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Review

Biphasic activation of nuclear factor-kappa B in chondrocyte death induced by interleukin-1beta: The expression of inducible nitric oxide synthase and phagocyte-type NADPH oxidase through immediate and monocarboxylate transporter-1-mediated late-phase activation of nuclear factor-kappa B

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ABSTRACT

Background: Degeneration of articular cartilage including reduced cellularity is often observed in the pathogenesis of osteoarthritis. We investigated nitric oxide (NO·)- and reactive oxygen species (ROS)-dependent cell death induced by interleukin-1β (IL-1β) in mouse chondrocyte-like ATDC5 cells and rat primary chondrocytes in vitro. Increased production of lactate was observed in IL-1β-treated ATDC5 cells before beginning of their death. Cell death was suppressed by introducing small interfering RNA (siRNA) for monocarboxylate transporter-1 (MCT-1), a membrane transporter for lactate and pyruvate distributed in plasma and mitochondrial inner membranes.

Highlight: MCT-1 gene silencing prevented IL-1β-induced expression of phagocyte-type NADPH-oxidase (NOX-2), an enzyme specialized for ROS production; there was no effect on the expression of inducible NO· synthase (iNOS). IL-1β-induced cell death was suppressed by NOX-2 siRNA, indicating involvement of this enzyme in cell death. Although phosphorylation and degradation of inhibitor of κBα (I-κBα) from 5 to 20 min after addition of IL-1β was not affected by MCT-1 siRNA, degradation of I-κBα and nuclear translocation of RelA/p65 observed in control cells from 36 to 48 h after exposure to IL-1β was not seen in MCT-1-silenced cells. Scavenging of ROS suppressed both late-phase I-κBα degradation and NOX-2 expression. MCT-1 siRNA lowered the level of ROS generated after 15 h exposure to IL-1β.

Conclusion: We found that MCT-1 contributed to the expression of NOX-2 via late-phase activation of nuclear factor κB in a ROS-dependent manner in cells exposed to IL-1β. Hence, MCT-1 could be a potential target for the treatment of degenerative joint diseases.

Contents

Abbreviations: ROS, reactive oxygen species; RNS, reactive nitrogen species; IL-1β, interleukin-1β; TNF-αα, tumor necrosis factor-; MCT, monocarboxylate transporter; NOX, NADPH oxidase; NOS, nitric oxide synthase; nNOS, neuronal NOS; iNOS, inducible NOS; eNOS, endothelial NOS; NF-κB, nuclear factor κB; AEBSF, 4-(2-aminoethyl)benzenesulfonyl fluoride; L-NAME, L-N^G-nitro-arginine methyl ester; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; I-κBα, inhibitor of κBα; IKK, I-κB kinase

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1. Introduction

Osteoarthritis, one of the most common diseases to develop in joints, occurs in almost 1 in 2 Japanese adults over the aged of 40. Aging as well as mechanical injury to the articular cartilage causes the onset of osteoarthritis [1,2]. Articular cartilage damage induces inflammation in tendons and/or synovial membranes, which causes exacerbation of degeneration of the cartilage including chondrocyte death, matrix loss, calcification, and osteophyte formation [3]. Swelling and pain in the joints reduce the quality of life of affected patients, and they are mainly given symptomatic treatment, such as exercise, use of technical aids, and administration of medicine including non-steroidal anti-inflammatory drugs, steroids, and hyaluronic acid [4]. Since articular cartilage scarcely regenerates unlike bone tissue, clarification of the mechanisms of pathological changes in osteoarthritis as well as progress in regenerative medicine for articular cartilage damage is anticipated for prevention and better treatment of patients with osteoarthritis.

Involvement of inflammatory cytokines and matrix metalloproteinases, as well as reactive oxygen species (ROS) and reactive nitrogen species (RNS), has been reported by a number of studies on the possible mechanisms of chondrocyte death, cartilage matrix decrease, and osteophyte formation in osteoarthritis [5–7]. Among the various causative factors of the disease, interleukin-1β (IL-1β) is a major inflammatory cytokine known to be involved in the pathogenesis of osteoarthritis [8]. We previously investigated the mechanism of ROS- and RNS-dependent cell death in chondrocytes exposed to IL-1β in vitro [9,10]. Our results showed that the monocarboxylate transporter (MCT)-1, a lactate and pyruvate transporter localized at the plasma membrane and mitochondrial inner membrane [11], is required for ROS- and RNS-dependent chondrocyte death. MCT-1 contributes to the expression of NADPH oxidase (NOX)-2, an enzyme specialized for ROS production, via late-phase activation of nuclear factor κB (NF-κB) in an ROS-dependent manner in chondrocytes exposed to IL-1β [10]. Here, we review our findings along with those of others regarding MCT-1-dependent chondrocyte death and related information.

2. ROS and nitric oxide (NO•) produced in biological systems

ROS is a general term for oxygen-atom-containing molecules that have higher chemical reactivity as compared to molecular oxygen (O_2). Some ROS are free radicals, while others are not. The hydroxyl radical ($HO\cdot$), superoxide anion radical ($O_2^{-}\cdot$) and its protonated form, hydroperoxyl radical ($HOO\cdot$), organic peroxyl radicals ($ROO\cdot$), and alkoxy radicals ($RO\cdot$) are examples of the former, and the $NO\cdot$ radical is also sometimes classified in this group. On the other hand, organic peroxides (ROOH), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$), and others are the latter type of ROS.

It is known that all of the above-mentioned ROS are produced in biological systems [12]. For example, the human body has several types of NOX, which are membrane-bound enzymes specialized for production of $O_2^{-}\cdot$ by reduction of O_2 coupled with oxidation of NADPH to $NADP^+$ [13]. H_2O_2 is produced by either a spontaneous or superoxide dismutase (SOD)-catalyzed dismutation reaction of $O_2^{-}\cdot$ [14]. In addition, $O_2^{-}\cdot$ and H_2O_2 are usually formed in mitochondria as a result of incomplete transport of electrons along the electron transport chain [15]. It is considered that $HO\cdot$, one of the most reactive and short-lived ROS, is mainly formed by Fenton's reaction, the iron- or copper-catalyzed reduction of H_2O_2 [$H_2O_2+Fe^{2+}$ (Cu^+)$\rightarrow HO\cdot+OH^-+Fe^{3+}$ (Cu^{2+})] [16,17]. $ROO\cdot$ such as lipid peroxyl radicals are formed through abstraction of the hydrogen atom ($H\cdot$ radical) from lipids by $OH\cdot$ ($HO\cdot+R\text{-}H\rightarrow H_2O+R\cdot$) and subsequent addition of O_2 to carbon-centered radicals ($R\cdot+O_2\rightarrow ROO\cdot$) [18], which leads to various chain reactions including that of $ROO\cdot$ with RH to produce ROOH and $R\cdot$ and decomposition of ROOH into $RO\cdot$ and $HO\cdot$. It is well known that neutrophils express myeloperoxidase, which catalyzes the reaction between H_2O_2 and the chloride anion (Cl^-) to produce HOCl ($H_2O_2+Cl^-\rightarrow HOCl+OH^-$) [19]. Photo-excitation of some biological molecules such as protoporphyrin and its subsequent return to ground state causes activation of O_2 to $^1O_2$, which is known to induce photohypersensitivity in the skin [20,21].

A biologically important system responsible for $NO\cdot$ production is comprised of the nitric oxide synthase (NOS) family enzymes that catalyze the formation of $NO\cdot$ using L-arginine as the substrate (L-arginine$+2O_2+3/2NADPH+3/2\ H^+\rightarrow$L-citrulline$+2H_2O+3/2NADP^+ +NO\cdot$). There are three types of NOS, namely neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3). Both nNOS and eNOS are constitutively expressed enzymes, whereas the expression of iNOS is induced under inflammatory conditions. While production of $NO\cdot$ by nNOS and eNOS is regulated by change in intracellular calcium concentration, iNOS lacks the same regulatory mechanism. The amount of $NO\cdot$ production by iNOS mainly depends on the amount of iNOS protein expressed, which causes prolonged production of $NO\cdot$ by cells expressing iNOS protein. It is known that activation of NF-κB is critical for transactivation of the iNOS gene, and the Jak-Stat-IRF pathway is also regarded as an important trigger of iNOS expression [22–24]. Hence, various types of cells express iNOS and produce $NO\cdot$ in response to stimulation by inflammatory cytokines in combination with that by type 1 or type 2 interferon [25,26]. In contrast, a characteristic of chondrocytes is the expression of iNOS after a single stimulation by IL-1β or tumor necrosis factor-α (TNF-α) [27]. Thus, chondrocytes are considered to be unique cells ready for the expression of iNOS and production of $NO\cdot$ in response to elevation of these pro-inflammatory cytokines in their milieu.

3. Simultaneous production of NO• and ROS is required for chondrocyte death induced by inflammatory cytokines

As described above, chondrocytes readily express iNOS and produce $NO\cdot$ after a single stimulation by IL-1β or TNF-α [27], which is thought to be the basic condition for inflammatory degeneration of cartilage. However, $NO\cdot$ itself is not highly cytotoxic. On the other hand, peroxynitrite ($ONOO^-$), a ROS/RNS formed by the radical–radical coupling reaction of $NO\cdot$ and $O_2\cdot^-$ radicals, is one of the most powerful initiator of $NO\cdot$-dependent cytotoxic effects [28]. $ONOO^-$ cytotoxicity is considered to be based on its chemical reactivity, as it oxidizes and nitrates various biological molecules [29]. Our group reported that