Clinical and Experimental Rheumatology 2007; 25: 453-456.

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

A. Pozzuoli[1], C. Valvason[2], D. Bernardi[3], M. Plebani[3], D. Fabris Monterumici[4], S. Candiotto[5], R. Aldegheri[1], L. Punzi[2]

[1]Department of Medical and Surgical Specialties, Orthopaedic Clinic, and [2]Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Padua; [3]Department of Laboratory Medicine, University-Hospital of Padua; [4]Spine Surgery Unit, Padua University General Hospital; [5]Orthopaedic and Traumatologic Surgery Unit, Hospital of Dolo (VE), Italy.

Assunta Pozzuoli, PhD; Chiara Valvason, PhD, Fellow in Rheumatology; Daniela Bernardi, MD; Mario Plebani, MD, FRCPath, Professor of Clinical Biochemistry, Director Dept. of Laboratory Medicine; Daniele Fabris Monterumici, MD; Sergio Candiotto, MD; Roberto Aldegheri, MD, Professor of Orthopaedics and Traumatology; Leonardo Punzi, MD, PhD, Professor in Rheumatology, Director of Rheumatology Unit.

Please address correspondence to: Assunta Pozzuoli, Department of Medical and Surgical Specialties, Orthopaedic Clinic, Via Giustiniani 3, 35128 Padova, Italy.
E-mail: assunta.pozzuoli@unipd.it

Received on August 5, 2006; accepted in revised form on January 18, 2007.

Key words: YKL-40, COX-2, NO, lumbar disc herniation, low back pain.

Competing interests: none declared.

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 analgesics 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

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% CO_2 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 (NO_2^-), 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.

Table I. Baseline demographic of patients and biochemical and histological findings of discs examined.

Patients	Age	Sex	Level	YKL-40 ng/ml/mg	COX-2 pg/ml/mg	NO μM/mg x 10^{-2}	Relative amount of inflammatory cells*
1	54	M	L4-L5	2.34	31.6	3	-
2	36	F	L4-L5	0.07	12.5	0.51	-
3	39	M	L5-S1	3.44	18.8	6	-
4	30	M	L5-S1	1.57	47.9	8.5	±
5	45	M	L4-L5	0.83	16.41	5.8	-
6	19	M	L5-S1	0.30	21.45	2	-
7	47	M	L4-L5	4.60	45.87	69	-
8	50	F	L4-L5	3.32	37.23	6	±
9	36	M	L5-S1	2.00	29.30	3	-
10	63	F	L4-L5	0.67	11.00	14	++
11	36	M	L4-L5	0.31	29.30	1	-
12	63	M	L4-L5	0.64	27.1	45	-
13	42	F	L5-S1	2.86	28.6	27	±
14	46	M	L5-S1	1.48	33.57	6	-
15	29	M	L5-S1	0.87	29.33	3	-
16	30	M	L5-S1	0.25	10.80	1.1	+
17	51	M	L4-L5	1.51	11.33	5.25	-
18	71	M	L4-L5	1.87	26.26	30	-
19	75	F	L4-L5	0.28	11.50	4.1	-
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.

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

Fig 1. Hematoxylin and eosin staining of chondrocytes' clusters (100x).

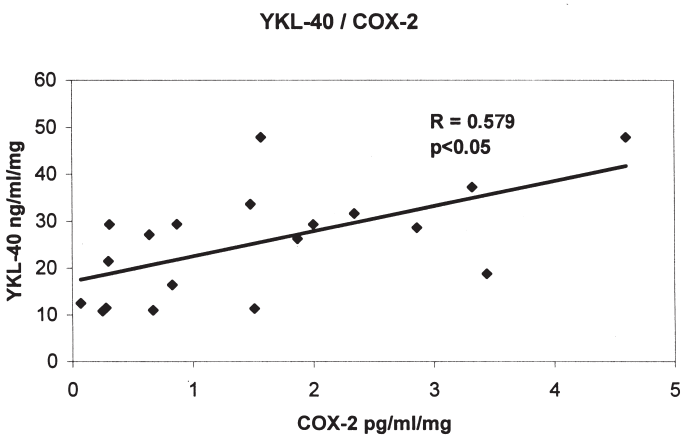

Fig 2. Correlation between YKL-40 and COX-2.

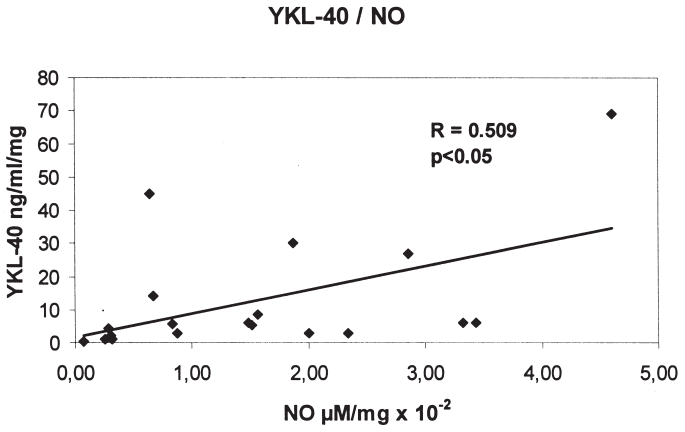

Fig 3. Correlation between YKL-40 and NO.

(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 in joints YKL-40 is secreted by synoviocytes and, mainly, by chondrocytes, especially when activated, its levels may reflect the degree of articular cartilage degradation and/or of synovial inflammation (13).

IVD degeneration is mainly characterized by tissue degradation, altered matrix composition, neoangiogenesis and local inflammation (2, 3). Although it is still unclear which is the actual triggering stimulus, it seems accepted that all these events act synergically to amplify the process. YKL-40 has been shown to act as a growth factor for connective tissue, and in particular for fibroblasts and chondrocytes (12, 13, 15). Since all these events have been found in IVD degeneration, YKL-40 seems an ideal candidate for having a crucial role in its pathogenesis.

The correlation between YKL-40 and COX-2, suggesting the possibility of reciprocal influences, may be interesting. COX-2 is an enzyme involved in PGE2 synthesis and therefore is related to pathogenesis of 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.

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 (11). NO is also implicated in the induction of apoptosis in chondroid tissues. YKL-40 is an enzyme involved in the matrix turnover and could have a synergist effect with NO that acts as a suppressor of proteoglycan synthesis in the intervertebral disc (13).

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

1. ANDERSSON GB: Epidemiological features of chronic low-back pain. *Lancet* 1999; 354: 581-5.
2. HURRI H, KARPPINEN J: Discogenic pain. *Pain* 2004; 112:225-8.
3. FREEMONT AJ, WATKINS A, LE MAITRE C, JEZIORSKA M, HOYLAND JA: Current understanding of cellular and molecular events in intervertebral disc degeneration: implications for therapy. *J Pathol* 2002; 196: 374-9.
4. THELANDER U, FAGERLUND M, FRIBERG S, LARSSON S: Straight leg raising test *vs* radiological size, shape and position of lumbar disc herniations. *Spine* 1992; 17: 395-9.
5. SAAL JA, SAAL JS, HERZOG RJ: The natural history of lumbar intervertebral disc extrusions treated nonoperatively. *Spine* 1990; 15: 683-6.
6. BOGDUK N: A narrative review of intra-articular corticosteroid injections for low back pain. *Pain Med* 2005; 6: 287-96.
7. KANG JD, GEORGESCU HI, LARKIN L, STEFANOVIC-RACIC M, DONALDSON WF, EVANS CH: Herniated lumbar intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6, and PGE_2. *Spine* 1996; 21: 271-7.
8. TAKAHASHI H, SUGURO T, OKAZIMA Y, MOTEGI M, OKADA Y, KAKIUCHI T: Inflammatory cytokines in the herniated disc in the lumbar spine. *Spine* 1996; 21: 218-24.
9. MATSUI Y, MAEDA M, NAKAGAMI W, IWATA H: The involvement of matrix metalloproteinases and inflammation in lumbar disc herniation. *Spine* 1998; 23: 863-8.
10. MIYAMOTO H, SAURA R, DOITA M, KUROSAKA M, MIZUNO K: The role of cyclooxygenase-2 in lumbar disc herniation. *Spine* 2002; 27: 2477-83.
11. HASHIZUME H, KAWAKAMI M, NISHI H, TAMAKI T: Histochemical demonstration of nitric oxide in herniated lumbar discs. A clinical and animal model study. *Spine* 1997; 22: 1080-4.
12. VOLCK B, JOHANSEN JS, STOLTENBERG M *et al*.: Studies on YKL-40 in knee joints of patients with rheumatoid arthritis and osteoarthritis. Involvement of YKL-40 in the joint pathology. *Osteoarthritis Cartilage* 2001; 9: 203-14.
13. JOHANSEN JS, OLEE T, PRICE PA, HASHIMOTO S, OCHS RL, LOTZ M: Regulation of YKL-40 production by human articular chondrocytes. *Arthritis Rheum* 2001; 44: 826-37.
14. PUNZI L, PODSWIADEK M, D'INCÀ R, ZANINOTTO M, BERNARDI D, PLEBANI M, STURNIOLO GC: Serum human cartilage glycoprotein-39 as a marker of arthritis associated with inflammatory bowel diseases. *Ann Rheum Dis* 2003; 62: 1224-6.
15. DE CEUNINCK F, GAUFILLIER S, BONNAUD A, SABATINI M, LESUR C, PASTOUREAU P: YKL-40 (cartilage gp-39) induces proliferative events in cultured chondrocytes and synoviocytes and increases glycosaminoglycan synthesis in chondrocytes. *Biochem Biophys Res Commun* 2001; 285: 926-31.