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Aldose Reductase, Oxidative Stress and Diabetic Cardiovascular Complications

Srinivasan Vedantham, Radha Ananthakrishnan, Ann Marie Schmidt, and Ravichandran Ramasamy*

Diabetes Research Program, Department of Medicine, New York University Langone Medical Center, New York, NY 10016, USA

Abstract

Cardiovascular disease represents the major cause of morbidity and mortality in patients with diabetes mellitus. Studies by us and others have implicated increased flux *via* aldose reductase (AR) as a key player in mediating diabetic complications, including cardiovascular complications. Data suggest that increased flux *via* AR in diabetics perpetuates increased injury after myocardial infarction, accelerates atherosclerotic lesion formation, and promotes restenosis *via* multiple mechanisms. Most importantly, studies have shown that increased generation of reactive oxygen species due to flux *via* AR has been a common feature in animal models of diabetic cardiovascular disease. Taken together, these considerations place AR in the center of biochemical and molecular stresses that characterize the cardiovascular complications of diabetes. Stopping AR-dependent signaling may hold the key to interrupting cycles of cellular perturbation and tissue damage in diabetic cardiovascular complications.

Keywords

Advanced glycation end products; Aldose reductase; Aldose reductase inhibitors; atherosclerosis; cardiovascular complications; Diabetes; hyperglycemia; ischemia reperfusion; Oxidative stress; Polyol pathway

INTRODUCTION

Diabetes and its cardiovascular complications such as atherosclerosis, restenosis, and cardiac ischemia are major causes of morbidity and mortality worldwide. The mechanisms that mediate the aberrant response to vascular injury remain to be fully clarified in these disease processes. Strident advances in molecular genetics made it possible to knock-in or knock-out specific genes in animal models and, thereby, to study functional significance of those genes in a disease perspective [1]. Increasing evidence supports a key role for hyperglycemia-mediated reactive oxygen species (ROS) generation in diabetic cardiovascular complications [2–8]. High levels of glucose are metabolized through Aldose Reductase (AR) and Sorbitol Dehydrogenase (SDH) to generate high intracellular levels of polyols and fructose. AR reduces glucose to sorbitol in the presence of NADPH, and SDH oxidizes sorbitol to fructose using NAD⁺. AR is ~35,900 Daltons cytoplasmic enzyme with

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*Address correspondence to this author at the Diabetes Research Program, New York University Langone Medical Center, Smilow Research Building Room 901, 550 First Avenue, New York, NY 10016, USA; Tel: 212-263-9475; Fax: 212-263-9497; ramasr02@nyumc.org.

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a triose phosphate isomerase structural motif that contains ten peripheral alpha-helical segments surrounding an inner barrel of beta-pleated sheet segments [9]. AR (E.C. 1.1.1.21; AKR1B1, ALD2) is a member of the aldo-keto reductase superfamily [10] and has been extensively studied [11, 12] in relation to diabetes and its secondary complications pertaining to the cardiovascular system, kidney and the eyes.

The primary focus of this review will be on AR, the rate limiting enzyme of the polyol pathway that channels the entry of excess glucose into the cytosol of cardiovascular cells, including cardiomyocytes and vascular cells. We will present evidences that polyol flux through AR plays a central role in mediating diabetic cardiovascular complications, in part *via* ROS, with special reference to ischemia-reperfusion injury, atherosclerosis, and restenosis. Finally we will discuss the clinical application of various AR inhibitors, their beneficial role in reducing these cardiovascular complications in diabetic and non-diabetic subjects.

AR AND HYPERGLYCEMIA IN CARDIOVASCULAR CELLS

Chronic elevation of glucose affects many biological processes in cardiovascular cells. The metabolic homeostasis of these cardiovascular cells is impacted by a number of key pathways - including the polyol pathway, the cytoplasmic redox state, the protein kinase C (PKC) pathway, the glucosamine biosynthesis pathway and production of advanced glycation end products (AGEs) [3, 13–16]. The increased activation of these specific pathways in hyperglycemia has specific functional repercussions. While in cultured bovine aortic endothelial cells, chronic hyperglycemia causes enhanced glycolytic and mitochondrial oxidative metabolism; [7] in rat chronic hyperglycemia inhibits glycolytic rates and alters substrate use of cardiovascular cells [17]. Excess glucose levels and/or the metabolic flux [14] associated with these cells leads to generation of excess intracellular superoxide and other mediators of oxidative stress, a phenomenon which is acknowledged to play an important role in the pathogenesis of diabetic complications [3, 18–20]. The primary source(s) of electrons that fuel superoxide production is still controversial and are likely to be multi-factorial. Williamson and colleagues demonstrated a strong link between polyol pathway flux and the ratio of free cytosolic NADH to NAD⁺, a factor critical to vascular function and redox homeostasis [21, 22]. There are a number of interactions of the polyol pathway and its coenzymes with other metabolic pathways. Cheng and Gonzalez [23] demonstrated that increased turnover of NADPH due to flux through AR competed with the antioxidant enzyme glutathione (GSH) reductase for the same pool of cytoplasmic NADPH. As detoxification of ROS and other peroxides are dependent on the availability of NADPH, any alteration and imbalance might lead to inability of the cells to protect against oxidative stress. The metabolic scenario created through depletion of NADPH and accumulation of reduced NAD leads to imbalance in the redox state creating a hypoxia-like response, or “pseudohypoxia” [21]. Increased NADH due to elevated activity of the polyol pathway leads to increased synthesis of diacylglycerol (DAG), which in turn activates phospholipase C and the protein kinase C family [3]. DAG-dependent activation has been shown to play critical role in smooth muscle cell proliferation induced by high glucose [24].

AR AND ATHEROSCLEROSIS

An important question has thus been to study the role of AR in cardiovascular disease. In recent years, there have been few studies which highlighted the role of AR in atherosclerosis. Inherently, mice display much lower levels of AR compared to human subjects. Hence, to develop a more relevant means to test the role of AR in murine models, a transgenic mouse line in which human AR (hAR) was expressed *via* a histocompatibility gene promoter [25] was used to study the effect of diabetes to accelerate atherosclerosis.

This transgene had AR activity comparable to that of humans. Upon crossing onto the atherogenic LDL receptor null background, the hAR transgene had an effect on acceleration of atherosclerosis particularly in streptozotocin induced diabetic mice [26]. In contrast, no significant effect of the hAR transgene was observed in non-diabetic mice. In parallel with increased atherosclerosis, the diabetic mice overexpressing hAR in the LDL receptor null background showed a decrease in anti-oxidant defenses, as observed by alteration of GSH. On the contrary, expression of hAR in high fat diet-fed mice with mild insulin resistance without hyperglycemia had no effect on vascular lesions, at least over the time course studied [27]. Thus, plausible evidence was shown to substantiate the fact that hyperglycemia is needed and might need to be sufficiently increased to provide substrate for AR in the acceleration of atherosclerosis.

In contrast, another study demonstrated increased lesion area in diabetic and non-diabetic AR knockout mice in apoE null background [28]. The lesions showed increased presence of 4-hydroxynonenal (4-HNE), an oxidative marker which correlated with the lesion size and this was attributed to defective clearance of toxic phospholipid aldehydes because of the AR deficient genotype. The lesions were shown to be highly stable as demonstrated by more collagen [28]. These contrasting reports lead to a paradox on the precise role of AR in cardiovascular disease. A plethora of questions arose regarding the ambiguity; wherein it was assumed either association of compensatory regulation in the AR null mice lead to alteration in vascular function or genetic over-expression simulates the disease pathophysiology. Emerging studies on the impact of aldehyde dehydrogenase-2 (ALDH-2) in detoxifying 4-HNE [29] adds further complexity to the interpretation of lipotoxic aldehyde levels in AR overexpressing and AR null mice. Thus, further investigation of the interplay between AR and ALDH-2 in detoxifying 4-HNE and other lipotoxic aldehydes in hAR and AR null mice is critical to resolving these contrasting findings.

Our recent study has elucidated that over-expression of hAR in apoE null mice made diabetic by streptozotocin is proatherogenic and that expression specifically in endothelial cells leads to increased pathology [30]. However, the highlight of the study was that use of a competitive inhibitor that reduces AR activity was found to reduce the lesion size significantly [30]. Overexpression of hAR led to endothelial dysfunction and increased expression of VCAM-1 and MMP-2. Diabetic hAR overexpressing mice aortic rings demonstrated decreased acetylcholine mediated endothelial vasorelaxation compared to the wild-type aorta. Endothelial cell specific overexpression of hAR imparted a similar effect [30]. In support of this study, other pharmacological studies of AR inhibition have shown improvement in acetylcholine induced relaxation of diabetic aorta in various murine models. These findings suggest that increased activity of the AR pathway in hyperglycemia is partly responsible for the abnormal endothelium-dependent relaxation in the diabetic blood vessel.

AR expression has been reported in CD68+ cells (monocytes/macrophages) in human atherosclerotic plaque macrophages [31]. Monocyte-derived macrophages isolated from human blood when incubated with oxLDL demonstrated increased AR gene expression and activity along with increased ROS. Inhibition of AR in these oxLDL-stimulated cells attenuated ROS generation. Similarly, endothelial function was improved and VCAM-1 and MMP-2 expression reduced by both pharmacological inhibition and targeted silencing of AR in ECs exposed to high oxLDL [30].

An additional mechanism proposed for AR-mediated accelerated atherosclerosis is through advanced glycation end (AGE) linked RAGE activation. In support of this, studies in smooth muscle cells incubated with AGE-bovine serum albumin (AGE-BSA) resulted in greater increments of ICAM-1 and monocyte chemoattractant protein-1 (MCP-1), migration and monocyte adhesion in AR transgenic versus wild-type cells [32]. These AGE adduct

mediated increases were suppressed either by pharmacological inhibition of AR using ARI (zopolrestat) or molecular intervention using AR antisense oligonucleotides [33].

AR IN THE MYOCARDIUM AND THE ROLE IN ISCHEMIA

Glucose flux *via* AR is known to be increased under ischemic conditions, irrespective of the presence or absence of diabetes [17, 34–37]. Several studies using isolated perfused hearts and transient occlusion of the left anterior descending coronary artery in mice and rats have shown enhanced glucose flux *via* AR [34, 35, 38, 39]. Pharmacological intervention using AR inhibitors reduced ischemic injury, attenuated ROS generation, improved glycolysis, increased ATP levels, and maintained the ionic balance (sodium and calcium homeostasis) in the heart [34, 35, 38, 39].

Studies performed in transgenic mice expressing human-relevant levels of AR showed increased cardiac injury during ischemia/reperfusion [35]. A more direct relationship of glucose flux *via* AR to oxidative stress was demonstrated in cardiac injury. AR influenced the opening of the mitochondrial permeability transition pore (MPTP) and was linked to generation of hydrogen peroxide and diminished antioxidant status as measured by GSH. Antioxidants or ARIs significantly reduced generation of ROS and inhibited MPTP opening in AR transgenic mitochondria after I/R [39]. Taken together, these studies implicate AR and ROS through the AR pathway as a key player in mediating I/R injury in the heart. In rabbit hearts subjected to I/R injury inhibition of AR was protective [40], although it has also been reported to abolish the cardioprotective effects of ischemic preconditioning [41]. Others [42] showed increases in AR activity during ischemia consistent with our earlier publication [34]; on the contrary they were unable to demonstrate cardio-protection with ARIs in a glucose-perfused isolated rat heart ischemia/reperfusion (I/R) model. Though reasons for these contrasting findings are not clear, it may be speculated that model-dependent variations and substrate availability during ischemia may underlie the apparent differences.

4-HNE accumulates during I/R. AR has been proposed to detoxify these aldehydes that accumulate during I/R. While studies from Chen *et al.* show reduction of 4-HNE by activation of ALDH2 and protection of hearts from ischemic damage, [29] other reports, including studies from our group, [43] have demonstrated increased injury, poor functional recovery and increased oxidative stress after myocardial I/R in mice hearts overexpressing AR. Further, mice expressing human AR displayed greater injury and higher malonyldialdehyde (MDA) content with reduced GSH [39]. Studies in rodent hearts subjected to I/R showed increases in the polyol pathway activity associated with oxidative damage. On the contrary, AR null mice were reported to have reduced oxidative stress and protection against ischemic injury [44]. In rat hearts subjected to I/R, increases in polyol pathway activity exacerbated oxidative damage [45]. Furthermore, AR inhibition in animals did not cause increases in lipid peroxidation products such as MDA [45, 46–49]. However further studies on comprehensive measurements of 4-HNE and the role of ALDH2 will enable us to understand the role of AR as a potential detoxifying enzyme in ischemic hearts.

Changes in AR expression have been demonstrated in failing hearts during diabetes. Type 2 diabetic rat hearts show increased substrate flux *via* AR and SDH [50]. Studies in dogs have linked changes in AR expression to progression of pacing induced heart failure [51]. In humans, increases in AR expression were observed in patients with ischemic cardiomyopathy and diabetic cardiomyopathy [52]. These reports underscore the importance of AR in the etiology of heart failure.

AR AND VASCULAR INJURY

AR is implicated in proliferation of smooth muscle cell (SMC) growth in a model of vascular repair. Intervention of AR prevents SMC growth in culture and *in situ* in balloon-injured carotid arteries [24, 53–57]. Increased glucose flux *via* the AR pathway was mediated through high-glucose-induced DAG accumulation and PKC activation in SMCs [24]. Inhibition of AR prevented high-glucose-induced stimulation of the extracellular signal-related kinase/mitogen-activated protein kinase and phosphatidylinositol 3-kinase [54], activated nuclear factor- κ B [55], TNF- α synthesis and release and vascular inflammation [58], decreased SMC chemotaxis and adhesion. These studies provide fundamental evidence for further evaluation of ARIs in diabetic patients undergoing angioplasty.

AR AND AGING

More recent studies have implicated altered glucose metabolism due to AR as one of the fundamental changes that contribute to age related vascular dysfunction and myocardial injury. Progressive increases in innate vascular dysfunction with aging have been demonstrated in humans and animals [59–67]. Alterations in metabolic and biochemical changes occur over time in aging vasculature, resulting in alterations in substrate metabolism and ATP levels, key factors that may contribute to vascular dysfunction [68–71]. Increased expression and activity of AR was observed in aged vasculature. Treatment of aged rats with an AR inhibitor improved the endothelial dependent vaso-relaxation [72]. More importantly, the study showed increased flux *via* the AR pathway was vulnerable to vascular disease partly *via* RAGE. AR driven AGEs were proposed to impact vascular function through RAGE [72].

Expression and activities of the polyol pathway enzymes mainly AR and SDH were significantly higher in aged vs. young rat hearts, and induction of ischemia further escalated the AR and SDH activity in the aged hearts [73]. Myocardial ischemic injury was significantly greater in aged rat and inhibition of AR reduced ischemic injury and improved cardiac functional recovery in those hearts [73]. Thus these studies provide the rationale for evaluating AR inhibitors as potential therapeutic adjuncts in treating myocardial infarction and vascular dysfunction in aging.

ARI_s AND TRANSLATIONAL APPLICATIONS IN HUMANS RELEVANT TO CARDIOVASCULAR STUDIES

Various studies in the murine and rodent hearts have shown beneficial effect of ARIs. Though novel classes of ARIs are in various stages of development, the classes of ARIs that belong to the carboxylic acid class and the hydantoins have been already studied [74–76]. Diabetic subjects (with neuropathy, n= 81) treated with zopolrestat (n=45) for one year displayed increased left ventricular ejection fraction (LVEF), cardiac output, left ventricle stroke volume and exercise LVEF [77]. In contrast, placebo-treated subjects demonstrated decreased exercise cardiac output, stroke volume and end diastolic volume [77]. In another clinical study, ARI treatment was associated with improved autonomic variability in diabetic patients with autonomic neuropathy [78]. These relatively small but key studies in human subjects with established diabetic complications underscore the promising potential of inhibiting AR in the heart in long-term diabetes.

Several other studies in type 1 and type 2 diabetic patients with nephropathy treated with ARIs have been reported. Administration of tolrestat for six months to type 1 diabetic subjects reduced urinary albumin excretion [79]. Similarly, type 2 diabetic subjects (n=35)

treated with epalrestat for five years had a similar clinical outcome [80]. In other studies, zopolrestat was administered to normotensive type 1 diabetic subjects (n=80) for one year and resulted in a progressive reduction in urinary albumin excretion that was not correlated with changes in glycosylated hemoglobin or blood pressure¹. These data strongly suggest that AR promotes diabetic cardiovascular and renal complications and provide key evidence in human subjects for the benefits of ARIs.

SUMMARY AND FUTURE PERSPECTIVES

AR is a central enzyme in the polyol pathway implicated in aberrant glucose metabolism and diabetic complications. Several studies demonstrate the critical role of AR in mediating ischemic myocardial injury, accelerating atherosclerosis, and vascular injury in diabetes and aging (Fig. 1). Though the boundaries to examine AR's physiologic actions in humans are open to debate, appropriate animal models are necessary to reproduce AR human biology to mimic the conditions representing human disease. Additional animal studies are required to understand various downstream targets of the AR pathway, specifically addressing ROS generation, source of the ROS at the sub-cellular level and inter-relationship with other pathways like the AGE-RAGE pathway in relevance to cardiovascular diseases. While there are on-going studies of ARIs in humans, the need of the hour is a well-designed large randomized multicenter human trial using an ARI, relatively free from side effects, which will help establish its therapeutic potential in cardiovascular diseases associated with diabetes, myocardial injury or even accelerated aging.

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ABBREVIATIONS

AGEs	Advanced Glycation End products
AR	Aldose Reductase
ARI	Aldose Reductase Inhibitor
DAG	Diacyl glycerol
4-HNE	4-Hydroxy Nonenal
I/R	Ischemia/Reperfusion
LVEF	Left Ventricular Ejection Fraction
MDA	Malondialdehyde
MPTP	Mitochondrial permeability Transition Pore
PKC	Protein Kinase C
RAGE	Receptor for Advanced Glycation End products
ROS	Reactive Oxygen Species
SDH	Sorbitol Dehydrogenase

¹Oates, P.J.; Klioze, S.S.; Schwartz, P.F.; Boland, A.D.; Group TZDNS. Aldose Reductase Inhibitor Zopolrestat Reduces Elevated Urinary Albumin Excretion Rate in Type 1 Diabetes Mellitus Subjects with Incipient Diabetic Nephropathy. *J. Am. Soc. Nephrol.*, 2008, 19, 642A.

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Fig. (1).

A schematic illustration of oxidative stress and AGEs generated by increased glucose flux *via* by AR plays a critical role in mediating myocardial ischemic injury, accelerating atherosclerosis, and vascular injury in diabetes and aging. Increased glucose flux *via* aldose reductase (AR) and sorbitol dehydrogenase (SDH) has several consequences, including 1) induction of osmotic stress due to increases in sorbitol and fructose pool size, 2) induction of glycativ stress due to generation of 3 deoxyglucosone (3-DOGzn- a precursor of advanced glycation end product), 3) elevation of cytosolic NADH/NAD⁺ ratio that leads to excess production of reactive xygen species including superoxide (O₂⁻) *via* cytosolic and mitochondrial NADH dependent pathways. Use of NADPH by AR may also impair glutathione levels. In some instances, increased substrate flux *via* hexokinase (HK) concomitant with a high NADH/NAD⁺ can also lead to increases in glyceraldehydes-3-phosphate (G3P) and methylglyoxal, another potent glycating agents. Furthermore, G3P conversion to α-glycerolphosphate, a precursor of diacylglycerol (DAG), results in activation of PKC. Abbreviations: 1,3PGA, 1,3-bis-phosphoglyceric acid; 2PGA, 2-phosphoglyceric acid; 3PGA, 3-phosphoglyceric acid; DHAP, dihydroxyacetone phosphate; F3P, fructose-3-phosphate; F6P, fructose-6-phosphate; FDP, fructose-1,6-diphosphate; G3PDH, glycerol-3-phosphate dehydrogenase; GSH, reduced glutathione; GSSG, oxidized glutathione; PEP, phosphoenolpyruvate; PFK, phsophofructokinase; PPP, pentose phosphate pathway. Modified from reference [14].