ANTI-INFLAMMATORY AND ANTI-APOPTOTIC FUNCTIONS OF HEME OXYGENASE-1 IN HUMAN ARTICULAR CHONDROCYTES

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INTRODUCTION:

Heme oxygenase-1 (HO-1) is an enzyme catalyzing physiologic breakdown of heme to biliverdin, CO, and iron, which is induced by oxidative or nitrosative stress, cytokines and other mediators produced during inflammatory processes in a variety of cells including endothelial, vascular smooth muscle cells, basophils, monocytes/macrophages, neutrophils, fibroblasts [1]. Recently it has been shown that HO-1 is present in human osteoarthritic (OA) cartilage [2], however, detailed function of HO-1 in OA chondrocytes is still unknown. The objective of this study was to characterize the HO-1 function in relation to lipopolysaccharide (LPS)-induced inflammation.

METHODS:

Cartilage specimens were obtained from 3 patients with OA undergoing total knee arthroplasty, and chondrocytes were isolated by enzymatic digestion under an approved IRB. Cells were cultured in DMEM/F12 supplemented with 10% FBS, 25 µg/mL ascorbic acid, and 25 µg/mL gentamicin. Serum containing medium was exchanged 24 hours before experiments. Hemin (Sigma), which is an established agent for HO-1 induction, was used in two different concentrations.

Exp.1: Anti-inflammatory effect of HO-1 on human OA chondrocytes. [Fig. 1] Human OA chondrocytes were exposed to either vehicle or low-dose Hemin (H, 1 uM), and high-dose Hemin (HH, 10 uM) prior to addition of LPS (50 ng/mL, 12 hours; Sigma). Total RNA was extracted by RNeasy mini kit (Qiagen), and 300 ng total RNA was amplified with GeneAmp kit (Applied Biosystems). Gene expression levels were quantified by real-time PCR (ABI Prism 7900HT Sequence Detection System, Applied Biosystems), and relative expressions of HO-1, aggrecan, type II collagen, IL-6, iNOS, and MMP-3 were compared with control with GAPDH as an internal control. Experiments were performed three times and the data represent mean±SD.

Exp.2: Anti-apoptotic effect of HO-1 on human OA chondrocytes. [Fig. 2] Human OA chondrocytes were exposed to either vehicle or low-dose Hemin (H, 1 uM), and high-dose Hemin (HH, 10 uM) prior to addition of LPS (50 ng/mL, 12 hours; Sigma). Cell lysates were separated by SDS-PAGE and blotted with anti-active Caspase-3 (Sigma).

Exp.3: Anti-apoptotic effect of HO-1 on human normal chondrocytes. [Fig. 3] For microscopic evaluation, normal human articular chondrocytes from the knee were obtained from Cambrex BioScience, and were seeded on a 0.5mm-thick plasma-treated PET strip. Under serum-free condition, the cells were exposed to either vehicle or low-dose Hemin (H, 1 uM), and high-dose Hemin (HH, 10 uM) prior to addition of LPS (50 ng/mL, 12 hours; Sigma). Each samples was fixed with 5% paraform aldehyde for 30 min at room temperature and stained with Vectashield mounting medium with DAPI (4', 6' Diamidino-2-Phenylindole Dihydrochloride) (VECTOR Laboratories). Number of compaction and fragmentation of the cell were counted under fluorescent microscope, and the apoptosis rate was calculated. Cell count was performed in three different regions on one slide and the experiments were repeated three times.

RESULTS:

Hemin upregulated HO-1 gene expression as a dose-dependent manner, and LPS itself downregulated HO-1. LPS downregulated aggrecan and type II collagen, and high concentration Hemin could reverse that. HO-1 mediated suppression of LPS-activated IL-6, iNOS, and MMP-3 [Fig. 1]. Active form of Caspase-3 which is a marker of apoptosis, was detected in control OA chondrocytes, LPS-treated group, and H+LPS group, while high concentration Hemin significantly reduced this [Fig. 2]. By using fluorescent microscope, chromatin compaction and DNA fragmentation were observed, and apoptotic cells were detected in cultured normal human articular chondrocytes. In LPS group, apoptosis rate was increased up to 69% compared with control. Both of low- and high-concentration Hemin significantly decreased the number of apoptotic cells [Fig. 3].

DISCUSSION:

Cytokines play a key role in chronic inflammatory diseases. While several cytokines including IL-4, IL-10, IL-13, IL-1ra are reported to be classified as anticatabolic / inhibitory [3], the protective mechanism of articular chondrocytes has not yet been clearly understood. In various type of cells, HO-1 can be rapidly induced in response to cytokines, NO and peroxynitrite, ROS, etc, and confers protection against oxidative injury or can lead to anti-inflammatory effects. Recently anti-inflammatory effects of IL-10 in murine macrophages are reported to be mediated by HO-1 [4], which might be the case in chondrocytes. Although detailed mechanisms underlying the action of the HO-1 still remains to be clarified, we have clearly shown in this study that HO-1 acts as anti-inflammatory and anti-apoptotic in human articular chondrocytes.

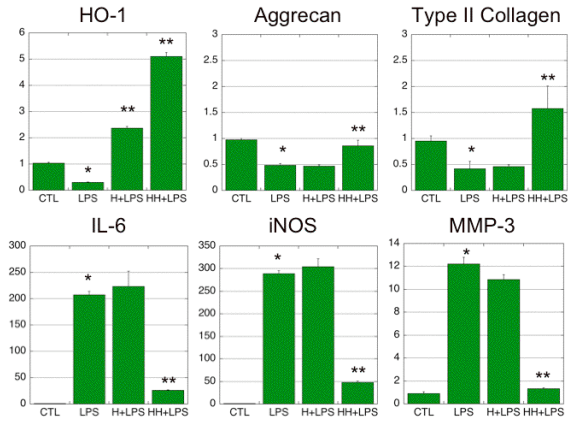

Fig. 1: Anti-inflammatory effect of HO-1 on human OA chondrocytes; Gene expression after LPS treatment. Data are shown as fold differences compared with control. (*: p < .01 vs. CTL, **: p < .01 vs. LPS)

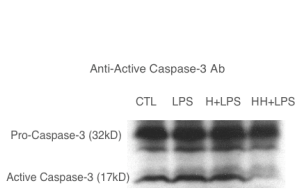

Fig. 2: Anti-Active Caspase-3.

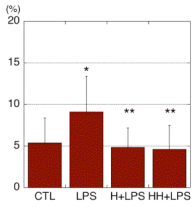

Fig. 3: Apoptosis rate

Western blotting using anti-active Caspase-3, and the apoptosis rate evaluated by fluorescent microscope. (*: p < .01 vs. CTL, **: p < .01 vs. LPS)

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