YKL-40 in human lumbar herniated disc and its relationships with nitric oxide and cyclooxygenase-2

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ABSTRACT

The basic pathophysiology of intervertebral disc degeneration and low back pain remains unclear. It has been hypothesized a role of biochemical mediators of inflammation and tissue degradation in intervertebral disc degeneration and herniation.

Chitinase 3-like protein 1 (YKL-40) is a glycoprotein mainly secreted by chondrocytes which has been proposed as a possible marker of inflammation and/or cartilage alterations.

Objective. To investigate the YKL-40 presence in human lumbar disc tissue culture and its possible relationships with some substances relevant in inflammation such as cyclooxygenase-2 (COX-2) and nitric oxide (NO).

Patients and methods. We analyzed lumbar discs from 19 patients who underwent surgery for lumbar disc herniation at L4-L5 or L5-S1 levels. The specimens were cultured and incubated for 72 hours. At the end of incubation, the supernatants were assayed for presence and concentration of YKL-40, COX-2 and NO.

Results. YKL-40 was detectable in all the samples analyzed. Mean (± SD) concentration was 1.54 ± 1.29 ng/ml/mg compared to dry weight. COX-2 and NO levels were 25.25 ± 11.42 pg/ml/mg and 1.3 ± 1.8 µM/mg x 10^2, respectively.

A correlation was found between YKL-40 and COX-2 (r = 0.579, p < 0.05) and YKL-40 and NO (r = 0.509, p < 0.05).

Conclusion. To our knowledge, this is the first report demonstrating YKL-40 release by intervertebral disc culture. It may contribute to better clarify the role of this protein in the pathophysiology of discal degeneration and inflammation as confirmed by its relationships with COX-2 and NO in disc tissue culture.

Introduction

Low back pain (LBP), with or without radiculopathy, represents an important clinical, economical and public health problem as about 70% of all people will experience this disease during their life (1). The most common origin of non-specific LBP seems to be discogenic, mainly due to an intervertebral disc (IVD) degeneration which, in turn, may be triggered and aggravated by herniation (2). There are no satisfactory pathogenetic hypotheses on IVD degeneration, thus LBP origin seems to be multifactorial, implying both mechanical and biochemical mechanisms (3). Although the role of mechanical factors seems crucial as a triggering event in most cases of acute LBP, some evidences lead to consider the mechanical hypothesis inadequate to completely explain IVD degeneration and LBP. Furthermore, radicular symptoms are not correlated to the size of disc herniation (4) and may sometimes disappear spontaneously (5); non steroidal anti-inflammatory drugs (NSAIDs) are more effective than analsgesics for the control of symptoms and local or systemic administration of steroids reduces pain even in patients with severe IVD degeneration (6).

IVD degeneration is characterized by tissue degradation and production of biochemical mediators of inflammation such as proinflammatory cytokines, matrix metalloproteinases (MMPs) and nitric oxide (NO) (7-9). Moreover, high levels of phospholipase A2 (PLA2) have been demonstrated in lumbar disc herniation. PLA2 plays a key role in the arachidonic acid cascade, the pathway of prostaglandins E2 (PGE2) synthesis in which cyclooxygenase-2 (COX-2) represents a rate-limiting enzyme (10). The presence of COX-2 in degenerated IVD seems in keeping with such hypothesis and suggests a potential role of this enzyme in the mechanism of discogenic pain (10). NO, a potent inflammatory mediator, implicated in vasoregulation, neurotransmission, and neuropathic pain, was also found in herniated lumbar disc (11). NO synthesis can be induced by different cytokines, e.g., tumor necrosis factor (TNF) alpha and interleukin (IL)-1beta.

YKL-40, a glycoprotein member of “mammalian chitinase-like protein”, has deeply been studied for its possible function in tissue remodelling. It is a major secretory protein of human chondrocytes, but it is also produced by synoviocytes, macrophages, neutrophils, cancer cells, endothelial cells, smooth muscle cells in blood.

Competing interests: none declared.
vessels, and by cells in the fibrotic liver (12, 13). Although its function is not well known, it has been proposed as a specific product of chondrocytes and consequently as a marker of cartilage turnover in osteoarthritis (OA) and rheumatoid arthritis (RA) (12). Serum and synovial fluid concentrations of YKL-40 are closely correlated in patients with joint diseases, suggesting that most of the protein found in serum may be produced within the joint. Moreover, mRNA YKL-40 is expressed in cartilage from RA or OA, but not in healthy adult cartilage (14, 15). Due to these properties, the determination of YKL-40 has been recently proposed as marker of arthritis associated with inflammatory bowel diseases (14).

The first aim of this study was to investigate the presence of YKL-40 in herniated disc, accordingly with its possible production by disc cells, in particular chondrocyte-like cells. Furthermore, we studied its relationships with some substances relevant in inflammation, such as COX-2 and NO, in order to better analyze the role of inflammatory mediators in IVD degeneration and herniation.

Materials and methods

Nineteen herniated lumbar IVD specimens were obtained from 19 consecutive adult patients undergoing surgical lumbar discectomy for disc herniation and persistent radicular symptoms. The mean age was 45 years (range 19-75) and there were 14 men and 5 women. Eleven discs were from L4-L5 level and 8 from L5-S1 (Table I).

We excluded from the study patients with tumors, infections, inflammatory or autoimmune systemic diseases and taking steroids or NSAIDs. These drugs should had to be withdrawal at least 15 days and 2 days before the study, respectively. For the pain control, the acetaminophen use was permitted. Informed consent was obtained from the patients.

Tissue culture

Herniated discs were immediately stored in sterile normal saline solution and then washed thoroughly with Gey’s balanced salt solution ( Gibco™, Grand Island, NY) to remove blood contaminations. Then the tissue was diced and incubated 200 mg tissue/2 ml of Neuman-Tytell serumless media (Gibco™) for 72 hours at 37°C in a 5% CO₂ atmosphere (7). At the end of incubation, the media were harvested and stored at –80°C.

Biochemical assays

YKL-40 and COX-2 were determined by ELISA methods, respectively YKL-40 (Metra Biosystems, USA, limit of detection 20ng/ml) and COX-2 (Assay Designs, Inc, USA, limit of detection 2,15 ng/ml). NO was measured as the concentration of its stable end product, nitrite (NO₂⁻), by the Griess reaction.

Histological analysis

The specimens were fixed in formalin 10% and then stained with hematoxylin and eosin to evaluate the presence of inflammatory cells.

Statistical analysis

The statistical analysis was done with SPSS® (Statistical Package for the Social Science) Software. Comparison and correlation between YKL-40 and COX-2 and NO were performed respectively by Student’s t test and the Pearson’s coefficient. A p value less than 0.05 was considered statistically significant.

Results

YKL-40 was detectable in all the samples analyzed (Table I); its mean concentration was 1.54 ± 1.29 ng/ml/mg compared to dry weight. Histological analysis demonstrates the presence of chondrocytes and very few inflammatory cells (Fig. 1). COX-2 and NO levels were, respectively, 25.25 ± 11.42 pg/ml/mg and 1.3 ± 1.8 μM/mg x 10⁻² compared to dry weight. Correlations were found between YKL-40 and COX-2 (r = 0.579, p < 0.05) (Fig. 2) and YKL-40 and NO

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<th>Patients</th>
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<th>COX-2 pg/ml/mg</th>
<th>NO μM/mg x 10⁻²</th>
<th>Relative amount of inflammatory cells*</th>
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Mean ± SD 45 ± 15

1.54 ± 1.29 25.25 ± 11.42 1.3 ± 1.8

*++ very commonly found; + commonly found; ± rarely found; - not found.
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(r = 0.509, p < 0.05) (Fig. 3). No correlations were observed between COX-2 and NO and between herniated IVD features (size, level and type) and the concentrations of YKL-40, COX-2 and NO.

Discussion

The complex mechanism responsible for the degeneration of the IVD is still incompletely clarified. However, an increasing body of the evidences pointed out that mechanical stress may stimulate, probably in predisposed individuals, inflammatory reactions which in turn may induce breakdown of the extracellular matrix (3). Biochemical substances produced during these processes, in particular proinflammatory cytokines, PGs and MMPs, are involved in the induction of most features associated with IVD degeneration (pain, loss of function, etc.). Although the links between these events and the progression of disc lesions are poorly established, it is possible that a role could be played by some substances produced by cells activated during inflammation and involved in the matrix metabolism, such as YKL-40 (12). In this study, detectable levels of YKL-40, COX-2 and NO have been found in lumbar disc tissue cultures of all 19 patients examined. To our knowledge, this is the first report demonstrating the presence of YKL-40 in this tissue. Although YKL-40 functions are not yet completely clarified, it has been suggested that it may play a role in remodeling or degrading the extracellular matrix and in modulating local inflammatory process as matrix-degrading enzyme, which is probably in keeping with its production from cells located in tissues with increased remodeling/degradation or inflammation of the extracellular matrix (12, 13). Since YKL-40 has a similar function as a matrix-degrading enzyme, the effects of these two substances may be synergised, possibly amplifying the process. Moreover YKL-40 correlates with NO. NO is an intra- and intercellular messenger which plays many roles in immunologic regulation and inflammation. It has been proposed as a possible element in the pathogenesis of LBP secondary to a lumbar disc herniation (10). Moreover, COX-2 could indirectly affect chondrocytes metabolism as prostaglandins inhibit aggrecan synthesis (15). Since YKL-40 has a similar function as a matrix-degrading enzyme, the effects of these two substances may be synergised, possibly amplifying the process.

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In our study, very few inflammatory cells were found, thus suggesting the main role of chondrocytes in the production of YKL-40, COX-2 and NO. Obviously, our observations need to be supported by specific studies on the expression of these substances by disc chondrocytes in culture, which represents our next aim. In conclusion, our results are consistent with the hypothesis that, like in human articular cartilage, YKL-40 plays a relevant role in IVD degeneration and herniation that are related to a local inflammation supported by COX-2 and NO presence.
References