THE ROLE OF CYCLOOXYGENASE-2 (COX-2) IN THE PATHOMECHANISM OF PAIN INDUCTION IN LUMBAR DISC HERNIATION (THE 2ND REPORT) - 6-METHOXY-2-NAPHTYL ACETIC ACIDS (6MNA), THE SELECTIVE INHIBITOR OF COX-2, INHIBITS PROSTAGLANDINE2 SYNTHESIS IN VITRO -

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INTRODUCTION
Lumbar disc herniation is the disease one of whose major complaints is pain. In terms of the pain, the role of various kinds of biochemical mediators has been focused though the precise pathomechanism remains unclear. It is well established that prostaglandinE2 (PGE2) is a strong mediator to induce pain or enhance sensitvity to pain-inducing substances in lumbar disc herniation. In the process of PGE2 synthesis, cyclooxygenase-2 (COX-2), one of the PGE2 synthase, has been noticed as a rate-limiting enzyme in inflammatory condition. COX-2 has been known to be induced by stimulation of the inflammatory cytokines in many kinds of cells, and at the 45th annual meeting of Orthopaedic Research Society, we reported that the induction of COX-2 and the release of the inflammatory cytokines might play important roles in pain induction of lumbar disc herniation due to PGE2 synthesis. However, it has been not fully discussed whether COX-2 may dominantly work at the site of lumbar disc herniation. Therefore, the purpose of the present study is to clarify in how much degree COX-2 may contribute to the pain induction of lumbar disc herniation.

MATERIALS AND METHODS
(1) Immunohistological Study
Seventeent herniated lumbar disc specimens were obtained from the patients underwent surgeries. In this series, 12 specimens were from the patients with radicular pain, and remaining 5 specimens were from those without any pain, whose main symptoms were neurological deficits mainly as motor disturbance. We detected the degrees of COX-2 expressions of these specimens by immunostaining using monoclonal antibody specific for human COX-2. Moreover, the correlations between the degrees of COX-2 expressions and the clinical data, SLR test or the duration of the disease etc were examined.

(2) In Vitro Study
Cell Culture
Five specimens were digested with collagenase. These cells were cultured with the stimulation of the inflammatory cytokines, 100U/ml of IL-1β or TNFα, in adding with or without 50ug/ml of 6-methoxy-2-naphtyl acetic acids (6MNA), which is an active metabolite of Nabumeton, selective inhibitor of COX-2, for 6 hours.

PGE2 Assay for the Culture Supernatants
The concentrations of PGE2 were measured by radioimmunoassay.

Detection of the Expressions of COX-2 mRNA
Gene expressions of COX-2 mRNA of the herniated lumbar disc-derived cells were detected by RT-PCR using specific primer for COX-2 and others.

RESULTS
Immunohistologically, the localization of COX-2 was detected in the cytosols of the chondrocyte cells constituting the discs obtained from all the patients with radicular pain (Fig.1). On the other hand, little expression of COX-2 was detected in the specimens obtained from the patients without any pain. However, the extent of COX-2 expression did not necessarily correlate with the any clinical data such as positive reaction for SLR test or the duration of the disease.

In culture supernatants, the inflammatory cytokine-stimulated cells, especially IL-1β, produced dramatically higher concentrations of PGE2 than the unstimulated. 6MNA distinctly inhibited this PGE2 synthesis (Fig.2). The expression of COX-2 mRNA were detected regardless of the presence or absence of 6MNA in the cytokine-stimulated cells though little expression in the unstimulated. COX-2 mRNA strongly expressed in the cells stimulated by IL-1β, which led the cells to secrete high concentration of PGE2. In the control, GAPDH, the housekeeping gene, were constitutively expressed (Fig.3).

DISCUSSION
Immunohistologically, all specimens from the painful patients expressed COX-2, in contrast little expression of COX-2 was observed in the specimens from the pain free patients. This result suggests that induction of COX-2 may play an important role in causing pain of lumbar disc herniation. In vitro study showed that 6MNA, the selective inhibitor of COX-2, distinctly inhibited the PGE2 synthesis though the inflammatory cytokine-stimulated cells without 6MNA produced dramatically higher concentrations of PGE2. Especially, IL-1β might have a strong capability to produce PGE2 secondary to COX-2 induction. 6MNA didn’t inhibit the expression of COX-2 mRNA, and this suggests that it may effect on the COX-2 enzyme itself not gene level. In the present study, therefore, it is suggested that COX-2 may dominantly work in causing pain of lumbar disc herniation due to regulate PGE2 synthesis.