Signaling Pathway in Mechanical Stress-Induce Inflammatory Responses in Human Ligamentum Flavum Fibroblasts
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INTRODUCTION
Inflammation has been proposed to constitute an important pathogenetic component in ligamentum flavum hypertrophy. However, the pathomechanisms of inflammation in ligamentum flavum remain unclear. An in vitro model of human ligamentum flavum fibroblasts subjected to mechanical stress to elucidate the effects of mechanical stress on cultured human ligamentum flavum fibroblasts and to further investigate the molecular and biochemical mechanisms by which mechanical stress induce inflammatory responses in vitro.

MATERIALS AND METHODS
Human ligamentum flavum fibroblasts were obtained from six patients undergoing lumbar spine surgery. Mechanical stress was applied by subjecting human ligamentum flavum fibroblasts in monolayer culture to different magnitude of centrifugal forces. Cell viability and cytotoxicity were assayed by colorimetric MTT and lactate dehydrogenase (LDH) Assay, respectively. Specific genes expression were determined by real-time PCR and doses of prostaglandin E2 (PGE2) and nitric oxide (NO) were measured in the culture supernatants. Cyclooxygenase-2 (COX-2), inducible NO synthase (iNOS), JNK, ERK and p38 MAPK were determined by Western blotting analysis.

RESULTS AND DISCUSSION

A. Fig. 1
Effects of centrifugal force on ligamentum flavum fibroblasts viability and cytotoxicity.

B. Fig. 2
Effects of centrifugal force on NO production in ligamentum flavum fibroblasts.

C. Fig. 3
Effects of centrifugal force on target genes expression in ligamentum flavum fibroblasts.

Fig. 4
Upregulation of iNOS and COX-2 level were shown after applying centrifugal force.

Fig. 5
Synthesis of PGE2 in ligamentum flavum fibroblasts after applying centrifugal force for 60 min.

Fig. 6
JNK and MAPK-p38 pathways are involved in centrifugal force-induced iNOS and COX-2 protein expression.

It was found that mechanical stress significantly suppressed cell viability without inducing cytotoxicity. Mechanical stress with 67.1 g/cm² for 60 min significantly increases the production of PGE2 and NO as well as pro-inflammatory cytokines, IL-1α, IL-1β and IL-6, gene expression. It also showed that mechanical stress-dependent induction of COX-2 and iNOS required JNK and p38 MAPK, but not ERK 1/2 activities, as indicated by the result of pretreatment with the JNK inhibitor SP600125 and p38 inhibitor SB203580.

Conclusion.
The results of this study suggested that mechanical stress induced inflammatory responses in human ligamentum flavum fibroblasts. The activation of both JNK and p38 MAPK mechanotransduction cascades are crucial intracellular mechanisms that mediate COX-2/PGE2 and iNOS/NO production.

REFERENCES

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