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

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Introduction

Pain of the nerve root is one of the major symptoms of the lumbar disc herniation. In terms of the pathogenesis of this pain, the role of various kinds of biochemical mediators are focused in addition to the direct mechanical compression to the nerve root though the precise pathomechanism remains still elusive. It is well established that protaglandin E2 (PGE2) is a strong mediator to induce the pain in inflammatory conditions including lumbar disc herniation. Recently, cyclooxygenase-2 (COX-2), the inducible isozyme of COX, has been identified as the key enzyme to regulate PGE2 synthesis in inflammatory conditions. There are many reports concerning the induction of COX-2 by stimulation with inflammatory cytokines to many kinds of cells. However, the role of COX-2 in pathogenesis of lumbar disc herniation has not been fully discussed. Therefore, the purpose of this study is to clarify the role of COX-2 and the inflammatory cytokines in the pathogenesis of lumbar disc herniation by using immunohistochemical and molecular biological methods.

Materials and Methods

Twelve herniated lumbar disc specimens were obtained from the patients who underwent the posterior surgery for lumbar disc herniation with certain radicular symptoms. Depending on the macroscopic finding during surgery, 9 specimens were classified as extension and the remaining were sequestration. Normal disc specimens were obtained from 3 patients who underwent the anterior surgery for idiopathic scoliosis or traumatic burst fracture.

(1) Immunohistochemical study of the herniated lumbar disc

The localizations of COX-2 in the surgical specimens were examined immunohistochemically using monoclonal antibody specific for human COX-2. The inflammatory cytokines were also detected using monoclonal antibody specific for human interleukin-1 (IL-1) and polyclonal antibody for human tumor necrosis factor-α (TNF-α).

(2) Expressions of mRNAs of COX-2, IL-1 and TNF-α of herniated lumbar disc-derived cells by stimulation with inflammatory cytokines in vitro

The monolayer culture of the disc cells were prepared from the surgically obtained herniated lumbar disc specimens by collagenase digestion. In the 6-well plates, 1.0x10⁵ attached cells/cm² were cultured in DMEM supplemented with 10% fetal calf serum in the presence of either 100 U/ml of IL-1α or TNF-α for 6 hours. Total cellular RNA was directly isolated from the cell monolayers using a protocol of AGPC method. Gene expression of either COX-2 or the inflammatory cytokines was detected by PCR using each specific primer for COX-2, IL-1β or TNF-α respectively after reverse transcription of obtained total cellular RNA.

(3) Analysis of the supernatants for PGE2 production

In order to detect the effect of the inflammatory cytokines on PGE2 synthesis of the herniated lumbar disc-derived cells, the concentrations of PGE2 in later 5 cases of the previous experiments were measured by ELISA.

Results

Immunohistochemically, the localizations of COX-2 and the inflammatory cytokines such as IL-1α and TNF-α were detected in cytosols of not only the inflammatory cells infiltrating along the margins of the herniated disc tissues but also the chondrocytic cells constituting the discs, though no expression in the control specimens (Fig.1). By RT-PCR, strong expression of COX-2 mRNA was detected when the herniated lumbar disc-derived cells were stimulated with either IL-1α or TNF-α, while little expression was shown in the unstimulated cells (Fig.2). Expressions of mRNAs of IL-1α and TNF-α were also detected by stimulation with the inflammatory cytokines by themselves (Fig.2). Moreover, in the culture supernatants of the stimulated cells by either IL-1α or TNF-α, significantly higher levels of PGE2 were demonstrated in comparison with unstimulated cells (Fig.3).

Discussion

Based on the findings of our experiments, it was demonstrated that the cells constituting the herniated lumbar disc secrete the inflammatory cytokines such as IL-1α and TNF-α autocrinely or paracrinely. It was also shown that these inflammatory stimulation induced the expression of COX-2, which was the key enzyme to regulate PGE2 synthesis. It is, therefore, suggested that both the induction of COX-2 stimulated by the inflammatory cytokines and autocrine or paracrine regulation of the inflammatory cytokines gene expression may play an important role in causing radiculopathy of lumbar disc herniation.

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