \textsuperscript{22}.
PG SYNTHESIS

As a measure of PG synthesis, the rate of sulphate incorporation was determined *ex vivo*23. After 1 h of pre-culture, 148 kBq Na$^{35}$SO$_4$ (Dupont, NEX-041-H, carrier free) in 10 μl DMEM was added to each sample. After 4 h, the cartilage samples were washed with cold PBS and digested with papaain for 2 h at 65°C. Glycosaminoglycans (GAGs) were precipitated by addition of cetylpyridium chloride (CPC), and $^{35}$SO$_4$ labelled GAGs were measured by liquid scintillation analysis. The total sulphate incorporation rate of each cartilage sample was calculated using the specific activity of the medium and was normalized to time and wet weight of the explants. Synthetic activity is expressed as nmoles of sulphate incorporated per h per g wet weight of the cartilage (nmol/h g).

PG RELEASE

For determination of the release of the newly synthesized PGs as a measure of retention of these PGs, the release of $^{35}$SO$_4$ labelled PGs in the medium was determined. After labelling (see above) the cartilage samples were rinsed three times for 45 min in 1.5 ml culture medium and then incubated in 200 μl fresh culture medium without sulphate label for 3 days. Thereafter the samples were washed with cold PBS and GAGs were precipitated from the medium and stained with Alcian Blue dye solution, as described previously23. The $^{35}$SO$_4$ labelled GAGs were measured by liquid scintillation analysis and the release was normalized to the specific activity of the medium and the wet weight of the explants. The release of newly formed PGs is normalized to the total amount of newly synthesized PGs and expressed as percentage release of newly formed PGs in 3 days (% new PG release).

For the total release of PGs, Alcian Blue staining of the medium was quantified photometrically with chondroitin sulphate (Sigma C4384) as a reference. The total amount of GAGs released (blue staining) is expressed as a percentage of the original tissue content (% GAG release).

PG CONTENT

As a measure of PG content of the cartilage samples the amount of GAG was determined as described previously24. The GAGs in the papain digest of cartilage samples were precipitated and stained with Alcian Blue as described above. The staining was quantified photometrically by the change in absorbance at 620 nm. Chondroitin sulphate (Sigma C4383) was used as a reference. Values were normalized to the wet weight of the cartilage explants (mg/g).

CALCULATIONS AND STATISTICS

Delta (histology) or percentage (PG turnover) change in mean values ± S.E.M (n = 10 animals) of femoral and tibial cartilage of experimental knees compared to control knees, respectively, is presented. The paired Student’s *t* test was used to compare data of the experimental and contralateral control joints within each group and the unpaired *t* test was used to analyse differences between the two groups of animals with different follow-up. *P* values less than 0.05 were considered statistically significant.

Results

CARTILAGE DAMAGE

Three weeks after induction of experimental OA, the affected knees clearly showed macroscopic damage of the articular cartilage of femoral condyles. No damage was found on the condylar cartilage of the control knees. Damage, although less pronounced, was also found on the tibial plateau. The macroscopic changes at 3 weeks appeared (not objectivated) less severe when compared to those seen 10 weeks post-surgery, both for femoral condyles and tibial plateau.

These macroscopic observations were confirmed by histological analysis (Fig. 1, Tables I and II). In the 3 weeks post-surgery group the average modified Mankin score of the cartilage degeneration in the experimental femoral condyles was mild but significantly different from that of the contralateral control joints (Table I). The grooves were clearly visible as depicted by a representative micrograph in Fig. 1 (top left). On average minor but statistically significant cartilage degradation of the experimental tibial plateau cartilage was seen in the 3 weeks post-surgery group (compared to the contralateral control; Fig. 1, middle-left, and Table I). At 10 weeks post-surgery histological cartilage damage was more severe as observed in both femoral condyles (Fig. 1, top-right) and the tibial plateau (Fig. 1, middle-right). On average this was statistically significant for both cartilage surfaces of the experimental joints (Table I). Most important paired analysis, as depicted in Fig. 2, left panel, the delta change in histological cartilage damage (experimental compared to the contralateral joints), of both femoral and tibial cartilage, was statistically significantly less severe in the 3 weeks post-surgery group than in the 10 weeks post-surgery group. The structural changes and the cellularity of the cartilage, contributed the most to this histological difference between 3 and 10 weeks (Table II).

SYNOVIAL INFLAMMATION

Macroscopic evaluation of the synovial tissue showed in both, the 3 weeks and the 10 weeks post-surgery group, very mild signs of inflammation in the experimental joints, which could be confirmed by light-microscopic examination (Table I, Fig. 1, lower panels and Fig. 2, right bars).

On average the delta change in synovial inflammation between the experimental joints and the contralateral control joints was not statistically significantly different for the 10 weeks post-surgery group compared to the 3 weeks post-surgery group (Fig. 2, right bars).

PG TURNOVER

Synthesis of PGs was increased in the experimental joints compared to the contralateral control joints at femoral condyles as well as at tibial plateaus both 3 and 10 weeks post-surgery (Table I). Although for femoral condyles the increase at 10 weeks was higher than at 3 weeks post-surgery [Fig. 3(A)] this apparent difference was not statistically significant.

The release of newly formed PGs, normalized to the synthesis of PGs (percentage new release), as a measure of retention of the newly formed PGs was not increased in the experimental femoral cartilage 3 weeks post-surgery (Table I). For tibial plateaus at 3 weeks and for both femoral condyles and tibial plateaus at 10 weeks post-surgery the
The percentage release of newly formed PGs was significantly increased (Table I). The increased release of newly formed PGs in condylar cartilage was significantly higher at 10 weeks than at 3 weeks [Fig. 3(B)]. The total amount of PGs released, normalized to the content and expressed as percentage release of PGs, of the experimental joints was enhanced in the 10 weeks post-surgery group but not in the 3 weeks post-surgery group (Table I). This difference between 3 and 10 weeks post-surgery was observed for the tibial plateau cartilage as well as condylar cartilage. For both cartilage surfaces this difference between 3 and 10 weeks was statistically significant [Fig. 3(C)].

As a result of the ineffective PG synthesis and enhanced PG release in the 10 weeks post-surgery group, the content of PGs was decreased significantly in the femoral cartilage as well in the tibial cartilage of the experimental joint compared to the contralateral joints. In contrast, PG content was not changed significantly in the 3 weeks post-surgery group for both the femoral and tibial cartilage [Fig. 3(D)]. The almost 20% decrease in the PG content of the femoral cartilage at 10 weeks post-surgery and 13% decrease in tibial cartilage were significantly different from the changes observed at 3 weeks [Fig. 3(D)].

Discussion

Many different in vivo animal models of OA have been described from mice models to equine models, from spontaneous to chemically induced models. The goal of studies using these models is extrapolation of results obtained in these models to the human situation, with respect to the pathogenic processes or effects of treatment. Each model has in this perspective its characteristics, advantages, and disadvantages. In the canine groove model chondral damage combined with forced loading of the experimental joint induces osteoarthritic features. This model appeared in short-term follow-up to induce very similar changes, with slightly more cartilage damage and slightly less inflammation than the ACLT model. This sequence of events is similar to that described for human OA. This demonstrates that completely different causes, i.e., chondral trauma and joint instability, can lead to very similar features of experimental secondary OA. Whether these features remain similar during further progression over the years remains to be established and needs a 4–5 year follow-up group for the groove model as has been reported for the ACLT model.
Table I
Histological and biochemical characteristics of experimental and contralateral control joints in the groove model, 3 and 10 weeks post-surgery

<table>
<thead>
<tr>
<th></th>
<th>Control (3 weeks)</th>
<th>Experimental (3 weeks)</th>
<th>P&lt;</th>
<th>Control (10 weeks)</th>
<th>Experimental (10 weeks)</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histological cartilage damage (modified Mankin grade)</td>
<td></td>
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<tr>
<td>Condyles</td>
<td>0.4 ± 0.1</td>
<td>2.1 ± 0.3</td>
<td>0.005</td>
<td>0.3 ± 0.1</td>
<td>3.4 ± 0.3</td>
<td>0.005</td>
</tr>
<tr>
<td>Plateau</td>
<td>0.8 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>0.005</td>
<td>1.3 ± 0.02</td>
<td>2.5 ± 0.4</td>
<td>0.02</td>
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<tr>
<td>Histological synovial inflammation (Goldenberg and Cohen grade)</td>
<td></td>
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<tr>
<td>Joint</td>
<td>1.2 ± 0.3</td>
<td>2.5 ± 0.2</td>
<td>0.01</td>
<td>0.7 ± 0.2</td>
<td>2.0 ± 0.3</td>
<td>0.01</td>
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<tr>
<td>PG synthesis (nmol/h g)</td>
<td></td>
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<tr>
<td>Condyles</td>
<td>5.7 ± 0.4</td>
<td>8.8 ± 1.6</td>
<td>0.03</td>
<td>9.3 ± 0.9</td>
<td>17.1 ± 1.8</td>
<td>0.003</td>
</tr>
<tr>
<td>Plateau</td>
<td>3.9 ± 0.3</td>
<td>5.9 ± 0.9</td>
<td>0.01</td>
<td>7.2 ± 0.5</td>
<td>11.0 ± 1.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Percentage release new PGs (%)</td>
<td></td>
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<tr>
<td>Condyles</td>
<td>9 ± 1</td>
<td>9 ± 1</td>
<td>ns</td>
<td>11 ± 1</td>
<td>19 ± 3</td>
<td>0.005</td>
</tr>
<tr>
<td>Plateau</td>
<td>18 ± 2</td>
<td>23 ± 2</td>
<td>0.005</td>
<td>16 ± 1</td>
<td>20 ± 1</td>
<td>0.005</td>
</tr>
<tr>
<td>Percentage release of total PGs (%)</td>
<td></td>
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<tr>
<td>Condyles</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>ns</td>
<td>7 ± 1</td>
<td>13 ± 1</td>
<td>0.005</td>
</tr>
<tr>
<td>Plateau</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
<td>ns</td>
<td>12 ± 1</td>
<td>16 ± 1</td>
<td>0.005</td>
</tr>
<tr>
<td>PG content (mg/g)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Condyles</td>
<td>38 ± 3</td>
<td>37 ± 2</td>
<td>ns</td>
<td>49 ± 3</td>
<td>39 ± 1</td>
<td>0.005</td>
</tr>
<tr>
<td>Plateau</td>
<td>37 ± 3</td>
<td>37 ± 2</td>
<td>ns</td>
<td>49 ± 3</td>
<td>42 ± 2</td>
<td>0.005</td>
</tr>
</tbody>
</table>

The absolute values of both condyles and tibial plateau are given for contralateral control knees and experimental knees (mean ± s.e.m.). Histological cartilage damage was graded according to modified criteria of Mankin (maximum score of 11). Synovial inflammation had a maximum score of 10 and was graded according to the modified criteria of Goldenberg and Cohen. PG synthesis was measured by $^{35}$S-sulfate incorporation (nmol/h g). % New PG release: release of the newly formed PGs normalized to PG synthesis (%) as a measure of retention of the newly formed PGs. % Total release: release of total amount of PGs in 3 days normalized to PG content of the cartilage (%). GAG content was determined as a measure of PG content (mg/g).

Although with respect to outcome the similarity between the ACLT model and the groove model is striking, it was never evaluated whether the observed characteristics were the results of experimental (secondary) OA or whether they were rather the expression of surgically applied chondral damage. The present results clearly show that the changes observed in the groove model of OA at 10 weeks are not observed at 3 weeks post-surgery. Thus, the characteristics observed 10 weeks after induction of joint degeneration according to the groove model are not just the expression of the surgically applied damage but are the result of progressive features of experimental secondary OA.

It is noticeable from Table I that the control values between the 3 and 10 weeks post-surgery group are not similar. This is explained by the fact that four different “batches” of animals were used. Although we realize that this is a potential bias, we feel that this is of less relevance to the present study because the data of the experimental joints were analysed in relation to their contralateral control values (paired observation, within each animal). This

Table II
Specification of histological characteristics of experimental and contralateral control cartilage in the groove model, 3 and 10 weeks post-surgery

<table>
<thead>
<tr>
<th></th>
<th>Control (3 weeks)</th>
<th>Experimental (3 weeks)</th>
<th>P&lt;</th>
<th>Delta change</th>
<th>Control (10 weeks)</th>
<th>Experimental (10 weeks)</th>
<th>P&lt;</th>
<th>Delta change</th>
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<tbody>
<tr>
<td>Histological cartilage damage structure</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Condyles</td>
<td>0.10 ± 0.05</td>
<td>0.78 ± 0.15</td>
<td>0.011</td>
<td>0.68 ± 0.15</td>
<td>0.08 ± 0.05</td>
<td>1.42 ± 0.17</td>
<td>0.005</td>
<td>1.34 ± 0.18</td>
</tr>
<tr>
<td>Plateau</td>
<td>0.53 ± 0.04</td>
<td>0.78 ± 0.07</td>
<td>ns</td>
<td>0.25 ± 0.10</td>
<td>0.73 ± 0.10</td>
<td>1.13 ± 0.18</td>
<td>ns</td>
<td>0.39 ± 0.21</td>
</tr>
<tr>
<td>Histological cartilage damage cells</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Condyles</td>
<td>0.00 ± 0.00</td>
<td>0.53 ± 0.11</td>
<td>0.011</td>
<td>0.53 ± 0.11</td>
<td>0.01 ± 0.01</td>
<td>1.13 ± 0.13</td>
<td>0.005</td>
<td>1.12 ± 0.13</td>
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<tr>
<td>Plateau</td>
<td>0.08 ± 0.03</td>
<td>0.10 ± 0.04</td>
<td>ns</td>
<td>0.03 ± 0.06</td>
<td>0.19 ± 0.07</td>
<td>0.70 ± 0.17</td>
<td>0.038</td>
<td>0.52 ± 0.20</td>
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<tr>
<td>Histological cartilage damage safranin-O staining</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Condyles</td>
<td>0.25 ± 0.06</td>
<td>0.78 ± 0.07</td>
<td>0.004</td>
<td>0.53 ± 0.03</td>
<td>0.21 ± 0.07</td>
<td>0.80 ± 0.10</td>
<td>0.005</td>
<td>0.59 ± 0.11</td>
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<tr>
<td>Plateau</td>
<td>0.18 ± 0.04</td>
<td>0.58 ± 0.06</td>
<td>0.004</td>
<td>0.40 ± 0.05</td>
<td>0.39 ± 0.11</td>
<td>0.77 ± 0.10</td>
<td>0.012</td>
<td>0.38 ± 0.10</td>
</tr>
</tbody>
</table>

Histological cartilage damage was according to the modified criteria of Mankin (maximum score of 11, see Materials and methods). Figures of each of the three components (structure, cellularity, and safranin-O staining) are depicted. The absolute values of both condyles and tibial plateau are given for contralateral control knees and experimental knees (mean ± s.e.m.) of the 3 and 10 weeks post-surgery groups. In addition delta changes (mean ± s.e.m.; experimental compared to control) of each post-surgery group are given. Statistical differences in delta changes between the post-surgery groups are shown in the far right column.
Fig. 2. Average change in histologically determined cartilage damage (modified Mankin score) for femoral condyles and tibial plateau (left y-axis) and average change in synovial tissue inflammation (modified Goldenberg and Cohen score; right y-axis), both calculated as delta change between experimental joint compared to contralateral control joint for each animal. Open bars represent the mean values ± S.E.M. of cartilage damage 3 weeks post-surgery (n = 10) and solid bars represent the mean values ± S.E.M. of cartilage damage 10 weeks post-surgery (n = 10). Asterisks indicate statistically significant (P < 0.05) change compared to contralateral controls. Statistically significant differences between groups are indicated.

excluding any biological variation between animals (animal groups). This is also the reason why data, in addition to absolute values (Table I), have been expressed as percentage changes (Figs. 2 and 3).

Within 3 weeks following surgically applied chondral damage an increased synthesis of PGs was observed, an apparent attempt to repair the damaged tissue. This increased synthesis was similar at 3 and 10 weeks post-surgery. However, in contrast, at 3 weeks this repair appeared, at least for the femoral condyles, to be effective. Retention of newly formed PGs was normal 3 weeks postsurgery, whereas it was significantly impaired 7 weeks later. Thus, between 3 and 10 weeks after the chondral trauma the enhanced synthesis becomes ineffective. As expected enhanced catabolism characterized by an increased breakdown and release of resident PGs causes a shift from normal to impaired retention of newly formed PGs. The breakdown and release of resident PGs has frequently been reported to be dependent on matrix-metalloproteinases (MMPs) and aggrecanases. Because, the groove model is associated with only very mild synovial inflammation, synovial fluid could seldom be aspirated. It was therefore impossible to measure accurately these proteins/aggrecanases.

However, it seems likely that this increase in proteinase/aggrecanase activity contributes to the damage in the tibial plateau which has not been surgically damaged. Hardly any histological damage (no damage of the articular surface and no changes in cellularity, Table II) or biochemically determined PG release [Fig. 3(C)] was seen 3 weeks post-surgery, whereas 10 weeks after the experimentally induced trauma the tibial cartilage was significantly adversely affected. Incongruity, as a result of movement of the roughened condylar surface on a smooth tibial plateau, may in addition add to the development of the osteoarthritic features at the tibial plateau. The development of catabolic activity between 3 and 10 weeks post-surgery is clearly represented in the PG content of the cartilage when biochemically determined. This does not corroborate the histological findings because there it were the structural and cellular changes (Table II) rather than the loss in safranin-O staining that determined the difference between 3 and 10 weeks post-surgery. It is, however, not unexpected that subtle changes (such as a 20% change) in safranin-O staining cannot be detected by histochemistry. Biochemically determined changes in PG content are more reliable in that respect. The increased synthesis without a significant increase in catabolic activity even resulted in a slight increase in PG content (although not statistically significant; week 3), whereas the development of catabolic activity between 3 and 10 weeks results in a clearly diminished PG content 10 weeks post-surgery. Whether, during this change from a net anabolic to a net catabolic activity a point of no return is crossed, remains to be established.

Studies on the spontaneous healing of articular cartilage defects have been performed in many animal models, including the dog. Defects fully contained in articular cartilage (chondral defects) may persist with no sign of reparative tissue filling the lesion or heal with limited filling by fibrous tissue, fibrocartilage, or hyaline cartilage. In some cases the reparative tissue that fills such defects displays the structure of articular cartilage although complete regeneration of chondral effects has never been reported. In our model there seems to be a repair process at 3 weeks post-surgery, whereas at 10 weeks post-surgery degeneration is evident. Apparently, possible reparative activities as observed in previous studies might nonetheless finally result in progressive damage later on. However, it might also be the intensified loading in our model that changes a potential repair process into progressive damage by forcing the process of joint degeneration to cross a point of no return. Future studies in this respect are needed to clarify the impact of chondral damage without forced loading on the development of OA.

The present results show that the characteristics observed 10 weeks after induction of joint degeneration in the groove model are not just the expression of the surgically applied damage but are the result of progressive features of experimental OA. The groove model adds to the existing animal models of OA with features resembling human OA. One point of distinction is that the degenerative changes are progressive while synovial inflammation is minimal and even diminish over time. Because of this, evaluation of cartilage protective effects of treatment is not hampered by inflammatory activity in the joint. This phenomenon makes the groove model especially suitable for evaluation of disease modifying osteoarthritic drugs. A second point of distinction is that there is no persistent trigger causing joint damage, which should render the model more sensitive to treatment. A persistent trigger for joint damage, such as joint instability used in the ACLT model, could counteract the possible beneficial effects of treatment. Moreover, assuming that cartilage repair is possible, the trigger, intrinsic to the cartilage damage itself, could be removed by treatment. Thus, the groove model might be suitable to monitor progression of OA in the long term after treatment is stopped, and may even be amenable to demonstrating a cure.

In conclusion, animal models are essential in research on OA aimed at better understanding of the pathophysiology of OA especially in its early phases and to study effects and mechanisms of treatment. The groove model of OA may have an additional value in this respect.
Fig. 3. Changes in PG synthesis rate (A), percentage release of newly formed PGs (B), percentage total release of PGs (C) and PG content (D) of femoral and tibial cartilage calculated as percent from the control femoral and tibial cartilage controls for each animal. Mean values (n = 10 ± S.E.M.) are presented for 3 weeks post-surgery group (open bars) and 10 weeks post-surgery group (solid bars). Asterisks indicate statistically significant (P < 0.05) change compared to contralateral controls. Statistically significant differences between groups are indicated.

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References