

## What is the Role of Lipid Peroxidation Product 4-hydroxynonenal in Inflammation?

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Inflammation is a complex system of host systemic and local responses to injury and infection. Inflammation contributes to almost all disease processes, including immunological and vascular complications, sepsis, cancer and metabolic injury. During Gram negative bacterial infections, lipopolysaccharide (LPS), a highly pro-inflammatory endotoxin is released from the surface of replicating Gram-negative bacteria into the circulation, where it is recognized by a variety of circulating cell types including macrophages, triggering gene induction of pro-inflammatory cytokines, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 (IL-1), and biosynthesis of prostaglandins (PGE2). These and other cytokines act in an autocrine or paracrine manner to induce and amplify the host cell response and defense systems that help to eliminate the bacterial infection. However, uncontrolled and excessive cytokine expression can induce acute or chronic inflammatory processes. It is well established that increased expression of cytokines elicits the cytotoxic actions in many inflammatory diseases. Moreover, cytokines play a critical role in several cardiovascular and neurological degenerative diseases as well as cancer. Hence elucidation of the mechanisms that mediate and regulate cytokine signals is of profound importance to understanding and managing a very large array of disease processes. Further, recent studies have provided important links between elevated cytokines such as TNF- $\alpha$  and IL-1 with oxidative stress during initial inflammatory processes [1,2], and have shown to alter redox equilibrium through a thiol-dependent mechanism [3]. Interestingly, antioxidants have been shown to down-regulate cytokine transcription and biosynthesis. Conversely, increased oxidative stress, e.g., depletion of GSH, can augment pro-inflammatory signals by up-regulating Reactive Oxygen Species (ROS). Hence, levels of reduced glutathione are a critical determinant of ROS signaling.

Under physiological conditions, there is a balance between generation of ROS and their removal by antioxidant systems. In general, oxidative stress occurs when this balance is disrupted, either directly by infectious agents or by cytokines released from inflamed cells that may lead to increased ROS generation and/or decreased antioxidant defense. Normally, ROS are involved in some of the essential cellular functions such as host cell defense, mitochondrial respiration, cytokine generation and cell proliferation/apoptosis. There are several potential sources of ROS in inflammation, one of which is the activation of NADPH oxidase in phagocytes, monocytes and most other inflammatory cell types. In fact, activation of NADPH oxidase has been observed upon exposure of various cell types to cytokines, growth factors and hyperglycemia [4]. Thus, NADPH oxidase is a key player in signal transduction. The NADPH oxidase (a membrane-bound holoenzyme with different flavocytochrome subunits) catalyzes the one electron reduction of oxygen, using NADPH or NADH as the electron donor and leading to the production of superoxide anions.

The superoxide anions thus generated can form other ROS (such as hydroxyl radicals and hydrogen peroxide) and cause tissue injury and alter gene expression. Several investigators have shown in multiple cell types that NADPH oxidase, rather than xanthine oxidase and other mitochondrial enzymes, is the main source of ROS [5,6]. LPS and various inflammatory cytokines such as TNF- $\alpha$ , IL-1 and IL-6

can activate NADPH oxidase to generate significant, sometimes toxic, amounts of ROS (initially  $O_2^{\cdot-}$ ) which propagate their signals that activate transcription factors. Besides cytokines, growth factors and hyperglycemia can also activate NADPH oxidase to propagate their signals. This is supported by the observations that cytokines, growth factors and hyperglycemic signals are interrupted by such antioxidants as N-acetyl cysteine and  $\alpha$ -tocopherol and glutathione (GSH), which can attenuate inflammation [7]. Therefore, when oxidants overwhelm the antioxidative capacity, lipid peroxides and toxic lipid-derived aldehydes (LDAs) such as 4-hydroxy-trans-2-nonenal (HNE) are formed by the process called lipid peroxidation. Lipid peroxidation is known to be a causative factor that contributes to the pathophysiology of inflammation [8]. The process of lipid peroxidation can be initiated by a variety of oxidants, including  $H_2O_2$ , superoxide and the highly reactive hydroxyl radicals. Lipid peroxidation can alter vital membrane protein structure and function, and if it proceeds unchecked could lead to cell death. Free radicals and lipid peroxides and their metabolites can also oxidize proteins and DNA within the cells, while extracellular free radical release can initiate damage to neighboring cells. Furthermore, some lipid peroxidation products may stimulate leukocyte recruitment to the site of inflammation.

The most active and abundant aldehyde generated during this process is HNE, which is largely responsible for pathogenesis during oxidative stress [9,10]. This aldehyde is highly reactive towards free sulfhydryl groups of proteins by generating thioether adduct that undergo further cyclization and hemiacetal formation. HNE also reacts with histidine and lysine residues of proteins to form stable Michael adduct [7]. This aldehyde induces heat shock proteins, inhibits cellular proliferation, and is highly toxic to cells. However, we and other investigators have shown that low concentrations of HNE stimulate proliferation of vascular smooth muscle cells, hepatic stellate cells and colon cancer cells, whereas HNE is apoptotic in endothelial cells and lens epithelial cells [11-13]. At high concentrations, HNE displays a variety of genotoxic and mutagenic effects [14]. Cytokines, growth factors and hyperglycemia are known to increase lipid peroxidation and generate HNE. Recent studies identified HNE as is one of the most potent physiological regulators of transcription factors such as NF- $\kappa$ B and AP1 which mediate inflammation, cellular immune response, influences a plethora of cellular functions and affects the functions of virtually every cell type [14].

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The ROS-sensitive transcription factor NF- $\kappa$ B is a critical inflammatory mediator. When inactive, it is sequestered in the cytosol in a complex with its inhibitor, I $\kappa$ B and stimulation of protein kinases such as PKC, MAPK, and IKK results in the activation of NF- $\kappa$ B via the phosphorylation of I $\kappa$ B. Several studies have shown that low concentrations of HNE activate NF- $\kappa$ B while high concentrations inhibit NF- $\kappa$ B, but the mechanisms are not clearly understood [15]. The other major redox-sensitive transcription factor, AP1, is formed by homo- or heterodimerization of members of the Jun and Fos families of proteins; ROS can regulate AP1 activity via several mechanisms. AP1 can be regulated via the c-Jun N-terminal kinase (JNK) cascade; JNKs are part of the mitogen-activated protein kinase (MAPK) superfamily of serine/threonine kinases that also includes the extracellular signal-regulated kinases ERK1/2 and p38MAPK. All MAPKs are activated via a cascade of phosphorylation reactions. The activation of NF- $\kappa$ B and AP1 during bacterial infections leads to increased synthesis of several proinflammatory cytokines and chemokines, and the biosynthesis of PGE2 by cyclooxygenase (COX) from arachidonic acid. However, the relationship of oxidative stress to the increased release of cytokines and chemokines and activation of COX-2 during inflammation remains unknown. One of the molecules involved in these processes may be HNE, which is generated by ROS-induced lipid peroxidation. It has been shown that HNE is cytotoxic, and elevated levels of HNE have been reported in various disease conditions. Indeed, HNE can be detected in inflammatory exudates, and increased HNE-proteins adducts were observed during LPS-induced apoptosis of placental cells and other inflammatory processes. HNE has been shown to increase the LPS-induced production of cytokines such as IL-1, IL-6 and TNF- $\alpha$  in human blood mononuclear cells. However, it is not clear how the lipid peroxidation-derived toxic aldehydes (such as HNE and its conjugate with GSH, GS-HNE) are involved in the inflammatory process. Because polyol pathway enzyme aldose reductase (AR) has been shown to reduce HNE and GS-HNE with a  $K_m$  of 10-30  $\mu$ M, it can be expected that AR is a key determinant of the cellular redox state and that inhibition of this Fenzyme will interrupt ROS signaling by increasing oxidative stress [16]. It is well known that the activation of PKC in response to growth factors, cytokines or environmental stress leads to cell hypertrophy, proliferation, migration, cell growth, or apoptosis. The PKC isozymes are activated by many extracellular signals, including HNE; these enzymes modify the activities of multiple effectors, such as cytoskeletal proteins, MAPKs, and transcription factors. Several lines of evidence suggest that PKC activation by HNE and related oxidants may be a cause of inflammation; however, it is not known which of the PKC isozyme(s) are responsible for inflammation, and how HNE regulates their function.

One of the most intriguing and still unsolved questions is how intracellular HNE levels might play a significant role in disease development, particularly in regulating an inflammatory response to bacterial infection and/or other challenges. Elucidation of the role of HNE in inflammation is an intimidating task, as it involves tight regulation and interaction of many cell types and signaling cascades, and injury and disease can result from chronic or overwhelming inflammation. Our recent publications clearly indicate that AR plays an important role in cell growth and differentiation and cytotoxicity [17]. The question before us is how AR could be involved in the redox metabolism that regulates cytotoxicity? We propose that one of the mechanisms by which AR regulates the cellular redox state could be via AR/NADPH-catalyzed reduction of lipid aldehydes and their conjugates with GSH. Indeed, our recent studies indicate that AR catalyzed GS-HNE reduced product GS-DHN could be signaling molecule [15].

This conclusion was drawn based on the observation that inhibition of AR prevents HNE and GS-HNE induced cytotoxic signals but not GS-DHN induced. However, more detailed studies are required to understand how HNE-glutathione conjugates GS-HNE and GS-DHN regulate various inflammatory signals. This observation has opened up a wide area of research to understand: (1) the role of lipid peroxidation, lipid aldehydes formation, and conjugation with GSH and reduction in the initiation and/or propagation of cell growth and differentiation signals; and (2) how AR that forms GS-DHN could mediate growth factor/cytokine signaling. Lipid peroxidation influences the extent of cell growth and apoptosis in various cells. Although the precise mechanism(s) of cell growth and differentiation are not known, based upon recent studies, it seems like intracellular concentration of HNE is a key event in this process. Hence, the biochemical mechanisms that maintain the intracellular levels of HNE are necessary to investigate in depth for understanding the pathophysiology of a number of disease processes. Effective wide range anti-oxidant therapies with strong bioavailability and potency are required to control the localized HNE levels that regulate various inflammatory processes. Although, enzymes that regulate intracellular levels of HNE such as GST, AR and ALDH1 have been shown to regulate inflammatory conditions and inhibition of these enzymes specifically AR has shown promising effects in targeting cytokines and various other oxidants-mediated cellular alterations *in vitro* and *in vivo* models of inflammatory pathologies, constructive pre-clinical and clinical studies need to be performed to validate these approaches as clinical therapies.

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