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Aldehyde dehydrogenase 2 in cardiac protection: a new therapeutic target?

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Abstract

Mitochondrial aldehyde dehydrogenase 2 (ALDH2) is emerging as a key enzyme involved in cytoprotection in the heart. ALDH2 mediates both the detoxification of reactive aldehydes such as acetaldehyde and 4-hydroxy-2-nonenal (4-HNE) and the bioactivation of nitroglycerin (GTN) to nitric oxide (NO). In addition, chronic nitrate treatment results in ALDH2 inhibition and contributes to nitrate tolerance. Our lab recently identified ALDH2 to be a key mediator of endogenous cytoprotection. We reported that ALDH2 is phosphorylated and activated by the survival kinase protein kinase C epsilon (PKCε) and found a strong inverse correlation between ALDH2 activity and infarct size. We also identified a small molecule ALDH2 activator (Alda-1) which reduces myocardial infarct size induced by ischemia/reperfusion in vivo. In this review, we discuss evidence that ALDH2 is a key mediator of endogenous survival signaling in the heart, suggest possible cardioprotective mechanisms mediated by ALDH2, and discuss potential clinical implications of these findings.

Introduction

Clinical interventions for acute myocardial infarction (AMI), such as angioplasty or thrombolysis, are aimed at disrupting the occlusion and restoring coronary flow. However, these treatments do not prevent myocardial tissue damage during ischemia, nor do they reduce reperfusion injury. Therefore, the identification of novel cardioprotective strategies remains a clinical priority. The mitochondrial enzyme aldehyde dehydrogenase 2 (ALDH2) is rapidly emerging as a crucial enzyme involved in protecting the heart from ischemic injury (Chen et al. 2008; Churchill et al. 2009; Doser et al. 2009; Lagranha et al. 2009; Perlman et al. 2009). ALDH2 is one of 19 members of the ALDH gene family which play a crucial metabolic role in the oxidation and detoxification of reactive aldehydes in a range of organs and cell types (Vasiliou and Nebert 2005). While probably best known for its role in catalyzing the oxidation of acetaldehyde to acetic acid in ethanol metabolism, ALDH2 is also a key metabolic enzyme involved in the detoxification of other reactive aldehydes such as 4-hydroxy-2-nonenal (4-HNE). In addition to its dehydrogenase activity, ALDH2 has an esterase activity that catalyses the conversion of nitroglycerin (glyceryl trinitrate, GTN) to 1,2 glyceryl dinitrate (1,2-GDN), and thus mediates bioactivation of GTN (Chen et al. 2005; Chen and Stamler 2006). ALDH2 is encoded in the nucleus and co-translationally imported

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into the mitochondrial matrix, owing to a 17 amino acid N-terminal mitochondrial localization sequence (Vasiliou and Nebert 2005). A common human polymorphism in ALDH2, in which a glutamate at amino acid 487 is replaced by a lysine (E487K), has the highest prevalence (40%) in the Asian population (Goedde et al. 1983). The functional ALDH2 enzyme is a tetramer, therefore if any of the 4 subunits contains the E487K form, the activity of the enzyme is severely compromised. Thus, relative to the wild-type homozygotes (ALDH2*1), E487/K487 heterozygotes (ALDH2*1/2) have 30–40% of the activity and K487 homozygotes (ALDH2*2/2) show negligible activity (Chen et al. 2008; Goedde et al. 1983). The ALDH2*2 carriers experience facial flushing after alcohol ingestion (due to impaired acetaldehyde oxidation) and have reduced vasodilation in response to GTN (due to impaired bioconversion of GTN) (Li et al. 2006; Mackenzie et al. 2005). In addition, carriers of the ALDH2*2 allele have increased susceptibility to certain cancers and neurodegenerative diseases (Vasiliou et al. 2000).

Our lab recently identified ALDH2 as a key mediator of endogenous cytoprotection against myocardial ischemia/reperfusion injury (Chen et al. 2008). We found an inverse correlation between ALDH2 activity and infarct size in a myocardial infarction model and, using a novel small molecule activator of ALDH2, confirmed that ALDH2 activation is sufficient to protect the heart from ischemic damage. In this review, we will suggest possible cardioprotective mechanisms mediated by ALDH2, and the potential clinical implications of these findings.

ALDH2 Phosphorylation and Activity Correlates with Cardioprotection

One of the most powerful methods of reducing myocardial infarct size is by “preconditioning” the heart with sub-lethal periods of ischemia and reperfusion prior to the onset of sustained ischemia (Murry et al. 1986). The observation that adenosine and other GPCR agonists can mimic ischemic preconditioning (IPC) and confer cardioprotection (Liu et al. 1991) led to the hypothesis that PKC, which lies downstream from these Gi-coupled receptors, was involved in transducing the cytoprotective signal to downstream end-effectors (Armstrong et al. 1994; Ytrehus et al. 1994). However, there are multiple PKC isozymes expressed in the heart, and individual PKC isozymes exert distinct and even opposing physiological functions. Our lab generated PKC-isozyme selective activators and inhibitors, based on the interaction between each PKC isozyme and its isozyme-selective anchoring protein, or RACK (Receptor for Activated C Kinase) (Mochly-Rosen 1995; Souroujon and Mochly-Rosen 1998). Using these PKC-isozyme-selective regulators, in combination with transgenic and knockout mice, it was found that PKC ϵ and PKC δ mediate opposing roles in survival signaling in the heart; ϵ PKC *activation* elicits cytoprotection and mimics IPC, whereas δ PKC *inhibition* protects against ischemia-reperfusion (Churchill et al. 2005; Dorn et al. 1999; Gray et al. 1997; Inagaki et al. 2003; Liu et al. 1999; Murriel et al. 2004; Ping et al. 1997; Saurin et al. 2002). A number of PKC ϵ -mediated cytoprotective mechanisms have since been proposed, including inhibition of the mitochondrial permeability transition (Baines et al. 2003) opening of mitochondrial ATP-sensitive K⁺ channels (Jaburek et al. 2006), regulation of gap junction permeance through phosphorylation of connexin 43 (Bowling et al. 2001) and regulation of ATP generation through phosphorylation of cytochrome C oxidase subunit IV (Ogbi et al. 2004).

To identify those PKC ϵ substrates involved in cytoprotection, we used an unbiased phosphoproteomic approach. We identified a 55-kDa protein whose phosphorylation increased during ischemia in rat hearts pre-treated with either ethanol [as a preconditioning agent (Chen et al. 2001)], or the PKC ϵ -selective agonist, $\Psi\epsilon$ RACK, and whose IPC-induced phosphorylation was prevented by the presence of the PKC ϵ -selective inhibitor, $\Psi\epsilon$ V₁₋₂. Mass spectrometry identified the protein as mitochondrial ALDH2 (Chen et al. 2008).

Activation of PKC ϵ in the ischemic heart with either ethanol or Ψ eRACK increased ALDH2 activity with a concomitant reduction in infarct size (Figure 1). Conversely, agents that inhibited PKC ϵ prevented ALDH2 activation and abolished cardioprotection. Further, in the presence of ALDH2 inhibitors, such as cyanamide or high levels of GTN (discussed below), PKC ϵ -mediated cardioprotection was lost. We found a striking inverse correlation between infarct size and ALDH2 activity ($R^2=0.95$), suggesting that ALDH2 plays a crucial role in protecting the heart from ischemic injury (Chen et al. 2008). We also found that PKC ϵ and ALDH2 physically interact, *in vivo*, and that PKC ϵ phosphorylation of ALDH2 results in increased ALDH2 activity (Chen et al. 2008). We investigated whether ALDH2 was also regulated by PKC δ , as we had previously identified mitochondrial substrates of PKC δ including pyruvate dehydrogenase kinase 2 (Churchill et al. 2005). However we found that PKC δ did not associate with ALDH2 *in vivo*, suggesting selective phosphorylation by PKC ϵ (Chen et al. 2008). We identified three putative PKC ϵ phosphorylation sites, located at Thr¹⁸⁵, Thr⁴¹² and Ser²⁷⁹ on human ALDH2 (Chen et al. 2008), Thr⁴¹² was also recently identified as an ALDH2 phosphosite that correlates with nitrate-induced cardioprotection *in vivo* (Perlman et al. 2009). Furthermore, in an independent phosphoproteomic study by Murphy and collaborators, increased ALDH2 phosphorylation was observed in female rat hearts, when compared to males, and correlated with increased cardiac protection associated with female gender (Lagranha et al. 2009). Taken together, it appears that ALDH2 phosphorylation plays a crucial role as a mediator of endogenous cardioprotection (Figure 2).

Activation of ALDH2 with Alda-1 Reduces Ischemic Damage to the Heart

It could be argued that increased ALDH2 activity simply reflects protected cardiac cells. Therefore, to confirm the direct cytoprotective role of ALDH2, we set out to identify pharmacological activators of ALDH2 using a high-throughput screen of a diverse library of small molecules. This led to the independent identification of N-(1,3-benzodioxol-5-ylmethyl)-2,6-dichlorobenzamide, or ALDH2 activator 1 (Alda-1) and two other halogen analogs of this compound, all with similar activities. Treatment with Alda-1 doubled wild-type ALDH2 activity over basal levels but had no effect on the related enzymes alcohol dehydrogenase (ADH), aldehyde dehydrogenase 1 (ALDH1) or aldehyde dehydrogenase 5 (ALDH5). Treatment with Alda-1 also increased activity of mutant ALDH2*2 ~10 folds and doubled activity of the heterotetramer (ALDH2*1/2), almost to the basal level of the wild-type ALDH2 (Figure 3). This finding was unanticipated, as it is uncommon to find drugs that restore activity to a catalytically inactive, mutant enzyme. Importantly, in an *in vivo* model of MI in rats, administration of Alda-1 (8 mg/kg) prior to ischemia, reduced infarct size by 60%, thus confirming the cardioprotective role of ALDH2 and suggesting therapeutic potential for Alda-1 in treatment of ischemia-reperfusion injury.

ALDH2 Mediates Detoxification of Reactive Aldehydes

Much of the cellular damage occurring during cardiac IR is due to reactive oxygen species (ROS) generation (Bolli et al. 1989), which leads to lipid peroxidation and the production and accumulation of multiple reactive aldehydes, including acetaldehyde (Cordis et al. 1993) and 4-HNE, in the ischemic heart (Blasig et al. 1995; Eaton et al. 1999). These reactive aldehydes are highly cytotoxic, forming protein adducts with cysteine, histidine and lysine residues *via* Michael's addition (Uchida and Stadtman 1992). For example, 4-HNE inhibits the function of key metabolic proteins such as GAPDH (Uchida and Stadtman 1993), the Na/KATPase (Siems et al. 1996) and the 20S proteasome (Farout et al. 2006), and is also a potent inducer of mitochondrial permeability transition (Kristal et al. 1996), thought to be a primary mechanism leading to necrotic cell death in reperfused myocardium (Baines et al. 2005; Nakagawa et al. 2005). Glutathione (GSH) can scavenge 4-HNE *via* Michael's

addition in a reaction catalyzed by glutathione-S-transferase (GST) (Petersen and Doorn 2004). 4-HNE is also metabolized by ALDH2 to the unreactive 4-hydroxynon-2-enoic acid (4-HNA) (Petersen and Doorn 2004). Thus, detoxification of reactive aldehydes is a likely cytoprotective mechanism mediated by ALDH2.

At high concentrations, such as those occurring during ischemia, 4-HNE directly inhibits ALDH2 (Chen et al. 2008; Doorn et al. 2006), further exacerbating toxic aldehyde accumulation. 4-HNE interacts covalently with Cys302, located at the ALDH2 active site, resulting in >90% enzymatic inhibition (Doorn et al. 2006). Alda-1 prevents this 4-HNE-induced inactivation of ALDH2, in addition to its ability to activate the enzyme (Chen et al. 2008) (Figure 4). Therefore, the mechanism of protection mediated by Alda-1 is likely due to a combination of direct activation of ALDH2 and prevention of ALDH2 inactivation by 4-HNE at high substrate concentrations. Indeed, accumulation of 4-HNE protein adducts were lower in IR hearts treated with Alda-1, than in untreated IR controls (Chen et al. 2008).

ALDH2 Mediates Bioactivation of Nitroglycerin

Nitroglycerin (glyceryl trinitrate, GTN) has been used clinically since the 1870s for the treatment of angina pectoris, myocardial infarction and heart failure. The beneficial effects of GTN and other nitrates are afforded by vasoactive nitric oxide (NO) or S-nitrosothiol (SNO), which activate soluble guanylate cyclase (sGC), leading to increased blood flow (Arnold et al. 1977; Gruetter et al. 1981; Moncada et al. 1991). Nitrates can also confer direct myocardial cytoprotection independent of their hemodynamic effect (Bolli 2007). Nitrate administration results in the nitrosylation and activation of protein kinases, including cGMP-dependent kinases and PKC ϵ (Vondriska et al. 2001) and has also been shown to result in opening of mitoK_{ATP} channels (Sasaki et al. 2000) and inhibition of mitochondrial permeability transition (Gori et al. 2008). In contrast, chronic continuous treatment with GTN can lead to free radical generation, disruption of endothelial function and increased cardiovascular morbidity (Gori and Parker 2008; Nakamura et al. 1999). However, GTN confers cardiac protection when prolonged GTN treatment is terminated at least 1 hour before the ischemic event (Chen et al. 2008). When administration of GTN overnight (5 μ g/min/kg body weight by transdermal patch) was terminated 3h before exposure to ischemia/reperfusion, cardiac infarct size decreased from 45% in controls to 33%. However, if the GTN patch was left on until immediately prior to ischemia, infarct size increased from 45% to 59% (Chen et al. 2008).

The dichotomous effect of GTN in cytoprotection is probably explained by recent findings that ALDH2 mediates bioactivation of GTN (Chen and Stamler 2006; Chen et al. 2002) and that prolonged treatment with GTN inhibits ALDH2 enzymatic activity (GTN-tolerance) (Beretta et al. 2008; Chen et al. 2002). All nitrate esters, including GTN, are pro-drugs requiring enzymatic metabolism to generate bioactive NO through an intermediate 1,2-glyceryl dinitrate (1,2 GDN) (Brien et al. 1988) that is generated by ALDH2 (Beretta et al. 2008; Chen et al. 2002). Inhibition of ALDH2 prevents GTN conversion to 1,2-GDN, blocks cGMP production, and attenuates GTN-induced vasodilatation (Chen et al. 2005). Furthermore, in aorta made GTN-tolerant, ALDH2 activity was inhibited and cGMP accumulation was abolished and production of cGMP and reduction in blood pressure induced by 0.1–1 μ M GTN treatment were substantially attenuated in ALDH2 $-/-$ mice (Chen et al. 2005). Although an ALDH2-independent bioactivation mechanism of GTN exists, it occurs at supra-maximal GTN concentrations (10 μ M) (Chen et al. 2005). Since therapeutic plasma concentrations of GTN rarely exceed 100 nM, it appears that ALDH2 is the major enzyme involved in the biotransformation of GTN and that inactivation of ALDH2 contributes to GTN-tolerance (Chen et al. 2005). GTN-induced ALDH2 inactivation may be due to either irreversible adduct formation at the active site (Chen and

Stamler 2006) or to GTN-induced superoxide production and ROS-mediated inhibition of ALDH2 (Sydow et al. 2004), or a combination of both.

The role of ALDH2 in GTN bioactivation has also been confirmed in humans (Li et al. 2006; Mackenzie et al. 2005). One of the early descriptions that GTN inhibits ALDH2 enzymatic activity was the clinical appearance of “disulfiram-like” effects in patients undergoing chronic nitrate therapy who consumed alcohol (Towell et al. 1985). This would be expected, as ALDH2 inhibited by GTN would be unable to metabolize acetaldehyde derived from ethanol ingestion. In healthy volunteers, treatment with the ALDH2 inhibitor, disulfiram, blocked the vasodilatory response to GTN, but not that induced by sodium nitroprusside (SNP) (which acts *via* direct release of NO) or by verapamil, a calcium channel blocker (acting *via* an NO-independent mechanism) (Mackenzie et al. 2005). Subjects with the ALDH2*2 mutation also have a significantly attenuated vasodilatory response to GTN, but respond as carriers of the wild-type allele to SNP or verapamil (Mackenzie et al. 2005). Interestingly, the response of the ALDH2*2 carriers to GTN was similar to wild-type patients treated with disulfiram (Mackenzie et al. 2005). These data confirmed that ALDH2 is involved in the activation of GTN in humans and that patients with ALDH2*2 have a reduced vasodilatory response to nitrates.

Clinical Implications of ALDH2 Inhibition During Ischemia

The finding that ALDH2 plays a key role in cytoprotective signaling may have important clinical ramifications. First, because ALDH2 activity correlates strongly with cardioprotection, interventions which reduce ALDH2 activity are likely to worsen injury in the event of an ischemic episode. This has particular relevance to the sustained use of nitrates in ischemic conditions. Organic nitrates are among the most commonly used therapies in treatment of patients with angina and heart failure. However, there is debate as to whether chronic GTN use may actually be deleterious (Gori and Parker 2008). Chronic use of GTN results in GTN-tolerance, which is currently considered simply as a loss of efficacy to the drug. However, given the importance of ALDH2 in cytoprotective signaling, enzymatic inactivation of ALDH2 may, in fact, exacerbate ischemic damage due to impaired detoxification of 4-HNE and other reactive aldehydes. Taken together, these findings suggest that the current clinical practice of GTN therapy in patients at risk for cardiac ischemia should be examined. This may be of particular importance to ALDH2*2 patients who already have a fraction of the wild type ALDH2 activity and have a limited clinical response to GTN (Li et al. 2006; Mackenzie et al. 2005). For these patients, the risk/benefit ratio of GTN administration may also need to be reassessed, as GTN treatment offers limited vasodilatory efficacy (Li et al. 2006; Mackenzie et al. 2005), while GTN-induced inhibition of ALDH2 may worsen outcome during an ischemic event.

Therapeutic Potential for ALDH2 Activators

Activators of ALDH2, such as Alda-1, may have therapeutic potential in a number of cardiovascular indications. First, our data that ALDH2 activation with Alda-1 can ameliorate ischemia/reperfusion injury *in vivo* suggests that ALDH2 activators may be useful in patients with acute myocardial infarction, cardiac bypass surgery or heart transplantation. In these indications, free radical generation, production of 4-HNE and mitochondrial dysfunction contribute to irreversible injury, which may be ameliorated by ALDH2 activation. Aldas may also be beneficial for patients with peripheral artery disease or angina who have become GTN-tolerant, *i.e.* by preventing GTN-induced ALDH2 inactivation. Further, the finding that Alda-1 restores activity of mutant ALDH2*2 suggests that these patients may become more responsive to GTN therapy following Alda-1 treatment. Finally, 1 in 3 alcoholics develop alcoholic cardiomyopathy, manifest as reduced contractile

dysfunction and cardiac hypertrophy, which is thought to be due directly to acetaldehyde accumulation (Hintz et al. 2003). In a recent study, it was demonstrated that transgenic overexpression of ALDH2 reduces myocardial hypertrophy and prevents contractile defects in mice subjected to chronic alcohol ingestion (Doser et al. 2009). Therefore, ALDH2 activators may be of therapeutic potential in the treatment of alcoholic cardiomyopathy.

Summary

Accumulating evidence suggest that mitochondrial ALDH2 plays a pivotal role in mediating cytoprotective signaling in the heart. ALDH2 may confer cardioprotection through metabolism of reactive aldehydes (such as 4-HNE) and through its role in the bioconversion of nitrates to NO. Therefore, ALDH2 agonists, such as Alda-1, may ultimately lead to novel therapies which limit injury during myocardial infarction or bypass surgery. While activation of ALDH2 is protective, agents that impair ALDH2 activity (including GTN) are likely to interfere with endogenous cardioprotective signaling if present during an ischemic event (model, Figure 5). Further research into the benefit of ALDH2 activation in GTN-treatment is warranted. These studies may be of particular importance to carriers of the ALDH2*2 mutation, who already have significantly impaired ALDH2 activity.

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Figure 1. ALDH2 activity correlates with cardiac protection

A) ALDH2 activity correlates with cardiac protection. Activation of PKC ϵ (with ethanol or $\Psi\epsilon\text{RACK}$) increased ALDH2 activity and reduced infarct size. Conversely, PKC ϵ -induced cardioprotection and the concomitant increase in ALDH2 activity were blocked when PKC ϵ was inhibited with ϵV_{1-2} . When ALDH2 was inhibited (with cyanamide or nitroglycerin), infarct size was increased. Linear regression demonstrates a strong inverse correlation between infarct size and ALDH2 activity ($R^2=0.95$). From Chen et al. Science (2008). Reprinted with permission from AAAS.

Figure 2. Scheme of PKC ϵ mediated activation of ALDH2

On activation (with ethanol, preconditioning or $\Psi\epsilon$ RACK) PKC ϵ translocates from the cytosol to the mitochondria and phosphorylates ALDH2, increasing enzymatic activity. PKC ϵ -mediated activation of ALDH2 increases detoxification of reactive aldehydes, such as 4-HNE, a cytotoxic aldehyde that accumulates during cardiac ischemia injury as a by product of ROS-induced lipid peroxidation. Enhanced ALDH2 activity reduces 4-HNE protein adduct formation and increases cell survival.

Figure 3. Alda-1 activates wild-type and mutant ALDH2

A) Activation of wild-type and mutant ALDH2 by Alda-1. Enzymatic activity of recombinant ALDH2 proteins (20µg) in the absence and presence of 100µM Alda-1 (n=3 for each, **p<0.01 vs. control). From Chen et al. Science (2008). Reprinted with permission from AAAS.

Figure 4. Alda-1 prevents 4-HNE-induced inactivation of ALDH2

Treatment with 200 μ M 4-HNE results in inactivation of recombinant ALDH2 and is blocked by co-incubation with 20 μ M Alda-1. Thus, Alda-1 prevents 4-HNE-induced ALDH2 inactivation. From Chen et al. Science (2008). Reprinted with permission from AAAS.

Figure 5. Scheme of Alda-1 mediated cardioprotection

ALDH2 plays a pivotal role in cardioprotective signaling through its dual role in the bioactivation of GTN to nitric oxide-related species (NO_x) and in the detoxification of reactive aldehydes (such as 4-HNE and acetaldehyde), to non-reactive acids (such as 4-HNA and acetic acid). Alda-1 treatment increases ALDH2 activity and also prevents inhibition of ALDH2 (by GTN or 4-HNE) and thus ensures continued detoxification of reactive aldehydes during ischemia-reperfusion or during chronic treatment with GTN.