Cyclic Mechanical Stress on 3-D Tissue of Human Synovium-derived Cells Induced Prostaglandin E2 Production via COX-2 and mPGES-1 Expression.

INTRODUCTION:

Prostaglandin E2 (PGE2) plays a significant role in development of osteoarthritis (OA) to cause pain, inflammation, and cartilage degradation (1-3). PGE2 is known to be produced by synovial fibroblasts or chondrocytes in response to proinflammatory cytokines such as IL-1β and/or TNF-α. However, synovitis induced by these proinflammatory cytokines is the secondary pathological event in contrast to the primary excessive mechanical stress on synovial joint in the progression of OA (4). The molecular mechanism of PGE2 production by mechanical stress is still unclear. The purpose of this study was to examine the expression of PGE2 by cyclic mechanical stress on three-dimensional (3-D) tissue of human synovium-derived cells.

METHODS:

Human synovium-derived cells were isolated enzymatically from human synovial membranes and the adherent cells were expanded until passage 3 to 7. Collected cells (5.0×10⁶/scaffold) were resuspended in the media and mixed with 1% collagen solution (Atelocollagen®, KOKEN JAPAN). The cell-collagen solution mixture was then seeded onto a collagen scaffold (AteloCell MIGHTY®, KOKEN JAPAN) to produce a 3-D construct. The 3-D constructs were incubated for 3 days before cyclic compressive loading according to our previous methods (5).

Firstly, cyclic compressive loading of 40kPa (≥10% strain) at 0.5Hz for 1hr was applied to the constructs with (n=8) or without the administration of COX-2 selective inhibitor (n=5). After 6 hours incubation, the concentrations of PGE2, IL-1β, TNF-α in supernatant were measured by homogeneous time-resolved fluorescence (HTRF) method, the mRNA expressions of COX-2 and mPGES-1 genes were examined by RT-PCR, and the protein expression of COX-2 was examined by Western blotting using anti-COX-2 monoclonal antibody.

Secondly, IL-1β (10mg/ml, n=3) or TNF-α (100mg/ml, n=4) was administered to the 3-D constructs after the same pre-incubation for 3 days. After 6 hours incubation, the concentration of PGE2 in supernatant was measured with HTRF. Each experiment was repeated three times.

RESULTS:

The concentration of PGE2 in supernatant was significantly higher in the mechanically-stressed group, but the concentration of IL-1β or TNF-α was unchanged by mechanical stress (Fig1). mRNA levels of COX-2 and mPGES-1 genes and protein level of COX-2 were up-regulated by cyclic mechanical stress (Fig2). By administering COX-2 selective inhibitor, either mRNA or protein level of COX-2 was not suppressed, while mRNA level of mPGES-1 gene was suppressed. The increased concentration of PGE2 by cyclic mechanical stress was impeded by COX-2 selective inhibitor (Fig3).

The administration of IL-1β or TNF-α also induced PGE2 production (Fig4).

DISCUSSION:

PGE2, COX-2, and mPGES-1 production was up-regulated by cyclic mechanical stress to the constructs, while pro-inflammatory cytokines such as IL-1β and TNF-α remained unchanged. In addition, the administration of IL-1β or TNF-α induced PGE2 production as previously reported in monolayer culture (6). These results indicate that PGE2 production by cyclic mechanical stress might not be induced by these cytokines (Fig4).

COX-2 selective inhibitor suppressed PGE2 induction by mechanical stress in dose-dependent manner, but not mRNA and protein level of COX-2. This 3-D culture system using cyclic compressive loading might be useful to reveal the molecular mechanism of PGE2 regulation by cyclic mechanical stress in vivo for better understanding of pathology and therapy of OA.

REFERENCES: