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Cellular Response to Bioactive Lipid Peroxidation Products

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Reactive aldehydes, such as 4-hydroxy-2-nonenal, have been implicated as inducers in generating intracellular reactive oxygen species and activation of stress signaling pathways, that integrate with other signaling pathways to control cellular responses to the extracellular stimuli. Here, I briefly summarize a novel signaling pathway in cellular response, in which aldehyde-stimulated detoxification response is mediated by cyclooxygenase metabolites. These findings argue that lipid mediators could induce a cellular process that represents a cellular defense program against toxic compounds.

Keywords: Reactive aldehydes, Lipid peroxidation, Prostaglandins, Cyclooxygenase, Detoxification enzymes

I dedicate this article to Professor Etsuo Niki on the occasion of his 60 ys birthday and retirement from the University of Tokyo.

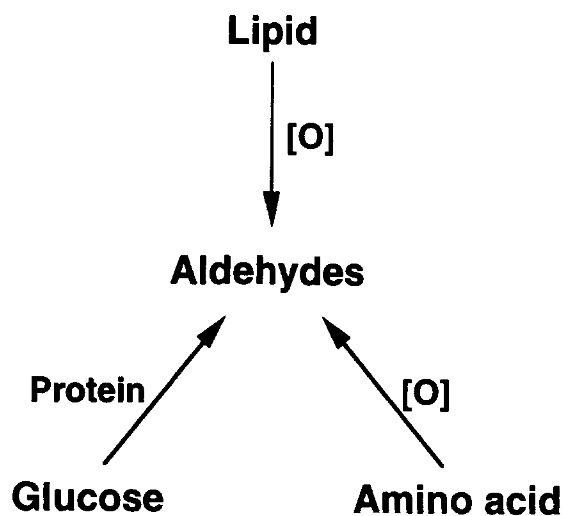

FIGURE 1 Potential sources of reactive aldehydes associated with oxidative stress

INTRODUCTION

A growing body of evidence suggests that the pathogenesis of many diseases are mediated by products of non-enzymatic reactions, such as the peroxidative degradation of polyunsaturated fatty acids ("lipid peroxidation") and glucose-protein or glucose-lipid interactions ("glycation"), as well as the oxidative modification of amino acids ("amino acid oxidation") (Fig. 1). These reactions lead to the formation of unstable, reactive aldehydic intermediates that readily form intra- and intermolecular covalent adducts

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with various biomolecules, such as proteins and phospholipids. Lipid peroxidation proceeds via a free radical chain reaction mechanism and yields lipid hydroperoxides as the major initial reaction products. The subsequent decomposition of lipid hydroperoxides generates a number of breakdown products that can undergo a wide variety of damaging actions. A number of reactive aldehydes derived from lipid peroxidation have been implicated as causative agents in cytotoxic processes, which are initiated by the exposure of biological systems to oxidizing agents[1]. Compared to free radicals, aldehydes are stable and can diffuse within or even escape from the cell and attack targets which are far from the site of the original event. Therefore, they are not only end products and remnants of lipid peroxidation and glycation processes, but also may act as "second cytotoxic messengers" for the primary reactions. Some of these aldehydes have been shown to exhibit a high level of reactivity with various biomolecules, including proteins, DNA, and phospholipids, generating stable products at the end of a series of reactions, that are thought to contribute to the pathogenesis of various diseases. In addition, it has recently been found that some of the aldehydes are responsible for the effects of lipid peroxidation and glycation on signaling / transcription regulation.

INTRACELLULAR OXIDATIVE STRESS INDUCED BY REACTIVE ALDEHYDES

Intracellular oxidative stress is increasingly seen as a major upstream component in the signaling cascade, which is involved in a variety of cellular functions, such as cell proliferation, inflammatory responses, stimulating adhesion molecule, and chemoattractant production. A number of agents appear to be potential sources of intracellular oxidative stress. We have recently shown that lipid peroxidation-derived aldehydes, such as 4-hydroxy-2-nonenal (HNE), induce the intracellular production of reactive oxygen species in cultured hepatocytes (Fig. 2)[2]. This finding is consistent with previous studies which show that HNE itself induces lipid peroxidation, as indicated by increased levels of MDA[3]. It is therefore likely that reactive aldehydes tend to trigger the formation of reactive oxygen species or are oxidants by themselves, thus potentiating oxidative stress in the cells. Excess oxidative stress is toxic, exerting cytostatic effects, causing membrane damage, and activating the pathways of cell death (apoptosis and / or necrosis). In addition, it has been suggested that some level of oxidative stress may be required in response to cytotoxic agents and that it is converted into the redox regulatory system as a downstream signaling pathway.

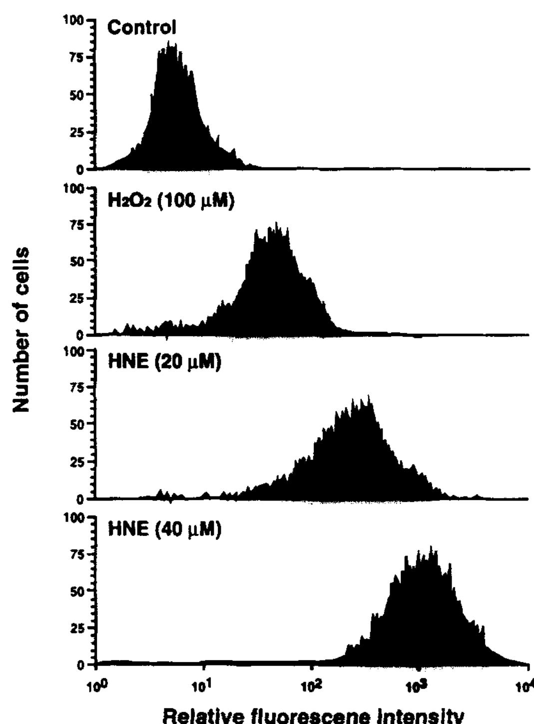

FIGURE 2 Changes in intracellular peroxide levels after exposure of RL34 cells to exogenous H_2O_2 or HNE

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ACTIVATION OF STRESS SIGNALING PATHWAY

One potential target for the intracellular oxidative stress may be the mitogen-activated protein kinase (MAPK) family. MAPKs, which are characterized as proline-directed serine/threonine kinases, are important cellular signaling components that convert various extracellular signals into intracellular responses through serial phosphorylation cascades[4]. At the present time, three distinct but parallel MAPK cascades, namely the extracellular-signal regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38, have been identified in mammalian cells[5]. Both JNK and p38 are characterized by their strong response to cellular stresses, such as UV light, osmotic stress, DNA-damaging drugs, and proinflammatory cytokines. Targets of these cascades include several transcription factors, including c-Jun, which become activated after exposure to cellular stresses. We found that the HNE treatment of the cells results in the depletion of intracellular GSH and in the formation of protein-bound HNE in plasma membranes[2]. A potent expression of c-Jun level occurred within 30 min of HNE treatment, which was accompanied by a time-dependent increase in activator protein-1 (AP-1) DNA binding activity. In addition, HNE caused an immediate increase in tyrosine phosphorylation in RL34 cells and strongly induced the phosphorylation of c-Jun N-terminal kinases (JNK) and p38 mitogen-activated protein kinases (MAP kinases). The phosphorylation of JNK was accompanied by a rapid and transient increase in JNK and p38 activities, whereas changes in the activity of ERK were negligible. It has thus become apparent that some of the reactive aldehydes, including HNE, could be potential inducers of this stress signaling pathway[2,6] and involved in the activation of AP-1[2,6–8]. Being the major component of the AP-1, c-Jun, once activated, may subsequently activate the transcription of several genes.

HNE-INDUCED GENE EXPRESSION OF COX-2 AND GST

It has been proposed that the activation of the stress signaling pathway may be an important mechanism for controling the genetic program which cells use to actively and immediately respond to various extracellular stimuli. Such a process involves the induction of proteins that are ultimately responsible for the detoxification of cytotoxic xenobiotics. Based on the observations that oxidative stress results in the up-regulation of cyclooxygenase-2 (COX-2), the rate-limiting enzyme in the production of prostaglandins and thromboxanes (Fig. 3), we evaluated the effect of oxidized lipids on COX-2 induction and identified HNE as the potential inducer of COX-2. In addition, the HNE-induced COX-2 expression was accompanied by the induction of glutathione S-transferase (GST) expression, while the HNE-induced COX-2 expression preceded that of GST, suggesting the existence of correlation between COX-2 and detoxification enzyme inductions. Indeed, the COX-2 inhibitors significantly suppressed the HNE-induced expression of GST, whereas the COX metabolite (PGD2) markedly up-regulated the enzyme. Several possible mechanisms exist for explaining the link between COX-2 and GST. Both COX-2 and GST are known to be up-regulated in transformed cells and various forms of cancer[9–13], and enhanced PG synthesis in a variety of tumors has also been reported[14,15]. Moreover, both enzymes are known to play a role in the inhibition of free radical-mediated oxidative damage[16]. It seems therefore likely that the elevated synthesis of COX-2 and GST may be required to prevent toxic compounds from accumulating in the cells and to inhibit oxidative injury.

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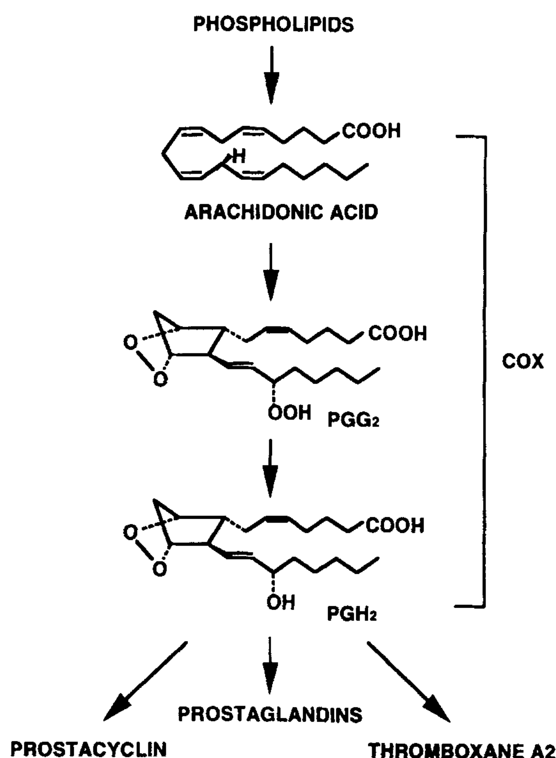

FIGURE 3 The mediation of arachidonic acid metabolism by COX

INDUCTION OF GSTP1 GENE EXPRESSION BY CYCLOPENTENONE PGS

On the other hand, we observed that PGD2 was readily converted to the cyclopentenone-type PGs of J series (Fig. 4), including PGJ_2 and 15-deoxy-$\Delta^{12,14}$-PGJ_2, which significantly induced GST expression *in vivo*. In addition, we screened diverse chemical agents relative to the induction of GST activity and found that, among the class of PGs examined, the PGs of the A and J series, which react enzymatically or nonenzymatically with thiols such as GSH, represent potential GST inducers[17]. It was found that 15d-PGJ_2 induced a drastic elevation of the mRNA level of Class π GST isozyme (GSTP1)[17]. We also were able to show that 15d-PGJ_2 stimulated the promoter activity of the 5′-flanking region of the GSTP1 gene and then induced GST mRNA and protein in RL34 cells[17]. The stimulation required the specific region which contained the GPEI which specifically responded to 15d-PGJ_2 activation. The GPEI was first identified as one of the multiple *cis*-regulatory DNA elements of the rat GSTP1[18]. The importance of this enhancer for the specific expression of the gene in early hepatocarcinogenesis has been established, using transgenic rats[19]. It has also been shown that the enhancer of GSTP1 expression is regulated by multiple factors, including AP-1 which is known to be a heterodimer composed of the products of the *jun* and *fos* oncogenes[20]. Indeed, we observed that c-Jun is involved in the formation of nuclear proteins-GPEI complexes induced by 15d-PGJ_2[17]. c-Jun is a member of a multiprotein family that has been implicated in a number of signal transduction pathways associated with cellular growth, differentiation, neuronal excitation, and cellular stress[21], Based on the fact that c-Jun is required for cellular defense against toxicity[22], it is possible that c-Jun functions as an important component that activates GPEI, followed by the expression of GSTP1. Whereas, another candidate of *trans*-acting factor(s) for the induction of GST and other Phase II enzymes has been recently identified. Venugopal and Jaiswal[23] have reported that the transcription factor Nrf2 positively regulates the ARE-mediated expression of Phase II detoxification enzyme genes. Itoh *et al.*[24] have also shown by gene-targeted disruption in mice that Nrf2 is a general regulator of the Phase II enzyme genes in response to electrophiles and reactive oxygens. More recently, the general regulatory mechanism which underlies the electrophile counterattack response has been demonstrated, in which electrophilic agents alter the interaction of Nrf2 with its repressor protein (Keap1), thereby liberating Nrf2 activity from repression by Keap1, culminating in the induction of the Phase II enzyme genes and antioxidative stress protein genes *via* AREs[25].

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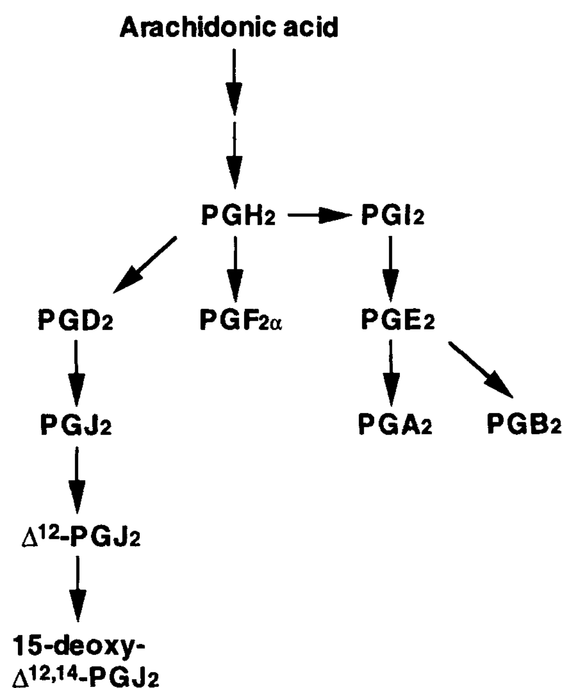

FIGURE 4 The prostaglandin biosynthetic pathway

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CONCLUSION

Reactive aldehydes are ubiquitously distributed in the environment. In addition to their use in the chemical industry and their occurrence as environmental pollutants, aldehydes are components of food, intermediates in mammalian metabolism, and endogenous products generated form lipid peroxidation, glycation, and amino acid oxidation reactions. They react with a variety of nucleophilic sites in DNA and protein, generating various types of adducts which represent putative markers of oxidative stress. The relative amount of adducts varies with the type of reactive aldehyde; it is largely determined by the yield and reactivity of aldehydes and the stability of the adducts. On the other hand, reactive aldehydes, once formed or activated, induce diverse aspects of severe cellular stress, including peroxide formation (oxidative stress), chromosomal aberrations, sister chromatid exchanges, point mutations, and cell killing. They can induce a specific program of gene expression, known as the cellular stress response, which is commonly activated by a variety of environmental cues, such as chemical carcinogens and UV irradiation. This program includes the activation of transcription factors, such as c-*fos* and c-*jun*, whose gene products have been proposed as being required for cellular defense against genotoxic agents. The induction of MAPKs followed by the activation of transcription factors by reactive aldehydes may, therefore, be an important mechanism for controling the genetic program which cells use to actively and immediately respond to environmental cues, via the induction of proteins that are ultimately responsible for the detoxification of cytotoxic aldehydes. As a part of the cellular response, HNE has been observed to induce COX-2 and GST gene expressions. Moreover, it has been shown that the GST induction by HNE is mediated by COX metabolites, PGs. The fact that cyclopentenone PGs represent the most potent inducers of GST suggests a potential mechanism underlying the electrophile-induced upregulation of a detoxification enzyme, in which the COX-2 induction, followed by enhanced PG synthesis, leads to the up-regulation of the detoxification enzyme gene expression via the production of the cyclopentenone PGs as the sources of intracellular oxidative stimuli (Fig. 5). Our findings suggest the presence of a novel signaling pathway in detoxification response mediated by locally produced lipid mediators.

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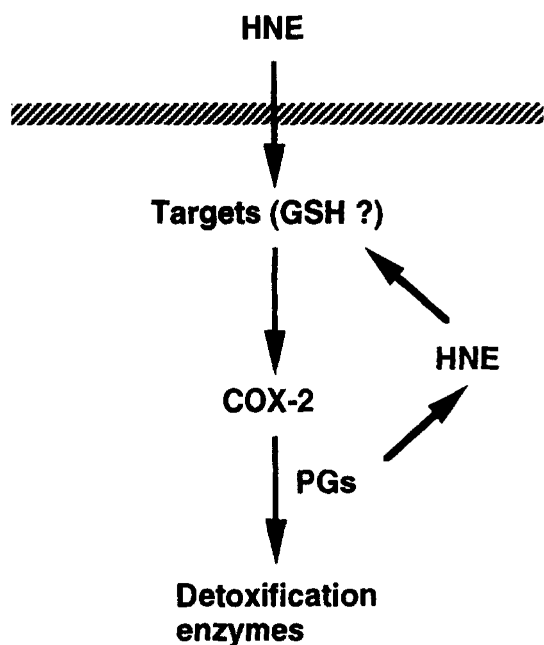

FIGURE 5 A proposed mechanism for the activation of the arachidonic acid cascade by HNE

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Univ.), Dr. Kimihiko Satoh (Hirosaki Univ.), and Dr. Masayoshi Imagawa (Osaka Univ.).

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