Cardioprotection by Hormetic Responses to Aldehyde

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Everyone encounters various stressors (causes of stress), such as psychological pressure, mental fluctuations, and physical burdens, in their everyday life. It is well accepted that the highest levels of perceived stress correlate with early onset of cardiovascular disease. Conversely, appropriate (mild to moderate) stressors, such as physical activity, have been shown to promote health. This bidirectional dose–response relationship of treatments that are beneficial at low levels but noxious at higher levels is referred to as “hormesis”. In the fields of toxicology, pharmacology, radiation biology, and medicine, the significance of the biological effects of low-level exposure to various agents has attracted considerable attention. It is very important to understand how biological systems respond to low levels of stress and their implications within society. Aldehydes, the major endproducts of lipid peroxidation, have been implicated in the pathogenesis of oxidative stress-associated diseases. In addition to the pathogenic effect associated with oxidative stress, sublethal levels of aldehydes interact with signaling systems to upregulate the expression of genes to counteract the stressor challenge and to re-establish homeostasis. The present review article discusses current discoveries regarding the hormetic response to aldehyde and its clinical significance in cardioprotection. (Circ J 2010; 74: 1787–1793)

Key Words: Antioxidants; Metabolism; Myocardial infarction; Oxidative stress; Stress

We all encounter various stressors (causes of stress), such as psychological pressure, mental fluctuations, and physical burdens, in everyday life. It is well accepted that the highest levels of perceived stress correlate with the early onset of cardiovascular disease. Chronic stress facilitates plaque formation in the coronary arteries; superimposed acute stress often triggers plaque rapture and thrombosis, leading to myocardial infarction, a major cause of sudden death.

Recent studies have demonstrated that stress affects health by modulating the rate of cellular aging. Telomeres are DNA–protein complexes that cap chromosomal ends and promote chromosomal stability. Telomeres become shorter with every replication of the cell and telomerase status and telomere length are well-established indices of cellular aging. For example, the telomere length in peripheral blood mononuclear cells (PBMC) from healthy premenopausal mothers of chronically ill children is significantly shorter than that in the PBMC of age-matched mothers of healthy children.

Conversely, appropriate (mild to moderate) stressors have been shown to promote health. For example, there is documented evidence of the significant health benefits of physical activity. Physical activity is known to decrease the risk of premature mortality in general and the risk of coronary artery disease, hypertension, colon cancer, and diabetes mellitus in particular. Beyond the cardiovascular health benefits of physical exercise, the positive effects of exercise on mental health and cognitive function have also come to public attention. There is a positive correlation between aerobic capacity and brain tissue density in middle-aged men and women, with physical exercise seemingly slowing brain aging. Such an effect in the human brain has major implications in terms of delaying the onset of conditions such as dementia and Alzheimer’s disease.

Recent studies have shown that exercise prevents the destruction of telomeres; however, the relationship between the level of physical activity and telomere length seems to be bidirectional. Moderate exercise results in significantly longer PBMC telomere length compared with very low exercise levels, but these benefits are not necessarily seen with higher levels of exercise.

These bidirectional dose–response relationships of treatments that are beneficial at low levels but noxious at higher levels are referred to as “hormesis”. In the fields of toxicology, pharmacology, radiation biology, and medicine, the significance of the biological effects of low-level exposure to various agents has attracted considerable attention. Stress response hormesis refers to the induction, by stressors, of an adaptive response that results in a general increase in stress resistance. It is very important to understand how biological systems respond to low levels of stress and the implications within society. The present review article discusses current discoveries regarding the hormetic response to aldehyde and its clinical significance in cardioprotection.
What Is Aldehyde?

Acetoaldehydes are produced following alcohol consumption. “Alcohol flushing” syndrome is attributable to elevated blood levels of acetaldehyde. However, even without the consumption of alcohol, aldehydes are produced endogenously as the major end products of lipid peroxidation. Reactive oxygen species (ROS) are inevitably produced as byproducts of mitochondrial oxidative energy production. Superoxide radicals are dismutated by superoxide dismutase (SOD) to produce hydrogen peroxides, which, in turn, are degraded into water and molecular oxygen by catalase, glutathione peroxidase, and peroxiredoxin. Hydroxyl radicals (OH•), which are the most potent ROS, are formed from hydrogen peroxide through the Fenton reaction. The OH• attack neighboring polyunsaturated fatty acids in the cell membrane, thereby triggering lipid peroxidation, which results in the generation of lipid hydroperoxides and α,β-unsaturated aldehydes, including 4-hydroxy-2-nonenal (4-HNE). These aldehydes are highly electrophilic and react with biomolecules such as proteins and nucleic acids to generate various adducts. By virtue of their high chemical stability, these lipid peroxidation products diffuse greater distances than their precursor ROS, so they can disseminate oxidative injury and amplify damage (Figure 1). Thus, it is now well accepted that much of the oxidative stress-associated damage can be attributed to aldehydes. Accumulation of aldehydes has been found in ischemic, hypoxic, and failing hearts, as well as in oxidized low-density lipoprotein atherosclerotic lesions, and the brains of patients with Alzheimer’s disease. Thus, aldehydes have been implicated in the pathogenesis of oxidative stress-associated diseases.

In addition to the pathogenic effect associated with oxidative stress, sublethal levels of aldehydes interact with signaling systems to upregulate the expression of genes to counteract the stressor challenge and to re-establish homeostasis (Figure 2).

Sublethal Concentrations of 4-HNE Protect Cardiomyocytes Against Oxidative Injury via an NF-E2-Related Factor 2 (Nrf2)-Dependent Mechanism

Investigations into whether 4-HNE, one of the most abundant aldehydes produced by lipid peroxidation in vivo, induces stress response hormesis in cultured cardiomyocytes found that at higher concentrations (ie, ≥20 μmol/L) 4-HNE was cytotoxic but at lower concentrations it had no appreciable cytotoxicity (Figure 3). Notably, pretreatment of cultured cardiomyocytes with a sublethal concentration of 4-HNE (5 μmol/L) for 14 h primed the cells to become resistant to subsequent exposure to cytotoxic concentrations of 4-HNE. Investigations into the mechanism underlying the cardioprotection mediated by 4-HNE revealed that, under normal (unstressed) conditions, Nrf2 is tethered to Keap1 in the cytoplasm. This complex directs Nrf2 polyubiquitination and degradation. During oxidative stress, Nrf2 is liberated from Keap1 and enters the nucleus, where it forms a heterodimer with the small Maf transcription factor, Nrf2, to induce the expression of genes for proteins that function as antioxidants and enzymes in phase II detoxification and glutathione biosynthesis. 4-HNE induces the nuclear translocation of Nrf2 and enhances the expression of γ-glutamylcysteine ligase (GCL) and the core subunit of the Xc high-affinity cysteine transporter, thereby increasing intracellular GSH levels 1.45-fold. Cardiomyocytes treated with either Nrf2-specific short

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**Figure 1.** Much of the oxidative stress-associated damage can be attributed to aldehydes. ROS, reactive oxygen species; 4-HNE, 4-hydroxy-2-nonenal; ALDH2, aldehyde dehydrogenase 2.
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Figure 2. Sublethal levels of aldehydes interact with signaling systems to upregulate gene expression to counteract stressor challenge and to re-establish homeostasis. ROS, reactive oxygen species.

Figure 3. Hormetic effect of 4-hydroxy-2-nonenal (4-HNE) on cardiomyocytes.

Figure 4. Pretreatment of mice with 4-hydroxy-2-nonenal (4-HNE) attenuated the increase in 4-HNE adduct proteins in response to ischemia–reperfusion injury.
interference (si) RNA or the GCL inhibitor L-buthionine sulf-oximine were less tolerant to 4-HNE; moreover, the cardioprotective effect of 4-HNE pretreatment against subsequent ischemia–reperfusion injury was completely abolished in these cells. The mechanism by which 4-HNE induces the nuclear accumulation of Nrf2 remains to be determined. Specific cysteine residues (Cys273/Cys288) in the Keap1 protein are known to act as sensors for oxidative stress and modification of these residues leads to a conformational change in Keap1, with consequent release of Nrf2. 16 4-HNE may induce a conformational change in Keap1 directly via adduct formation or indirectly by increasing the production of mitochondrial ROS. 17

The cardioprotective effect of 4-HNE can be reproduced in vivo. When 4-HNE is injected via the retro-orbital vein, sufficient reactive 4-HNE reaches the heart. Cardiac Nrf2 is activated 60 min after systemic administration of 4-HNE, with subsequent Nrf2-dependent upregulation of cardiac GSH and mRNA levels for antioxidant enzymes. In Langendorff-perfused mouse hearts, systemic administration of 4-HNE significantly improved the recovery of left ventricular function during ischemia–reperfusion. Consistent with these findings, levels of total lactate dehydrogenase released into the perfusate during reperfusion were significantly lower in 4-HNE-pretreated hearts than in control hearts. Ischemia–reperfusion significantly increased the levels of 4-HNE adduct proteins in the Langendorff-perfused hearts; notably, systemic administration of 4-HNE prior to ischemia significantly attenuated the increase in 4-HNE adduct proteins during reperfusion (Figure 4). This type of cardioprotection was not seen in Nrf2-2-knockout mice following the systemic administration of 4-HNE. 18

Growing evidence indicates that a brief ischemic insult in 1 organ releases endogenous factors that protect other organs against a prolonged ischemic insult. This phenomenon is known as "remote ischemic preconditioning". 19 The exact nature of signal transduction from a remote tissue to the target organ remains to be elucidated. Although aldehydes conjugate easily with receptive nucleophiles (such as glutathione) in the circulating blood, these conjugates can stimulate stress resistance pathways in remote organs. These findings raise the possibility that aldehydes and/or their metabolites act as humoral mediators to mediate remote organ protection.

**Aldehyde Dehydrogenase (ALDH) 2 Is a Major Aldehyde-Detoxifying Enzyme in the Mitochondria**

In mammalian cells, reactive aldehydes are detoxified by oxidation to carboxylates, a reaction catalysed by ALDHs. The ALDHs are a superfamily of NAD(P)⁺-dependent enzymes and, to date, 19 distinct ALDH genes have been identified in the human genome. 20 ALDH2 is localized to the mitochondria, a major source of ROS and a target of membrane lipid peroxidation. ALDH2 has been identified as a major aldehyde-detoxifying enzyme in the mitochondria (Figure 1). Recently, the mitochondrial translocation of protein kinase Cε and the subsequent phosphorylation (activation) of ALDH2 were shown to constitute the final common pathway for cardioprotection induced by ischemic preconditioning. Indeed, administration of a small-molecule activator of ALDH2 (ie, Alda-1) to rats before an ischemic event reduced infarct size by 60%. 21–23

There is a polymorphism in the ALDH2 gene specific to north-east Asian populations. The mutant allele ALDH2*2 has a single point mutation of the active ALDH2*1 gene, acting as a dominant negative gene. ALDH2 acts as a homo- or heterotrimer, and all tetramers that contain at least 1 ALDH2*2 subunit are inactive. People homozygous for the ALDH2*2 allele (~8% of the Japanese population) do not have any ALDH2 activity, whereas activity in individuals heterozygous for the ALDH2*2 allele (~40% of the Japanese population) is as low as one-sixteenth that in ALDH2*1 (wild-type) homozygous individuals. 24 In addition to an association with alcohol flushing syndrome (commonly seen in people of Asian descent), the ALDH2*2 allele is also associated with increased serum levels of lipid peroxides 25 and an increased risk of late-onset Alzheimer’s disease. 26 However, favorable effects of the ALDH2*2 allele have also been documented: the prevalence of proliferative retinopathy in Japanese patients with non-insulin-dependent diabetes mellitus is lower in the inactive ALDH2 compared with the active ALDH2 group, 27 although it remains to be clarified whether these correlations can be attributed to the hormetic effects of aldehydes in the retina.

**Cardioprotection Can Be Achieved Within the Setting of Chronic Exposure to Aldehydes Throughout Life**

To investigate whether a hormetic effect could be induced within the setting of the chronic exposure to aldehydes that persists throughout life, we created a loss-of-function model of Aldh2 by overexpressing Aldh2*2. 28 Consistent with the mitochondrial localization of the Aldh2*2 protein, levels of 4-HNE adduct proteins were increased in the mitochondrial, but not cytosolic, fraction of Aldh2*2 transgenic (Tg) hearts. Interestingly, despite significant accumulation of 4-HNE adduct proteins in the mitochondrial matrices, left ventricular function in the Aldh2*2 Tg mice was equivalent to that in their wild-type littermates until at least 2 years of age. Furthermore, the Aldh2*2 Tg hearts exhibited greater tolerance to acute oxidative stress induced by ischemia–reperfusion than did wild-type hearts.

The expression of most major antioxidant enzymes, such as catalase, SOD, and GPx, was unaltered in Aldh2*2 Tg hearts. Instead, there was upregulation of genes encoding enzymes involved in amino acid biosynthesis and transport in Aldh2*2 Tg hearts. This included upregulation of genes encoding 3-phosphoglycerate dehydrogenase, phosphoserine aminotransferase, and phosphoserine phosphatase, all of which are involved in the 3-step conversion of 3-phosphoglycerate (a glycolytic intermetabolite) to serine, and upregulation of genes involved in various metabolic pathways that eventually converge on glutathione biosynthesis, such as serine hydroxymethyltransferase 1/2, which catalyses the conversion of serine and tetrahydrofolate (THF) to glycine and 5,10-methylene THF; methylenetetrahydrofolate dehydrogenase, which catalyses the interconversion of 5,10-methylene THF and 10-formyl THF; and cystathionase, which is involved in cysteine biosynthesis in the trans-sulfuration pathway (Figure 5). Consequently, intracellular concentrations of glutathione were increased 1.37-fold in Aldh2*2 Tg compared with wild-type hearts. Consistent with this transcriptome analysis, metabolome analysis indicated that glucose uptake was upregulated in Tg hearts and that glucose biotransformation was shifted from glycolysis towards the pentose phosphate pathway to generate NADPH and amino acid biosynthesis, 29,30 which ultimately provide precursor amino acids for glutathione biosynthesis. Enhanced supply of NADPH via the pentose
Figure 5. Activation of amino acid metabolism plays a key role in cardioprotection against chronic oxidative stress. Cys, cysteine; Gly, glycine; Glu, glutamate; GSH, glutathione; GSSG, oxidized glutathione; Gnmt, glycine N-methyltransferase; Hcy, homocysteine; Cth, cystathionase; Met, methionine; Phgdh, 3-phosphoglycerate dehydrogenase; Psat1, phosphoserine aminotransferase; Psph, phosphoserine phosphatase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; Ser, serine; Shmt1, serine hydroxymethyltransferase 1; TCA, tricarboxylic acid; THF, tetrahydrofolate. Red arrows indicate enzymes that show significant increases in the Aldh2*2-Tg heart.

Figure 6. Mitochondrial oxidative stress activates retrograde signaling and confers enhanced tolerance to ischemia–reperfusion injury via metabolic remodeling. HNE, 4-hydroxy-2-nonenal; PPP, pentose phosphate pathway; GSH, glutathione; eIF2α, eukaryotic initiation factor 2α; ATF4, activating transcription factor 4.
phosphate pathway helps in the recycling of oxidized glutathione.

This work has extended the concept of cardioprotection by preconditioning. According to the current understanding of cardioprotection by preconditioning, the mitochondria are the targets for protection from cell death. During sustained ischemia–reperfusion, the opening of the mitochondrial permeability transition pore (MPTP) induces mitochondrial swelling, depolarization, and ultimately cell death. Cardioprotection by preconditioning induced by brief non-lethal episodes of ischemia–reperfusion activates a variety of signaling cascades, all of which culminate in the inhibition of MPTP opening. We have shown that mitochondrial retrograde signals (ie, signals originating from the mitochondria) play a key role in cardioprotection (Figure 6). A shift towards the oxidative state in mitochondrial matrices sends a signal to the nucleus to change nuclear gene expression, enabling the cells to adapt to, and thus compensate for, mitochondrial oxidative stress.

The eukaryotic initiation factor (eIF) 2α–activating transcription factor 4 (Atf4) pathway provides the key mitochondrial retrograde signals in response to mitochondrial aldehyde stress. Chronic mitochondrial aldehyde stress triggers phosphorylation of eIF2α and the combined transcriptional and translational activation of Atf4 upregulates the gene expression of enzymes involved in amino acid biosynthesis and transport that ultimately provide precursor amino acids for glutathione biosynthesis, thereby increasing intracellular glutathione levels. Indeed, heterozygous knockout of Atf4 blunted the increased expression of cardiac genes involved in amino acid metabolism and the increase in intracellular glutathione levels in Aldh2*2 Tg hearts, thereby attenuating the oxidative stress-resistant phenotype. These findings indicate that Atf4-dependent activation of amino acid metabolism and glutathione biosynthesis are causally involved in the oxidative stress-resistant phenotype observed in Aldh2*2 Tg mice.

The study described above is limited by uncertainty as to whether the cardioprotection observed for Aldh2*2 Tg hearts actually exists in individuals carrying the ALDH2*2 allele. More generally, it would be of interest to determine whether a mitochondrial retrograde response transduced via the eIF2α–ATF4 pathway is involved in cardioprotection during human aging and in age-related diseases. Aging is accompanied by increased ROS production, with subsequently increased oxidative damage to mitochondrial DNA, proteins, and lipids. In addition, loss of cardioprotection in aged hearts is likely to be a consequence of an age-associated reduction in retrograde response signaling. Notably, a paradoxical decline in relative levels of eIF2α Phosphorylation and ATF4 expression have been demonstrated in aged rat tissues, including the heart, suggesting that the eIF2α–ATF4-mediated retrograde response to mitochondrial dysfunction operates less efficiently in the aged heart. How this retrograde regulation is affected by aging and age-related diseases is an important area for future research.

Conclusions

The cardiac stress response to aldehydes is accomplished by the activation of 2 basic leucine zipper transcription factors, namely Nr2f2 and ATF4, at the posttranscriptional level. These 2 transcription factors act in a coordinated manner to regulate glutathione biosynthesis and the glutathione redox cycle at different time points. Mimetic triggers of hormesis may be a promising approach to prevent the onset of oxidative stress-associated heart diseases and cardiac senescence itself without the risk of the overwhelming damage that is associated with the use of aldehydes themselves.

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References


