Human group II phospholipase A₂ in normal and diseased intervertebral discs

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Abstract

We measured calcium-dependent phospholipase A₂ (PLA₂) activity and immunoreactive group II PLA₂ levels of 54 normal discs obtained from cadavers and 73 disc samples surgically obtained from patients with spinal disorders, including intervertebral disc herniations, spondylosis, and spondylolisthesis. Both cadaveric and surgical disc specimens contained about two-fold greater PLA₂ activity than the ileal mucosa, one of the richest sources of group II PLA₂. Discs of middle-aged cases had significantly higher activity than those of younger and elder cases. In cadaveric normal discs, calcium-dependent PLA₂ activity was significantly higher in females than in males. Annulus fibrosus and nucleus pulposus contained the same PLA₂ levels. In diseased discs, herniated fragments that had extruded or protruded out of the discs possessed lower activity than other parts of discs in the intervertebral space. Immunoreactive group II PLA₂ levels of intervertebral discs closely correlated with PLA₂ enzymatic activity. We purified a PLA₂ from human intervertebral disc to homogeneity to further identify the isozymic nature of discal PLA₂. Its NH₂-terminal amino acid sequence and molecular weight were identical to those of human group II PLA₂. Immunohistochemical analysis using a monoclonal anti-group II PLA₂ antibody showed that in both annulus fibrosus and nucleus pulposus chondrocytes contained intense group II PLA₂ immunoreactivity in their cytoplasm, and that the matrix contained no substantial immunoreactivity. These results suggest that group II PLA₂ in chondrocytes has important physiological roles in discal ordinary metabolism, maintaining discal homeostasis.

Keywords: Phospholipase A₂; Intervertebral Disc; Cartilage; Chondrocyte; Spine; (Human)

1. Introduction

Phospholipase A₂ (PLA₂) hydrolyzes the sn-2 fatty acyl ester bond of glycerophospholipids to produce free fatty acids and lysophospholipids. This enzyme plays important roles in remodeling membrane phospholipids, in regulating icosanoid biosynthesis, and in mediating inflammatory reactions [1,2].

Recent findings of high levels of PLA₂ activity in herniated nucleus pulposus suggested that PLA₂ within the nucleus pulposus can cause nerve injury and that activation of PLA₂ within the intervertebral disc contributes to the process of disc degeneration [3]. However, the precise roles played by PLA₂ in the pathogenesis of disc herniation remain to be clarified, because the properties and the origin of PLA₂ within the discs normal and diseased are unknown.

In this study we purified a PLA₂ from human intervertebral discs to homogeneity and confirmed that its N-terminal amino acid sequence was identical to human group II PLA₂. The location of group II PLA₂ in discs was immunohistochemically determined with specific monoclonal antibodies raised against human group II PLA₂. We measured PLA₂ activity and immunoreactive group II PLA₂ (IR-group II PLA₂) levels of uninflamed intervertebral disc samples obtained from normal fresh cadavers and
from patients with a variety of spinal disorders. We further compared \( \text{PLA}_2 \) levels of disc portions remaining in the original disc space with those of extrudedly and protrudedly herniated portions of discs.

2. Materials and methods

2.1. Disc materials

Human intervertebral discs \((n = 73)\) were obtained from 45 patients, 26 females and 19 males, aged 12-80 years (mean 41.4), who underwent discectomy from the anterior or posterior approach (Table 1). Erythrocyte sedimentation rates, serum C-reactive protein concentration, and leukocyte counts of the patients were normal. Severity of clinical symptoms was assessed using respective evaluation scores for cervical and lumbar lesions proposed by the Japanese Orthopaedic Association (JOA score). To assess disc degeneration, magnetic resonance imaging (MRI) findings of the patients were evaluated using the grading scale of Eyre et al. [4]. After surgical removal, disc samples were immediately frozen and stored at \(-80^\circ\text{C}\).

Since it is ethically impossible to obtain normal disc samples from patients and healthy subjects, intervertebral discs \((n = 54)\) were obtained from 10 fresh human cadavers, aged 19–73 years (mean 46.2), which had been dissected at the Osaka Medical Examiner’s Office. None of the deceased had an obvious history of spinal disorders and autopsy confirmed that they had not suffered from any inflammatory disorders. A portion of each disc was fixed in formalin and stained with hematoxylin-eosin and toluidine blue. All of the discs were histologically normal with some age-dependent changes [5]. The results of preliminary experiments showed that incubation of cadaveric disc homogenates did not affect \( \text{PLA}_2 \) activity for at least 8 h.

Hence, we used cadaveric discs as control specimens. Annuli and nuclei of C6/7, L1/2 and L4/5 interval discs were removed and stored at \(-80^\circ\text{C}\).

<table>
<thead>
<tr>
<th>Spinal disorders</th>
<th>Number of cases</th>
<th>Mean age (range)</th>
<th>Number of discs</th>
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<tr>
<td>Intervertebral disc herniation</td>
<td>28</td>
<td>39 (13–70)</td>
<td>37</td>
</tr>
<tr>
<td>Spondylolysis</td>
<td>5</td>
<td>53 (35–80)</td>
<td>12</td>
</tr>
<tr>
<td>Spondylolysis</td>
<td>4</td>
<td>51 (45–59)</td>
<td>5</td>
</tr>
<tr>
<td>Idiopathic scoliosis</td>
<td>2</td>
<td>12, 16</td>
<td>7</td>
</tr>
<tr>
<td>OPLL (^b)</td>
<td>2</td>
<td>44, 75</td>
<td>5</td>
</tr>
<tr>
<td>Traumatic fracture/dislocation</td>
<td>2</td>
<td>21, 23</td>
<td>3</td>
</tr>
<tr>
<td>Neurenteric cyst</td>
<td>1</td>
<td>48</td>
<td>3</td>
</tr>
<tr>
<td>Metastatic spinal tumor</td>
<td>1</td>
<td>59</td>
<td>1</td>
</tr>
<tr>
<td>Total number</td>
<td>45</td>
<td></td>
<td>73</td>
</tr>
</tbody>
</table>

\(^a\) In several cases, more than two disc samples were obtained from one patient.

\(^b\) Ossification of posterior longitudinal ligament of cervical spine.

2.2. Assay for \( \text{PLA}_2 \) activity

Each disc sample (40–80 mg) was thawed at room temperature and then homogenized in 40 vol. of 25 mM Hepes-HCl buffer (pH 7.4) on ice with a glass homogenizer. An equal volume of 2 M KBr was added to the homogenate. After 30-min incubation at 4°C under continuous stirring, the mixture was centrifuged at 15,000 \( \times g \) for 10 min at 4°C. An aliquot of the supernatant (2 \( \mu l \)) was used for the assay. \( \text{PLA}_2 \) activity was determined as reported previously [6,7]. Briefly, the assay mixture (50 \( \mu l \)) contained 0.8 mM 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG, Avanti Polar Lipids), 5 mM cholate, 0.1 M Tris-HCl (pH 8.5), 0.1 M NaCl, 5 mM CaCl\(_2\), and the enzyme. The reaction was stopped by adding 200 \( \mu l \) of the Dole’s reagent, and then fatty acids were extracted by the method of Dole and Meinertz [8]. Heptadecanoic acid (5 nmol) (Nacalai Tesque, Kyoto) was used as an internal standard. Extracted fatty acids were labeled with 9-anthryldiazomethane (Funakoshi Co., Tokyo), and then each of the derivatized fatty acids was quantitated by reverse-phase high-performance liquid chromatography (HPLC) with UV detection at 254 nm. Calcium-dependent \( \text{PLA}_2 \) activity was estimated as the difference between the activity assayed in the presence of 5 mM CaCl\(_2\) and that in the presence of 10 mM EDTA. Protein concentrations were determined with bicinchoninic acid protein assay reagent (Pierce).

2.3. Assay for immunoreactive group II \( \text{PLA}_2 \)

IR-group II \( \text{PLA}_2 \) in the KBr extracts from intervertebral discs was measured by a sensitive radioimmunoassay kit specific to human group II \( \text{PLA}_2 \), using a monoclonal antibody against human splenic group II \( \text{PLA}_2 \) (donated by the Diagnostic Science Department, Shionogi and Co., Settsu, Osaka) [9].

2.4. Purification of \( \text{PLA}_2 \) from human intervertebral discs

Human intervertebral disc (5.4 g wet wt.) was cut into thin slices and then homogenized in 70 ml of 10 mM Tris-HCl (pH 7.4) with a glass homogenizer. After 20 min of incubation on ice under continuous stirring, the homogenate was centrifuged at 20,800 \( \times g \) for 20 min at 4°C. The pellet was homogenized in 70 ml of 10 mM Tris-HCl (pH 7.4) containing 1 M KBr and kept on ice under continuous stirring for 30 min to extract \( \text{PLA}_2 \) activity. The homogenate was centrifuged at 20,800 \( \times g \) for 20 min at 4°C. The supernatant (KBr extract) was diluted 10-fold with 10 mM Tris-HCl (pH 7.4), and applied to a SP Sepharose column (4 \( \times 3 \) cm, Pharmacia Biotech) pre-equilibrated with the same buffer. The column was washed with 150 ml of 10 mM Tris-HCl (pH 7.4) containing 0.15 M NaCl, and then \( \text{PLA}_2 \) activity was eluted with 10 mM Tris-HCl (pH 7.4) containing 1 M NaCl and 0.1% Triton.
X-100. The eluent (SP Sepharose-1) was diluted 10-fold with 10 mM Tris-HCl (pH 7.4) and applied again to a SP Sepharose column (2 × 1.5 cm) pre-equilibrated with 10 mM Tris-HCl (pH 7.4) containing 0.1% Triton X-100. After the column was washed with the same buffer, PLA2 activity was eluted with 10 mM Tris-HCl (pH 7.4) containing 1 M NaCl and 0.1% Triton X-100. The eluent was further concentrated to about 1.8 ml with a Microcon 30 microconcentrator (Amicon). Aliquots (100–200 µl) of the concentrated PLA2 solution were repeatedly (15 times) injected to a TSK gel G2000SWxL (7.8 × 150 mm, Tosoh) pre-equilibrated with 10 mM Tris-HCl (pH 7.4) containing 0.3 M NaCl and 0.1% Triton X-100. Elution was performed with the same buffer at a flow rate of 0.5 ml/min. The absorbance at 295 nm was continuously monitored. The pooled PLA2-active fractions (Gel filtration) were concentrated using an SP Sepharose column (1 × 1 cm), as described above. The resultant solution (20 ml, SP Sepharose-2) was subjected to a Cosmosil 5C4-300 column (4.6 × 50 mm, Nacalai Tesque) pre-equilibrated with 0.1% trifluoroacetic acid (TFA) in water. The column temperature was maintained at 20°C. Elution was performed with the linear gradient of acetonitrile in 0.1% TFA: 0–25% in 5 min, 25–35% in 40 min, and then 35–100% in 5 min at the flow rate of 0.3 ml/min.

2.5. Polyacrylamide gel electrophoresis

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed on a 15% gel according to the method of Laemmli [10]. Proteins were stained with 0.25% Coomassie Brilliant Blue R-250 in 45% methanol-9% acetic acid. The marker proteins were purchased from Boehringer Mannheim.

2.6. NH2-terminal amino acid sequence and molecular weight determination

NH2-terminal amino acid sequence analysis was performed by an Applied Biosystem 473A and a 120A PTH analyzer. Matrix-assisted laser desorption mass spectra were obtained on a Shimadzu/Kratos laser ionization time-of-flight (TOF) mass spectrometer (KOMPACT MALDI III). α-Cyano-4-hydroxycinnamic acid was used as a matrix-forming material and myoglobin was used for mass calibration.

Table 2

<table>
<thead>
<tr>
<th>Step</th>
<th>Total vol. (ml)</th>
<th>Total protein (mg)</th>
<th>Total act. (µmol/min)</th>
<th>Specific act. (µmol/min per mg protein)</th>
<th>Yields (%)</th>
<th>Purification (×-fold)</th>
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<tr>
<td>KBr extract</td>
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<td>75.5</td>
<td>2.87</td>
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<td>1</td>
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<tr>
<td>SP Sepharose-1</td>
<td>50.0</td>
<td>6.0</td>
<td>72.5</td>
<td>12.1</td>
<td>96</td>
<td>4.2</td>
</tr>
<tr>
<td>Gel filtration</td>
<td>11.0</td>
<td>0.572</td>
<td>24.9</td>
<td>43.5</td>
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<td>15.2</td>
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<td>HPLC</td>
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<td>0.014</td>
<td>5.8</td>
<td>420</td>
<td>7.7</td>
<td>146</td>
</tr>
</tbody>
</table>

2.7. Immunohistochemistry

Formalin-fixed and paraffin-embedded sections of disc specimens that were obtained at autopsy and surgery were reacted with mouse monoclonal anti-group II PLA2 antibody. The primary immunoreaction was visualized using an ABC kit (Vector Laboratories, Burlingame, CA, USA) according to the manufacturer's instructions [11]. For control, the primary antiserum was omitted or was replaced by anti-group I (pancreatic) PLA2 antibody. Cartilage of the femoral condyle from a 59-yr-old female suffering from rheumatoid arthritis was also examined to compare stainability of articular cartilage with that of disc cartilage.

2.8. Statistical analysis

Data were expressed as mean ± standard deviation (S.D.). Data of PLA2 activity and IR-group II PLA2 levels were analyzed by Mann-Whitney test. Regression analysis was used to determine the relationships between PLA2 activity and IR-group II PLA2 levels, MRI grades or JOA scores. Results of P < 0.05 were considered significant.

3. Results

3.1. Characterization of PLA2 purified from human intervertebral discs

With minor modifications of the HPLC method [6,7,12], a PLA2 (14 mg) was purified to homogeneity from human intervertebral discs (5.4 g wet wt.) with the overall recovery of 7.7% and about 150-fold purification (Table 2). At each chromatographic step during purification, PLA2 activity was eluted as a single peak (data not shown). The specific activity of 420 µmol/min per mg was obtained with the mixed micelles of POPG and cholate as the substrate, and is similar to that of group II PLA2 purified from human ileal mucosa [12]. The purified enzyme migrated as a single band with an apparent molecular mass of 14.9 kDa on SDSPAGE and cholate as the

matrix-forming material and myoglobin was used for mass calibration.

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</table>
identical to that of human group II PLA$_2$ purified from spleen [13].

3.2. PLA$_2$ activity and immunoreactive group II PLA$_2$ levels in intervertebral discs

Table 1 summarizes 73 disc samples obtained from patients with spinal disorders. The disc samples ($n = 19$) obtained from patients with idiopathic scoliosis, OPLL, trauma, neurenteric cyst, and metastatic tumor were apparently normal.

All examined discs contained PLA$_2$ activity and IR-group II PLA$_2$ levels greater than those of other human group II PLA$_2$-rich organs, e.g., the ileum mucosa (51 nmol/min/mg protein) [14] (Fig. 1). Discal PLA$_2$ activity correlated with IR-group II PLA$_2$ levels ($r = 0.887, P < 0.0001$).

PLA$_2$ activity in surgical and cadaveric specimens were 87.3 ± 68.3 nmol/min/mg protein ($n = 73$) and 101 ± 71 nmol/min/mg protein ($n = 54$), respectively; and IR-group II PLA$_2$ levels in surgical and cadaveric specimens were 878 ± 908 ng/mg protein and 1005 ± 857 ng/mg protein, respectively. In both PLA$_2$ activity and IR-group II PLA$_2$ levels, there was no significant difference between discs obtained from patients and those from cadavers.

As shown in Fig. 2, discs of the middle-aged (30–59 years old, 110 ± 70 nmol/min per mg protein) had significantly higher activity than those of the second and third decades (12–29 years old, 60.3 ± 55.0 nmol/min per mg protein, $P < 0.0002$) and those of elders ($> 60$ years old, $70.3$ ± 49.2 nmol/min per mg protein, $P < 0.03$). Similarly, discal IR-group II PLA$_2$ levels in the middle-aged (1153 ± 981 ng/mg protein) were significantly greater than those of the second and third decades (570 ± 564 ng/mg protein, $P < 0.002$) and those of elders (513 ± 386 ng/mg protein, $P < 0.002$).

In cadaveric normal discs, PLA$_2$ activity ($P < 0.04$) and IR-group II PLA$_2$ levels ($P < 0.04$) were significantly higher in females (118 ± 78 nmol/min/mg protein, and 1255 ± 987 ng/mg protein, respectively) than in males (76.7 ± 52.4 nmol/min per mg protein, and 637 ± 429 ng/mg protein, respectively) (Fig. 3). Ages of female group (19–72) matched those of male groups (24–73). No
significant differences were found between PLA₂ activities in annulus fibrosus (109.8 ± 81.7 nmol/min per mg protein) and those in nucleus pulposus (92.3 ± 58.5 nmol/min per mg protein) (Fig. 4). The PLA₂ activity in intervertebral disc had no correlation with spinal levels.

3.3. PLA₂ activity and immunoreactive group II PLA₂ levels in diseased discs

In surgical specimens, the PLA₂ activity and IR-group II PLA₂ levels tended to be higher in females than in males, as recognized in cadaveric specimens, but there was no statistical significance. Both PLA₂ activity and IR-group II PLA₂ levels did not correlate with degenerative grades of MRI findings of discs or with the clinical severity as assessed by JOA scores (data not shown). Both extruded and protruded portions of herniated discs [15] had lower PLA₂ activity (P < 0.05) and IR-group II PLA₂ levels (P < 0.05) than the disc materials that remained at their original site. Ages of patients with disc herniations matched those of patients with other spinal disorders, and those of cadavers (Fig. 4).

Of all discs examined, thoracic disc materials surgically obtained from two patients with idiopathic scoliosis, aged 12 and 16, had significantly low PLA₂ activity and IR-group II PLA₂ levels (P < 0.01). On the contrary, cervical disc materials obtained from a 48-year-old female suffering from a neurenteric cyst in the spinal canal had significantly high activity and IR-group II PLA₂ levels (P < 0.05) (Fig. 1).

3.4. Immunohistochemistry

All of the chondrocytes examined in both annulus fibrosus and nucleus pulposus exhibit group II PLA₂ immunoreactivity in their cytoplasm (Fig. 5). Unlike articular chondrocytes (Fig. 6), the chondrocyte lacunae and the matrix of discs had no IR-group II PLA₂. Control sections using anti-group I (pancreatic) PLA₂ antibody were not stained at all.

The staining patterns of articular cartilage differ from those of disc cartilage. There was a large amount of IR-group II PLA₂ in the deep layer adjacent to subchondral bone, while there was its small amount in the superficial layer near the joint space. In agreement with previous results [16], the chondrocyte lacunae and surrounding cartilage matrix, as well as the cytoplasm of the chondrocytes, contained IR-group II PLA₂ (Fig. 6).

4. Discussion

In this study we found high PLA₂ activity and IR-group II PLA₂ levels in all intervertebral discs obtained from patients with disc herniations and other spinal disorders, and normal discs obtained from cadavers. Mean enzymatic activity and IR-group II PLA₂ levels per the protein concentration in the intervertebral discs were greater than that found in normal terminal ileal mucosa, one of the richest source of group II PLA₂. In comparison with activity in various rat tissues assayed by the same method, human intervertebral discs showed a level of activity as
high as in rat pancreas or spleen [7]. Mean IR-group II PLA₂ levels was 25 times higher than those of inflamed colonic mucosa of patients with Crohn's disease and ulcerative colitis [14]. It is surprising that normal intervertebral discs with sparse cellularity showed higher IR-group II PLA₂ levels than inflamed mucosa having much higher cellularity. Since the discs contain abundant extracellular proteins and have greater protein contents than colonic and ileal mucosas, the amount of group II PLA₂ per a cell in the discs is much greater than those of intestinal mucosal cells.

We purified a PLA₂ with a molecular mass of 13888 Da from human intervertebral discs, and the sequence of its NH₂-terminal 25 amino acid residues was identical to that of human group II PLA₂ purified from spleen [13]. At all the chromatographic steps during purification, PLA₂ activity was eluted as a single peak. The discal PLA₂ activity closely correlated with IR-group II PLA₂ levels. These results indicate that group II PLA₂ is responsible for measurable calcium-dependent PLA₂ activity in intervertebral discs.

IR-group II PLA₂ was localized in the cytoplasm of chondrocytes in both annulus fibrosus and nucleus pulposus. All chondrocytes in discs were uniformly stained and the chondrocyte lacunae and disc matrix had no group II PLA₂ immunoreactivity. These results suggest that the PLA₂ is stored in and secreted from chondrocytes in response to stimulations.

Recently articular cartilage was found to contain large quantities of group II PLA₂ in both osteoarthritis and rheumatoid arthritis [16–21]. A previous immunohistochemical study [16] showed that in articular, laryngeal and auricular cartilage the peripheral areas of the chondrocyte lacunae and surrounding cartilage matrix contain IR-group II PLA₂ and that there were two distinct populations of PLA₂-containing and PLA₂-deficient chondrocytes. We confirmed these findings in this study, and demonstrated that the localization of group II PLA₂ in intervertebral discs and the articular cartilage is quite different. This may be interpreted that chondrocytes in the disc store the enzyme in the secretory granule, like intestinal Paneth cells (Tojo et al. unpublished results), whereas chondrocytes in the articular cartilage are continuously releasing the enzyme into the extracellular matrix after its synthesis.

Chondrocytes in the deep layer of articular cartilage adjacent to subchondral bone had a larger amount of IR-group II PLA₂ than in the superficial layer near the joint space. These results coincide with the fact that deep layers of articular cartilage show higher PLA₂ activity than superficial ones in rheumatoid arthritis and osteoarthritis [19], and that cultured chondrocytes obtained from the deep layers of the cartilage released more PLA₂ than those obtained from the superficial layers [20].

Discs of middle-aged patients and cadavers had significantly high PLA₂ activity and IR-group II PLA₂ levels as compared with those of younger and elder cases. For instance, discs of two young girls with idiopathic scoliosis had significantly low PLA₂ activity and IR-group II PLA₂ levels, while discs of a middle-aged patient with neurenteric cyst had significantly high PLA₂ activity and IR-group II PLA₂ levels. Since the histological appearance and MRI findings of these cases were normal, the measured difference in the activity and IR-group II PLA₂ levels may be due to physiological age-dependent changes in expression levels of the enzyme. In this context, Recklies and White reported that PLA₂ activity was not detectable in cartilage from newborns [21]. These findings suggest that group II PLA₂ develops postneonatally, that is, its amounts in discs gradually increase during adolescence, reach the peak of synthesis at middle age, and then decrease with aging. A decrease in the number of viable chondrocytes in senescent discs [5] may cause reduction of PLA₂ activity. An immunohistochemical study [22], however, suggests that the fetal gastric mucosa contained a large amount of IR-group II PLA₂, but not the adult mucosa.

Cadaveric discs of females had higher PLA₂ activity and IR-group II PLA₂ levels than those of males, although serum IR-group II PLA₂ levels did not depend on sex (H. Tojo, unpublished results). It remains to be clarified whether or not the expression of group II PLA₂ in intervertebral discs is hormonally regulated.

The nucleus pulposus consists of gel-like materials that are rich in proteoglycans, whereas the annulus fibrosus is mainly composed of collagen fibers. Since there was no difference in the PLA₂ activity and IR-group II PLA₂ levels between nucleus pulposus and annulus fibrosus, the difference in local environments of cartilage matrix may not affect the degree of expression of group II PLA₂ in the chondrocytes.

Herniated disc fragments had lower PLA₂ activity and IR-group II PLA₂ levels than disc materials remaining within the site. When a disc fragment has herniated out of...
its original site, the ordinary nutritional pathway of chondrocytes may be obstructed, leading to decreasing production of PLA₂. This result is compatible with the fact that the amounts of prostaglandin released from sequestered discs as measured by radioimmunoasay, were very low, compared with those from lumbar facet cartilage and bone [23]. Alternatively, there is a possibility that certain stimuli in herniated discs increase the secretion of group II PLA₂, leading to depleting the enzyme store.

Prostaglandins and leukotrienes stimulate neural elements and cause pain [24,25], and, in addition, activation of PLA₂ produces mechanical hyperalgesia [26]. Thus, PLA₂ activity in discs seems to affect the severity of symptoms. However, in this study, the activity did not correlate with clinical features of patients assessed by JOA scores. The PLA₂ activity measured by the present method may not reflect PLA₂ activity around nerves in the vicinity of herniated discs, or it may be unrelated to i-cosanoid production. Since herniated discs contain low levels of PLA₂ and prostaglandins, immunologic reactions might be more responsible for pathophysiology of radiculopathy [23].

PLA₂ liberates arachidonic acid from membrane phospholipids at the site of inflammation, and extracellular group II PLA₂ expression has been linked to the potentiation and perpetuation of inflammatory reactions [27]. However, because morphologically normal cadaveric discs had the same group II PLA₂ levels and activity as surgical specimens, we conclude that group II PLA₂ is constitutively produced and plays important physiological roles in discal ordinary metabolism to maintain cartilage homeostasis. In the metabolism of chondrocytes, prostaglandins are implicated in the regulation of cartilage differentiation: prostaglandin E₂ stimulates the development of chick limb and chondrogenesis [28], and inhibitors of prostaglandin synthesis inhibit chondrogenesis in limb cells in vitro [29]. Mineralization of cartilage adjacent to the growth zone is associated with elevated levels of alkaline phosphatase and PLA₂ of matrix vesicles in cartilage [30]. These facts suggest that PLA₂ may participate in diverse metabolic processes. Further investigations are necessary to elucidate the function of group II PLA₂ in the metabolism of intervertebral discs.

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References