Introduction

The last decade has seen assays developed which permit the quantification of collagenous and noncollagenous macromolecules derived from bone, cartilage, and synovial tissues. This development has greatly enriched knowledge of matrix composition and metabolism of connective tissues as well as of the underlying pathophysiologic processes involved in disturbances of cartilage turnover.

The ability to use biochemical markers to predict disease progression and identify patients most likely to progress is a top priority in the future management of osteoarthritis (OA). Ultimately, it would enable much more rapid assessment of structure-modifying therapies in clinical trials. It may also allow the identification of patients at highest risk of progression, allowing the efficient testing of new treatments.

Although its role in the pathophysiology of disease is not fully understood, aging is one of the main risk factors for the development of OA. Age-related changes in articular cartilage are therefore likely to play a role in the etiology of OA.

Key words: Pentosidine, Cartilage loss, MRI, Knee osteoarthritis.

Summary

Objective: Age-related changes in articular cartilage are likely to play a role in the etiology of osteoarthritis (OA). One of the major changes in the extracellular matrix of cartilage is the age-related accumulation of advanced glycation end products (AGEs). Pentosidine, an AGE cross-link, is one of the few characterized AGEs and is considered an adequate marker for the many AGEs that are formed in vivo. We used data from a longitudinal observation study to determine if urinary pentosidine could serve as a marker to predict cartilage loss.

Methods: We conducted a prospective analysis of data from the Boston Osteoarthritis of the Knee Study (BOKS); a completed natural history study of knee OA. All subjects in the study met American College of Rheumatology (ACR) criteria for knee OA. Knee magnetic resonance imaging (MRI) images were scored for cartilage in 14 plates of the knee using the Whole Organ Magnetic Resonance Imaging Score (WORMS) semi-quantitative grading scheme. Within the BOKS population, a nested sample of 127 subjects (39% of the whole sample) who had both baseline pentosidine and longitudinal magnetic resonance imaging (MRI) measurements (MRIs performed at baseline and 30 months later) was assessed. Urinary pentosidine was assayed and normalized to creatinine to account for differences in urine concentrations. We analyzed the data using three different methods to assess if baseline measures of pentosidine predicted subsequent cartilage loss on MRI. These were (1) analysis 1: logistic regression with the outcome cartilage loss in any plate; (2) analysis 2: proportional odds model where the outcome was defined as 0 = no cartilage loss, 1 = cartilage loss in one plate, 2 = cartilage loss in two plates, and 3 = cartilage loss in at least three plates; and (3) analysis 3: Poisson regression with the outcome the number of plates with cartilage loss. All analyses were adjusted for age, sex and Body Mass Index (BMI).

Results: At baseline the mean (standard deviation) age was 67 (9) years and 54% were male. The results for the three analytic steps are as follows: Analysis 1: the odds ratio for cartilage loss is 1.01 (95% confidence interval (CI) 0.93–1.09) with 1 unit increase in pentosidine. Analysis 2: the odds ratio for more cartilage loss is 0.99 (95% CI 0.92–1.06) with 1 unit increase in pentosidine. Analysis 3: the relative number of plates with cartilage loss decreased was 1.00 (95% CI 0.95–1.03) with a 1 unit increase in pentosidine.

Conclusion: Urinary pentosidine does not predict knee cartilage loss. Previous studies have suggested that local content within cartilage of AGEs is elevated in persons at high risk for progression. Our data suggest that these changes are not measurable systemically. Alternatively, urinary pentosidine levels reflect cartilage degradation in all joints (thus whole body cartilage breakdown) and may therefore not relate to OA severity in a single knee joint.

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Key words: Pentosidine, Cartilage loss, MRI, Knee osteoarthritis.
One of the major changes in the extracellular matrix of cartilage is the age-related accumulation of advanced glycation end products (AGEs). AGEs result from nonenzymatic glycation: the spontaneous reaction of a reducing sugar with proteins. Consequently, AGEs accumulate in all tissues with age, but this accumulation is most pronounced in tissues with long-lived proteins. Articular cartilage is a tissue with relatively low turnover and therefore contains high AGE levels when compared with other tissues. Pentosidine, an AGE crosslink that is relatively straightforward to measure, is one of the few characterized AGEs and is considered a good marker for the many AGEs formed in vivo. Pentosidine levels in articular cartilage increase as much as 50-fold from age 20 to 80. This increase in AGE levels coincides with the age-related increase in the incidence of OA, suggesting that accumulation of AGEs may be involved development of the disease.

Accumulation of AGEs in cartilage affects the biomechanical, biochemical and cellular characteristics of the tissue, potentially making cartilage vulnerable to loading and increasing the risk for damage. In addition to measuring AGEs in cartilage, pentosidine can also be measured in synovial fluid, serum and urine. To date, however, no study in humans has investigated whether urinary pentosidine predicts cartilage loss on magnetic resonance imaging (MRI). We took advantage of a natural history study of symptomatic OA to investigate whether higher levels of AGEs are associated with an increased risk for cartilage loss.

Materials and methods

STUDY SAMPLE

We conducted a prospective analysis of data from the Boston Osteoarthritis of the Knee Study (BOKS): a completed natural history study of knee OA. To be eligible for the study, a person had to have knee pain, aching or stiffness on most days within the last month, and they had to have reported that a physician had told them that they had arthritis in the knee. If they met both of those criteria, then they underwent radiography (weight bearing fluoroscopic PA, lateral and skyline views) and if, on any of these views they had a definite osteophyte in the symptomatic knee, they were eligible for the study. In addition, if they screened positive for another form of arthritis or were using medications that were appropriate for rheumatoid or other forms of arthritis, they were excluded. Thus, all subjects in the study had primary clinical knee OA and met American College of Rheumatology (ACR) criteria for this disorder.

Of 324 subjects who entered the study, 86% completed a full comprehensive follow-up at a later time-point (either 15 and/or 30 months). Those lost to follow-up did not differ substantially from the other participants in age, or weight, but were more likely to be men and to have higher WOMAC pain and disability scores at baseline. The comprehensive examinations performed at each visit consisted of an MRI of the more affected knee, and a comprehensive set of radiographs including a semi-flexed fluoroscopically positioned PA radiograph using the method of Chaisson et al. and Block et al.

This study was a nested sample selected from participants who had longitudinal MRI. Within the BOKS population we selected approximately equal numbers of subjects with cartilage loss and subjects without cartilage loss for the purposes of the nested case control study. Assays were conducted on this nested sample. The study was powered based upon a previous published study (OA and COMP), where the mean score of COMP (log transformed value) was 7.08 with standard deviation (SD) of 0.40 among subjects with grades 3, 4 OA, and 6.91 with SD of 0.33 among subjects without OA. We assumed that the difference in biochemical markers between subjects with cartilage loss defined by MRI and subjects without cartilage loss is the same as that detected by radiographs.

MAGNETIC RESONANCE IMAGING

All studies were performed with a Signa 1.5 T MRI system (General Electric Corp., Milwaukee, WI) using a phased-array knee coil. A positioning device was used to ensure uniformity of positioning among patients. The imaging protocol included sagittal spin-echo proton density- and T2-weighted images (repetition time (TR), 2200 ms; time to echo (TE) 20/80 ms) with a slice thickness of 3 mm, a 1-mm interslice gap, one excitation, a field of view (FOV) of 11–12 cm, and a matrix of 256 × 192 pixels; and coronal and axial spin-echo fat-suppressed proton density- and T2-weighted images (TR 2200 ms; TE 20/80) with a slice thickness of 3 mm, a 1-mm interslice gap, one excitation, and with the same FOV and matrix.

Tibiofemoral (TF) cartilage on MRI was scored paired and unblinded to sequence on 14 plates (anterior, central and posterior femur; anterior, central and posterior tibia; medial and lateral patella), using the Whole Organ Magnetic Resonance Imaging Score (WORMS) semiquantitative method. Both cartilage signal and morphology were scored using a 0–6 scale: 0 = normal thickness and signal; 1 = normal thickness but increased signal on T2-weighted images; 2 = solitary focal defect of less than 1 cm in greatest width; 3 = areas of partial-thickness defects (<75% of the plate) with areas of preserved thickness; 4 = diffuse partial-thickness loss of cartilage (<75% of the plate); 5 = areas of full-thickness loss (<75% of the plate) with areas of partial-thickness loss; 6 = diffuse full-thickness loss (<75% of the plate). Intraobserver intraclass correlation coefficient on agreement for cartilage readings ranged from 0.75 to 0.97. The interobserver agreement was 0.62. Reliability was based upon a random sample of 10 films. Films were read paired and unblinded to sequence by two readers (AG, MG (see Acknowledgments) who are both musculoskeletal radiologists) using MRI sequence data from the sagittal and coronal places.

In WORMS, grade 1 does not represent a morphologic abnormality but rather a change in signal in cartilage of otherwise normal morphology. Grades 2 and 3 represent similar types of abnormality of the cartilage, focal defects without overall thinning. Therefore, to create a consistent and logical scale for evaluation of cartilage morphologic change, we collapsed the WORMS cartilage score to a 0–4 scale, where the original WORMS scores of 0 and 1 were collapsed to 0, the original scores of 2 and 3 were collapsed to 1, and the original scores of 4, 5 and 6 were considered 2, 3 and 4, respectively, in the new scale.
Cartilage loss was defined as a change in the score at any plate compared to baseline.

We selected subjects who attended the baseline and final visits with an intervisit duration generally over 30 months. Within the BOKS population, pentosidine and cartilage loss on serial MRI were available on 127 participants.

Elevated blood sugar can potentially affect pentosidine levels. We conducted additional analyses in the 111 subjects without self-reported diabetes. The diagnosis of diabetes was based upon self-report. The question asked “Do you have diabetes (high blood sugar)?

PENTOSIDINE

Urinary pentosidine was measured by HPLC (Spark Endurance autosampler and Dionex P580 pump) in diluted nonhydrolysed urine (1:1 in 0.025% H2SO4) following injection of 50 µl on a Whatman partisil 10 SCX column (250 × 4.6 mm)19. Pentosidine was eluted using an isocratic gradient of 0.025% H2SO4 and 100 mM A2(SO4)3 in 0.025% H2SO4. Column temperature was kept at 60°C and eluted pentosidine detected by a Jasco FP-1520 fluorimeter at 328 nm (ex) 378 nm (em). Purified pentosidine calibrated by mass spectroscopy served as a reference. Limit of detection: <2.4 pmol/ml, intra-assay variation: 5.6%, and interassay variation: 6.2%, with no freeze–thaw effects (pentosidine is stable, even upon acid hydrolysis at 110°C). Urinary pentosidine values were normalized vs urinary creatinine to account for urinary dilution. No degradation of pentosidine will occur in urine (nor in serum), since the molecule is stable, even to overnight 110°C in 6 M HCl. Assays were conducted at TNO, The Netherlands.

STATISTICAL ANALYSIS

We analyzed the data using three different methods to assess if baseline measures of pentosidine predicted subsequent cartilage loss on MRI (ascertained from baseline to 30 month visit). These were (1) analysis 1: logistic regression with the outcome the number of plates with cartilage loss; (2) analysis 2: proportional odds model where the outcome was defined as 0 = no cartilage loss, 1 = cartilage loss in one plate, 2 = cartilage loss in two plates, and 3 = cartilage loss in at least three plates; and (3) analysis 3: Poisson regression with the outcome the number of plates with cartilage loss. All analyses were adjusted for age, sex and Body Mass Index (BMI).

Results

At baseline the mean (SD) age was 67 (9) years and 54% were male. The remainder of the demographic characteristics are displayed in Table I. Seventy-six percent of the subjects had radiographic TF OA (K&L ≥ 2), while the remainder had patellofemoral OA. Further descriptive characteristics for the participants are provided according to whether they lost cartilage in any plate during the course of 30 month follow-up or not. Compared to those that did not lose cartilage at follow-up there was an overrepresentation of women and persons with PF OA (as opposed to TF OA K&L ≥ 2) in participants who lost cartilage at follow-up.

There was no difference in pentosidine levels between the two groups at baseline.

The results of analysis 1 (logistic regression with the outcome cartilage loss in any plate) are displayed in Table II. The unadjusted and adjusted odds ratios for cartilage loss were 1.01 (95% confidence interval (CI) 0.93–1.09) per 1 unit increase in pentosidine.

The results of analysis 2 (proportional odds model where the outcome was defined as 0 = no cartilage loss, 1 = cartilage loss in one plate, 2 = cartilage loss in two plates, and 3 = cartilage loss in at least three plates) are displayed in Table II. Both unadjusted and age, sex and BMI adjusted odds ratios for cartilage loss was 0.99 (95% CI 0.92–1.06) per 1 unit increase in pentosidine.

The results of analysis 3 (Poisson regression with the outcome the number of plates with cartilage loss) are displayed in Table II. The relative number of plates with cartilage loss was 1.00 (95% CI 0.95–1.03) with 1 unit increase in pentosidine. The results of the unadjusted analyses are similar to those of the adjusted.

We restricted analyses to 111 subjects without self-reported diabetes and found the results were similar.

Conclusion

Urinary pentosidine, an AGE crosslink does not predict knee cartilage loss in subjects with symptomatic OA. Previous studies have suggested that the local content of AGEs within cartilage is elevated in persons at high risk for progression. Our data suggest that these changes are not measurable systemically.

Whilst the results of our findings may suggest that pentosidine does not predict cartilage loss, there are a number of potential alternate explanations. Synovial fluid is often recommended as the preferable body fluid in which to analyze

Table I

<table>
<thead>
<tr>
<th>Table I</th>
<th>Baseline characteristics of study population, N = 127</th>
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<tbody>
<tr>
<td></td>
<td>Whole sample</td>
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<tr>
<td>Age, mean ± SD</td>
<td>67 ± 9.05</td>
</tr>
<tr>
<td>Gender (male%)</td>
<td>54.33</td>
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<tr>
<td>BMI, mean ± SD</td>
<td>31 ± 5.20</td>
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<tr>
<td>% K&amp;L ≥ 2</td>
<td>76.19</td>
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<tr>
<td>History of knee joint injury (%)</td>
<td>44.4</td>
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<tr>
<td>Pentosidine/Cr, mean ± SD (µmol/ml)</td>
<td>1.85 ± 4.66</td>
</tr>
<tr>
<td>Cartilage loss (no. of plates with cartilage loss), Mean, range</td>
<td>1.02 (0, 5)</td>
</tr>
<tr>
<td>Median number of plates with cartilage loss (range)</td>
<td>1 (0, 5)</td>
</tr>
<tr>
<td>Proportion of participants with cartilage loss in one plate</td>
<td>25.20</td>
</tr>
<tr>
<td>Proportion of participants with cartilage loss in two plates</td>
<td>16.54</td>
</tr>
<tr>
<td>Proportion of participants with cartilage loss in three or more plates</td>
<td>11.82</td>
</tr>
<tr>
<td>Proportion of subjects with diabetes</td>
<td>12.90</td>
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</tbody>
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biochemical markers, as serum and urine measurements reflect systemic disturbances in turnover rather than local pathological changes in OA joints. The urinary measure was used as a less invasive and more available means to obtain this marker level. It may be, however, that urinary measurement of systemic pentosidine levels is not sufficiently sensitive to detect local alterations in AGEs in a single joint. Urinary pentosidine is not highly correlated with synovial fluid or serum levels and thus our findings cannot specifically be related to urinary pentosidine measures only and further work needs to be done on serum and or synovial fluid to ascertain if they predict OA progression.\(^13\)

Previous studies of AGEs and pentosidine more specifically have focused at the articular level. An alternate explanation for our results is that urinary pentosidine levels may reflect cartilage degradation in all joints (thus whole body cartilage breakdown) and may therefore not relate to OA severity in a single knee joint.

In addition, pentosidine is present in almost all connective tissues. Its absolute level within a tissue primarily depends on the turnover rate of that tissue. As was demonstrated by Sell and Monnier\(^12\), the highest pentosidine levels are found in dura mater and cartilage. But also meniscus, intervertebral disk, tendons, ligaments, skin and bone contribute to excreted pentosidine levels. This causes the background level above which a change due to elevated tissue destruction in the knee joint may rise. Based on our analyses we may not be able to differentiate this change.

Previous studies on the accumulation of AGEs in cartilage have demonstrated that it affects the biomechanical, biochemical and cellular characteristics of the tissue. At the biomechanical level, increased AGE levels are accompanied by increased stiffness of the tissue\(^9\). Elevated AGE levels not only increase tissue stiffness but also increase cartilage brittleness, indicating that AGE accumulation leads to increased susceptibility of articular cartilage to mechanical damage\(^21\). On the cellular level, accumulation of AGEs was shown to decrease the synthesis of cartilage proteoglycans\(^8,10\) and type II collagen\(^12\). In addition, degradation of extracellular matrix constituents was impaired at increased AGE levels\(^11,12\). In combination, the decreased synthesis and degradation of the cartilage matrix result in tissue that possesses decreased capacity to adapt to changes in its environment (such as a different loading pattern). Moreover, these data also suggest that decreased cartilage turnover is likely to result in decreased repair capacity of the tissue. In combination these studies suggested that the increased tissue brittleness and decreased extracellular matrix turnover, due to the accumulation of AGEs, results in an articular cartilage that is more prone to damage, and by this mechanism, the age-related increase in cartilage AGEs could have explained the age-related increase in the incidence of OA. These studies were not conducted on systemic measures of AGEs such as pentosidine but rather on tissue samples \textit{in vivo}.

A number of clinical studies have investigated the potential role of pentosidine in small clinical samples. Pavelka et al.\(^22\) examined the prognostic value of different markers for progression of knee OA. Eighty-nine persons with knee OA and 20 healthy volunteers (not age matched) as controls were examined. In persons with knee OA there were higher serum levels of pentosidine compared with healthy control subjects, and baseline pentosidine was correlated with further joint space narrowing. It is not clear if any of the analyses are adjusted for age.

A cross-sectional study of 38 persons with knee OA and 38 healthy volunteers (again not age matched) were examined for serum and synovial fluid concentrations of pentosidine to determine whether there was a relationship with cartilage oligomeric matrix protein\(^23\). Pentosidine in synovial fluid and in serum was correlated with synovial fluid COMP. Both pentosidine and COMP concentrations did not correlate significantly with the radiological stage of the disease. Due to the differences in age between the OA and healthy sample it is difficult to know if the differences found are due to age or disease.

Miyata et al.\(^24\) examined the concentrations of pentosidine in plasma and synovial fluid from 22 patients with rheumatoid arthritis and compared their levels with those in 17 patients with OA, 26 diabetic patients, and 25 normal subjects. Pentosidine levels in plasma and synovial fluid from RA patients were significantly higher than those in OA patients, diabetic patients, and normal subjects. Chen et al.\(^17\) found similarly that penotisidine levels in persons with rheumatoid arthritis were considerably higher than those with OA.

There are some limitations of this work that warrant mentioning. Age-related increases are commonly seen in biochemical markers and these may produce variation in both AGE level and cartilage loss\(^25\). Efforts were made to adjust for age in analyses. The BOKS study assessed the local structural changes in participants knees only. For the purposes of this study we investigated the knee that had an MRI performed. It may be that other studies that investigate the total body burden of OA including other joint areas such as the hands, hips and spine or even the other knee may be able to detect an association with urinary pentosidine. Another potential explanation for our null findings is that we have insufficient power; given how null our findings are this possibility is unlikely. The diagnosis of diabetes was based upon self-report. Whilst self-report is likely to introduce bias in classification, particularly systematic underreporting the results of our analyses is unlikely to be any different.

In sum, urinary pentosidine does not predict cartilage loss in persons with symptomatic knee OA. Further work needs to be done to explore if serum or synovial fluid measures of pentosidine predict cartilage loss.

\textbf{Conflict of interest statement}

Nothing to declare. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

\begin{table}[h]
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\begin{tabular}{|l|c|c|}
\hline
& \textbf{Unadjusted OR (95\% CI)} & \textbf{Adjusted OR* (95\% CI)} \\
\hline Analysis 1: logistic regression with the outcome of cartilage loss in any plate & 1.00 (0.92–1.07) & 1.01 (0.93–1.09) \\
Analysis 2: proportional odds model for cartilage loss & 0.99 (0.93–1.07) & 0.99 (0.92–1.06) \\
Analysis 3: Poisson regression (relative risk) & 1.00 (0.96–1.04) & 1.00 (0.95–1.03) \\
\hline
\end{tabular}
\caption{Results of three analytic methods to determine if baseline measures of pentosidine predicted subsequent cartilage loss on MRI}
\end{table}
Role of funding source

The study sponsor was not involved in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or the decision to submit the paper for publication.

Acknowledgments

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References