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Interleukin-1 beta affects cyclooxygenase-2 expression and cartilage metabolism in mandibular condyle

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ABSTRACT

Extracellular matrix degradation in mandibular condylar cartilage is mediated by various cytokines in the temporomandibular joint (TMJ). Interleukin-1 beta (IL-1β) is detected in joint structures with pathologic status, and participates in catabolic action in the extracellular matrix. The purpose of this study was to investigate the effects of IL-1β on cyclooxygenase-2 (COX-2) expression and cartilage metabolism using cultured chondrocytes from mandibular condyle. Articular chondrocytes from the porcine mandibular condylar cartilage around the surface were cultured and treated with 0–10 ng/ml IL-1β or 0–1000 ng/ml prostaglandin (PGE_2) for 0–24 h. The mRNA levels of COX-2, MMP-1, -3, and -13 were evaluated by real-time PCR analysis. The protein levels of PGE_2 and MMPs were examined by ELISA and Western blot analysis, respectively. The expression levels of COX-2 and PGE_2 were enhanced by exogenous IL-1β in chondrocytes. The mRNA levels of MMP-1, -3, and -13 were up-regulated by PGE_2 treatment dose-dependently. It is shown that the expression of COX-2/PGE_2 was enhanced by IL-1β in articular chondrocytes from mandibular condyle, and that MMP-1, -3, and -13 were induced by PGE_2, suggesting that IL-1β-induced COX-2/PGE_2 play a crucial role in catabolic processes of mandibular condylar cartilage under inflammatory conditions.

1. Introduction

Temporomandibular joint-osteoarthritis (TMJ-OA) is a multifactorial joint disease characterized by degenerative changes such as deterioration and abrasion of articular cartilage due to the loss of proteoglycan and collagen network, and occurrence of thickening and remodelling of the underlying bone,[1] resulting in painful and impaired occlusal function with limited jaw movement.

Excessive mechanical stress is believed to work as an initial stimulus for the cascade leading to articular cartilage degradation.[1,2] With respect to TMJ-OA, the mechanism of overloading is probably the same as that in other synovial joints. Since cartilage metabolism is maintained by a balance between anabolic and catabolic processes in normal joints, disruption of the balance would lead to loss of cartilage integrity.[3] Matrix metalloproteinases (MMPs) are likely to play a crucial role in an increased degradation of collagen and proteogylcan in OA articular cartilage.[4] Stimulation of chondrocytes by proinflammatory cytokines increases the production of MMPs.[5–7]

In previous studies, proinflammatory cytokines have been suggested to play important roles in the progress of OA.[8,9] In particular, IL-1β is a key mediator for the destruction of TMJ structures,[3,10–12] exhibiting an ability to enhance the catabolic action, and interacts frequently with cell surface receptors.[13]

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In contrast, blocking of IL-1β receptors was found to result in inhibition of the destructive effects of IL-1β on cartilage matrix.[14] The expression of IL-1β is enhanced in both synovial membrane[15] and articular cartilage[10,16] of limb joints in patients with OA. In the synovial fluid of patients with TMJ-OA, IL-1β was also detected at high levels.[17–20]

Various proinflammatory mediators and enzymes including cyclooxygenase (COX) are involved in the IL-1β-induced catabolic cascade.[3,12] Two isoforms of COX, COX-1 and COX-2, catalyse the rate-limiting step in the conversion of arachidonic acid to prostaglandins (PGs) and thromboxane A_2, both of which are considered crucial for physiological metabolism in various tissues.[21] In addition, COX-2 is induced by various stimuli, including chemical mediators, and plays crucial roles in the inflammation process. In limb articular cartilage of patients with OA, enhanced IL-1β was found to induce the expression of COX-2.[22–24]

Previous studies demonstrated that induction of COX-2 by IL-1β enhanced PGE_2 production in articular chondrocytes of limb joints in patients with OA.[25–27] It is thus assumed that selective COX-2 inhibitors decrease PGE_2 production, leading to the suppression of inflammation and pain in OA patients.[28,29] PGE_2 plays a crucial role in the remodelling and homeostasis of bone and cartilage, [30–32] whereas PGE_2 has been suggested to contribute to the IL-1β-mediated pain and degradation of cartilage matrix in OA joints.[23,33] It is thus confirmed that PGE_2 exhibits both catabolic and anabolic functions for bony structures; however, it is unclear how different functions are initiated. Mandibular condylar cartilage differs from limb articular cartilages in terms of histologic architecture. Mandibular condylar cartilage consists largely of fibrocartilage, not hyaline cartilage, with thick multi-layers composed of several collagen fibre zones on the surface.[34] Type I collagen is distributed widely in the superficial layer of the mandibular condylar cartilage of foetal calf, whereas type II collagen is also abundant in the matured cartilage layer beneath the fibrous layer.[35] In addition, an immunohistological study revealed that both type IX and XI collagens are present in the mandibular condylar cartilage of neonatal mammalian.[36] Because of the compositional differences between fibrocartilage and hyaline cartilage, mandibular condylar cartilage would be needed to examine the degradation of cartilage matrix in TMJ.

In this study, in order to elucidate the COX-2/PGE_2-related mechanism of cartilage degradation in the mandibular condyle under inflammatory conditions, we investigated the effects of IL-1β on the expression of COX-2 and PGE_2 and induction of MMPs using cultured articular chondrocytes from the porcine mandibular condyle.

2. Materials and methods

2.1. Cell isolation and culture

The protocol for a series of experiments was approved by the Animal Care and Use Committee at Hiroshima University. TMJs were obtained from female pigs of 6 to 9 months old with a body weight of 100–110 kg in a slaughterhouse of Japan Agriculture (Miyoshi, Japan). Mandibular condylar cartilage around the surface (surface to a depth of approximately 0.5 mm) was carefully dissected from the mandible, and the cartilage pieces were digested with 0.1% actinase in Dulbecco's modified Eagle's medium (DMEM) for 1 h and 0.02% collagenase in DMEM for 12 h.

Isolated chondrocytes were seeded at a density of 1×10^5 cells/well in 6-well culture plates (35 mm diameter; Corning, New York, NY, USA). The cultures were maintained in 2 ml of DMEM supplemented with 10% foetal bovine serum (FBS, Biological Industries, Kibbutz Bet-Haemek, Israel) and 60 μg/ml kanamycin under an atmosphere of 5% CO_2 in a humidified incubator at 37 °C until confluence.

2.2. Treatment of the chondrocytes with IL-1β or PGE_2

The chondrocytes from porcine mandibular condyle were cultured to confluence with a medium containing 10% FBS. They were then cultured in a medium with 0.5% FBS for 12 h and incubated in non-FBS fresh medium with 0.1–10 ng/ml IL-1β (recombinant human IL-1β, R&D Systems, Minneapolis, MN, USA) for 0–24 h. For another series of experiments, the chondrocytes were treated with human recombinant PGE_2 (Cayman Chemical, Ann Arbour, MI, USA) at a concentration of 0–1000 ng/ml for 0–24 h.

2.3. Quantitative real-time polymerase chain reaction (PCR) analysis

The mRNA levels of COX-2 and MMP-1, -3, and -13 were examined by quantitative real-time PCR analysis using a LightCycler® system (Roche Diagnostics, Basel, Switzerland) and QuantiTectTM SYBR® Green PCR Master Mix (QIAGEN, Tokyo, Japan). Total RNA was extracted from cultured chondrocytes derived from the dissected mandibular condylar cartilage using Trizol® Reagent (Gibco BRL, Gaithersburg, MD, USA). First-strand cDNA was synthesized from 1 μg of total RNA using Rever Tra Ace-α (Toyobo, Osaka, Japan).

The primer sequences are listed in Table 1. Each signal of the proteins was evaluated in a quantitative manner, relative to the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) signals. Normalized cycle threshold (C_t) values are expressed relative to those of the controls.

2.4. Quantification of PGE_2

The expression level of PGE_2 was quantified using a Prostaglandin E_2 EIA Kit-Monoclonal® (Cayman Chemical) following

Table 1 – The nucleotide sequences of primers for real-time PCR.

Gene	Sequence 5′-*3′
COX-2	Forward: CTT ACT GGA ACA TGG CAT CAC
	Reverse: CTC TGC TCT GGT CGA TTG A
MMP-1	Forward: CTA CAC TTC GGG GAG AAC TA
	Reverse: CGG ACT TCA TCT CTA TCG G
MMP-3	Forward; ACT GGA TTT GCC AAG AAG TG
	Reverse: GCA TAG GCA TGA GCC AAA AC
MMP-13	Forward: AAG CCT TCA AGG TGT GGT CT
	Reverse: GAA AGC GTG AGC CAA CAG A
GAPDH	Forward: TCA TCC CTG CTT CTA CCG
	Reverse:CAG ATC CAC AAC CGA CAC