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THE ROLE OF CYCLOOXYGENASE-2 AND INFLAMMATORY CYTOKINES IN PAIN INDUCTION OF HERNIATED LUMBAR INTERVERTEBRAL DISC

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INDEXING WORDS

lumbar disc herniation; radiculopathy; cyclooxygenase-2; inflammatory cytokine; prostaglandinE2

SYNOPSIS

Lumbar disc herniation (LDH) is the disease which is the major cause of radiculopathy. In terms of the pathogenesis of disease, it is reported that prostaglandinE2 (PGE2) plays an important role to induce radiculopathy. Arachidonate cascade, which is the process of PGE2 synthesis, is mainly regulated by two kinds of enzymes, phospholipaseA2 (PLA2) and cyclooxygenase (COX). Previously, PLA2 was recognized as the rate-limiting enzyme of this cascade, and some authors reported the clinical significance of PLA2 at the site of LDH concerning the radicular pain. Recently, COX was elucidated to consist of 2

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types of isoform, a constitutive form of COX-1 and an inducible form of COX-2. COX-2 has been focused as a key enzyme to regulate PGE2 synthesis and plays an important role in inflammation, because COX-2 was induced in many types of cells by the stimulation of inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factorα (TNFα). However, it is not fully discussed whether or not, COX-2 is induced in lumbar disc tissue and if it plays a significant role in the pathogenesis of LDH.

To clarify the role of COX-2 in the pathomechanism of radiculopathy of LDH, we have investigated the expression of COX-2, IL-1β and TNFα in herniated lumbar disc tissue. Immunohistologically, they were detected in the cytosol of chondrocytes constituting the disc tissue. RT-PCR showed that herniated lumbar disc-derived cells expressed mRNA of COX-2, IL-1β and TNFα in the presence of inflammatory cytokines in vitro. The disc-derived cells also produced much PGE2 by stimulating of inflammatory cytokines at the same time and this PGE2 production was distinctly suppressed by a selective inhibitor of COX-2, 6-methoxy-2-naphtyl acetic acids (6MNA).

These results suggest that COX-2 and inflammatory cytokines might play a causative role in the radiculopathy of LDH through upregulating PGE2 synthesis.

INTRODUCTION

Radiculopathy is the major complaint of the patients with lumbar disc herniation (LDH). Persistent pains deteriorate the activities of daily living (ADLs) of the patients. To clarify the pathomechanism of radiculopathy in LDH it could contribute to relief this pain and improve limited ADLs of the patients. In terms of the pathogenesis of LDH, various kinds of mechanism, including direct compression of the nerve root,[7,26] lower pH,[6,19] immune[2,3] and inflammatory reaction[9-12,16,18,20-25,27,28,30] have been discussed as an etiology of this pain.

Especially, prostaglandinE2 (PGE2), one of the inflammatory mediators, has been noticed as an important factor to cause this radicular pain and enhance the sensitivity to pain-inducing substances at the site of LDH.[9,10,18,21,28,30]

Two kinds of enzyme, phospholipaseA2 (PLA2) and cyclooxygenase (COX) regulate an arachidonate cascade, which is the process of PGE2 synthesis. PLA2 was previously recognized as the rate-limiting enzyme of this cascade and some references emphasized the importance of the role of PLA2 in the pathogenesis of LDH.[11,12,27] Recently, COX-2, an inducible form of COX, has been shown to be upregulated by the stimulation of the inflammatory cytokines including interleukin-1β (IL-1β) and tumor necrosis factorα (TNFα) in many types of cells such as synoviocytes[4] or articular chondrocytes.[8] Thus, COX-2 is focused as an important enzyme to regulate PGE2 synthesis in inflammation. However, its role in LDH was not fully investigated at present.

Therefore, we have examined the localization of COX-2 and inflammatory cytokines immunohistochemically. Also it was investigated whether COX-2 and inflammatory cytokines were induced in lumbar disc-derived cells and whether COX-2 played a significant role in PGE2 synthesis using cellular and molecular biological methods in order to clarify the significance of COX-2 in the pathogenesis of LDH.

MATERIALS AND METHODS

Thirteen specimens of herniated lumbar disc were obtained from the patients who underwent the posterior surgery for LDH combined with radiculopathy. 10 were male and 3 female, with a mean age of 41years (18-59 years). According to the macroscopic findings during surgery, these specimens were classified as 10 extrusion types and 3 sequestration types. As a normal control, disc tissues were obtained from 3 patients who underwent the anterior surgery for traumatic burst fracture of lumbar vertebrae. All patients had no

history of administration or injection of steroid hormones or non-steroidal anti-inflammatory drugs within less than two weeks prior to their surgeries.

(1) Immunohistological study of the herniated lumbar disc specimens

Air-dried cryostat thin sections of herniated disc specimen were fixed in cold acetone and endogenous peroxidase activity was quenched using a 3 % hydrogen peroxide in phosphate-buffered saline. Cellular antigens were determined using specific antibodies as follows. Monoclonal antibodies were utilized for human COX-2 (Santa Cruz, AL, USA) and IL-1β antibody (Genzyme, MA, USA). To detect the localization of TNFα, polyclonal antibody (Genzyme, MA, USA) was used. Cellular antigen was indicated as a brown colored spot developed by incubation with avidin-biotin-peroxidase conjugate.

(2) Cell culture

Besides the histological examination, remaining tissues were digested with 0.05 % collagenase for 2 hours. Obtained cells were then cultured in a monolayer fashion in Dulbecco's Modified Eagle Medium (DMEM; IWAKI, Chiba, Japan) supplemented with 10 % heat-inactivated fetal bovine serum (FBS; SIGMA chemical Co., MO, USA) and antibiotics. Reaching the confluence in the culture flask ($75cm^2$), disc-derived cells were seeded into 6-multiwell plates (5.0×10^5 cells/well) and cultured in 10% FBS-DMEM. Two days after, attached monolayer culture was subjected to following experiments.

(3) Detection of mRNA of COX-2, IL-1β and TNFα

Isolation of total cellular RNA

Cells obtained from 8 different patients were treated with either 100

U/ml of IL-1β or TNFα for 6 hours to examine the mRNA expression of COX-2 and inflammatory cytokines. Total cellular RNA was isolated from the cell layer using a suggested protocol as below: Cells were lysed directly in the culture dish by adding 1 ml of RNAzol^{TM} B (TEL-TEST, INC., TX, USA). After adding 0.2 ml of chloroform to cell lysate, samples were mixed vigorously and placed on ice for 5 minutes. After 12000-g centrifugation at 4 °C for 15 minutes, the upper aqueous phase of each sample was transferred and mixed with an equal volume of iso-propanol (Wako pure chemical industries Ltd., Osaka, Japan). After each sample was stored at 4 °C for 15 minutes, RNA precipitate was obtained by centrifugation for 15 minutes at 12,000 g.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

One μg of RNA sample was incubated at 70 °C for 5 minutes in combination with 1 μl of Oligo d(T)16 primer (50 μM) and 14 μl of RNase free distilled water. This sample was then mixed with 2 μl of 10x PCR buffer, 2 μl of dNTP mix (2 mM each), 4 μl of $MgCl_2$ (25 mM), 1 μl of RNase Inhibitor (20 U/ml) and MulV Reverse Transcriptase (50 U/ml). All reagents for RT-PCR except for the specific primers were purchased from Perkin Elmer in USA. Reverse transcription was performed at 37 °C for 120 minutes. Then, cDNA was amplified by 3-step PCR using the specific primers described below.

COX-2: Sense: TTCAAATGAGATTGTGGGAAAATTGCT,
Anti-Sense: AGATCATCTCTGCCTGAGTATCTT,

IL-1β: Sense: AAACAGATGAAGTGCTCCTTCCAGG,
Anti-Sense: TGGAGAACACCACTTGTTGCTCCA,

TNFα: Sense: CAGAGGGAAGAGTTCCCCAG
Anti-Sense: CCTTGGTCTGGTAGGAGACG

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH):
Sense: GTGAAGGTCGGAGTCAACG,

Anti-Sense: GAGATGATGACCCTTTTGGC

The reaction cycle of 3-step PCR was denaturing at 95 °C, annealing at 55 °C and extending at 72 °C for 1 minute respectively. Thirty cycles were applied to detect COX-2 and GAPDH mRNA and 35 cycles were IL-1β and TNFα mRNA. PCR amplicon was electrophoresed using 1.2-% agarose gels and the magnitude of mRNA expression of COX-2, IL-1β or TNFα was compared semiquantitively with that of GAPDH mRNA as an internal control.

(4) Analysis of the culture supernatants for PGE2 synthesis

In order to examine whether PGE2 synthesis in lumbar disc tissue is due to COX-2 or not, the monolayer culture obtained from latter 5 herniated lumbar disc specimens (extrusion type; 3 cases, sequestration type; 2 cases) was cultured for 6 hours with 100U/ml of IL-1β or TNFα in adding with or without 50μg/ml of 6MNA, which is an active metabolite of Nabumeton, selective inhibitor of COX-2 (Funakoshi, Osaka, Japan).[5,14,15,17] The concentration of PGE2 in culture supernatants was measured by radioimmunoassay. At the same time, expressions of mRNA of COX-2 and GAPDH were detected by RT-PCR. The procedures of the cell culture, the isolation of total cellular RNA, and RT-PCR were same as described above.

RESULTS

(1) Immunohistological study

Immunohistologically, COX-2 was detected in the cytosol of the chondrocytes constituting the lumbar disc. Inflammatory cytokines, IL-1β and TNFα were also detected in the cytosol of chondrocytes (Fig.1 and 2-a, b). On the

other hand, localization of neither COX-2 nor the inflammatory cytokines was observed in the control specimens (data not shown).

(2) Expressions of mRNA of COX-2, IL-1β and TNFα of the herniated lumbar disc-derived cells

The results were obtained from all 8 specimens and a representative result is shown in Fig. 3. The lumbar disc-derived cells expressed COX-2 mRNA by stimulating of IL-1β and TNFα compared with the unstimulated cells while mRNA of GAPDH was constitutively expressed regardless of the inflammatory stimulation. Expression of the inflammatory cytokine mRNA was also augmented by stimulating of the inflammatory cytokines themselves. The magnitude of mRNA expression of both COX-2 and the inflammatory cytokines was stronger when cells were stimulated with IL-1β compared with the stimulation of TNFα.

(3) Analysis of the culture supernatants for PGE2 synthesis

When the monolayer culture was stimulated with the inflammatory cytokines, it released a significant amount of PGE_2 in supernatant respectively. A remarkably higher concentration of PGE_2 was detected when cells were stimulated with IL-1β. This augmentation of PGE_2 synthesis was accompanied with mRNA induction of COX-2 by the inflammatory stimulation and inhibited by adding 6MNA (Figure 4), which might indicate that PGE_2 synthesis of this monolayer culture was mainly mediated by COX-2.

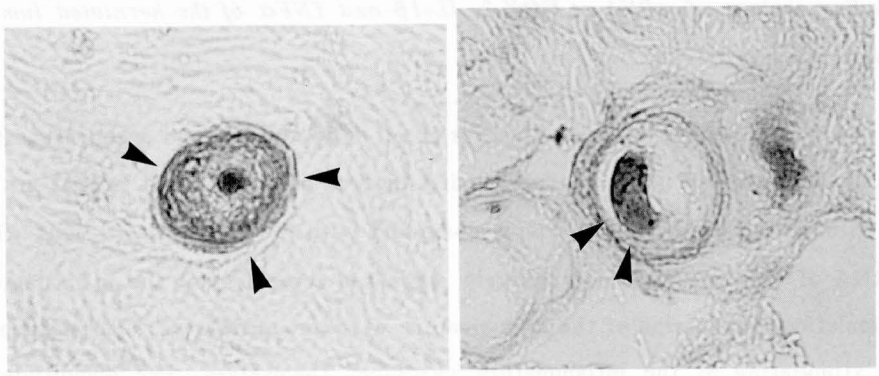
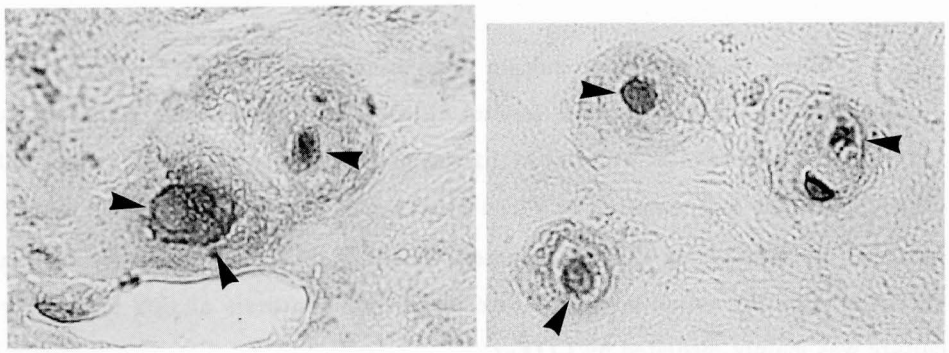


Figure 1. COX-2 in the cytosol of chondrocyte constituting the lumbar disc (original magnification x200).



(a) (b)

Figure 2. The localization of IL-1β (a) and TNFα (b) in the cytosol of chondrocytes in lumbar disc (original magnification x200).

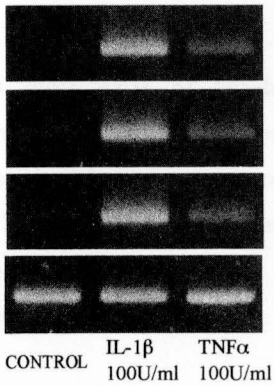


Figure 3. Herniated lumbar disc-derived cells expressed mRNA of COX-2, IL-1β and TNFα by stimulation of IL-1β or TNFα, while little expression was shown in the unstimulated cells.

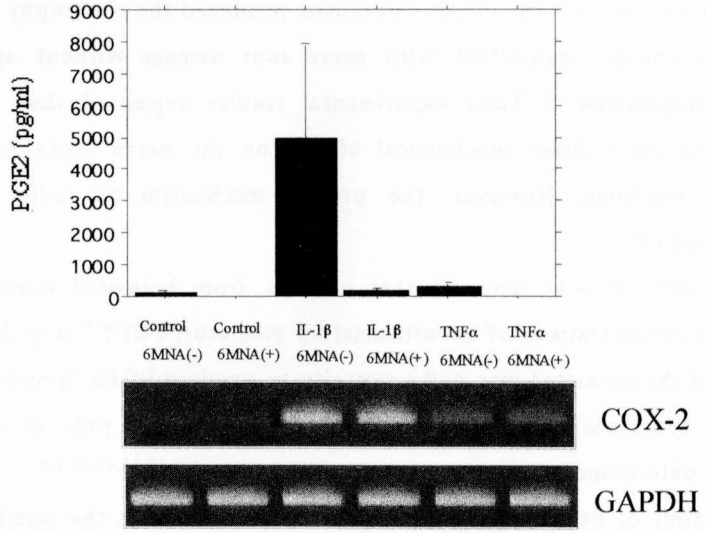


Figure 4. The inflammatory cytokine-mediated PGE2 synthesis by disc-derived cells. They produced remarkably high concentrations of PGE2 while little expression in the unstimulated in the cytokine-stimulated cells. This PGE2 synthesis was inhibited by 50 μg/ml of 6MNA and 6MNA did not affect any expression of COX-2mRNA.

DISCUSSION

In general, radicular pain has been considered to arise from the inflammatory condition around the herniated disc tissues. Traditionally, the direct mechanical compression of the nerve root by disc tissue was reported to induce this pain in LDH.[7,26] However, this mechanical compression seems not to explain the cause of radiculopathy completely, because it was elucidated that there was little correlation between the size of a herniated disc and the magnitude of the patient's complaint concerning the pain in LDH.[11,12,22]

Mccarron reported that autogenous nucleus pulposus transplanted into the lumbar epidural space in dog models induced an inflammatory response.[16] Olmarker also showed that the nucleus pulposus produced the electrophysiologic and histologic changes consistent with nerve root damage without apparent mechanical compression.[22] Their experimental results suggested that nucleus pulposus might have direct biochemical effects on the nerve roots including inflammatory reactions. However, the precise mechanism of radiculopathy remains still unclear.

Recently, it was reported that extracts from herniated lumbar disc contained high concentrations of an inflammatory mediator, PGE2[21] and that both the normal and the herniated disc had a capacity to produce PGE2 in response to inflammatory cytokines.[10] PGE2 has been noticed to cause pain or enhance sensitivity to pain-inducing substances at the site of LDH.[9,10,18,21,28,30]

In terms of PGE2 synthesis, PLA2 was recognized as the rate-limiting enzyme of arachidonate cascade. Saal showed that a high activity level of PLA2 was detected in the herniated lumber disc specimens.[27] Kawakami also showed that, using a rat model, the allograft of the disc materials transplanted in the lumbar epidural space resulted in increasing the sensitivity to painful stimuli with expressing PLA2 histochemically[11] and that the injection of PLA2 selective inhibitor improved the threshold of pain to a normal level.[12]

THE ROLE OF COX-2 AND INFLAMMATORY CYTOKINES

Another PGE2 synthase, COX-2, was identified[13,31] and noticed as a more important enzyme to regulate arachidonate cascade in the inflammatory process. COX-2 has been reported to be induced by stimulation of inflammatory cytokines in many types of cells such as synoviocytes[4] and articular chondrocytes[8] in contrast with COX-1, which is constitutively expressed to regulate cellular metabolism physiologically through PGE2 synthesis. In articular chondrocyte, Geng reported that PGE2 synthesis enhanced by IL-1β and TNFα was mediated through upregulation of COX-2.[8] Amin also reported that superinduction of PGE2 production coincides with the upregulation of COX-2 in human osteoarthritis-affected cartilage.[1] However, the role of COX-2 in the pathogenesis of LDH has never been discussed in the literature.

This is the first report concerning the localization of COX-2 in disc tissue and the induction of COX-2 mRNA by stimulating of the inflammatory cytokines. A high concentration of PGE2 was also detected in the cytokine-stimulated culture supernatant, whereas 6MNA,[5,14,15,17] the selective inhibitor of COX-2, markedly inhibited this PGE2 synthesis. 6MNA did not affect the expression of COX-2 mRNA, which suggested that 6MNA might suppress the COX-2 enzyme activity but not the gene expression of COX-2. These results suggest that COX-2 may play an important role in causing radiculopathy of a lumbar disc herniation through upregulating PGE2 synthesis.

In the present study, we also referred to the role of the inflammatory cytokines in pathogenesis of the LDH. As previously reported, it was demonstrated that the expression of the inflammatory cytokines such as IL-α, IL-1β, IL-6 and TNFα were detected in the herniated lumbar disc specimens immunohistologically.[28] And it was also shown that high concentrations of these cytokines were observed in the culture supernatant of the herniated disc specimens.[9,10] Moreover, it was reported that nucleus pulposus-derived cells had the capacity to secrete IL-1β and IL-6 in cell culture.[25] We detected the expressions of IL-1β and TNFα in the specimen immunohistologically and showed

that the expression of the inflammatory cytokine mRNA was induced by stimulating of the inflammatory cytokines themselves autocrinely and paracrinely. In rheumatoid arthritis, it was reported that the synovium-derived cells stimulated by IL-1β produced the same IL-1β autocrinely.[29] This might be an important point to explain the propagation of inflammatory reaction in the pathogenesis of rheumatoid arthritis. We would suggest that autocrine and paracrine regulation of the inflammatory cytokines synthesis might play an important role not only to induce pain but also to raise an inflammatory "vicious circle" at the site of LDH.

Based on the results of the present study, we hypothesize the mechanism of inducing radiculopathy in LDH (Fig.5). When the herniated lumbar disc is exposed into the epidural space, it may induce the inflammatory condition in conjunction with adjacent tissue including macrophages. Then, these cells released the inflammatory cytokines, which might lead to cytokine release by

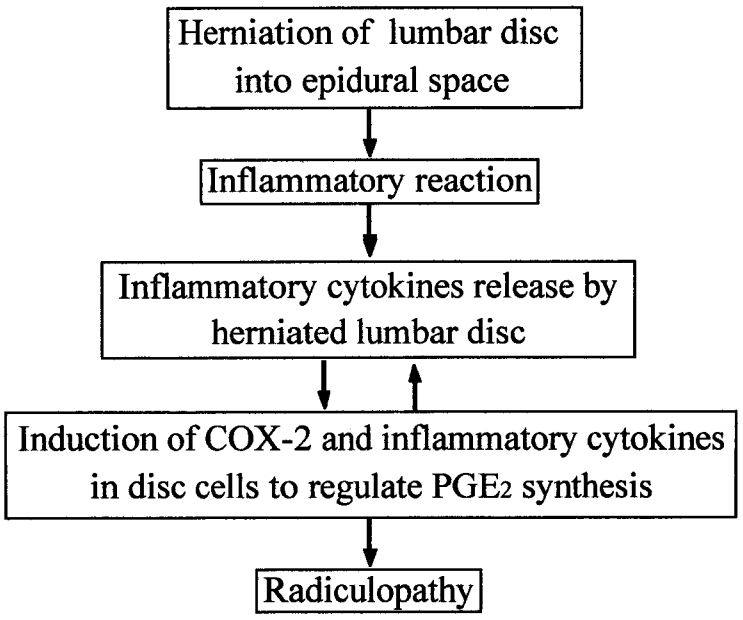


Figure 5. The mechanism of radiculopathy in lumbar disc herniation (hypothesis).

herniated lumbar disc tissue autocrinely or paracrinely. Finally, COX-2 was induced in disc cells and the increase of PGE_2 synthesis by COX-2 might cause radiculopathy in LDH.

In conclusion, the induction of COX-2 and the release of the inflammatory cytokines may play an important role in causing radiculopathy of lumbar disc herniation through upregulating PGE_2 synthesis.

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REFERENCES

1. Amin, A.R., Attur, M., Patel, R.N., Thakker, G.D., Marshall, P.J., Rediske, J., Stuchin, S.A., Patel, I.R., and Abramson, S.B.: J. Clin. Invest. 1997. 99. 1231/1237. Superinduction of cyclooxygenase-2 activity in human osteoarthritis-affected cartilage.
2. Bisla, R.S., Marchisello, P.J., Locksin, M.D., Hart, D.M., Marcus, R.E., and Granda, J.: Clin. Orthop. 1976. 123. 149/154. Auto-immunological basis of disk degeneration.
3. Bobechko, W.P. and Hirsch, C. J.: Bone Joint Surg. 47B. 1965. 574/580. Auto-immune response to nucleus pulposus in the rabbit.
4. Crofford, L.J., Wilder, R.L., Ristimaki, A.P., Sano, H., Remmers, E.F., Epps, H.R., and Hla, T.J.: Clin. Invest. 1994. 93. 1095/1101. Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues. Effects of interleukin-1 beta,

phorbol ester, and corticosteroids.

5. DeWitt, D.L., Maede, E.A., and Smith, W.L.: Am. J. Med. 1993. 9. 40S/44S. PGH synthase isoenzyme selectivity: the potential for safer nonsteroidal antiinflammatory drugs.
6. Diamant, B., Karlsson, J., and Nachemson, A.: Experientia. 1968. 15. 1195/1196. Correlation between lactate levels and pH in discs of patients with lumbar rhizopathies.
7. Garfin, S.R., Rydevik, B., Lind, B., and Massie, J.: Spine. 1995. 20. 1810/1820. Spinal nerve root compression.
8. Geng, Y., Blanco, F.J., Cornelisson, M., and Lotz, M.: J. Immunol. 1995. 155. 796/801. Regulation of cyclooxygenase-2 expression in normal human articular chondrocytes.
9. Kang, J.D., Georgescu, H.I., McIntyre-Larkin, L., Stefanovic-Racic, M., Donaldson, W.F., and Evans, C.H.: Spine. 1996. 21. 271/277. Herniated lumbar intervertebral discs spontaneously produce matrix metalloprotainases, nitric oxide, interleukin-6, and prostaglandin E_2.
10. Kang, J.D., Stefanovic-Racic, M., McIntyre, L.A., Georgescu, H.I., and Evans, C.H.: Spine. 1997. 22. 1065/1073. Toward a biochemical understanding of human intervertebral disc degeneration and herniation.
11. Kawakami, M., Tamaki, T., Weinstein, J.N., Hashizume, H., Nishi, H., and Meller, S.T.: Spine. 1996. 21. 2101/2107. Pathomechanism of pain-related behavior produced by allografts of intervertebral disc in the rat.
12. Kawakami, M., Tamaki, T., Hashizume, H., Weinstein, J.N., and Meller, S.T.: Spine. 1997. 22. 1074/1079. The role of phospholipase A_2 and nitric oxide in pain-related behavior produced by an allograft of intervertebral disc material to the sciatic nerve of the rat.
13. Kujubu, D.A., Fletcher, B.S., Varnum, B.C., Lim, R.W., and Herschman, H.R.: J. Biol. Chem. 1991. 266. 12866/12872. TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue.

14. Laneuville, O., Breuer, D.K., Dewitt, D.L., Hla, T., Funk, C.D., and Smith, W.L.: J. Pharmacol. Exp. Ther. 1994. 271. 927/934. Differential inhibition of human prostaglandin endoperoxide H synthases-1 and -2 by nonsteroidal anti-inflammatory drugs.

15. Meade, E.A., Smith, W.L., and DeWitt, D.L.: J. Biol. Chem. 1993. 25. 6610/6614. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs.

16. Mccarron, R.F., Wimpee, M.W., Hudkins, P.G., and Laros, G.S.: Spine. 1987. 12. 760/764. The inflammatory effect of nucleus pulposus.

17. Melarange, R., Gentry, C., O'Connell, C., Blower, PR., Neil, C., Kelvin, AS., and Toseland, CD.: Agents Actions. 1992. Spec No. C82/3. Anti-inflammatory and gastrointestinal effects of nabumetone or its active metabolite, 6MNA (6-methoxy-2-naphthylacetic acid): comparison with indomethacin.

18. Muramoto, T., Atsuta, Y., Iwahara, T., Sato, M., and Takemitsu, Y.: Int Orthop. 1997. 21. 172/175. The action of prostaglandin E2 and triamcinolone acetonide on the firing activity of lumbar nerve roots.

19. Nachemson, A.: Acta Orthop. Scand. 1969. 40. 23/42. Intradiscal measurements of pH in patients with lumbar rhizopathies.

20. Nygaard, Ø.P., Mellgren, S.I., and Østerud, B.: Spine. 1997. 22. 2484/2488. The inflammatory properties of contained and noncontained lumbar disc herniation.

21. O'Donnell, J.L. and O'Donnell, A.L.: Spine. 1996. 21. 1653/1656. Prostaglandin E2 content in herniated lumbar disc disease.

22. Olmarker, K., Rydevik, B., and Nordborg, C.: Spine. 1993. 18. 1425/1432. Autologous nucleus induces neurophysiologic and histologic canges in porcine cauda equina nerve roots.

23. Olmarker, K., Blomquist, J., Stromberg, J., Nannmark, U., Thomsen, P., and Rydevik, B.: Spine. 1995. 20. 65/69. Inflammatogenic properties of nucleus pulposus.

24. Olmarker, K. and Larsson, K.: Spine. 1998. 23. 2538/2544. Tumor necrosis factor alpha and nucleus-pulposus-induced nerve root injury.

25. Rand, N., Reichert, F., Floman, Y., and Rotshenker, S.: Spine. 1997. 22. 2598/2602. Murine nucleus pulposus-derived cells secrete interleukin-1-β, -6, and -10 and granulocyte-macrophage colony-stimulating factor in cell culture.

26. Rydevik, B., Brown, M.D., and Lundborg, G.: Spine. 1984. 9. 7/15. Pathoanatomy and pathophysiology of nerve root compression.

27. Saal, J.S., Franson, R.C., Dobrow, R., Saal, J.A., White, A.H., and Goldthwaite, N.: Spine. 1990. 15. 674/678. High levels of inflammatory phospholipase A2 activity in lumbar disc herniations.

28. Takahashi, H., Suguro, T., Okazima, Y., Motegi, M., Okada, Y., and Kakiuchi, T.: Spine. 1996. 21. 218/224. Inflammatory cytokines in the herniated disc of the lumbar spine.

29. Temime, N., Joliviere, A., Lando, D., Teyton, L., and Charron, D.: Hum. Immunol. 1991. 31. 261/270. Autocrine stimulation of interleukin 1 in human adherent synovial lining cells: down regulation by interferon gamma.

30. Willburger, R.E. and Wittenberg, R.H.: Spine. 1994. 19. 2068/2070. Prostaglandin release from lumbar disc and facet joint tissue.

31. Xie, W., Chipman, J.G., Robertson, D.L., Erikson, R.L., and Simmons, D.L.: Proc. Natl. Acad. Sci. USA. 1991. 88. 2692/2696. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing.