IMMUNOHISTOLOGICAL EVALUATION OF DISTRIBUTION OF CYCLOOXYGENASE-1 AND -2 IN HERNIATED INTERVERTEBRAL DISC
(WHICH CELL TYPES CONTRIBUTE TO THE PATHOGENESIS OF LUMBAR DISC HERNIATION, INFLAMMATORY CELLS IN GRANULATION OR THE CHONDROCYTES OF THE DISC?)

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Introduction It has been reported that a variety of inflammatory mediators such as prostaglandinE2 (PGE2) might contribute to pain induction of lumbar disc herniation (LDH). Herniated lumbar disc is a mixture tissue which consists of the nucleus pulposus, the annulus fibrosus and inflammatory granulation infiltrating along the margin of the disc. In vitro studies revealed that the cultured chondrocytes from the disc has a capability to induce strong inflammatory agents (1). However, little histological study has been performed to investigate whether or not the chondrocytes of the disc can express these inflammatory mediators despite many attention has been paid on the inflammatory mediator of the granulation tissues in vivo. That is, there is no consensus of which type of cell can participate in the pathogenesis of LDH. Arachidonate cascade, the pathway of PGE2 production, has a rate-limiting enzyme, cyclooxygenase-2 (COX-2). There is another isoform of COX named COX-1, 64% of whose amino acid sequence is identical with COX-2. Despite the similarity of their structures, COX-1 and COX-2 subserve different physiologic functions because of striking differences in their tissue expression and regulation. COX-1 is constitutively expressed in almost all types of cells and appears to be responsible for the PG production that are important for homeostatic function. On the other hand, COX-2 is the inducible product of an “immediate-early” gene which is upregulated just during inflammation, and might play an important role in LDH (2). Therefore, the purpose of this study is to evaluate the expression of COX-2 in herniated lumbar disc specimens in order to clarify the dominant cell types which participate the pathogenesis of LDH in contrast with that of COX-1.

Methods Twenty herniated lumbar disc specimens were obtained from patients (18-59, mean age: 37 years old) who underwent posterior surgery for LDH combined with sciatica. The specimens were classified as 15 extrusion type and five sequestration type. Five disc tissues, obtained from patients who underwent anterior surgery for traumatic burst fracture of lumbar vertebrae (20-38 years old) were used as controls. Immunostaining was carried out using monoclonal antibodies utilized for COX-1 and -2. Immunohistological preparations were evaluated according to homogeneous criteria (4 grades; homogeneous, moderate, focal, negative) by three researchers in a blind fashion. When the granulation tissues were detected, this evaluation was performed separately in the chondrocytes and the cells of the granulation.

Results The block sections showed that the cells embedded in disc matrix were morphologically compatible with chondrocytes. The inflammatory granulation tissues were detected in 55% (11 of 20 cases) of the specimens from patients with LDH, and the cells constituting the granulation were clearly distinguished from the chondrocytes in the matrix. It was impossible to specify the origin of these chondrocytes from which part of the disc, the nucleus pulposus or the annulus fibrosus. Cells positive for COX-1 were observed in LDH specimens as well as controls (Figure 1, A, B). 60% of LDH specimens and 80% of control discs showed moderate or strong intensity in the semiquantitative evaluation. Anti-COX-2 reactivity was significantly detected in the specimens of LDH (Figure 1, C). All LDH specimens showed moderate or strong intensity. In cases where the granulation tissues were detected, the distribution of these reactivity was in not only the cells of the granulation tissues but also the chondrocytes of the disc materials (Figure 2). In the evaluation of the degree of the staining with anti-COX-2, significant correlation was found between the chondrocytes and the cells from the granulations, and 91% of the evaluation matched between these two cell types. On the other hand, low intensity of staining with anti-COX-2 was observed in control discs. Neither moderate nor strong intensity was found in controls.

Discussion This study has shown that not only the cells constituting the inflammatory granulation which infiltrated along the disc but also the chondrocytes included in the disc material were apparently positive for COX-2 in LDH specimens. Takahashi et al. (3) and Hashizume et al. (4) reported that the majority of the cells which expressed the inflammatory cytokines and inducible nitric oxide synthase (iNOS), which might be related to the inflammatory agents of LDH, were the cells constituting the inflammatory granulation. On the other hand, Furusawa et al. (5) reported that iNOS was detected in not only the cells of the granulation but also the chondrocytes of the cervical herniated disc. Rydevik et al. (6) also reported that the chondrocytes within the disc could be affected by the inflamed tissues such as the synovium and articular cartilage of the facet joint and subsequently synthesize inflammatory mediators, which suggested that the chondrocytes of the disc could contribute to exacerbation of inflammation. This study has shown that the degree of the reactivity with anti-COX-2 had a good correlation between the cells of the granulation and the chondrocytes of the disc, which suggests that both cell types might interact with each other leading to exacerbated inflammation. Recently, clinical application of selective inhibitors of COX-2 has been considered as one of the key strategies in treating inflammatory diseases with the expectation of fewer side effects than former NSAIDs have because they inhibit not physiological parts in which COX-1 plays a pivotal role, but inflammatory parts in which COX-2 does. Therefore, it would be noteworthy to clarify the pathomechanism of the disease in which COX-2 might play an important role.

Figure 1. The expression of COX-1 in the chondrocytes of the control disc(A) and the herniated lumbar disc (B). Anti-COX-2 reactivity was detected in the chondrocytes of the herniated lumbar disc (C).

Figure 2. The distribution of the expression of COX-2 in the herniated lumbar disc specimen. Not only the cells of the inflammatory granulation (white arrows) but also the chondrocytes of the disc (black arrows) expressed COX-2.

References