Review

Aldose reductase: new insights for an old enzyme

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Abstract

In the past years aldose reductase (AKR1B1; AR) is thought to be involved in the pathogenesis of secondary diabetic complications such as retinopathy, neuropathy, nephropathy and cataractogenesis. Subsequently, several AR inhibitors have been developed and tested for diabetic complications. Although these inhibitors have found to be safe for human use, they have not been successful in clinical studies because of limited efficacy. Recently, the potential physiological role of AR has been reassessed from a different point of view. Diverse groups suggested that AR, in addition to reducing glucose, also efficiently reduces oxidative stress-generated lipid peroxidation-derived aldehydes and their glutathione conjugates. Because lipid aldehydes alter cellular signals by regulating the activation of transcription factors such as NF-κB and AP1, inhibition of AR could inhibit such events. Indeed, a wide array of recent experimental evidence indicates that the inhibition of AR prevents oxidative stress-induced activation of NF-κB and AP1 signals that lead to cell death or growth. Furthermore, AR inhibitors have been shown to prevent inflammatory complications such as sepsis, asthma, colon cancer and uveitis in rodent animal models. The new experimental *in vitro* and *in vivo* data has provided a basis for investigating the clinical efficacy of AR inhibitors in preventing other inflammatory complications than diabetes. This review describes how recent studies have identified novel plethoric physiological and pathophysiological significance of AR in mediating inflammatory complications, and how the discovery of such new insights for this old enzyme could have considerable importance in envisioning potential new therapeutic strategies for the prevention or treatment of inflammatory diseases.

Keywords: aldose reductase; cancer; diabetes; inflammation; oxidative stress; sepsis; uveitis.

Introduction

Aldose reductase (AKR1B1; AR) is a monomeric reduced NAD phosphate (NADPH)-dependent cytosolic enzyme that belongs to a superfamily of aldo-keto reductases (AKR). The story of AR was started five decades back when it was first identified as a protein with glucose reducing activity in 1956 by Hers (1). Subsequently, van Heyningen (2) reported that high levels of sorbitol and galactitol accumulated in the diabetic and galactosemic rat ocular lens is due to increased AR activity. Following this observation, Kinoshita et al. (3–5) have shown that inhibition of this enzyme with flavinoids and pharmacological inhibitors such as sorbinil and tolrestat prevented the cataractogenesis in diabetic rats. Furthermore, several series of studies showed that the pathophysiological conditions attributed to hyperglycemia are believed to be caused by the accumulation of sorbitol in tissues via polyol pathway hyperactivity (Figure 1) (5–8). Sorbitol being impermeable through the biological membranes accumulates inside the tissues leading to osmotic stress. This results in ionic imbalance and protein insolubilization leading to secondary diabetic complications such as diabetic cataractogenesis, retinopathy, nephropathy, and neuropathy. Extensive investigations have been performed in the identification and development of potential AR inhibitors that could suppress sorbitol accumulation and prevent secondary diabetic complications. Several *in vitro* and experimental animal models indicate that drugs with varying AR inhibiting efficacy show significant protection against diabetic complications [see Refs. (9–11) for recent reviews]. The involvement of AR in diabetes is further supported by the demonstration that its overexpression in transgenic animals enhances hyperglycemic injury to specific target organs (12, 13). Such mice develop cataracts more rapidly during hyperglycemia compared to non-transgenic litter mates. In addition, polymorphism of the AR gene is a genetic risk marker for diabetic nephropathy reported to date (14), and AR gene expression is increased in peripheral blood mononuclear cells obtained from insulin-dependent diabetes mellitus patients with nephropathy (15). Together, these observations provide compelling evidence pointing to a significant role of AR in mediating hyperglycemic injury. However, in clinical trials some of the AR inhibitors have yielded uncertain results, in part, owing to lack of efficacy, skin allergic reactions, and liver toxicities. Despite three decades of intense investigations, including some clinical studies, the details of AR-mediated hyperglycemic injury remain unclear. In particular, the mechanisms which control and regulate the expression of the AR gene and the catalytic activity of AR protein remain poorly understood. Recent studies from the past decade or so suggest that reducing glucose might not be the major physiological function of AR. This is supported by studies which show prevention of diabetic cataracts by using antioxidants without affecting sorbitol levels (16–18). Furthermore, reports have also suggested that the increased activity of AR
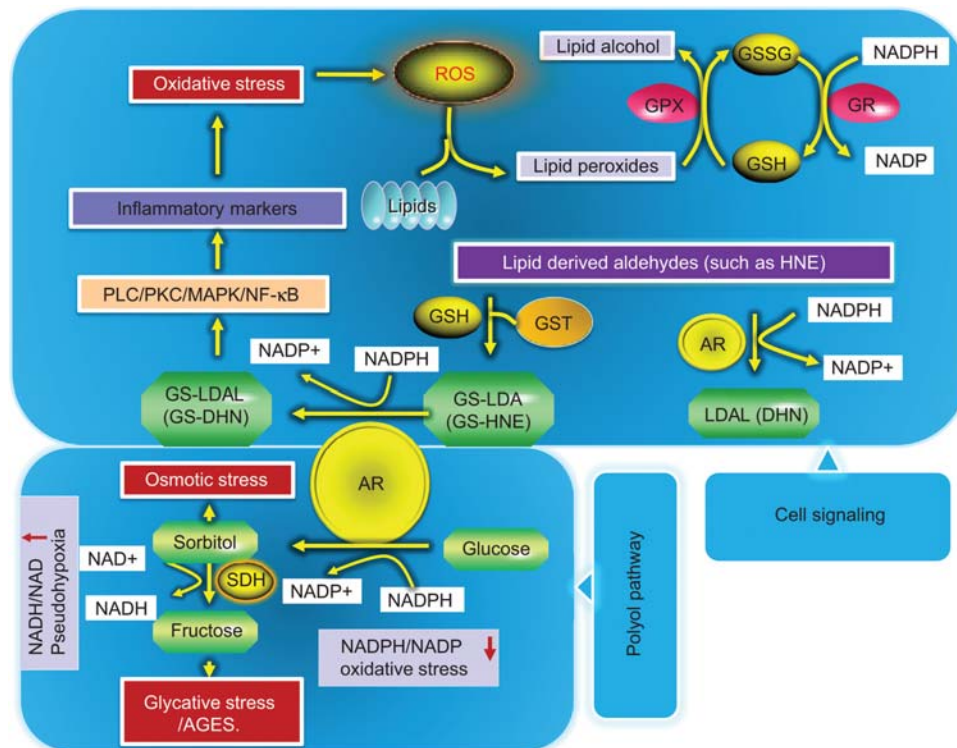
Figure 1   Aldose reductase regulates polyol pathway of glucose metabolism and lipid aldehyde mediated cell signaling.
During hyperglycemia AR reduces glucose to sorbitol using NADPH as a cofactor and later sorbitol dehydrogenase (SDH) reduces sorbitol to fructose using NAD as a cofactor. AR causes oxidative stress by decreasing the ratio of NADP/NADPH and also competing with glutathione reductase (GR) for NADPH. Increased accumulation of sorbitol could cause osmotic stress. Metabolites of fructose increase AGE formation and cause glycative stress. Furthermore, during oxidative stress conditions, AR catalyzed lipid aldehyde reaction products mediate cellular signals via activation of redox sensitive transcription factors.

under hyperglycemia leads to the depletion of cellular NAD-PH, which compromises antioxidative defenses, because NADPH is an essential cofactor for reduction of oxidized glutathione (GSSG) by glutathione reductase (11).

The isolated, homogeneous enzyme has a poor affinity for glucose (K_m of 50–100 mM), and its kinetic and structural properties are unlike those of other glucose-metabolizing enzymes. In the mid-1990s, the structural studies and X-ray analyses of AR crystals indicated that the active site of AR lacked the ionic residues characteristic of polyol-binding proteins but revealed highly plastic and hydrophobic residues at its active site (19). These studies thus indicate that the high hydrophobicity of the substrate-binding domain essentially precludes efficient carbohydrate reduction, and suggests that hydrophobic aldehydes are likely to be the preferred substrates. From there on, several studies were directed to identify potential physiological substrates of AR.

The most obvious endogenous source of hydrophobic aldehydes is lipid peroxidation, which generates high concentrations of long-chain aldehydes. Because several of these are unsaturated, such as 4-hydroxynonenal (HNE) the most abundant, they display high toxicity, owing to their ability to bind cellular glutathione (GSH). In the early 2000s, simultaneous reports from two groups indicated that AR efficiently catalyzes lipid peroxidation-derived aldehydes (LDAs) such as HNE (20, 21). Later, our studies show that recombinant

human AR catalyzes the reduction of a large series of saturated and unsaturated aldehydes with 1000-fold higher efficiency than glucose (22–25). Furthermore, AR is particularly efficient in catalyzing medium- to long-chain (C-6 to C-18) aldehydes generally generated during lipid peroxidation (Figure 1). In addition to aldehydes, this enzyme exhibited a higher efficiency in catalyzing the reduction of the GSH conjugates of unsaturated aldehydes than that of their parent free aldehydes (23–25). Thus, our studies show that AR is an important metabolic route for the detoxification of lipid-derived aldehydes. This conclusion is supported by the observations that (a) homogeneous AR catalyzes the reduction of HNE and its conjugate GS-HNE (to DHN and GS-DHN, respectively) with an affinity which is four orders of magnitude higher than that for glucose (20); (b) the generation of GS-DHN in perfused rat hearts (26), lens (27), and erythrocytes (28) exposed to HNE is prevented by AR inhibition; (c) inhibition of AR exacerbates the toxicity of HNE to the ocular lens, isolated cardiac myocytes, and vascular smooth muscle cells (VSMCs) in culture; and (d) exposure of VSMCs to HNE leads to marked upregulation of AR (29). Taken together, these observations provide firm support for the concept that metabolism of LDAs is a significant *in vivo* role of AR (Figure 2). Because LDAs are known to alter the cellular function by regulating the oxidative stress signals mediated by NF-κB and AP1 (30–32), it was hypothesized

that AR regulates cellular function by altering the oxidative stress signals. This hypothesis is supported by the studies which indicate that the inhibition of AR prevents HNE-, growth factor- and cytokine-induced cytotoxicity in cultured cells (33–37). Most importantly, our studies indicating inhibition of AR prevents endotoxin, allergen, cytokine, and growth factor-induced activation of NF-κB signals made a solid foundation in recapitulating the novel role of AR in the pathophysiology of various disease processes (Figure 2). Very recent and currently ongoing studies indicate that the inhibition of AR prevents various inflammatory diseases such as sepsis, asthma, and colon cancer in experimental animals. In this review, we recapitulate the novel role of AR in the pathophysiology of inflammatory diseases.

The polyol pathway hypothesis of diabetic complications

The polyol pathway enzyme AR has been implicated in the development of secondary diabetic complications in general and diabetic nephropathy, in particular. The polyol pathway consists of two enzymes (Figure 1): AR, which catalyzes the NADPH-mediated reduction of glucose to sorbitol, and sorbitol dehydrogenase, which catalyzes the conversion of sorbitol to fructose, utilizing nicotinamide adenine dinucleotide (NAD). The net result of the polyol pathway is the formation of fructose from glucose and the transfer of reducing equivalents from NADPH to NAD. Under normoglycemic conditions the polyol pathway affects <3% of glucose flux. However, under hyperglycemia this pathway can account for 25–30% of the total glucose metabolism (11). It has been suggested that the hyperglycemia-induced increase in activity of the polyol pathway (and the attendant metabolic changes) are the primary cause of hyperglycemic injury. In agreement with a crucial role of AR in mediating hyperglycemic injury, it was demonstrated that synthetic inhibitors of AR prevent, delay and in some cases even reverse tissue injury owing to several secondary diabetic complications, and AR inhibitors decrease elevated urinary albumin excretion. Although overwhelming evidence derived from inhibitor studies, transgenic animals and genetic susceptibility analysis suggest a crucial role of AR in diabetic nephropathy, the lack of a clear mechanistic understanding made the data remain only correlative. Based on a high accumulation of polyol in diabetic and galactocemic lens, it was initially suggested that the AR perturbs cell structure, function, and ion balance by inducing osmotic stress owing to membrane-impermeable polyols. However, later experiments demonstrated that even under extreme and prolonged hyperglycemia the sorbitol concentrations are not osmotically relevant. Although several other explanations have been put forward to account for its injurious effects (e.g., depletion of myoinositol and NADPH, as well as generation of pseudohypoxia), the role of AR in
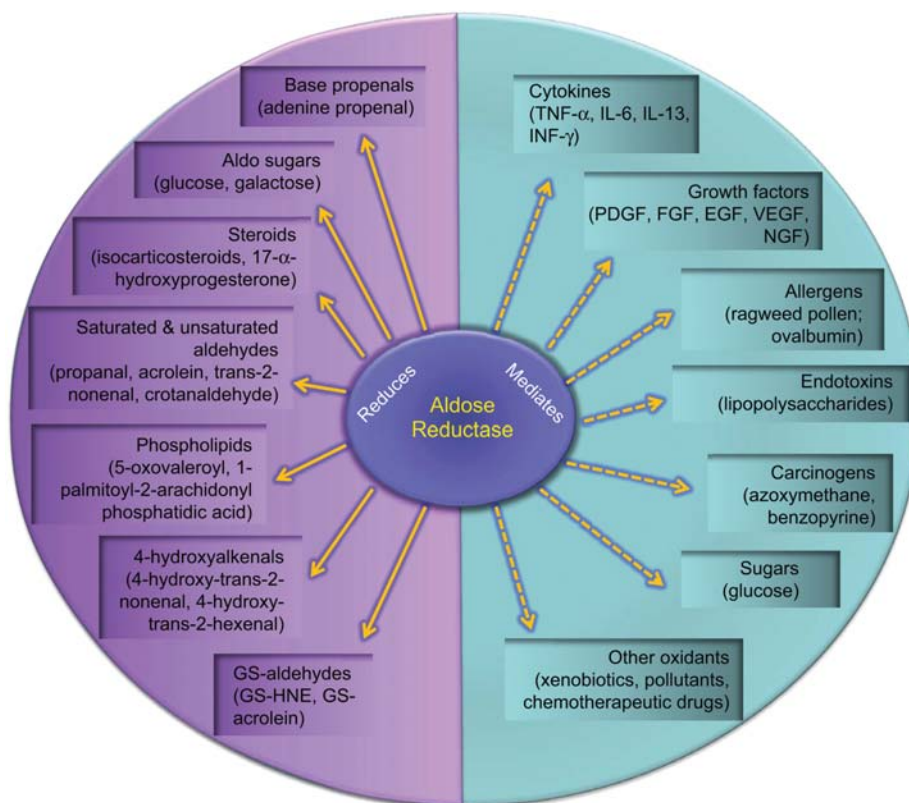

Figure 2 Aldose reductase catalyzes a wide array of substrates and regulates cellular signals initiated by various oxidants.

mediating hyperglycemic injury remains obscure. However, even from the beginning, several features of AR were disconcerting. The isolated, homogeneous enzyme has a poor affinity for glucose (K_m of 50–100 mM), and its kinetic and structural properties are unlike those of other glucose-metabolizing enzymes. The high hydrophobicity of the substrate-binding domain of AR essentially precludes efficient carbohydrate reduction and suggests that hydrophobic aldehydes are likely to be the preferred substrates. Indeed, we and others have shown that AR efficiently catalyzes lipid aldehydes and their GSH conjugates (20–27). Specifically, we have also found that the AR catalytic site has more affinity towards GS-aldehyde conjugates than parent aldehydes alone. By using site-directed mutagenesis experiments, we have found that the AR active site has residues responsible for binding to glutathione moiety (24). Indeed, we have recently solved the crystal structure of GSH-analog bound to AR, and our structural studies confirmed that AR has a specific GS-aldehyde binding site in its active site (38). Taken together, these observations provide firm support for the concept that metabolism of LDAs is a significant *in vivo* role of AR. Based on this identification, it was concluded that upregulation of AR during diabetes could be a response to oxidative stress, which appears to be an important feature of long-term diabetes, given the increased generation of reactive oxygen species (ROS) and increased accumulation of lipid peroxidation products in tissues from diabetic animals and humans. The significance of AR in diabetes is also strengthened by identification of genetic polymorphisms associated with the human AR gene. Ko and colleagues identified that (A–C)n dinucleotide repeat domain at the 5′ end of the AR gene is associated with early-onset diabetic retinopathy in non-insulin-dependent diabetes mellitus patients (39). Later, several studies indicated that AR Z-2 (A–C)n microsatellite polymorphism in the AR gene is associated with several secondary diabetic complications, including retinopathy, neuropathy, and nephropathy (40–46). Thus, studies performed in different population groups have established the association of AR gene polymorphism and diabetic complications.

Significance of AR in oxidative stress signaling

It is well established that ROS generated in response to cytokines, growth factors, lipopolysaccharide (LPS), and hyperglycemia cause lipid peroxidation and form LDAs. The LDAs and their GSH conjugates are excellent AR substrates. Because ROS is an essential mediator of intracellular signaling under a variety of conditions, some of the mitogenic and cytotoxic effects of ROS can be mediated by LDAs and their GSH conjugates. Indeed, at low levels HNE is a potent smooth muscle cell mitogen, and at high concentrations it induces apoptosis in several cell types (47–50). Moreover, inhibition of HNE metabolism by inhibiting AR prevents the growth of vascular lesions (29). To build on these observations, our laboratory undertook a systematic study to delineate the role of AR in mediating the cytotoxic signals of cytokines. These studies indicate that reduction of LDAs and

their GSH conjugates is essential for transducing the cytotoxic signals (47). Increased oxidative stress and lipid peroxidation are key features of inflammation-induced cytotoxicity and activation of redox sensitive transcription factors such as NF-κB and AP1, which stimulate the expression of genes that transcribe inflammatory cytokines and chemokines. Uncontrolled and excessive production of inflammatory mediators causes cytotoxicity in an autocrine and paracrine manner. The ROS-sensitive transcription factor NF-κB is a crucial mediator of oxidative stress-induced inflammation initiated by bacterial infections, xenobiotics, environmental pollutants, and autoimmune diseases (51, 52). When inactive, it is sequestered in the cytosol as a complex with its inhibitor, IκB; stimulation of protein kinases such as protein kinase C (PKC), mitogen-activated protein kinase (MAPK), and IκB kinase (IKK) results in the activation of NF-κB via phosphorylation of IκB (51, 52). Several studies have shown that ROS activate NF-κB, but the mechanisms are not clearly understood. 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 MAPK superfamily of serine/threonine kinases that also includes the extracellular signal-regulated kinases ERK1/2 and p38MAPK (53–55). All MAPKs are activated via a cascade of phosphorylation reactions. We have recently shown that inhibition or ablation of AR attenuates the phosphorylation of p38 and JNK in endothelial cells and macrophages (56, 57).

Lipid peroxidation has been suggested to be a major contributor to the pathophysiology of inflammation (31). At low concentrations lipid peroxidation products such as HNE can stimulate proliferation of VSMCs and apoptosis in vascular endothelial cells (VECs) and at high concentrations HNE is genotoxic and mutagenic (47, 58, 59). Thus, as discussed above, the enzymes that can detoxify HNE could be involved in mediating oxidative stress-induced signals, including those of cytokine and LPS that activate transcription factors. The enzymes that regulate the concentration of HNE and its metabolites are known to modify the activities of multiple cytoskeletal proteins, MAPKs and transcription factors (60). Several reports suggest that PKC activation by HNE and its metabolites cause inflammation (61–64). Increased generation of ROS by growth factors, cytokines, chemokines, and LPS could be an essential step for cell growth because overexpression of antioxidants such as catalase and super oxide dismutase (SOD) or treatment with N-acetylcysteine are known to diminish growth factor and cytokine-stimulated cell growth (65–67). It has been demonstrated that through ROS, growth factors stimulate redox-sensitive transcription factors such as NF-κB, AP1, CREB and ATF2 (Figure 3) (68). Of these, NF-κB is the major transcription factor activated by oxidative stress (69). Because LPS signals are propagated by autocrine and paracrine effects to generate excessive amounts of cytokines and growth factors during the inflammatory response, antibodies against cytokines such as interleukin-18 (IL-18), tumor necrosis factor-α (TNF-α)

and IL-6 have been shown to attenuate the progression of LPS-induced cytotoxicity (70–72).

The association of ROS and AR is supported by the observation that inhibitors of AR attenuate glucose-induced oxidative stress and superoxide production in retinal pericytes, bovine aortic endothelial cells, and rabbit aortas (73–75). The strongest evidence that AR is involved in mediating growth comes from our studies showing that inhibition of AR prevents proliferation of cultured VSMCs in response to fibroblast growth factor, high glucose, and thrombin (29, 34, 76). The observation that the AR inhibitor, epalrestat, prevents initial thickening in the coronary arteries of galactose-fed beagle dogs provides additional support for the role of AR in abnormal VSMC growth (77, 78). We recently demonstrated that AR plays a pivotal role in the proliferation of VSMCs, apoptosis of VECs, and restenosis of rat carotid arteries after balloon injury (33, 57, 76). In addition, a significant decrease in neointima formation in balloon-injured rat carotid arteries, inhibition of AR diminished *in situ* activation of NF-κB during restenosis as well as in cultured VSMCs (35, 76, 79, 80). The inhibition of AR has been

shown to regulate cell growth and death by modulating the cell cycle events at the G1/S transition in VSMCs as well as in colon cancer cells (81, 82). Our studies also indicated that AR mediates high glucose-induced VSMC growth by regulating the high glucose triggered release of TNF-α, a major proinflammatory cytokine, in VSMCs via activation of PKC and TACE (83, 84). Our recent observations show that AR mediates the mitogenic and cytotoxic signals of cytokines and growth factors. We have further shown that inhibition or ablation of AR attenuates TNF-α- and growth factor-induced IκB-α phosphorylation, degradation, and activation of NF-κB, and PKC, proliferation of VSMCs and apoptosis of VECs, human lens epithelial cells (HLECs), and macrophages (24, 57). These findings are consistent with our hypothesis that AR, via modulation of NF-κB, is involved in the regulation of many genes during inflammation induced by cytokines (TNF, IL-1, IL-8, IL-6), cell adhesion proteins such as ICAM-1, MHC genes, enzymes such as nitric oxide synthase (NOS), Cox and Mn-SOD, and endotoxins such as LPS (Figure 3). In addition, AR inhibition has also been shown to regulate the expression of glucose transporter pro-
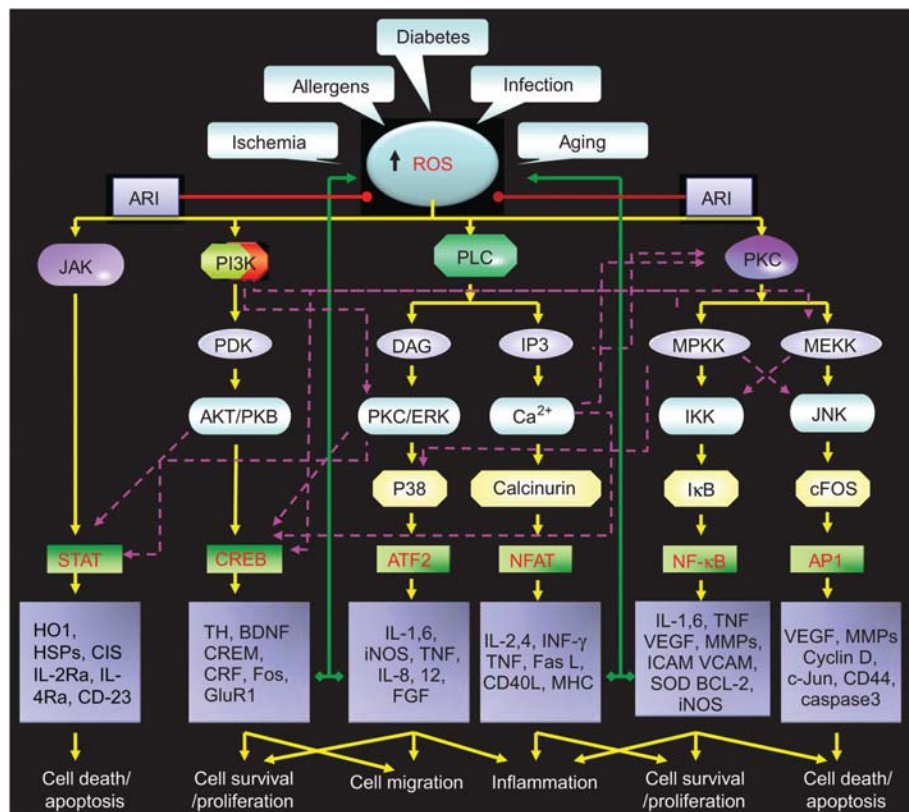

Figure 3 Aldose reductase prevents inflammatory complications by preventing the oxidative stress-induced inflammatory signals downstream to reactive oxygen species.
Various disease conditions such as sepsis, diabetes, and infections by allergens could cause oxidative stress by generating the reactive oxygen species. The increased ROS levels are well known to mediate inflammatory signaling by activating various protein kinases such as JNK, PI3K, PKC, and PLC, etc., which activate redox sensitive transcription factors such as STAT, CREB, NF-κB, AP1, NFAT, and ATF2 via a series of signaling events transduced by other kinases such as MAPK, ERK, and JAK. The activation of transcription factors lead to the transcriptional activation of inflammatory cytokines, chemokines, and growth factors which in an autocrine and paracrine manner could amplify inflammatory complications. Aldose reductase inhibitors (ARIs) could prevent the inflammatory signaling by preventing pathways downstream to ROS as well as autocrine/paracrine mediated formation of ROS by inflammatory proteins.

teins in macrophages. Reddy et al. (85) have shown that AR inhibitors prevent the LPS-induced activation of transcription factor CREB via regulating the ROS/cAMP/PKA pathway. Hwang et al. (86) have also shown that ROS activated JAK2/STAT5 pathway in ischemic hearts was inhibited by AR inhibition, indicating that AR also mediates the JAK/STAT signaling pathway. These studies indicate that AR inhibition works downstream of ROS formation and also blocks ROS generation in an autocrine and paracrine manner. Because various oxidant stimuli such as LPS, cytokines, growth factors, and high glucose are known to cause oxidative stress and increase the synthesis of inflammatory cytokines and chemokines, AR-catalyzed reaction products should play an important role in eliciting oxidative stress-induced cytotoxicity and inflammation. This is further substantiated by our demonstration that HNE, GS-HNE, and GS-DHN promote cultured VSMC growth (87). AR inhibition or ablation of AR by siRNA prevents HNE- and GS-HNE-induced growth, but has no effect on the GS-DHN-induced VSMC proliferation. These studies indicate that it is the reduced form of lipid-aldehyde glutathione conjugates (GS-DHN) that are involved in the oxidative stress-induced LDA-mediated signaling. Furthermore, studies are required to investigate how GS-DHN activates gene transcription. Nevertheless, our observations have opened a wide area of research into the role of lipid peroxide and lipid aldehyde formation in oxidative stress signaling.

Significance of AR in inflammatory complications

Inflammation is a complex system of a host systemic and local response to injury and infection. Inflammation contributes to almost all disease processes, including immunological and vascular pathology, sepsis, and chemical and metabolic injury. In inflammation, the regulation of the immune response by macrophages plays a central role which triggers gene induction of proinflammatory cytokines, such as TNF-α, 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 infection. However, uncontrolled and excessive cytokine expression can induce acute or chronic inflammatory processes. Recent studies indicate that inhibition of AR prevents cytokine-, growth factor- and high glucose-induced apoptosis of HLECs, VECs, macrophages, and proliferation of VSMCs and colon cancer cells. As shown in Figure 4, ROS generated during growth factor and cytokine signaling induce lipid peroxidation, which in turn leads to the generation of a wide range of cytotoxic aldehydes such as HNE. These aldehydes react readily with reduced GSH to form glutathionyl aldehydes such as GS-HNE. We have found that AR efficiently reduces GS-HNE to GS-DHN and that the GS-DHN formed in turn activates phospholiase C (PLC) via an unidentified mechanism. This
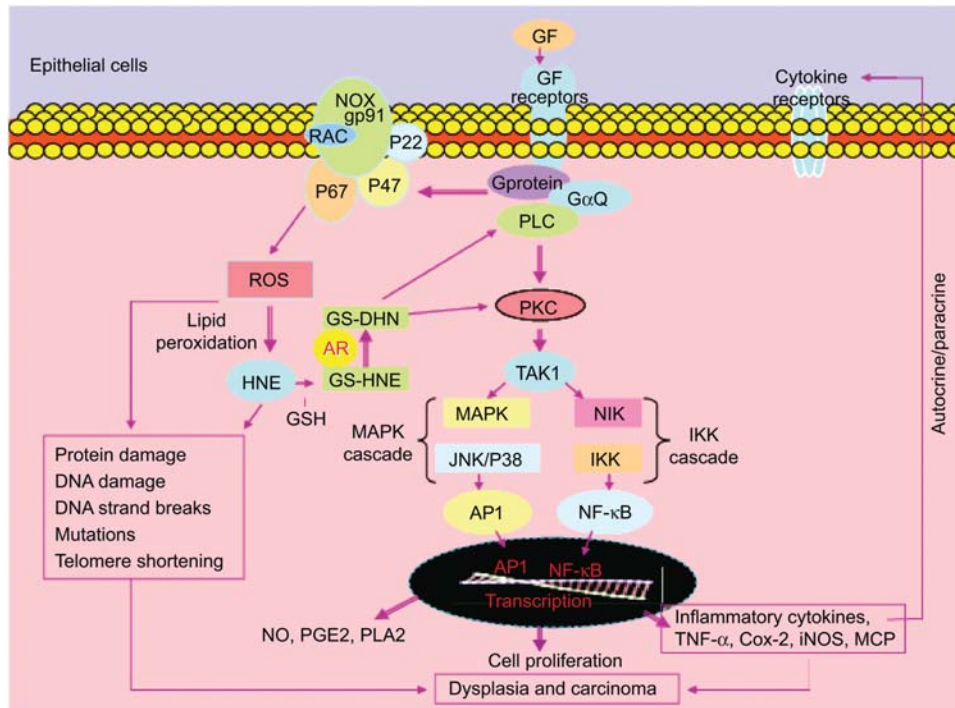

Figure 4 Aldose reductase mediates growth factor-induced inflammatory signals.
Growth factor stimulated oxidative stress generates lipid peroxidation-derived lipid aldehydes such as highly toxic HNE. HNE being highly electrophilic conjugates with cellular GSH to form GS-HNE. AR catalyzes the reduction of GS-HNE to GS-DHN. The GS-DHN has been shown to mediate oxidative stress signals upstream to PLC/PKC leading to activation of transcription factors such as NF-κB and AP1 which transcribes inflammatory genes. The inflammatory proteins propagate the carcinogenic signals and cause tissue damage, dysfunction leading to uncontrolled tumor growth.

leads to the activation of signaling cascades that involve the activation of such transcription factors as NF-κB and AP1 via PKC/PI3K/MAPK/IKK. NF-κB and AP1 stimulate transcription of various inflammatory cytokines (TNF-α, interleukins), chemokines (MCP-1, MIP-1), and inflammatory mediators such as cycloxygenase-2 (Cox2) and inducible NOS (iNOS). Uncontrolled productions of these inflammatory markers cause cytotoxicity leading to tissue damage and dysfunction leading to pathologies such as cancer and inflammatory response syndrome. We have shown that AR inhibitors such as sorbinil, tolrestat, zopolrestat, and fidarestat, as well as ablation of AR by siRNA, effectively block the ROS-induced formation of GS-DHN and downregulate stress signals that activate NF-κB and AP1 in vascular cells and macrophages (87). Furthermore, AR inhibitors also prevented the activation of caspase-3 and degradation of nucleosomal histones by high glucose or TNF-α in HLECs and by LPS in macrophages (35, 56). These results raised the interesting and significant question of how AR regulates the signaling events initiated by cytokines and growth factors, and how inhibition of AR prevents cytokine and growth factor signaling. Understanding this role of AR should provide pharmacological tools for eventual therapeutic interventions to control cell proliferation, apoptosis, tissue repair, and to prevent the cytotoxicity of cytokines and chemokines, which are increased during oxidative stress. More importantly, these studies will provide a mechanistic link between oxidative stress and inflammation. We have extended our investigations from cultured cells to various animal models of inflammatory diseases such as colon cancer, sepsis, asthma, and uveitis and have shown that inhibition of AR prevents these inflammatory disorders. These studies backed by strong evidence obtained using cellular as well as animal models suggest that AR plays a pivotal role in the pathophysiology of inflammation.

Aldose reductase in sepsis

During sepsis and related systemic inflammatory response syndrome, the chemical or biological (bacterial and viral) agents cause severe toxicity by increasing oxidative stress (88, 89). The acute uncontrolled inflammatory response can lead to extensive tissue injury and multiple organ failure. Studies from our laboratory have indicated that the toxic effects of uncontrolled inflammation can be effectively prevented or significantly ameliorated by inhibiting AR either by pharmacological AR inhibiting drugs or by genetic ablation of AR message (87). Despite mechanistic ambiguities, our demonstration that AR mediates cytokine-induced activation of NF-κB suggested to us an entirely new modality for prevention and treatment of the acute inflammatory episodes. Therefore, to examine the role of AR in a cellular model of inflammation, we investigated how AR mediates the lipopolysaccharide (LPS)-induced release of inflammatory mediators in RAW264.7 murine macrophages and peritoneal macrophages (87, 90). Pharmacological inhibition or siRNA ablation of AR prevented the biosynthesis of cyto-

kines in LPS-activated RAW264.7 cells. Inhibition or ablation of AR significantly attenuated LPS-induced activation of PKC and PLC, nuclear translocation of NF-κB, and phosphorylation and proteolytic degradation of IκB-α in macrophages, suggesting that inhibition of AR prevents key steps in the development of inflammation. Given our results with macrophages in culture, we tested whether inhibition of AR would also prevent acute inflammatory events in vivo. For this, we chose to study endotoxin-induced sepsis complications in mice (91). Our results show that as in LPS-treated macrophages administration of AR inhibitor in the mice prevented the serum, liver, spleen, and heart inflammatory cytokines in response to LPS challenge (87). Treatment with AR inhibitor blunted the activation of PKC, JNK, and p38-MAPK, as well as phosphorylation of IκB-α, IKK, and PLC. These changes were associated with decreased myocardial NF-κB and AP1 activity, PGE2 production, induction of Cox2, and iNOS. Furthermore, our studies demonstrate that inhibition of AR prevented the LPS-induced functional recovery in myocardial fractional shortening in vivo and preserved contractile function of isolated perfused hearts, indicating that AR inhibition prevents LPS-induced cardiomyopathy (91). Most importantly, inhibition of AR increased survival in mice injected with lethal doses of LPS. Similarly, AR inhibition also prevented the inflammatory cytokine levels in a cecum ligation and puncture model of polymicrobial sepsis, which closely mimics the sepsis syndrome in humans (92). These observations provided a promising demonstration of the potentially high therapeutic efficacy of AR inhibitors in treating sepsis and other acute inflammatory syndromes.

Aldose reductase in asthma pathogenesis

Asthma is one of the most common chronic respiratory diseases, with more than 100 million sufferers worldwide (93). This inflammatory disorder is caused by a hypersensitive immune system that results from several triggers, such as dust, pollen, viruses, and changes in the weather. Although it is not clear how asthma is initiated in the setting of chronic inflammation, accumulating evidence strongly support the association of airway inflammation with asthma (94). Furthermore, the increase in inflammation in the bronchial epithelium leads to eosinophil infiltration, an increase in mucus production, and most importantly upregulation of cytokines such as TNF-α, IL-4, IL-5, IL-6, and IL-13, chemokines such as MCP-1, and MIP-1, adhesion molecules such as ICAM-1, and E- and P-selectins (94). Thus, exposure of nearby cells to inflammatory cytokines and chemokines can trigger various autocrine/paracrine effects, leading to Th2 immune response and inflammatory cell accumulation. Our studies indicate that inhibition of AR prevents expression of inflammatory markers in human small airway epithelial cells, indicating that AR inhibition could prevent asthma (95–97). Indeed, we examined the efficacy of AR inhibitors in prevention of allergen-induced airway inflammation in mouse models of asthma. We found that AR inhibition prevents ragweed pollen extract and ovalbumin-induced allergic res-

ponses, airway inflammation and hyper-responsiveness in mouse models of asthma. Our studies also indicate that AR inhibitors significantly prevent the number of eosinophils and mucin levels in bronchoalveolar lavage (BAL) fluid of allergen-treated mice. These results indicate that AR inhibition could significantly prevent the pathophysiological effects of allergen-induced respiratory complications.

Aldose reductase in uveitis

Uveitis is a systemic inflammatory response syndrome characterized by excessive production of inflammatory cytokines generated in response to bacterial infections (98). Our investigations indicating that AR plays an obligatory role in mediating bacterial endotoxin-stimulated inflammatory signaling suggest that inhibition of AR could be a useful approach for attenuating maladaptive host responses and for treating acute ocular inflammation due to uveitis. To determine whether inhibition of AR prevents ocular inflammation *in vivo*, we examined the effects of AR inhibitor on NF-κB signaling pathways and ocular inflammation in a rat model of LPS-induced uveitis (99). Inhibition of AR prevents inflammatory marker levels in the aqueous humor of uveitis rat eyes. AR inhibition also suppressed the inflammatory cells infiltration and protein concentration in the aqueous humor of uveitis rat eyes. Similarly, the increase of inflammatory cytokines such as TNF-α, NO and PGE2 levels in the aqueous humor of uveitis rat eyes was significantly attenuated by AR inhibition. Similarly, the increased expression of TNF-α, iNOS and Cox2 proteins in the ciliary body, corneal epithelium, and retinal wall was significantly prevented by AR inhibition. In addition to pharmacological inhibitors of AR, natural compounds such as benfotiamine and guggulsterone which prevent the expression of AR and the activation of NF-κB also ameliorate endotoxin-induced uveitis in rats (100–102). Thus, based upon these results, AR inhibitors could be used therapeutically to treat patients with uveitis and its associated complications that have the potential of stimulating the inflammatory signals.

Aldose reductase in cancer

Colon cancer is the third most common form of cancer and the second leading cause of cancer-related deaths in Western countries, including the United States (103). Epidemiological and experimental studies indicate that colon cancer is usually mediated by dietary and environmental factors and is more pronounced in genetically predisposed subjects (103, 104). Recent studies indicate that inflammation plays a major role in colon carcinogenesis (105). Results from our investigations have established the role of AR in the carcinogenic signaling induced by growth factors and cytokines, and provided new insights into the physiological role of this enzyme in colon cancer cell mitogenicity as well as inflammation associated with colon carcinogenesis. Our recent studies indicate that ROS-induced signaling that activates NF-κB and transcribes genes responsible for tumor progression is pre-

vented by AR inhibition in human colon cancer cells (37, 83, 106). Similarly, inhibition of AR also prevented tumor growth in nude mice bearing human adenocarcinoma (SW480 cell) xenografts. Furthermore, we have identified that reduced lipid aldehyde glutathione conjugate catalyzed by AR is a novel signaling intermediate in the transduction of ROS-initiated cell signals leading to mitogenicity in colon cancer cells. We have found that AR knockout mice are resistant to chemically induced colon cancer in the azoxymethane-induced mouse model (107). Thus, our results suggest that AR inhibitors could be used therapeutically to prevent colon cancer and its associated complications.

Expert opinion

Our demonstration that AR also efficiently reduces lipid aldehydes and their conjugates with GSH has opened new dimensions in understanding the detoxification of reactive aldehydes generated during lipid peroxidation. Using kinetic, structural, and physiological studies, we have investigated the mechanisms by which AR selectively recognizes and catalyzes the reduction of LDAs and their GSH conjugates. We have also shown that AR activity can be regulated by lipid aldehydes, as well as by NO. To our surprise, we have found that AR-catalyzed lipid aldehyde products are obligatory mediators of cytokine-, chemokine-, growth factor- and LPS-induced cellular cytotoxicity as measured by decreased cell growth or apoptosis. Recent studies demonstrate that AR plays a pivotal role in inflammation (108, 109). Understanding this role of AR has provided pharmacological tools for eventual therapeutic interventions to control cell proliferation, apoptosis, tissue repair, and to prevent the cytotoxicity of cytokines, which are increased during infections and inflammation. More importantly, these studies provided a mechanistic link with oxidative stress-induced toxicity, especially in inflammatory pathologies where oxidative stress is known to cause toxicity through the expression of proinflammatory cytokines and chemokines. Thus, based upon these results, AR inhibitors could be used therapeutically to treat patients with inflammatory diseases such as asthma, colon cancer, uveitis, sepsis, burn, and other injuries such as those caused by viruses and bioterrorism that have the potential of stimulating the immune system and generating large amounts of inflammatory cytokines and chemokines. These AR inhibitors could also be used to prevent inflammation mediated by cytokines and chemokines, irrespective of the source.

Outlook

Inflammatory complications, including sepsis, cancer, and asthma, remain huge clinical problems worldwide despite improved health care and specific treatment approaches. Accordingly, there is an ongoing need for development of new therapeutic strategies in the treatment of such diseases. Elucidation of cytokine signaling is crucial for understanding multiple diseases, including infection, atherosclerosis, and cancer, and for developing therapeutic interventions for min-

imizing their inflammatory components. Hence, investigating the mechanisms that normalize inflammatory signals has intense importance for understanding and managing a wide array of disease processes. As described in the present review, extensive research during recent years has identified that AR plays a major role in mediation of oxidative stress-induced inflammatory signals via PLC/PKC/IKK/MAPK/NF-κB/AP1. Inhibition of AR prevents inflammatory diseases such as uveitis, sepsis, colon cancer, atherosclerosis, and asthma in experimental animal models. We expect that these results will shed new light on the fundamental mechanisms regulating inflammation as well as lay down the foundation for future studies to devise strategies for clinical implications. Accordingly, potential strategies that prevent AR and retard the progression of inflammatory complications still need to be evaluated. The challenge for future research will be to unravel these complex interactions that AR mediates between cellular metabolism, inflammation, and cancer. A better understanding of the signaling pathways engaged by AR-catalyzed lipid peroxidation products and their glutathione conjugates will help in understanding the causes of tissue and organ dysfunction. The future search for new potential pathways and the development of rational therapeutic options for better management of inflammatory complications could be facilitated by using newly developed tools such as microRNA technology, nanoparticle based drug delivery, and microarrays to identify the signaling pathways mediated by AR.

Highlights

- AR catalyzes the first and rate limiting step of the polyol pathway of glucose metabolism.
- AR has been shown to be involved in secondary diabetic complications.
- Many AR inhibitors have gone to phase III clinical studies for diabetic neuropathy, but failed owing to lack of efficacy.
- AR in addition to reducing glucose efficiently reduces lipid peroxidation-derived lipid aldehydes and their conjugates with glutathione.
- AR-catalyzed reaction product such as GS-DHN could mediate oxidative stress signals.
- AR regulates cytokine, chemokine, growth factor, endotoxin, hyperglycemia signals by regulating PKC/MAPK/IKK/NF-κB and AP1 pathways.
- AR is now shown to be involved in the pathophysiology of inflammatory complications such as atherosclerosis, colon cancer, sepsis, asthma, and uveitis.
- AR inhibitors could be anti-inflammatory, antimitogenic, and chemotherapeutic agents and their potential efficacy needs to be investigated at the translational levels.

Acknowledgments

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