Glycoconjugate markers of joint diseases

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Abstract

A number of different types of glycoconjugate are found associated with joint tissue and fluids, comprising glycoproteins, glycolipids and glycosaminoglycans. Oligosaccharide chains of glycoconjugates are degraded by exoglycosidases, and the dominant exoglycosidase found in human blood, synovial fluid, the synovial membrane and chondrocytes of articular cartilage is HEX (N-acetyl-β-hexosaminidase). HEX is localized mostly intracellularly in synovial cells. Serum activity of HEX may be used to monitor the course and efficiency of treatment of Lyme arthritis, and activity of HEX, above 10 μkat/kg of protein in the synovial fluid, suggests rheumatoid disease. There is a shortage of HEX inhibitors able to penetrate synoviocytes, so the development of drugs which inhibit synthesis and/or the activity of HEX will be a promising field for future investigations.

Introduction

Joint diseases cause serious medical and social problems affecting millions of people in the world, which is why WHO (World Health Organization) has designated the first decade of the new millennium as the Decade of the Bone and Joint.

In joint diseases, the major clinical symptoms and disability of patients are caused by an irreversible enzymatic destruction of the two major components of the cartilage matrix: collagen II and aggrecan [1,2]. Degradation of cartilage is a complex process due to the action of a wide range of enzymes [3–5]. The destruction of joint cartilage involves the degradation of matrix, molecules which are released as fragments into joint fluid, blood and urine, where they may be detected. It has been suggested that fragments of matrix molecules of cartilage matrix or cells of the synovial membrane could be used as markers for diagnosis, prognosis and monitoring response to therapy. Many publications have described the increased release of markers of cartilage degradation, bone and synovial metabolism into the body fluid compartment during joint diseases, but these remain to be fully validated [6–8].

In the 1970s, several groups of investigators reported elevated levels of exoglycosidases in serum and synovial fluid in rheumatic diseases [9–11]. Unfortunately these investigations were not expanded upon for a number of years.

Based on previous literature and on our results, we present the behaviour of some glycosaminoglycans and exoglycosidases in healthy and diseased joints. We propose a new role for glycoconjugates and associated degrading enzymes as markers of joint diseases.

Synovial fluid markers in RA (rheumatoid arthritis) and OA (osteoarthritis)

Joint diseases involve the loss of the physiological balance between degradation and replenishment of extracellular matrix cartilage and other tissues of the joint. The released fragments of a cartilage matrix shed into joint fluid may be detected by biochemical or immunochemical assays. It has been hypothesized that such molecular ‘markers’ could be used for the diagnosis, prognosis and monitoring of joint diseases. These goals have not yet been reached; however, as we gain a better understanding of these markers, we learn more about the molecular pathogenic mechanism of the diseases and the balance between matrix degradation and synthesis [6,8].

Ortutay et al. [12] found that β-D-glucuronidase and β-D-N-acetylglucosaminidase activities, if measured at the original pH of the synovial fluid, could serve as significant predictors for RA.

Shinmei et al. [13] suggested that the levels of chondroitin sulfate isomers and the di-6S/di-4S ratio in joint fluid reflects the proteoglycan metabolism of joint tissues. Saxne and Heinegard [14] found that patients with early-stage RA who had high concentrations of aggrecan fragments in joint fluid progressed more rapidly towards joint destruction than patients with low aggrecan concentrations.

Our team [15,16] have focused on the concentration of individual glycosaminoglycans in the synovial fluid of patients with OA and RA. In RA we observed a reduction in the relative amount of glycosaminoglycans in relation to proteins. The highest concentration of hexosamines in synovial fluid (205.5 μg/ml) was in RA patients.

Exoglycosidase activity in the synovial fluid and serum of healthy people

Exoglycosidases release single monosaccharides from the non-reducing end of oligosaccharides. The main exoglycosidases of serum, synovial fluid and healthy joint
Activity of exoglycosidases in the synovial fluid and serum of healthy individuals

The localization of exoglycosidases in joint tissues

Table 1 | Activity of exoglycosidases (nmol/ml per min) in synovial fluid and serum of healthy individuals

<table>
<thead>
<tr>
<th>Exoglycosidase</th>
<th>Synovial fluid</th>
<th>Serum</th>
<th>SF/S ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEX (total)</td>
<td>17.3 ± 1.06</td>
<td>13.89 ± 1.33</td>
<td>1.25</td>
</tr>
<tr>
<td>HEX-A</td>
<td>8.01 ± 1.01</td>
<td>7.29 ± 1.34</td>
<td>1.1</td>
</tr>
<tr>
<td>GluA</td>
<td>5.52 ± 1.25</td>
<td>3.67 ± 0.55</td>
<td>1.5</td>
</tr>
<tr>
<td>GAL</td>
<td>3.4 ± 0.67</td>
<td>1.4 ± 0.45</td>
<td>2.4</td>
</tr>
<tr>
<td>α- MAN</td>
<td>2.55 ± 0.34</td>
<td>1.5 ± 0.4</td>
<td>1.7</td>
</tr>
<tr>
<td>FUC</td>
<td>3.29 ± 1.01</td>
<td>2.35 ± 0.8</td>
<td>1.4</td>
</tr>
</tbody>
</table>

In Table 1 one can see that exoglycosidase activity is higher in synovial fluid than in serum. HEX demonstrates the highest activity among the exoglycosidases, which is stable in serum of healthy subjects up to 40 years of age, whereas in older people (more than 40 years of age) levels significantly increase [22].

Exoglycosidases are produced by chondrocytes, neutral granulocytes, mastocytes, cells of the synovial membrane and subsynovial connective tissue [23–25].

RA and JIA (juvenile idiopathic arthritis) are chronic autoimmune inflammatory diseases primarily affecting the synovial membrane leading to joint damage and destruction. RA affects approx. 1.5% of the world population [26]. The pathogenesis of RA is unknown. Only a few laboratory markers, such as C-reactive protein and the ESR (erythrocyte sedimentation rate), correlate well with the inflammatory activity in RA, although those parameters are frequently elevated in other conditions, such as infections [4].

A number of papers have been published regarding lysosomal exoglycosidases, their role in cartilage degradation and the possibility of using them in the diagnosis of joint diseases [4, 10, 11, 27]. Ortutay et al. [12] suggested some kind of co-operation between proteases and exoglycosidases in the destruction of knee-joint cartilage. They believe that protease action increases the accessibility of cleavage sites for exoglycosidases. Berenbaum et al. [28] reported that serum HEX activity was 35% higher in RA patients than in healthy controls. These authors found that the serum HEX concentration was significantly higher in destructive RA than in inflammatory RA. Berenbaum et al. [28] hypothesized that the degradation of cartilage in RA depends, at least in part, on enhanced HEX activity, which can be reflected by serum HEX activity.

We [22, 25, 29] have found that the specific activity of HEX was significantly increased in comparison with controls in the serum of RA patients, in agreement with data reported by Berenbaum et al. [28]. Sohar et al. [4] have suggested that the increase in HEX activity in the serum of RA patients may depend on the increase in HEX activity of RA leucocytes.

Our results support this suggestion, as we observed a moderate increase in HEX activity, 25.46 and 17.3%, in the serum of patients with JIA and OA respectively [29]. In the case of patients with knee injury, the specific activity of HEX and its isoenzymes in serum was found to be similar to controls [29]. Our recent investigation [30] showed that exoglycosidase activity in serum and synovial fluid may be used to differentiate JIA, RA and LA. While an increase in the serum activity of lysosomal exoglycosidases points at inflammatory or autoimmunological process, i.e. JIA, RA and LA, an increase of the mean SF/S (synovial fluid/serum) ratio above 2.0 points more specifically to LA.

We suggest that measurement of total HEX activity in the synovial fluid of patients with RA may have some value as a diagnostic marker. In the synovial fluid of patients with JIA and RA, the specific activity of HEX was 29.2 ± 16.4 μkat/kg of protein and 27.4 ± 11.2 μkat/kg of protein respectively, and was significantly elevated in comparison with patients with OA (5.3 ± 3.2 μkat/kg of protein) and a control group (4.2 ± 0.21 μkat/kg of protein). We found that in rheumatoid diseases, the secretion of HEX from joint tissues to synovial fluid is much higher than in osteoarthritis and healthy controls. This may be of diagnostic value in children with prolonged exudate in the knee joint, resistant to pharmacological and physiotherapeutical treatment. In these cases we advise determining levels of HEX in the synovial fluid, where values above 10–13 μkat/kg of protein suggest rheumatoid disease. It is noteworthy that specific activity of HEX in the synovial fluid of patients with RA demonstrates a broad distribution which probably depends on destructive or inflammatory processes in the joint. However, significantly elevated HEX activity points towards inflammatory or autoimmunological process within the joint.
Profiles of the exoglycosidases in the synovial tissue of the knee joints of patients with RA, JIA and a control group are presented in Table 2 [29].

Our results indicate a similar pattern of exoglycosidase activity in normal and inflamed synovial tissue, with a significant predominance of HEX activity. Our results are in agreement with the data of Shikhman et al. [28], in which HEX was the dominant enzyme released from chondrocytes cultivated in tissue culture. In the synovial tissue of RA and JIA patients, we found approx. 10-fold higher HEX activity than in the synovial tissue of the control group. The increase in activity of GluA, GAL, MAN and FUC in RA and JIA (in comparison with controls) was moderate: it was no more than twice that of the control group. High activity of exoglycosidases in synovial tissue of RA and JIA patients suggest the utility of synovectomy in treatment of these diseases.

Comparative analysis of extracellular compared with intracellular activity of exoglycosidases in cultured human articular chondrocytes was performed by Shikhman et al. [28] and in cultured human synovial cells by our team [31]. The patterns of compartmental distribution of exoglycosidase activity in cultured synovial cells of RA and JIA patients are presented in Table 3 [31].

The activity of GAL, MAN and FUC in extracellular compartments of cultured synoviocytes was not detectable. It is most likely that those exoglycosidases were released extracellularly in small quantities, below the limits of detection of our colorimetric procedure. However, in the tissue sections of the synovial membrane, the activity of all exoglycosidases was easy to determine colorimetrically, and in rheumatoid diseases this was significantly higher than the control group (Table 2). Shikhman et al. [28] have demonstrated similar results in cultured human articular chondrocytes.

It is well-documented that most of the glycosaminoglycan-degrading exoglycosidases have optimum activity in the pH range from 3.5 to 5.5, and that this activity significantly decreases at neutral pH [19,32,33]. The discussion above suggests that the degradation of glycosaminoglycans predominantly proceeds in the intracellular compartment. The high levels of activity of exoglycosidases in the intracellular compartment of cultured synoviocytes indirectly confirms this suggestion. It should be remembered that extracellular degradation of glycosaminoglycans may also be important in joint physiology. Shikhman et al. [28] demonstrated that despite the almost 80% drop in activity at neutral pH, HEX still expressed measurable activity. Woynarowska et al. [34] also supported the importance of extracellular HEX activity in glycosaminoglycan degradation.

IL-1 and TNF-α (tumour necrosis factor-α) are key pro-inflammatory cytokines whose concentration significantly...
increases in rheumatoidal synovial fluid and joint tissues. Clarris et al. [35] reported that IL-1 significantly increased the activity of HEX in extracellular compartments of cultured healthy human fibroblasts, and noted a tendency to increase HEX activity in intracellular compartments by exposure to IL-1. Similar results were reported by Lecomte et al. [36]. Shikhman et al. [28] found that IL-1β selectively up-regulated the activity of extracellular hexosaminidase in chondrocyte cell cultures. Similar results were reported by Solavagione et al. [37] in the cell cultures of healthy rabbit chondrocytes.

In our research, we have established a profile of exoglycosidases in cell cultures stimulated by IL-1β of inflamed (RA, JIA) and post-injury human synoviocytes. Stimulation of synoviocytes by IL-1β in cell cultures significantly increased the activity of HEX, HEX-A and GluA in both compartments (mainly intracellular), as well as that of GAL, MAN and FUC in the intracellular compartment. Stimulation of rheumatoid synoviocytes by IL-1β increased the activity of HEX and HEX-A by 128–201% in the intracellular compartment and by 33–72% in the extracellular compartment.

In contrast with our results, Clarris et al. [35] observed a significant increase in extracellular HEX activity after stimulation of cultured synoviocytes derived from healthy individuals by IL-1β, and Shikhman et al. [28] noted a similar increase after stimulation of chondrocytes with IL-1β. The mechanism of selective stimulation of HEX by IL-1β is not known. Shikhman et al. [28] suggested that cytokines are involved in the secretion of HEX from chondrocytes and stated that IL-1β could selectively up-regulate HEX synthesis and facilitate intracompartmental transport of HEX from lysosomes/ endosomes into the extracellular space by modifying the mannose-6-phosphate receptor system.

Rheumatoid synoviocytes exhibit altered morphology and show certain similarities to tumour cells [38]. Our data confirmed the observation that synoviocytes obtained from patients with JIA and RA are more active in the synthesis and secretion of exoglycosidases than synoviocytes obtained from patients with injured knees or healthy joints [35].

Despite its huge public health impact, the conservative treatment of joint diseases (particularly of osteoarthritis) [39,40], is limited to a few classes of medication which provide primarily symptomatic relief. Inhibition of hexosaminidase activity may represent a potentially novel strategy to treat RA and OA. Liu et al. [40] have synthesized and investigated a series of iminocyclitols designed as transition-state analogue inhibitors of extracellular human hexosaminidase. Our team [41] is focusing on pyrimethomine which contributes to the regulation of HEX gene expression in synovial cells.

Conclusions
There has been a slight increase in conservative treatment and significant progress in the surgical treatment of joint diseases. To improve conservative treatment of joint diseases it is necessary to conduct an intensive study on the composition and metabolism of joint glycoconjugates, including glycoconjugate markers of joint diseases. It is quite clear that no markers have yet been formally validated to monitor joint diseases. Validation will require access to substantial well-characterized patient cohorts for randomized and prospective intervention studies in addition to sensitive and specific assays. We hypothesize that determining HEX activity in the synovial fluid of patients with suspected idiopathic juvenile arthritis will justify these investigations.

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References