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Celecoxib down-regulates mechanically induced ADAMTS-4 gene expression in 3D cultured tissue of human synovium-derived cells at lower concentration than indomethacin

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Cyclic mechanical load is thought to play a major role in temporomandibular disorder (TMD). We developed the cyclic loading bioreactor, in which matrix metalloproteases (MMPs) and a disintegrin, and metalloproteinase with thrombospondin motifs (ADAMTSs) are induced by mechanical stimulation to three-dimensional (3D) cultured tissue. The purpose of this study was to examine the effects of NSAIDs on the gene expressions of ADAMTSs, MMPs, and the protein expression of PGE2. Human synovial cells were seeded onto a collagen scaffold to construct 3D cultured tissue. Celecoxib or indomethacin were added to the culture media before cyclic compressive load. Cyclic compression was then applied to 3D tissues. After 6 hours, the media were assayed for PGE2 by HTRF®. The mRNA expression of the 3D tissue was quantitatively determined by real time RT-PCR.

We found that mechanical stress to the human synovial cells in 3D culture induced PGE2 production and up-regulated ADAMTS-4 and MMP-1, -3 gene expression. Celecoxib and indomethacin suppressed PGE2. Celecoxib down-regulated mechanically induced ADAMTS-4 gene expression at a lower concentration than indomethacin. These results indicate that celecoxib and indomethacin may have an inhibitory effect on cartilage destruction caused by mechanical stimulus. In addition, celecoxib may contribute to inhibition of cartilage destruction at a lower concentration than indomethacin. (J Osaka Dent Univ 2014 ; 48(1) : 55–59)

Key words : NSAIDs ; MMPs/ADAMTSs ; Mechanical stress ; 3D cultured tissue ; Human synovial cell

INTRODUCTION

Temporomandibular disorder (TMD) is a disease or disorder of the temporomandibular joint and masticatory muscles[1] that is sometimes caused by anterior displacement of the articular discs. When clenching the teeth, compressive force is applied directly to the synovium in the disc, which is displaced anteriorly, causing synovitis. Increased levels of inflammatory modulators, such as cytokines, nitric oxide, and cartilage matrix catabolites, as well as matrix metalloproteinases (MMPs), are found in the synovial fluid with TMD.[2–4] MMPs derived from fibrocartilage and cartilage cells are considered key enzymes that are responsible for extracellular matrix (ECM) breakdown in the TMJ.[5] Further, in recent years, not only MMPs, but also a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) have been attracting attention as proteinases that degrade the ECM of cartilage. Among them, especially ADAMTS-4 and ADAMTS-5 are known to degrade aggrecan in the extracellular matrix.[6, 7]

Until now, an arthritis model was produced by stimulation of IL-1β,[8] tension stress,[9] and hydrostatic fluid pressure.[10] We have developed a novel arthritis model using a cyclic loading bioreactor (CLS-7J® ; Technoview, Osaka, Japan),[11] and cultured human synovial cells in a three-dimensional (3D) cell-scaffold

construct (collagen scaffold). This model applys cyclic mechanical loading to 3D culturation of human synovial derived cells.[11]

In clinical situations, the inflammation and pain of arthritis are treated by NSAIDs or steroid. Celecoxib has been developed based on molecular design targeting of cyclooxygenase (COX)-2, and selectively inhibits COX-2 enzyme activity.[12–14] However, it is still unknown whether or not NSAIDs have a pharmaceutical role in osteoarthritis (OA). This study aimed to examine the effects of celecoxib and indomethacin on the expression level of ADAMTSs and MMPs genes in our synovitis model.

MATERIALS AND METHODS

Human synovial cell culture

Surgical specimens of human synovial membranes were obtained during arthro-scopic knee surgeries. Patients gave informed consent for the use of the specimens for experiments. This study was accepted by the Ethics Committee in Osaka University. Excised synovia were digested with 0.2% collagenase in Dulbecco's modified Eagle's medium (DMEM). The liberated cells were resuspended in medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were then cultured in a monolayer at 37°C in 5% CO_2 atmosphere. Cells from the third to the sixth passage were used for experiments.

Collagen scaffold preparation

A porous collagen sponge (Atelocollagen sponge Mighty®, KOKEN, Tokyo, Japan) 5 mm in diameter and 3 mm thick was prepared to fit a 96-well cell culture plate. It was produced as described previously.[15]

Cell seeding in collagen scaffold and 3D culture of cell-scaffold constructs

3D tissue was produced as described previously.[15] Briefly, collected cells (5.0×10^5/scaffold) were resuspended in 1 × DMEM containing 10% FBS, 1% antibiotics, and 0.5% collagen solution (Atelocollagen gel, KOKEN), and then seeded into a collagen scaffold in a 96-well cell culture plate by centrifugal force. After gelation, 3D cultured tissues were incubated for 3 days before being subjected to cyclic compressive load.

Reagents

Celecoxib was provided by Pfizer (New York, NY, USA), and indomethacin was purchased from Sigma-Aldrich (St. Louis, MO, USA). 3D cultured tissues were washed twice with phosphate-buffered saline (PBS) and various concentrations of celecoxib or indomethacin were added (0, 10^{-2}, 10^{-1}, 1 or 10 μM) in serum-free DMEM before cyclic loading.

Cyclic loading system and loading protocols

Cyclic dynamic loading was carried out using a cyclic loading bioreactor (CLS-7J®; Technoview, Osaka, Japan), described previously.[15] In this study, cyclic compression was applied to 3D constructs at 0 (no-loaded) or 40 kPa at 0.5 Hz for 1 hour. After loading, 3D cultured tissues were incubated at 37°C in 5% CO_2 atmosphere for 6 hours.

PGE2 measurements

Conditioned media were collected and immediately frozen at –80°C. Prostaglandin E2 (PGE2) concentration in the media were assayed by HTRF® (Cisbio ; Codolet, Gard, France).

Measurement of mRNA expression level

After collection of conditioned media, 3D cultured tissues were washed twice with PBS. RNA was extracted by TRIzol from the 3D cultured tissues. Single-strand cDNA was synthesizes by reverse-transcriptase (Prime-Script® RT reagent Kit ; TaKaRa Bio, Shiga, Japan). The final cDNA concentration was 1 ng/μL. The mRNA expression of the 3D cultured tissues was quantitatively determined by real time reverse transcriptase polymerase chain reaction (real time RT-PCR) using ABI PRISM® 7900 HT (Applied Biosystems, Carlsbad, CA, USA) with associated enzyme and reagents (SYBR® Premix Ex TaqTM Perfect Real Time ; TaKaRa Bio). Primer sequences for ADAMTS-4, ADAMTS-5, MMP-1 and MMP-3 were designed using Primer 3 published by MIT on the internet (http : //frod.wi.mit.edu/primer3) (Table 1). PCR amplification of the mRNA samples was carried out by

Table 1 Primers used in real-time RT-PCR

Gene	Forward primer	Reverse primer
GAPDH	tctctgctcctcctgttcgac	gttgactccgaccttcaccttc
ADAMTS-4	gtgccattgtggaggatgat	cttggagttgtcatggagca
ADAMTS-5	cttcactgtggctcacgaaa	tttggaccagggcttagatg
MMP-1	cccaaaagcgtgtgacagtaag	cttccgggtagaagggatttg
MMP-3	cgtgaggaaaatcgatgcag	cttcagctatttgcttgggaaag

initial denaturation for 30 sec at 94°C, and annealing for 30 sec at 60°C for 40 cycles (n = 9).

Statistical analysis

All experiments were carried out in triplicate and repeated three times. The multiple comparison test was used for statistical analysis. Tukey-Kramer was used at a significance of $p < 0.01$.

RESULTS

Concentration of PGE2 in culture supernatant by HTRF®

The concentration of PGE2 in the culture supernatant was significantly increased by cyclic mechanical stress to the 3D constructs from 0.33 ± 0.09 μg/mL to 4.87 ± 2.07 μg/mL. The PGE2 production was suppressed significantly by celecoxib and indomethacin at $10^{-2}\mu$M, $10^{-1}\mu$M, 1 μM and 10 μM (Fig. 1).

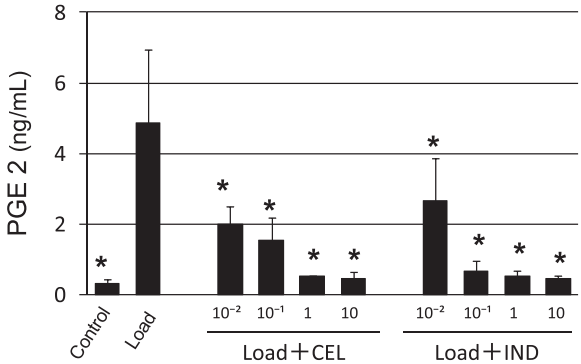

Fig. 1 Concentration of PGE2 for various concentrations of CEL or IND (0, 10^{-2}, 10^{-1}, 1 or 10 μM) added to 3D constructs in serum-free DMEM before cyclic loading (40 kPa, 0.5 Hz, 1 hour). The collected media were assayed with PGE2 after 6 hours.
CEL : Celecoxib, IND : Indomethacin, Mean ± SD ; *n = 9, *p < 0.01 compared with loaded sample (Tukey-Kramer)*.

Quantitative analysis of mRNA expression levels by real-time RT-PCR

Expression of mRNA in 3D cultured tissue of human synovium-derived cells was analyzed by real-time RT-PCR. The mRNA expression level for ADAMTS-4 was siginificantly increased with cyclic mechanical stress by 3.8-fold. The mechanically induced mRNA level of ADAMTS-4 was siginificantly supressed by celecoxib at all concentrations and by 1 μM and 10 μM of indomethacin (Fig. 2). The mRNA expression level for ADAMTS-5 was unchanged by mechanical loading or administration of NSAIDs. The mRNA expression level for MMP-1 was siginificantly increased by cyclic mechanical stress to the 3D constructs. The increased mRNA level of MMP-1 was unchanged by administration of NSAIDs. The mRNA expression level for MMP-3 was significantly increased by cyclic

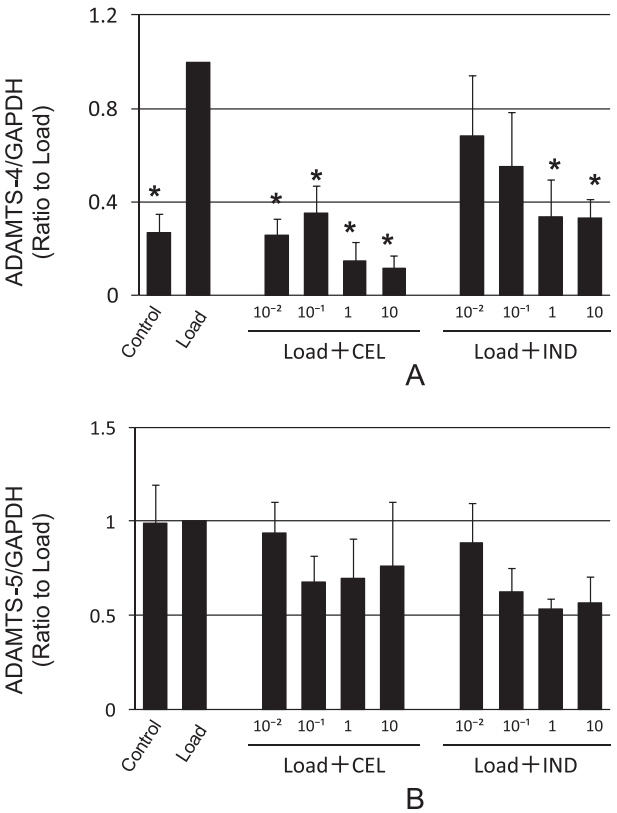

Fig. 2 The ratio to load of ADAMTSs/GAPDH for various concentrations of CEL or IND (0, 10^{-2}, 10^{-1}, 1 or 10 μM) added to 3D constructs in serum-free DMEM before cyclic loading (40 kPa, 0.5 Hz, 1 hour). The mRNA expression of the 3D cultures was quantitatively determined by real time RT-PCR after 6 hours.

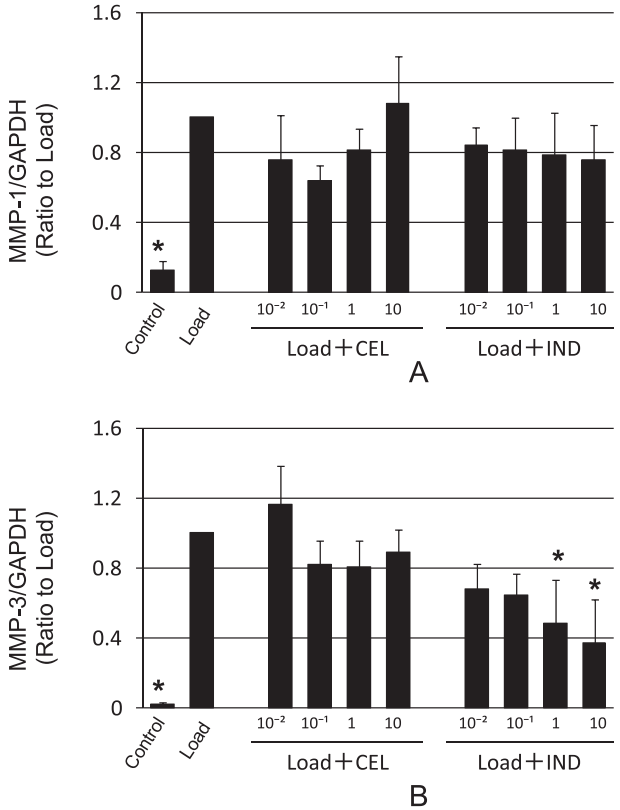

Fig. 3 The ratio to load of MMPs/GAPDH for various concentrations of CEL or IND (0, 10^{-2}, 10^{-1}, 1 or 10 μM) added to 3D constructs in serum-free DMEM before cyclic loading (40 kPa, 0.5 Hz, 1 hour). The mRNA expression of the 3D cultures was quantitatively determined by real time RT-PCR after 6 hours.

mechanical stress. However, it was significantly supressed by indomethacin at 1 μM and 10 μM, but not by celecoxib (Fig. 3).

DISCUSSION

Cartilage consists of a relatively small amount of chondrocytes, embedded in abundant ECM that contains numerous macromolecules, which are collagen fibrils and large aggregating proteoglycan aggrecan. The destruction of the cartilage matrix is induced primarily by elevated production of ADAMTSs and MMPs which are produced by chondrocytes and synovial fibroblasts as well as by macrophages.[16] The degradation of aggrecan is mediated by various matrix proteinases, mainly the aggrecanases which are members of the family of ADAMTS characterized by their ability to cleave the Glu 373-Ala 374 bond in the interglobular domain of aggrecan.[17]

It had been reported that in models of murine OA induced by antigen or surgical joint destabilisation, ADAMTS-4-null mice did not show any protective effect on cartilage aggrecan loss compared with wild-type mice, whereas there was a marked protective effect in ADAMTS-5-null mice,[18–20] suggesting that ADAMTS-5 seems to be related to pathology in murine OA. In contrast, Naito *et al* reported the predominant expression of ADAMTS-4 in human osteoarthritic cartilage, while ADAMTS-5 was constitutively expressed in OA and normal cartilage.[21] In this study, the cyclic compression load on human synovial 3D tissue culture increased gene expression of ADAMTS-4, but did not change that of ADAMTS-5. These results may indicate that ADAMTS-4 is induced by cyclic mechanical loading in human synovial tissue and might be related to the significance of ADAMTS-4 in the development of human OA.

In this synovitis model, we examined the effect of NSAIDs for PGE2 production and gene expression of ADAMTS-4, ADAMTS-5, MMP-1 and MMP-3. NSAIDs significantly supressed PGE2 increased by cyclic compressive loaded. Celecoxib inhibited mechanically induced ADAMTS-4 gene expression at lower concentration than indomethacin. Celecoxib has been designed to molecularly target COX-2, and has a high and selective ability to inhibit COX-2 compared with COX-1.[12–14] There is a difference in structure between celecoxib and indomethacin. It has been reported that celecoxib supresses of proteoglycan destruction and promotes proteoglycan synthesis.[22] For this reason it has been suggested that celecoxib might be more effective at protecting joints than indomethacin.

One drawback of this study is that it was only confirmed by gene expression of ADAMTSs and MMPs. Protein expression of these genes should be examined to explore clinical significance. Another drawback is that, we produced 3D constructs with cells from synovial tissue. It is necessary to produce a 3D construct of chondrocytes to confirm the effect of the mechanical stimuli on cartilage.

CONCLUSION

Mechanical stress to the human synovial cells in 3D

culture induced PGE2 production and up-regulated ADAMTS-4 and MMP-1, -3 gene expression. Celecoxib and indomethacin suppressed PGE2. Celecoxib down-regulates mechanically induced ADAMTS-4 gene expression at lower concentration than indomethacin. These results indicated that celecoxib and indomethacin may have an inhibitory effect on cartilage destruction caused by machanical stimulus, and that celecoxib may contribute to inhibition of cartilage destruction at a lower concentration than indomethacin.

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