Arthroscopic evaluation of potential structure modifying activity of hyaluronan (Hyalgan®) in osteoarthritis of the knee

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Summary

Objective: Several reported studies suggest that repeated intra-articular injections of hyaluronan result in sustained relief from pain and functional disability in patients with knee osteoarthritis. Several in vivo data suggest that hyaluronan might have a beneficial structural effect in osteoarthritis. The objective of the study was to evaluate the potential structure-modifying effects of Hyalgan (500–730 kDa molecular weight), a highly-purified sodium hyaluronate.

Design: Patients with painful knee osteoarthritis (ACR criteria) were enrolled in a prospective, controlled study of 1-year duration. After randomization, either conventional therapy or three cycles (every 3 months) of three intra-articular injections of Hyalgan (once a week during 2 weeks) were given. Clinical outcome was added using pain visual analog score (VAS), functional impairment: Lequesne’s index, quality of life: arthritis impact measurement scale (AIMS2) and structural outcome using X-rays: joint space narrowing and arthroscopy: global assessment using VAS, SFA scoring and grading systems.

Results: Of the 39 recruited patients, 36 completed the 1-year trial (19 in the Hyalgan group and 17 in the control group). There was no difference between groups at entry. Between-group comparison for changes in clinical parameters reached statistical significance for the quality of life index (AIMS2: –0.4 ± 0.7 vs +0.2 ± 0.9 in the Hyalgan and control groups respectively, P < 0.05). Deterioration in the structural parameters was less in the Hyalgan group, with a statistically significant difference for two of the three evaluated parameters (overall assessment of chondropathy: +5.1 ± 12.7 vs 16.7 ± 18.3, P = 0.016; SFA scoring system: +3.7 ± 7.3 vs +9.0 ± 11.5, P = 0.05) in the Hyalgan and control groups, respectively.

Conclusions: This study supports existing data concerning the favorable symptomatic effect of intra-articular injections of Hyalgan in osteoarthritic of the knee and suggests that repeated intra-articular injections of Hyalgan might delay the structural progression of the disease. Other studies are required to confirm these results and to determine the long-term monitoring of osteoarthritic patients using such local therapy.

Key words: Knee osteoarthritis, Arthroscopy, Hyaluronan.

Introduction

DRUGS considered to be active in the treatment of osteoarthritis may be classified as symptomatic and/or structure-modifying agents [1, 2]. Monitoring the effects of symptomatic agents in patients suffering from osteoarthritis of the knee is usually based on clinical measurements of changes in pain and/or function [3, 4]. Such outcome measures may be influenced by effective symptomatic agents whatever their mechanism of action [5, 6] but do not permit the evaluation of the potential of these drugs to act directly on the cartilage. The International League of Association for Rheumatology has defined chondroprotective agents as agents capable of preventing, delaying, or even reversing cartilage lesions due to osteoarthritis in humans. However, the outcome measures for cartilage lesions are not clearly established. Radiographic assessment of affected knees has been reported as an objective standard for long-term evaluation of knee osteoarthritis [7, 8] but is still tainted by false-positive and false-negative findings [9].

It was reported in a previous study [10] that (1) arthroscopic evaluation of articular cartilage of the knee was not simple, but was feasible and well-tolerated as an outpatient procedure under...
local anesthesia with a small arthroscope; (2) the quantification of chondropathic severity was feasible using either the physician’s overall assessment with a 100 mm visual analog scale (VAS) or the scoring systems proposed by the French Society of Arthroscopy which are composite indices taking into account the three main characteristics of chondropathy, i.e., localization, depth and size [11, 12]. Previous studies have shown that this method fulfills some of the guidelines proposed for outcome measures: validity, reproducibility and clinical relevance [10–12].

Hyaluronan (hyaluronic acid) forms the central axis of the various proteoglycan aggregates necessary for the functional integrity of cartilage and other extracellular matrices [13]. Hyaluronic acid is responsible for the unique viscoelastic properties of the synovial fluid [14–16] and is thought to play an important role in biological activities as diverse as cell proliferation and activation [17–20]. Its clinical use was suggested by the finding that hyaluronic acid was reduced in concentration and in chain length in the synovial fluids of arthritic patients [14]. The term ‘viscosupplementation’ has been used to describe the effects of high-molecular-weight hyaluronan [21]. However, this concept is based only on the mechanical properties of the molecule while there is a considerable body of evidence that the therapeutic efficacy of hyaluronan is due to a combination of both physicochemical and pharmacological properties [22].

Several studies with various preparations of hyaluronan of different molecular weights have been conducted [23–25 and reviewed in 26], and there has been a broad consensus in the studies published that this treatment is well-tolerated and results in sustained relief of pain and functional disability of patients suffering from knee osteoarthritis. This clinical improvement is usually observed after three to five injections performed at 1-week intervals. Moreover, a carry-over effect for at least 3 months after discontinuation has been suggested.

Animal model studies suggest that hyaluronan affects the progression of chondral lesions [27, 28]. To our knowledge, no data have been published in the literature suggesting that intra-articular injections of hyaluronic acid might interfere with the natural course of the cartilage lesions observed in knee osteoarthritis in humans. Therefore, it seemed interesting to conduct the following pilot study in order to evaluate, by arthroscopy, the effects of repeated intra-articular injections of hyaluronan.

**Patients and Methods**

**PATIENTS**

Patients suffering from knee osteoarthritis fulfilling the American College of Rheumatology criteria [29] were enrolled in the study. Other inclusion criteria were the following:

1. Primary osteoarthritis as defined by the American College of Rheumatology [29];
2. Clinical involvement of the medial compartment (pain localized at the medial part of the knee);
3. Active disease (pain and disability) justifying local therapy (lavage) at entry (based on the opinion of the investigator);
4. Absence of contraindication of arthroscopy (anti-coagulant therapy, cutaneous lesion of the knee, allergy to lidocaine);
5. Absence of advanced disease defined by a radiological grade IV according to the classification proposed by Kellgren and Lawrence [30];
6. Presence of chondropathy of the medial compartment at arthroscopy defined by the presence of chondropathy at least grade II according to the classification proposed by Beguin and Locker [31] and observed on at least 10% of the evaluated surface.

 Patients were not included in the study if (1) any intra-articular surgery was performed during the past 5 years (including any arthroscopic procedure). (2) Any intra-articular treatment was prescribed i.e., lavage, intra-articular injection of corticosteroids during the past 3 months. Any concurrent symptomatic treatment, i.e., analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), physiotherapy had to be stable for at least 1 month before the study.

**STUDY MEDICATION**

Hyalgan®, supplied by Fidia S.p.A., is a concentrated (20 mg/2 ml) solution of 500–730 kDa molecular weight, highly-purified sodium hyaluronate extracted from rooster combs.

**STUDY DESIGN**

The study was a single-center, prospective, randomized, controlled study of 1-year duration approved by the ethics committee of Cochin Hospital, Paris. All patients underwent knee arthroscopy during which lavage (2 l saline serum) was performed. After the arthroscopy procedure, and after written informed consent was obtained, the patients entered one of the two study groups...
according to a randomization scheme. The control group did not receive any intra-articular injection of hyaluronan during the 1 year of the study. The treated group received three intra-articular injections of Hyalgan once a week during 2 weeks (three injections) every 3 months, for a total of nine injections. The first injection was performed 1 month after arthroscopy and the last 3 months before the final visit. Before each injection, any synovial fluid was aspirated.

DATA COLLECTION

Clinical characteristics

Demographic data and parameters of osteoarthritis were recorded at entry for each patient, and included age, sex, body mass index, date of onset of osteoarthritis, and number of joints affected by osteoarthritis. Moreover, clinical, radiological and arthroscopical variables were collected at entry and after 1 year.

Clinical activity of osteoarthritis was assessed using the following variables: (1) global pain in the previous 48 h evaluated on a 100 mm length VAS and (2) functional disability, evaluated via (a) Lequesne's functional index [32], which consists of 10 questions regarding the presence of pain or disability, and (b) the French version of the revised arthritis impact measurement scale (AIMS2) [33].

These clinical parameters were collected by a physician who was aware of the patient group, i.e., using a non-blinded procedure. Moreover during the study, any requirement for concomitant therapy, e.g., analgesics, NSAID was recorded. The amount of this rescue treatment was calculated using the following methods: (1) an equivalent NSAID score, previously reported [34], in order to calculate a mean, daily NSAID score. For example, a patient taking 100 mg indomethacin daily has a score of 10, which is the same score if he/she takes 150 mg diclofenac or 20 mg piroxicam, (2) an equivalent analgesic score defined by the number of pills taken daily by the patient considering that one pill of paracetamol is equivalent to one pill of dextropropoxyphene.

Radiological variables

Radiological evaluation consisted of antero-posterior weight-bearing knee X-rays. The structural severity of osteoarthritis was determined by evaluating the joint space narrowing according to a seven-grade scale, previously reported [35]. Moreover, joint space width was evaluated by the interbone distance between the medial femoral condyle and the tibial plateau at the narrowest point in millimeters [8]. The paired radiographs of each patient at entry and after 1 year were analyzed by a single investigator (V.L.) using a blind procedure in which the investigator was unaware of patient identity and the chronology of the radiographs.

Arthroscopy

Arthroscopy of the knee was performed under local anesthesia, without tourniquet hemostasis, with a 2.7 mm Storz arthroscope (Storz, Paris, France) having a 30° fore oblique lens, using the inferolateral approach. Each arthroscopy was recorded on a ¼ inch U-MATIC video-cassette (Sony, Paris, France). All the videotapes were analyzed by a single investigator (X.A.) using a blinded procedure in which the investigator was unaware of patient identity and chronology. Three scoring methods were used: the overall assessment of the investigator, the revised SFA scoring and the SFA grading systems [10–12]. The overall assessment of chondropathy was evaluated using a VAS 100 mm in length, in which 0 indicates the absence of chondropathy and 100 the most severe chondropathy. In the second and third methods, i.e., the SFA systems for scoring and grading chondropathy [11, 12], the first step is recording, on an articular diagram of the knee, the observed chondropathies with their main baseline parameters: (1) localization: medial femur and medial tibia, lateral femur and lateral tibia, patella and trochlea; (2) grade based on the classification of chondropathy proposed by Beguin and Locker [31] in which grade 0 indicates normal cartilage, grade I swelling and/or softening, grade II superficial fibrillation, grade III deep fibrillation, and grade IV exposure of subchondral bone; and (3) size from 0–100% of the involved articular surface. Size of lesions is estimated by the investigator as a percentage of the whole articular surface and is reported on a special form [10–12].

The revised SFA scoring is a continuous variable, between 0 and 100, obtained as follows [10]: Revised SFA scoring = surface (%) of grade I lesions × 0.14 + surface (%) of grade II lesions × 0.34 + surface (%) of grade III lesions × 0.65 + surface (%) of grade IV lesions × 1.00.

The SFA grading is an ordinal variable including four grades for the medial compartment [12].
Table I

Baseline characteristics of the 39 patients suffering from knee osteoarthritis enrolled in the study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Treatment group</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hyaluronan N=20</td>
<td>Control N=19</td>
</tr>
<tr>
<td>Demographic data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years) (mean, s.d.)</td>
<td>60 ± 7</td>
<td>64 ± 8</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>11/9</td>
<td>15/4</td>
</tr>
<tr>
<td>Body mass index (kg/m²) (mean, s.d.)</td>
<td>27.5 ± 3.8</td>
<td>26.6 ± 3.6</td>
</tr>
<tr>
<td>OA characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration (years) (man, s.d., range)</td>
<td>4 (0-26)</td>
<td>2 (0-15)</td>
</tr>
<tr>
<td>Synovial fluid effusion (yes/no)</td>
<td>10/20</td>
<td>10/19</td>
</tr>
<tr>
<td>Number of joints effected by OA (mean, s.d., range)</td>
<td>2.6 ± 1.7</td>
<td>2.6 ± 1.2</td>
</tr>
<tr>
<td>Concomitant NSAID therapy during the previous 6 months (number of patients)</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

*Statistical significance determined by the nonparametric Mann-Whitney U test for the quantitative variables and by the Chi-square test for the qualitative variables. NS = not significant.

Statistical analysis

Statistical analyses were performed to compare by treatment group the baseline characteristics of patients and the changes which occurred in the clinical, radiological and arthroscopical variables during the study. For the continuous efficacy parameters, the analysis of covariance (ANCOVA) using the corresponding baseline (week 0) assessment as the covariate was performed. Categorical variables were analyzed by chi-square test. All statistical tests for the efficacy variables were performed two-sided with a level of significance \( \alpha = 0.05 \).

Arthroscopic parameters have been chosen as the \textit{a priori} primary efficacy variables. However, at the time the protocol was designed, no particular scale had been chosen. In this manuscript, we report the statistical analyses concerning the three arthroscopic parameters, i.e., the overall assessment of chondropathy using a 100 mm length VAS, the SFA scoring system, the SFA grading system. Some additional analyses were performed on the effect of concomitant therapy. NSAID treatment could potentially interact with progression of the structural disease [35] and NSAID intake might depend on the efficacy of the study treatment (i.e., the higher the treatment effect, the lower the expected NSAID intake). Due to the lack of independence between the effect of the treatment and the need for concomitant treatments, only exploratory analyses were carried out. The aims of these analyses were to study the main effect of NSAID intake on outcome and the effect of the interaction between this variable and treatment on outcome.

Table II

Symptomatic severity of knee osteoarthritis by treatment groups in the 36 patients who completed the 1 year study

<table>
<thead>
<tr>
<th>Clinical variables</th>
<th>Entry visit</th>
<th>Final visit</th>
<th>Difference from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hyaluronan N=19</td>
<td>Control N=17</td>
<td>Hyaluronan N=19</td>
</tr>
<tr>
<td>Pain (VAS, mm)</td>
<td>49.2 ± 20.1</td>
<td>52.1 ± 16.8</td>
<td>32.4 ± 25.5</td>
</tr>
<tr>
<td>Functional impairement</td>
<td>8.9 ± 4.1</td>
<td>9.4 ± 3.5</td>
<td>7.2 ± 4.8</td>
</tr>
<tr>
<td>Lequesne's index</td>
<td>2.6 ± 1.3</td>
<td>2.2 ± 0.7</td>
<td>2.2 ± 1.3</td>
</tr>
</tbody>
</table>

*ANCOVA analysis.
Table III
Changes in radiological and arthroscopic variables evaluating the structural severity of knee osteoarthritis by treatment groups in the 36 patients who completed the 1 year of the study (hyaluronan group: N = 19 control group: N = 17)

<table>
<thead>
<tr>
<th>Chondropathy</th>
<th>Entry visit</th>
<th>Final visit</th>
<th>Differences from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hyaluronan</td>
<td>Control</td>
<td>Hyaluronan</td>
</tr>
<tr>
<td><strong>Radiological variable</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Joint space narrowing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25%</td>
<td>7</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>≥25%; &lt;33%</td>
<td>5</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>≥33%; &lt;50%</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>&gt;50%; ≤66%</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>&gt;66%; ≤90%</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>≥90%</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Joint space width (mm)</td>
<td>4.5 ± 1.7</td>
<td>3.5 ± 1.2</td>
<td>4.0 ± 1.8</td>
</tr>
<tr>
<td><strong>Arthroscopy</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Overall assessment†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(VAS), mm</td>
<td>41.8 ± 24.3</td>
<td>52.6 ± 30.6</td>
<td>47.0 ± 26.6</td>
</tr>
<tr>
<td><strong>SFA scoring system†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>

*Statistical significance determined by ANCOVA analysis or Chi-square test (inter-group comparison).
†See methods section for explanation of the variables.
‡Statistical significance (P < 0.05) (intra-group comparison) determined by ANCOVA analysis or Chi-square test.
Results

PATIENTS AND STUDY COURSE

The baseline characteristics of the 39 patients enrolled in the study are summarized in Table I and the parameters evaluating the symptomatic severity and/or the structural severity of osteoarthritis using clinical, radiological and arthroscopical parameters are summarized in Tables II and III.

At entry, there was no statistically significant difference between the two studied groups with regard to clinical and radiological variables (see Tables I and II). However, at arthroscopy, there was a trend in favor of more severe disease in the control group which was close to statistical significance for the variable SFA scoring (P-value of 0.058 when using ANOVA analysis).

Three patients withdrew from the study during the 1 year of follow up: one in the hyaluronan group (the patient refused to continue the treatment due to lack of pain) and two in the control group [one because of moving, one because of surgery of the evaluated knee (osteotomy)]. Therefore, 36 patients completed the 1 year of the study.

Efficacy

Clinical parameters

Improvement was observed in both groups for pain and functional impairment but the differences between groups did not reach statistical significance (see Table II). A statistically significant difference between the two groups in favor of Hyalgan was found in the quality of life assessment. Moreover, 30% (six of 20) and 68.4% (13 of 19) in Hyalgan and control groups, respectively, received a NSAID during the study with the difference between groups being statistically significant (P = 0.016). The mean daily intake was 0.65 ± 2.17 and 2.77 ± 3.56 in the Hyalgan and the control group, respectively, (P = 0.044). A nonstatistically significant trend in favor of a more important analgesic rescue in the control group was observed. The paracetamol mean daily intake was 0.22 ± 0.41 and 0.49 ± 1.01 in the hyaluronan and control group, respectively.

Structural parameters

The changes in severity of chondropathy evaluated by both X-rays and arthroscopy are summarized in Table III. The changes observed in structural variables indicate deterioration in both groups but to a lower extent in the hyaluronan group. For the arthroscopic evaluation, the between-groups difference for the SFA scoring system and the overall assessment using the VAS were statistically significant in favor of Hyalgan, and this difference was close to statistical significance for the SFA grading system (P = 0.052). Evaluation of joint space narrowing and joint space width by radiography showed that the between-group differences for these parameters did not reach statistical significance although deterioration was less in the Hyalgan group.

The imbalance in the concomitant therapies prompted a post-hoc exploratory analysis in order to clarify the possible influence of NSAID intake on the between-treatments difference. Results of simple regression analysis on the SFA score, including the treatment and the NSAID consumption as main effects, and the treatment by NSAID interaction effect, showed that the effect of the treatment was still statistically significant, and neither the NSAID effect or the interaction effect was statistically significant.

Acceptability

One patient in the hyaluronan group refused to continue the course of intra-articular injections because he was free from pain. Moreover, eight out of the 20 hyaluronan treated patients (40%) reported pain during or immediately after the injection for at least one of the nine injections for a total of 17 events (180 hyaluronan injections were performed during the study). In all cases, pain was limited to the moment of injection or lasted for a few minutes after the injection. No acute hydralthrodial flare of osteoarthritis occurred during the study.

Discussion

This study supports existing data concerning the beneficial symptomatic effects of intra-articular injections of hyaluronan in osteoarthritis of the knee, and suggests that repeated intra-articular injections of hyaluronan might delay structural progression of the disease. It also suggests that arthroscopy might be able to identify chondromodulating agents; and confirms the feasibility of using arthroscopy as an outcome measure of knee osteoarthritis.

Arthroscopy, even simplified, can be considered an aggressive method. However, in this study, the high percentage (36 out of 39; 92%) of patients completing the trial may be considered an
argument favoring the good acceptability of this technique.

The results obtained in this study suggest that this technique is capable of demonstrating statistically significant changes in chondropathy in a relatively low number of patients (N = 17) and over a 1-year period. Sensitivity to change is one of the major characteristics of an outcome variable. The results obtained in this study suggest that arthroscopic parameters are more sensitive than radiological parameters to evaluate the changes in structural damage observed in osteoarthritis.

Although this was not its primary objective, this study confirms the beneficial clinical effects of intra-articular injections of hyaluronan in knee osteoarthritis. However, the study design (open procedure to collect the clinical parameters, systematic articular lavage in all patients at entry) may have influenced the final results.

The choice of the number of injections within each course (three) and the interval between each course (3 months) was based on data previously reported in different clinical studies suggesting that at least three injections (once a week during 2 weeks) are necessary to improve the clinical manifestations of osteoarthritis, and that this improvement persists several months after cessation of treatment [23-35 and reviewed in 26].

It should be noted that the intra-articular injections were performed in the hyaluronan treatment group even though the patients were painless. The acceptability of this procedure appeared good as only one patient refused to continue the injections because he was free from pain.

Comparison of the changes in severity of chondropathy by treatment group during the 1 year of the study suggests that repeated intra-articular injections might delay cartilage lesions due to osteoarthritis in humans. Although these results have been observed using rigorous methodology, a number of aspects must be considered before drawing definite conclusions: (1) the imbalance in the severity of chondropathy observed at entry; (2) the small number of evaluated patients; (3) the imbalance in the amount of rescue treatment. For example, one can argue that the progression of the disease is related to its severity at entry and/or that NSAID intake might have a deleterious structural effect in osteoarthritis, as has been recently suggested [35]. We conducted various statistical analyses in order to take into account the imbalance in both the severity of chondropathy and the amount of rescue treatment in the interpretation of the results. The results obtained suggest that in this study NSAID intake alone might not represent an explanation for the level of cartilage deterioration after 1 year of study. However, the possible role of other patient conditions and the suggested effect of the treatment in reducing access to concomitant therapies impose cautious consideration of this result. A direct answer concerning the possible effect of the NSAID alone, or in association with other treatments can only be provided by another, randomized factorial study where the two experimental factors (NSAID and other treatments) and their combinations are compared.

Moreover, these results were obtained in a very small subgroup of patients. This small sample size cannot exclude that the results obtained are due to chance alone. The particular characteristics of the recruited patients preclude any extrapolation of the results to the general population of patients with knee osteoarthritis.

In case of a real effect of hyaluronan intra-articular injections on structural damage, it might be of interest to evaluate different schedules of administration. In this study, we used a 3 month repetition of cycles of three intra-articular hyaluronan injections to severely symptomatic patients. It might be interesting to evaluate the effects of repetition of injection cycles performed only in case of a clinical flare of the disease.

In the light of these considerations, further studies are required in order to confirm our results and to evaluate long-term monitoring of osteoarthritis patients using such local (intra-articular) therapy.

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