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Review Article

HNE as an inducer of COX-2

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ABSTRACT

Cyclooxygenase-2 (COX-2), an inducible isoform responsible for high levels of prostaglandin (PG) production during inflammation and immune responses, mediate a variety of biological actions involved in vascular pathophysiology. COX-2 is induced by various stimuli, including proinflammatory cytokines, to result in PG synthesis associated with inflammation and carcinogenesis. 4-Hydroxy-2-nonenal (HNE) is one of a group of small molecules that can induce COX-2 expression. The mechanistic studies have revealed that the HNE-induced COX-2 expression results from the stabilization of COX-2 mRNA mediated by the p38 mitogen-activated protein kinase signaling pathway and uniquely requires a serum component, which is eventually identified to be modified low-density lipoproteins (LDLs), such as the oxidized form of LDLs. It has also been shown that HNE-induced COX-2 expression is mechanistically linked to the expression of transcription factor p53 and that the overexpression of COX-2 is associated with down-regulation of a proteasome subunit, leading to the enhanced accumulation of p53 and ubiquitinated proteins and to the enhanced sensitivity toward HNE. Thus, the overall mechanism and pathophysiological role of the COX-2 induction by HNE have become increasingly evident.

1. Introduction

Atherosclerosis is a disorder of the lipid metabolism as well as a chronic inflammatory disease. Monocyte-derived macrophages play a prominent role in the formation and progression of atherosclerotic plaque, particularly after their transformation into foam cells. When activated by inflammatory stimuli, the macrophages synthesize and secrete various mediators, which cause the clinical manifestations and acute clinical complications of atherosclerosis. The eicosanoids derived from the metabolism of arachidonate, among those mediators, have been extensively investigated because several studies have focused on their close relation to atherogenesis.

Cyclooxygense (COX) is a key enzyme catalyzing the rate-limiting step that converts free arachidonic acid to prostaglandin (PG) H_2 on the arachidonic cascade [1]. COX exists in two distinct isozymes (COX-1 and COX-2), one of which, COX-2, is primarily responsible for inflammation [2]. COX-2 is not normally present under the basal conditions or is present in very low amounts; however, it is rapidly induced in response to a wide variety of cytokines, growth factors, and ligands of G protein-coupled receptors. COX-2 is responsible for high levels of PG production during inflammation and immune responses and mediates a variety of biological actions involved in vascular pathophysiology. The induction of the COX-2 gene expression is regulated at both transcriptional (promoter-based) and post-transcriptional levels [3–5]. Both mitogen-activated protein kinase (MAPK) and nuclear factor-kB (NF-kB) signaling pathways have been shown to mediate the COX-2 gene expression [6].

There is considerable evidence that low-density lipoproteins (LDLs) is oxidatively modified in vivo, and that this modification results in an increase in its proinflammatory and proatherogenic properties. Several oxidized fatty acids generated in the oxidized LDLs have been shown to play a role as important signaling molecules in the context of the atherosclerotic lesion. In this article, I will illustrate a comprehensive summary of our studies on the activation of pro-inflammatory signaling mechanisms by one of the most studied products of lipid peroxidation, 4-hydroxy-2-nonenal (HNE).

2. Identification of HNE as an inducer of COX-2

In view of the observation that liver injury associated with oxidative stress is accompanied by increased PG synthesis, it is hypothesized that lipid peroxidation products may be involved in the up-regulation of the PG biosynthesis. Indeed, in an alcohol-fed rat, a model of alcoholic liver disease, alcohol over-intake increases the formation of HNE-modified proteins and is associated with the COX-2 and proinflammatory cytokine TNF-α expression [7,8]. In addition, the HNE-specific epitopes have been detected in foamy macrophages within human atheromatous lesions [9] where the pro-inflammatory responses, including COX-2 expression, are being accelerated. To determine if lipid peroxidation could be involved in the COX-2 expression, Kumagai et al. [10]

Abbreviations: COX, cyclooxygenase; HNE4, hydroxy-2-nonenal; LDLs, low-density lipoproteins; MAPK, mitogen-activated protein kinase; NF-kB, nuclear factor-kB

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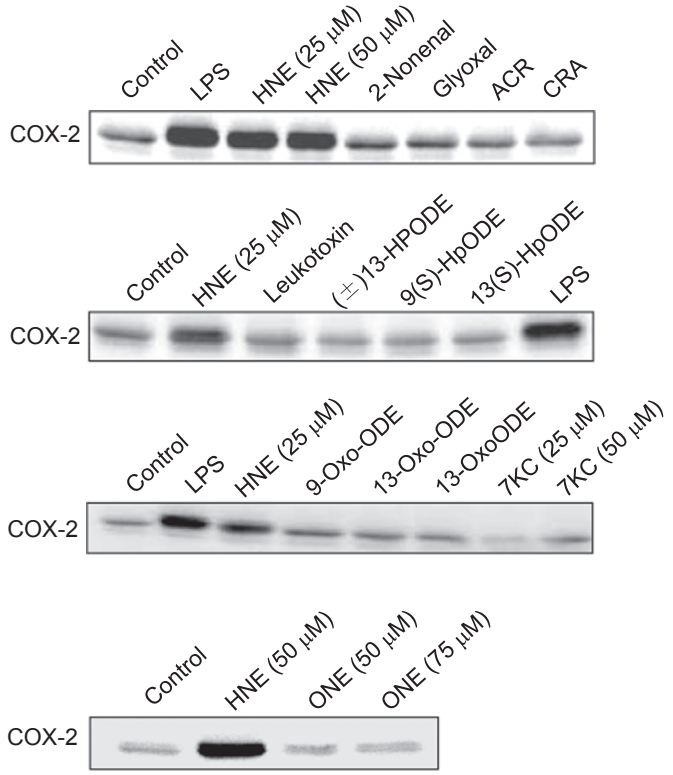

Fig. 1. Identification of HNE as the most active inducer of COX-2 in RAW264.7 macrophages (adapted from ref. [13]). RAW264.7 macrophages were treated for 6 h with 50 μM of the indicated compounds and COX-2 induction was examined by an immunoblot analysis. *Abbreviations*: ACR, acrolein; CRA, crotonaldehyde; ONE, 4-oxo-2-nonenal; 9(R)-HODE, 9R-hydroxy-10E,12Z-octadecadienoic acid; (+)13-HODE, (+)13-hydroxy-9Z,11E,-octadecadienoic acid; 9(S)-HpODE, 9S-hydroperoxy-10E,12Z-octadecadienoic acid; 13(S)-HpODE, 13S-hydroperoxy-9Z,11E-octadecadienoic acid; 9-OxoODE, 9-oxo-10E,12Z-octadecadienoic acid; 13-OxoODE, 13-oxo-9Z,11E-octadecadienoic acid; 7KC, 7-ketocholesterol; (+)13-HODE cholesteryl ester, (+)13-hydroxy-9Z,11E-octadecadienoic acid cholesteryl ester; Leukotoxin, (+)9(10)epoxy-12Z-octadecenoic acid.

conducted a screen of oxidized fatty acids on COX-2 induction in rat liver epithelial RL34 and mouse macrophage RAW264.7 cell lines and demonstrated that HNE could specifically stimulate the COX-2 expression (Fig. 1). They have also shown that the depletion of the GSH pools in the cells with L-buthionine-*S,R*-sulfoximine significantly reduced the HNE-induced expression of COX-2 whereas the *N*-acetylcysteine pretreatment reversely led to a dose-dependent enhancement of the COX-2 expression [10]. These findings suggest the intracellular GSH status may be strictly related to the HNE-induced COX-2 expression. Of interest, they also observed that the α,β-unsaturated aldehydes, such as acrolein, crotonaldehyde, and 2-nonenal, possessing an analogous functionality to HNE, were all inactive on the COX-2 induction. These studies represent a first demonstration of a link between COX-2 and HNE.

3. Involvement of p38 MAPK pathway

The NF-κB signal transduction cascade is a major stress response signaling pathway for the COX-2 gene expression. In mice and humans, the COX-2 promoter has binding sites for many transcription factors, including NF-κB in the 5′ region of the COX-2 gene [11], and the requirement of the activation of NF-κB to induce the expression of COX-2 in the lipopolysaccharide-stimulated macrophages has been described [6]. Based on the discovery of HNE as a potential inducer of COX-2, several studies focusing on the HNE-induced signaling mechanisms for the COX-2 expression have been performed [12,13]. Initially, it was anticipated that the NF-κB-dependent signaling pathway might mediate the HNE-stimulated COX-2 induction. However, no significant change

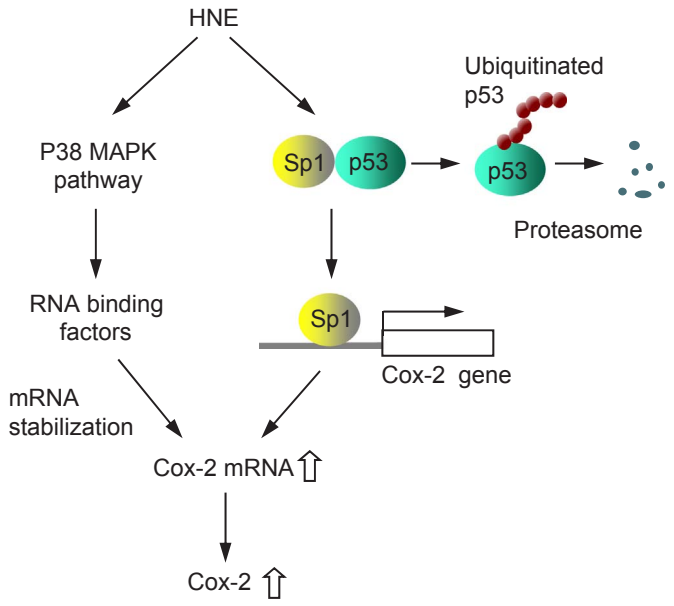

Fig. 2. Model for mechanisms by which HNE up-regulates COX-2 (adapted from ref. [14]). HNE stabilizes COX-2 mRNA through the p38 MAPK signaling pathway, leading to the up-regulation of COX-2. On the other hand, the present work suggests an alternative mechanism, by which HNE induces COX-2 gene expression through down-regulation of p53 followed by the activation of Sp1.

in the IκB and NF-κB levels after treatment with HNE was observed. Kumagai et al. [12,13] found that, instead of the NF-κB pathway, HNE elicited a rapid and significant phosphorylation of p38 mitogen-activated protein kinase (MAPK) and activate MAPK kinase (MKK)3/MKK6, a specific MAPKK of p38 MAPK. In addition, the relationship between COX-2 mRNA stability and HNE-activated p38 MAPK pathway was also revealed (Fig. 2). Involvement of a Src-dependent p38, ERK/c-Jun pathway was recently proposed as a major regulator of HNE-induced COX-2 expression in YPEN-1 cells [14]. Thus, the HNE-induced COX-2 gene expression is, at least in part, regulated at post-transcriptional levels via the p38 MAPK pathway.

4. Involvement of p53 and Sp1

To investigate transcriptional regulation of the COX-2 gene in response to HNE, Kumagai et al. [15] examined whether the HNE-induced COX-2 expression was mechanistically linked to the expression of p53, a transcription factor that regulates the response to a variety of stimuli, and found that the COX-2 levels were inversely correlated with the p53 levels. In addition, the down-regulation of p53 with the antisense oligonucleotides against p53 significantly enhanced the expression of COX-2 mRNA and protein. These findings and the fact that COX-2 protein is undetectable in normal epithelial cells suggest that mutations of p53 may contribute to the increased expression of COX-2. Of interest, 4-oxo-2-nonenal, an analog of HNE, is unable to induce COX-2 while it activates a p53 signaling pathway [16].

On the other hand, it was hypothesized that Sp1, a general transcription factor that is involved in various inducible and constitutive gene expressions, might also be involved in the induction of COX-2 in response to HNE [15]. This speculation was based on the facts that (i) p53 suppresses various gene expressions through preventing Sp1 activity, (ii) The rat COX-2 promoter region has no putative p53-binding elements, and a minimal promoter region required for the basal transcription of the human COX-2 gene has been demonstrated to contain GC-rich proximal sequences that are specifically bound by Sp1, and (iii) p53 negatively regulates Sp1 through the formation of a p53-Sp1 heterocomplex. The immunoprecipitation experiments indeed showed that p53 bound to Sp1 in intact cells under normal conditions