

Current Prospective of Aldose Reductase Inhibition in the Therapy of Allergic Airway Inflammation in Asthma

K.V. Ramana¹, U.C.S. Yadav¹, W.J. Calhoun² and S.K. Srivastava^{*1}

¹Department of Biochemistry and Molecular Biology, and ²Internal Medicine-Pulmonary/Critical Care, University of Texas Medical Branch, Galveston, TX, USA

Abstract: The prevalence of asthma and costs of its care have been continuously increasing, but novel therapeutic options to treat this inflammatory disease have not been brought to the US market. Current therapies such as inhaled steroids, long-acting beta-agonist bronchodilators, antihistamines and immunomodulators may control the symptoms of allergic asthma but fail to modify the underlying disease. Excessive use of steroids and other immunosuppressants alter the patient's quality of life, produce undesirable toxicities, and increase the risk of other pathologies such as diabetes. Hence novel therapeutic options to manage asthma are desirable. In the present review, we have discussed the role of the polyol pathway enzyme aldose reductase (AR) in the amplification of allergic airway inflammation. Recent studies have indicated that AR inhibition prevents the NF- κ B-dependent generation of pro-inflammatory cytokines and chemokines in mouse models of allergic airway inflammation indicating the potential use of AR inhibition as a novel tool to control allergic responses. Since orally available AR inhibitors have already undergone phase III clinical trials for diabetic neuropathy and appear to have a manageable side effects profile, they could be readily developed as potential new drugs for the treatment of asthma and related complications.

Keywords: Asthma, airway inflammation, aldose reductase, polyol pathway.

INTRODUCTION

Asthma is characterized by inflammation of the airway, variable bronchospasm, airway obstruction and airway hyperresponsiveness, which collectively cause symptoms of wheezing, cough, chest tightness, and shortness of breath. In the past century understanding of the asthma pathogenesis has heavily relied on cellular and molecular biology, immunology, microbiology, and genetics/genomics as well as pathophysiology. These studies helped define asthma heterogeneity in quantitative and molecular stipulations and in determining the precise role of the airway inflammation in disease initiation and progression [1]. The inflammatory component of asthma was broadly recognized in later part of 20th century which led to the addition of anti-inflammatory medications in the treatment and management of the disease [1]. Besides growing understanding and modern therapeutic interventions, the number of asthma patients is on rise and they still suffer from activity limitations, which suggest that the understanding of asthma pathogenesis is still incomplete, and there may be mechanisms which are obscure or not fully defined. Oxidative stress-induced inflammation has been identified in a number of pathologies and understanding of its role in asthma pathogenesis has just begun. Here we will discuss how inhibition of oxidative stress signaling may be a unique and novel therapeutic approach in asthma.

PATHOGENESIS OF ASTHMA

Epidemiologic Considerations

Asthma affects approximately 300 million individuals world-wide [2]. More than 22 million people suffer from asthma-related pathologies and approximately 5000 people die due to asthma each year in the United States [3]. The annual direct medical expenditure attributable to asthma treatment increased from approximately 11.3 billion U.S. dollars in 1998 to an estimated \$37.2 billion in 2007 [4]. Beside the growing trend in the prevalence of asthma, the uncertainty remains regarding the cause of asthma [5] because asthma pathogenesis is multi-factorial in nature and involves neural, immune, allergic, viral, inflammatory, environmental, and genetic paradigms [1].

The Inflammatory Nature of Asthma

It is becoming evident that decrease in the airway antioxidant capacity and resultant increased oxidative stress either due to exposure to the external stimuli such as environmental pollutants, allergens, gases, particulate matters, pets, exercise, and cold or intrinsic components such as inappropriate immune response, genetic predisposition or compromised host-pathogen interaction could play a significant role in asthma pathogenesis [6-11]. The mechanisms that regulate the airway inflammatory response are not completely elucidated, but the intensity of the response could be related to the redox status of the airway via enhanced generation of pro-inflammatory cytokines and the activation of redox-sensitive molecular signaling. Inflammatory mediators and cytokines may be generated by resident cells within the airways and

*Address correspondence to this author at the Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX 77555-0647, USA; Tel: (409) 772-3926; Fax: (409) 772-9679; Email: ssrivast@utmb.edu

lungs, as well as by cells that infiltrate into the airway, especially Th2 polarized T-helper cells, that play a major role in asthma pathogenesis [12]. The release of pro-inflammatory mediators such as chemokines including G-CSF, MCP-1, IL-8, and KC (in murine models) acts as chemoattractants for the inflammatory cells in the lung and attracts eosinophils, T-lymphocytes, macrophages, and neutrophils. The Th-2 cytokines including IL-4, IL-5, and IL-13, primarily secreted by infiltrated Th-2 lymphocytes, can induce airway hyper-responsiveness, metaplasia of epithelial cells, and airway remodeling [13-15]. Additionally, these mediators may further help in the recruitment and activation of additional inflammatory cells, which would augment the inflammatory cascade.

Mechanisms of Asthma and the Use of Anti-Inflammatory Agents

The etiology of asthma is though not clearly understood, oxidative stress has been implicated in asthma pathogenesis. Besides asthma, oxidative stress has also been implicated in many other respiratory diseases including pulmonary fibrosis, and chronic obstructive pulmonary disease (COPD) [1]. Oxidative stress in the airway could be induced by multiple factors including environmental pollutants, allergens, and infectious agents such as bacteria, virus, and fungus, chronic inflammation, obesity and emotional stress [16-25]. The influence of viral infections, particularly those early in life, has been well demonstrated, as risk factor for the development of asthma [26, 27]. ROS which include reactive free radicals with at least one unpaired electron are the main mediators of oxidative stress-induced inflammation. The ROS are highly reactive molecules which react readily with various biomolecules including cellular lipids and proteins and form toxic byproducts such as lipid aldehydes and their protein adducts resulting in pathophysiological changes [28-30]. Additionally, the altered redox status of the cell leads to activation of signaling kinases that eventually activate redox-sensitive transcription factors, which translocate to the nucleus and transcribe various inflammatory genes [31, 32]. The synthesis of excessive amounts of inflammatory cytokines, chemokines and growth factors leads to a vicious cycle of activation of signaling intermediates and further synthesis of inflammatory markers causing disease. We and others have demonstrated that redox-sensitive transcription factors such as NF- κ B and AP1 regulate the expression of an array of pro-inflammatory genes associated with asthma pathogenesis [33, 34], suggesting that antioxidants and other drugs that could inhibit inflammation may become valuable therapeutic agents to treat asthma pathogenesis [35-37].

Currently corticosteroids are the main drugs for asthma management, since in a large percentage of asthmatics they effectively prevent inflammation and ameliorate acute asthma exacerbation. However, a significant percentage of asthmatics besides

developing severe side effects become steroid-resistant. Additionally, anti-inflammatory effects of steroids are not disease specific and they also cause adverse side effects. Since IgE, IL-5 and IL-13 are known mediators of inflammation and play a major role in exacerbation, eosinophils infiltration and mucus cell metaplasia in allergic asthma, antibodies against these key mediators have been tried in patients. For example, omalizumab, anti IgE antibodies, has been demonstrated to be effective in preventing exacerbation and eosinophils infiltration in difficult-to-control-allergic asthma [38]. However, its effect on intrinsic asthma is not known. Similarly, humanized anti-IL-5 antibodies, mepolizumab, had mixed results in preventing the eosinophils infiltration and prevention of exacerbation of asthma even when given along with anti-inflammatory corticosteroids. In one clinical study involving broad population of asthma patients, mepolizumab had no clinically significant effect on lung functions [39]. In a selected group of patients with prednisone-dependent refractory eosinophilic asthma, mepolizumab significantly reduced exacerbation rates [40, 41]. Anti IL-9 antibodies have also been shown to inhibit airway inflammation and hyper-responsiveness in ovalbumin-induced asthma in mice [42]. Suppressors of the effects of IL-13, a key regulator of asthma pathogenesis by regulating IgE synthesis, mucus hypersecretion, subepithelial fibrosis and eosinophil infiltration, could be potential candidates for the therapy or prevention of asthma [43]. Although investigators have shown some positive outcomes when IL-13 suppressors were used in animal model [44-46], the clinical trials are awaited. While these approaches have potential in controlling asthma, their effects on the upstream inflammatory cascade that mediates oxidative stress-induced pathology remains unknown.

Oxidative stress is well known to induce inflammation by activating and up-regulating pro-inflammatory signaling intermediates [47-49]. Therefore, antioxidant(s) or other agents that could block the inflammatory signals and/or the transcription of pro-inflammatory markers could be excellent agents in the prevention and therapy of asthma [50]. Some improvement in the lung function in asthmatics has been demonstrated upon dietary intake of an antioxidant, ascorbic acid [51]. Similarly, in one study antioxidant therapy using N-acetylcysteine [NAC] has been found to be effective in decreasing human eosinophils functions [52]. However, other studies suggest that NAC increases airway resistance and exacerbates asthma [53, 54]. Various other anti-inflammatory agents that inhibit specific kinases and/or transcription factors have also been tried for the amelioration of airway inflammation in asthma [55-61]. However, these efforts have not resulted in the development of effective therapeutic interventions. Moreover, all the known antioxidants at therapeutic doses act as oxidants which may exacerbate the inflammation and aggravate the pathology.

We have recently shown that AR inhibitors have excellent anti-inflammatory effect in various models of

inflammatory disorders including asthma [62]. Inhibition of AR prevents the activation of oxidative stress-induced signaling cascade that activates redox sensitive transcription factors such as NF- κ B and AP-1 which results in the prevention of the transcription of inflammatory mediators and prevents inflammation. In the following sections we will discuss the potential of AR inhibitors in the prevention and therapy of asthma.

ALDOSE REDUCTASE IN ALLERGIC AIRWAY INFLAMMATION

Biochemical Considerations

Aldose reductase (AR; AKR1B1) is the rate limiting enzyme of polyol pathway that catalyzes the conversion of glucose into sorbitol at the expense of NADPH. Hyperglycemia in diabetes results in increased flux of glucose through polyol pathway that leads to increased levels of intracellular sorbitol. The sorbitol, being relatively impermeable through the biological membranes, accumulates inside the cells and causes osmotic stress, which has been implicated as a major contributor to diabetic complications. In late 90s we and others have demonstrated that besides reducing glucose ($K_m > 50\text{mM}$), AR efficiently (K_m 10-30 μM range) reduces a wide array of aldehydes and their glutathione conjugates to corresponding alcohols which could then be transported out of the cells [63, 64]. These evidences suggest that lipid aldehydes are better substrates for AR than glucose. Therefore, increased expression and activation of AR under oxidative stress, which cause lipid peroxidation, point towards a potential detoxification role of this enzyme.

Regulation of Aldose Reductase Expression

Extensive evidences have been presented during the past decade which suggest that AR mediates inflammatory signals that are induced during oxidative stress [64]. Inflammation is involved in the pathophysiology of diseases such as diabetes, cardiovascular, bacterial sepsis, autoimmune disorders, and tumorigenesis. It has been observed by several investigators that abundance of the AR mRNA and protein is significantly increased in response to growth stimuli e.g. Fibroblast growth factor (FGF)-1 has been shown to increase the expression of AR in NIH 3T3 cells [65, 66]; FGF and epidermal growth factor (EGF) induce AR expression in astrocytes [67, 68]; high glucose, TNF- α , thrombin, and FGF-2 also enhance the expression of AR in vascular smooth muscle cells (VSMC) [69]. In addition, various oxidants such as H_2O_2 , oxidized low-density lipoprotein (ox-LDL), 4-hydroxy *trans*-2-nonenal (HNE) have also been shown to induce AR in VSMC [70]. HNE also induces AR expression in T-lymphocytes and macrophages as has been shown *in vivo* during giant cell arthritis [71]. Further, bacterial infection and bacterial endotoxin, LPS also stimulate AR in animal models [72]. These evidences suggest that AR plays a critical role in regulation of redox signals.

Efficacy of AR Inhibition in the Ragweed Pollen Model of Airway Inflammation

Because of the consequences of AR activation, including facilitation of NF- κ B signaling, we postulated that products of the AR pathway might participate in the pathogenesis of allergic inflammation. Accordingly, we have recently demonstrated that allergic airway inflammation caused by ragweed pollen extract (RWE)-in mice could be ameliorated by treatment with AR inhibitors. AR inhibition significantly prevents RWE-induced allergic responses, airway inflammation, and hyper-responsiveness in a murine model of allergic asthma [73]. Mice were sensitized on day 0 and 4 and were treated without or with AR inhibitor before they were challenged with RWE or carrier on day 11 followed by determination of various pathological and physiological parameters of airway inflammation. A robust airway inflammation, as measured by accumulation of inflammatory cells in BAL fluid and airway sub-epithelium, was observed in RWE-sensitized and challenged animals. When the mice were treated with AR inhibitor before challenge, the peri-vascular and peri-bronchial inflammation and eosinophils accumulation in the BAL fluid induced by RWE-challenge were significantly reduced [73]. A principal pathology in human asthma exacerbations is mucus hypersecretion. In our experimental model, the mucin level increased ~25-folds in BAL fluid in RWE-challenged mice as compared to control and treatment with AR inhibitor significantly reduced the mucin level in BAL fluid [73]. Similarly, the number of mucin producing goblet cells in the airway epithelia, as assessed by periodic acid Schiff (PAS)-staining of lung sections, was also significantly reduced in mice treated with AR inhibitor [73]. The polarization of T cells towards Th2 phenotype which secrete Th2 cytokines is characteristic of allergic asthma. Therefore we determined the expression of Th2 cytokine genes in the lung tissues approximately 16 h after RWE-challenge. The levels of mRNA of inflammatory cytokines increased in RWE-challenged mice and AR inhibitor treatment significantly reduced these changes (Fig. 1). Additionally, a quantitative non-invasive measurement of airway hyper-responsiveness in response to methacholine inhalation showed a dose-dependent increase in enhanced pause (Penh) in RWE-sensitized and challenged mice, and in mice treated with AR inhibitor a significant decrease in the Penh value was observed which was similar to that of control mice at all the methacholine doses [73]. These data demonstrate that patho-physiological effects of allergic asthma caused by a common allergen relevant to human disease, ragweed pollen, could be prevented by AR inhibition in murine model.

Efficacy of AR Inhibition in the Ovalbumin Model of Airway Inflammation

In order to confirm that efficacy of AR inhibitors was not allergen specific; we have also investigated the effectiveness of AR inhibitors, sorbinil and fidarestat in

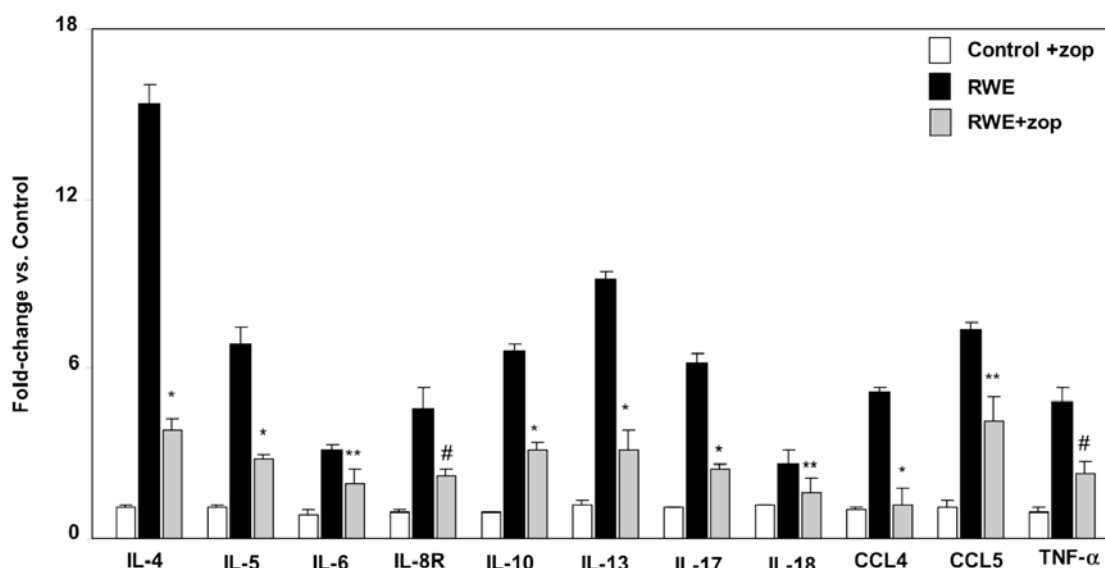


Fig. (1). Prevention of RWE-induced mRNA expression of inflammatory cytokines and chemokines in lung tissues by AR inhibition. The mice were sensitized with two intra-peritoneal (i.p.) injections of endotoxin-free RWE 150 µg/100µl, combined with Alum adjuvant in a 3:1 ratio, on days 0 and 4. On days 9 and 10, animals were administered AR inhibitor dissolved in DMSO (i.p. 25 mg/kg body weight) every 12 h for a total duration of 48 h. On day 11, mice were challenged intranasally with RWE (100 µg). Control groups of mice were challenged with equivalent volumes of PBS. Eighteen hours after the RWE-challenge, mice were killed and lungs were harvested. The total RNA was isolated from lungs and one microgram of total RNA from each sample was transcribed into first-strand cDNA and amplification of selected genes was quantified by RT-PCR using specific forward and reverse primers. The levels of RNA for the target sequences were determined by melting curve analysis. The bars (SEM± SD) represent fold-change over the control. (n=6); * $p<0.001$; ** $p<0.05$; # $p<0.01$ Vs RWE-challenge. RWE, ragweed pollen extract; ARI, aldose reductase inhibitor.

ovalbumin (OVA)-induced airway inflammation in mice [74]. The sensitization and challenge with OVA induced a marked infiltration of eosinophils in mice lungs and increased the levels of OVA-specific IgE in BAL fluid which were significantly reduced when AR inhibitor, administered before the challenge. Further, AR inhibitor treatment prior to OVA-challenge significantly ($p<0.005$) reduced the levels of Th2 cytokines including IL-4, IL-5, IL-13 and chemokines such as keratinocyte-derived chemokine (KC), Granulocyte colony stimulating factor (G-CSF) and MCP-1 in the BAL fluid as compared to untreated mice (Fig. 2). As observed in the RWE model, AR inhibitor significantly decreased the airway hyperresponsiveness in OVA-challenged mice as they exhibited a reduced Penh value in response to methacholine challenge compared to untreated mice [74]. Thus our results from two distinct animal models of allergic airway inflammation demonstrate that inhibition of AR ameliorates allergen-induced airway inflammation and presents a novel mechanism for therapeutic intervention in asthma pathogenesis in human.

Efficacy of AR Inhibition in the Prevention of Goblet Cell Metaplasia

Mucous glycoconjugate-containing goblet cells are the important source of mucous hypersecretion in patients with acute asthma. In general, the airways of healthy subjects contain very few goblet cells, but their

numbers are significantly increased in the airways of patients who die of asthma. However, the mechanisms that control the differentiation of epithelial cells into goblet cells are not clear. Our recent studies have demonstrated that AR inhibitors prevent differentiation of epithelial cells into mucous secreting goblet cells in cultured SAEC as well as in RWE-induced metaplasia in mice [73, 75]. Stimulation of SAEC with IL-13 transformed majority of the epithelial cells into mucus-filled goblet cells which lacked cilia. However, when the airway cells in monolayer were incubated with AR inhibitor prior to IL-13 stimulation, the number of cells with PAS staining and over-expressing Muc5AC were significantly decreased [75]. Further, SAEC in monolayer maintained ciliated morphology indicating that AR inhibitors prevent goblet cells metaplasia. Prevention of goblet cell metaplasia by AR inhibition observed in SAEC was further confirmed by using a mouse model of RWE-induced allergic asthma. Our results indicate that the population of PAS positive goblet cells and expression of Muc5AC in the RWE-challenged mice lung were significantly decreased in the mice treated with AR inhibitor, fidarestat [75]. Similarly, our studies also indicate that AR-knockout mice were resistant to RWE-induced goblet cell metaplasia in airway epithelium. Furthermore, we have shown that AR inhibition prevents goblet cell metaplasia by regulating the redox-signals mediated by JAK-1, ERK1/2 and STAT-6. Thus our studies indicate potential therapeutic role of AR inhibition in prevention

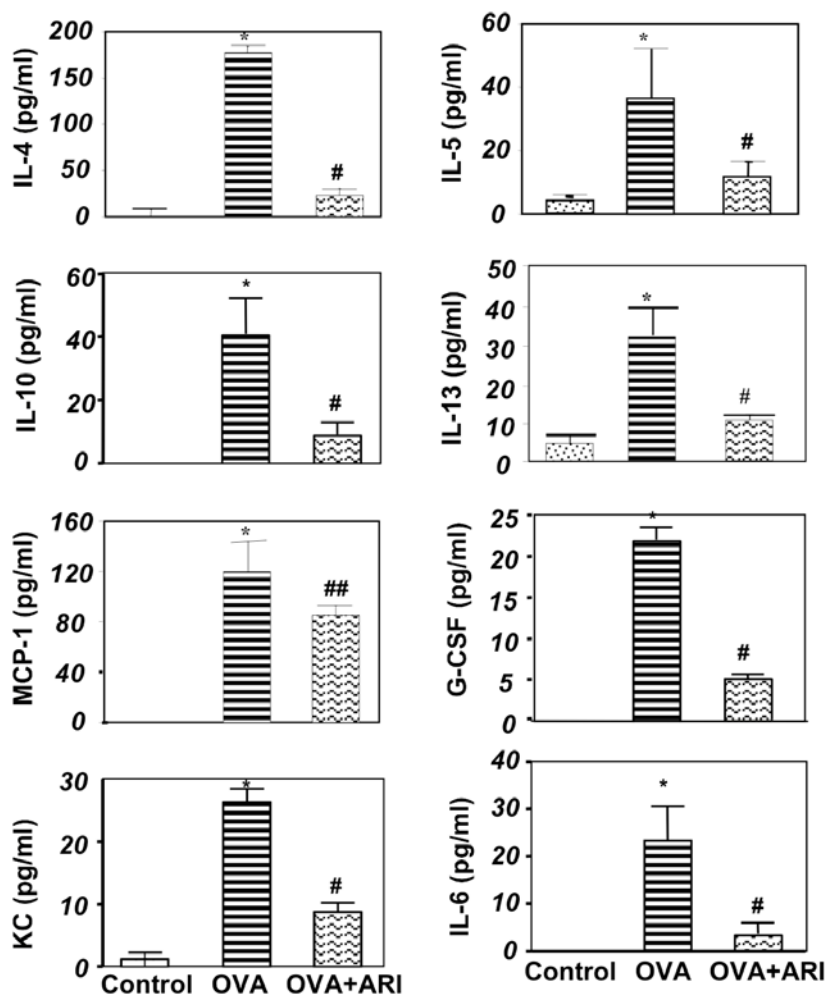


Fig. (2). Prevention of OVA-induced increase in inflammatory cytokines and chemokines in BAL fluid by AR inhibition. The mice were sensitized with injections (i.p.) of 100 µg Grade V chicken OVA, mixed with 2 mg aluminum hydroxide in saline, once a week for two consecutive weeks followed by challenge with aerosolized OVA, a week after the second sensitization. The mice in control groups were not sensitized or challenged. In Ova +ARI group, the mice received an injection of AR inhibitor (i.p.; 25 mg/kg body weight) before challenge. Animals were killed by CO₂ asphyxiation and lungs were subjected to bronchoalveolar lavage (BAL) using PBS. The collected BAL fluids were centrifuged at 1000 × g for 10 min at 4°C, and the supernatants was stored immediately at -80°C until used for cytokines and chemokines analysis. The levels of cytokines and chemokines in the BAL fluid were measured using the Bio-Rad Bioplex system and specific ELISA kits. Data are given as means ± SD (*n* = 4). **p* < 0.001 vs unchallenged mice; #*p* < 0.005 vs OVA-challenged mice; ##*p* < 0.05 vs OVA-challenged mice. OVA, Ovalbumine; ARI, aldose reductase inhibitor.

of goblet cell metaplasia [75], which would significantly ameliorate asthma pathogenesis.

The Effects of AR Inhibition in other Inflammatory Models

In the acute airway inflammation models various other investigators have also implicated the significance of AR. Ravindranath *et al.* (2009) have demonstrated that transgenic mice over-expressing AR showed a greater inflammatory response in cecal ligation and puncture (CLP) model as evaluated by increased circulating cytokine levels, neutrophil accumulation in the lungs, and activation of Rho kinase in lung endothelial cells (ECs) [76]. In response to bacterial endotoxin, LPS, lung cells from AR transgenic

mice produced more IL-6 and showed augmented JNK activation compared to wild-type. Further, AR activity was crucial for fMLP-induced CD11b activation, respiratory burst, and shape changes in human neutrophils, and TNF- α -induced activation of the Rho kinase/MKK4/JNK pathway and IL-6 production in human pulmonary microvascular ECs as well as neutrophils adhesion to TNF- α -stimulated ECs [76]. These evidences attribute an important role to AR in the mediation of acute inflammation in lung.

Our recent findings with AR null mice showed that inflammatory cells infiltration and eosinophilia in the lung, and airway hyperresponsiveness by RWE sensitization and challenge were significantly less in AR-null mice as compared to wild type mice

(unpublished data). These results corroborate our earlier results with AR inhibitors suggesting that AR indeed is involved in the allergen-induced airway inflammation, and thus could be an important therapeutic target to ameliorate airway inflammation.

Human *In vitro* Studies

Using SAEC, we have shown that inhibition or genetic ablation of AR could prevent RWE-, TNF- α - and LPS -induced oxidative stress and subsequent inflammatory changes. Incubation of SAEC with various stimulants such as RWE, TNF- α and LPS with or without AR inhibitors showed that inhibition of AR could prevent SAEC-death induced by RWE, TNF- α or LPS [73, 74]. Further, inhibition of AR also prevented the RWE-, TNF- α - or LPS-induced increase in inflammatory markers such as iNOS, Cox-2, Prostaglandins, IL6 and IL8. Since the expression of these inflammatory markers is dependent on the activation of transcription factor NF- κ B, the effect of AR inhibition on RW-, TNF- α - or LPS-induced NF- κ B activation was examined. It was observed that the inhibition of AR significantly prevented RW-, LPS- or TNF- α -induced activation of NF- κ B as well as its upstream signaling kinases such as MAPK, ERK, JNK and PKC [73, 74].

Suppression of Airway Inflammation by AR Inhibition

It is well known that cytokines and growth factors play an important role in accelerating inflammation. Cytokines such as IL-4, IL-5 and TNF- α , and growth factors such as EGF, FGF and VEGF play key role in inflammation by increasing ROS [77, 78]. The ROS, generated by cytokines and growth factors *via* NADPH oxidase, attack the cell membrane lipids resulting in the formation of lipid-derived aldehydes (LDAs) [79]. Several reports have implicated LDAs as contributors to oxidative stress-induced patho-physiologies such as proliferation, apoptosis, and activation of signaling pathways which could lead to airway inflammation in asthma and its associated complications [80-83]. We have demonstrated that in the presence of NADPH, AR readily reduces LDAs, including the most abundant and toxic lipid aldehyde, 4-hydroxynonenal (HNE), to respective alcohols with a K_m value of 10-30 μ M [38]. Further, the that reduction of glutathione (GSH)- lipid aldehyde conjugates by AR is 10 to 100-fold more efficient than their parent aldehydes because active site of AR has high affinity for GSH-conjugates [64, 84]. We have also demonstrated that besides carbonyl binding site in the active site of AR, there is a specific binding site for GSH [85]. Several studies have suggested that xenobiotics, cigarette smoke and environmental pollutants cause pathophysiological changes in COPD and asthma through generation of ROS, which increases lipid peroxidation resulting in increased levels of lipid aldehydes such as HNE in cells [16, 17, 86, 87]. Our hypothesis is that AR plays a pivotal role in airway inflammation *via* oxidants-induced

lipid aldehydes signals that activate a cascade of protein kinases leading to NF- κ B activation which in turn transcribes a number of pro-inflammatory genes (Fig. 3). We have indeed demonstrated that GS-DHN, a representative of GS-lipid alcohols, which are AR-catalyzed reduced products of GS-lipid aldehydes, mediates NF- κ B-dependent expression of inflammatory markers in vascular smooth muscle cells and macrophages [88, 89].

Ever increasing evidences suggest the role of ROS-induced lipid aldehydes in various inflammatory pathologies. Since oxidants and allergens such as RWE, and bacterial endotoxin, LPS, are known to cause increase in inflammation *via* increased generation of ROS and lipid aldehydes [72, 73], we reasoned that AR-catalyzed reaction products should play an important role in eliciting allergen-induced airway cytotoxicity and inflammation. Since AR inhibitors prevent the formation of inflammatory markers as well as their cellular effects and inflammation is one of the major component of asthma, we contemplate that AR inhibitors should be excellent drugs to treat airway inflammation irrespective of the source or causative factor.

POSSIBLE CLINICAL IMPLICATIONS FOR AR INHIBITOR THERAPY IN ASTHMA

Several lines of evidence suggest a potential place for AR inhibition therapy in the armamentarium of asthma therapeutics, and support proof-of-concept trials in human asthma. First, the mechanism of action has biologic plausibility – that is, NF- κ B activation leads to transcription and secretion of inflammatory mediators relevant to the pathogenesis of asthma, and inappropriate activation of NF- κ B by means of sorbitol, lipid alcohols, or other intracellular consequences of AR activity might plausibly lead to a coordinated program of inflammatory cytokine secretion and subsequent airway inflammation. Second, although mechanisms and findings in animal models have not been perfectly predictive for human disease, the remarkable suppression of eosinophilic inflammation in our murine model of allergic airway inflammation, both in the setting of ovalbumin sensitization and challenge (an extensively studied model) [74], and in the RWE (relevant to pollen allergy) [73] suggests a strong possibility of a similar effect in human asthma. Third, the magnitude of suppression of allergic inflammation by AR inhibitors treatment is comparable to that observed with corticosteroids, the most potent anti-inflammatory agents used in asthma. This degree of anti-inflammatory effect exceeds by 3-6 fold the suppression of inflammation achieved with anti-leukotrienes or omizumab, an anti-IgE monoclonal antibody and is therefore of potential clinical importance [90, 91]. Fourth, these agents are small molecules, suitable for oral administration. This route of delivery has several advantages for asthma management, including treatment of the entire airway from large to small and simple oral dosing not requiring

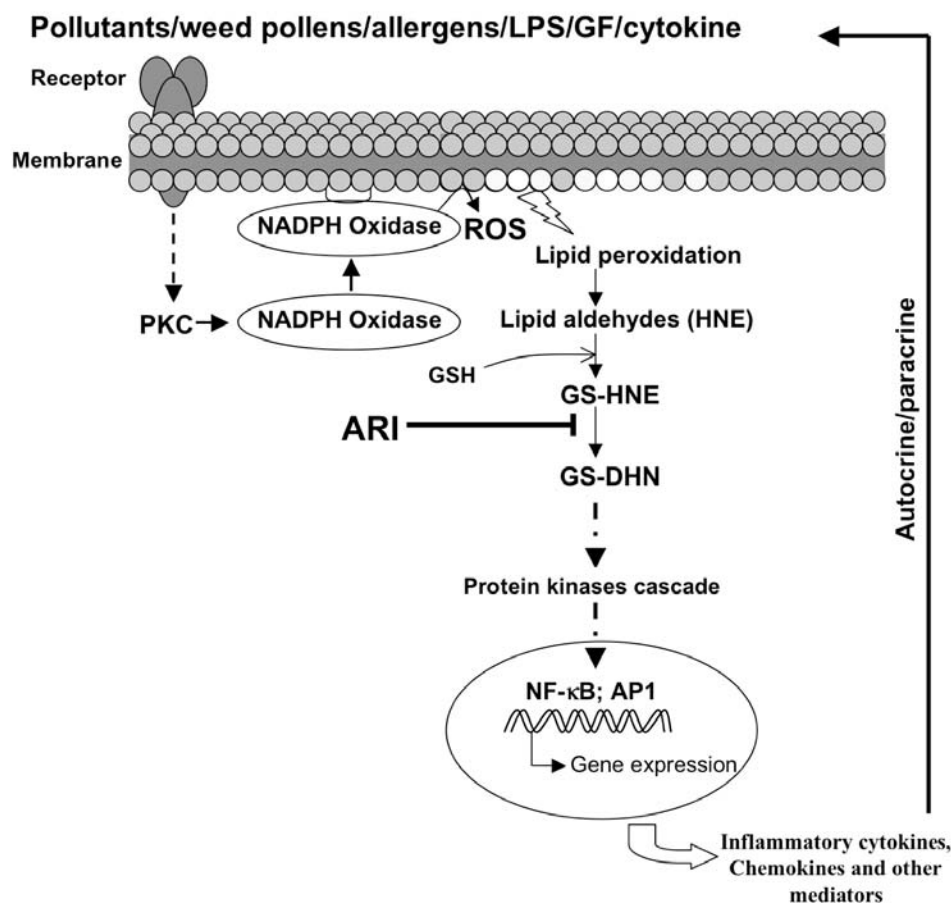


Fig. (3). Prevention of redox signaling by AR inhibition. We have discovered that AR is the key mediator of the signaling initiated by different stimuli including pollens, allergens, growth factors, cytokines and bacterial endotoxins which are known to induce increased ROS generation inside the cells. The ROS react with membrane lipids and oxidize them into lipid aldehydes such as 4-hydroxynonenal (HNE), which readily react with glutathione and forms GS-lipid aldehyde conjugates (GS-HNE). AR catalyzes the reduction of GS-HNE to GS-DHN with great efficiency ($K_m=10\text{--}30\text{ }\mu\text{M}$). GS-DHN is known to activate PLC, PKC and various other downstream protein kinases, eventually activating transcription factors NF- κ B and AP-1. The activated transcription factors then translocate to the nucleus and transcribe various inflammatory genes. The newly formed inflammatory markers then start a vicious cycle by an autocrine and/or paracrine manner and cause inflammation and pathogenesis. Inhibition of AR could block this cascade upstream of various kinases and shunt the inflammatory circuitry.

an inhaler. Finally, at least one AR inhibitor has been studied in humans for the prevention and treatment of diabetic neuropathy, so a robust dataset confirming the relative safety of that compound in humans over a period of a year or more is available [92]. Accordingly, an appropriately powered proof-of-concept clinical trial of AR inhibitor in mild asthma, using standard asthma endpoints in addition to measures of airway inflammation, may provide compelling new data on which the development of a new class of therapy for asthma may be based.

CONCLUSIONS AND FUTURE PERSPECTIVES

Use of corticosteroids, specifically inhaled corticosteroids as first-line choice drugs to treat asthma and COPD is now considered unpleasant as they cause adverse systemic effects including increase in the risk of diabetic onset and progression. Hence,

novel therapeutic approaches are mandatory in controlling respiratory complications. Recent studies have demonstrated that polyol pathway enzyme AR plays a pivotal role in the pathophysiology of airway inflammation leading to asthma pathogenesis in experimental animal models. Further, studies from our laboratory and others have also shown the therapeutic potential of AR inhibitors in preventing many inflammatory diseases such as sepsis, colon cancer and uveitis besides diabetic complications [6, 11-13]. These studies indicate entirely novel and unanticipated participant, AR, in many inflammatory pathologies including allergic asthma. The findings could be readily translated from 'bench to bedside' in a relatively short time, since AR inhibitors have already undergone Phase-III clinical studies for diabetic neuropathy and been found to be relatively safe even for long-term use. Because of their low toxicity, these drugs are ideally suited for future translational studies to devise

strategies for preventing and treating inflammatory pathologies including asthma.

ACKNOWLEDGEMENTS

This study was supported in parts by grants from American Asthma Foundation and NIH/NIDDK DK36118 (to SKS) and NIH/NIGMS GM71036 to KVR. Dr. Satish Srivastava is a William Bowes Senior Fellow of American Asthma Foundation.

ABBREVIATIONS

AR	= Aldose reductase
ARI	= Aldose reductase inhibitor
AP-1	= Activating protein-1
BAL	= Bronchoalveolar lavage
COX-2	= Cyclooxygenase-2
ERK	= Extracellular signal-regulated kinase
IL	= Interleukin
I κ B	= Inhibitor of NF- κ B
IKK	= I κ B kinase
IFN- γ	= Interferon- γ
IP-10	= Inducible protein-10
JNK	= c-Jun N-terminal kinase
MAPK	= Mitogen-activated protein kinases
NO	= Nitric oxide
NOS-2	= Inducible nitric oxide-synthase
LPS	= Lipopolysaccharide
LXR	= Liver X receptors
MIP-1a	= Macrophage inflammatory protein-1a
MMPs	= Matrix metalloproteases
NF- κ B	= Nuclear transcription factor-kappa B
OVA	= Ovalbumine
RWE	= Ragweed pollen extract
PGE2	= Prostaglandin E2
STAT	= Signal transducers and activators of transcription
TLRs	= Toll-like receptors
TNF- α	= Tumour necrosis factor- α
COPD	= Chronic obstructive pulmonary disease

REFERENCES

- [1] Walter MJ, Holtzman MJ. A Centennial History of Research on Asthma Pathogenesis. *Am J Respir Cell Mol Biol* 2005; 32: 483-9.
- [2] Masoli MD, Fabian SH, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 2004; 59: 469-78.
- [3] Akinbami L. Office of Analysis and Epidemiology, CDC National Center for Health Statistics E-stat, Asthma Prevalence, Health Care Use and Mortality: United States, 2003-05, Available at: <http://www.cdc.gov/nchs/data/hestat/asthma03-05/asthma03-05.htm#fig1> [accessed Aug 31, 2010].
- [4] Kamble S, Bharmal MJ. Incremental direct expenditure of treating asthma in the U.S. *J Asthma* 2009; 46: 73-80.
- [5] Nauta AJ, Engels F, Knippels LM, et al. Mechanisms of allergy and asthma. *Eur J Pharmacol* 2008; 58: 5354-60.
- [6] D'Amato G, Cecchi L, D'Amato M, et al. Urban air pollution and climate change as environmental risk factors of respiratory allergy: an update. *J Investig Allergol Clin Immunol* 2010; 20: 95-102.
- [7] Agrawal DK, Shao Z. Pathogenesis of allergic airway inflammation. *Curr Allergy Asthma Rep* 2010; 10: 39-48.
- [8] Parsons JP, Mastrorade JG. Exercise-induced asthma. *Curr Opin Pulm Med* 2009; 15: 25-28.
- [9] Robinson DS. Regulatory T cells and asthma. *Clin Exp Allergy* 2009; 39: 1314-23.
- [10] Holloway JW, Yang IA, Holgate ST. Genetics of allergic disease. *J Allergy Clin Immunol* 2010; 125: S81-94.
- [11] Hopkin J. Immune and genetic aspects of asthma, allergy and parasitic worm infections: evolutionary links. *Parasite Immunol* 2009; 31: 267-73.
- [12] Holgate ST. Pathogenesis of asthma. *Clin Exp Allergy* 2008; 38: 872-97.
- [13] Kim HY, DeKruyff RH, Umetsu DT. The many paths to asthma: phenotype shaped by innate and adaptive immunity. *Nat Immunol* 2010; 11: 577-84.
- [14] Barrett NA, Austen KF. Innate cells and T helper 2 cell immunity in airway inflammation. *Immunity* 2009; 31: 425-37.
- [15] Elias JA, Zhu Z, Chupp G, Homer RJ. Airway remodeling in asthma. *J Clin Invest* 1999; 104: 1001-6.
- [16] D'Amato G, Liccardi G, D'Amato M, Holgate S. Environmental risk factors and allergic bronchial asthma. *Clin Exp Allergy* 2005; 35: 1113-24.
- [17] Patel MM, Miller RL. Air pollution and childhood asthma: recent advances and future directions. *Curr Opin Pediatr* 2009; 21: 235-42.
- [18] Csillag A, Boldogh I, Pazmandi K, et al. Pollen-induced oxidative stress influences both innate and adaptive immune responses via altering dendritic cell functions. *J Immunol* 2010; 184: 2377-85.
- [19] Boldogh I, Bacsai A, Choudhury BK, et al. ROS generated by pollen NADPH oxidase provide a signal that augments antigen-induced allergic airway inflammation. *J Clin Invest* 2005; 115: 2169-79.
- [20] Rada B, Leto TL. Redox warfare between airway epithelial cells and *Pseudomonas*: dual oxidase versus pyocyanin. *Immunol Res* 2009; 43: 198-209.
- [21] Mochizuki H, Todokoro M, Arakawa H. RS virus-induced inflammation and the intracellular glutathione redox state in cultured human airway epithelial cells. *Inflammation* 2009; 32: 252-64.
- [22] Castro SM, Guerrero-Plata A, Suarez-Real G, et al. Antioxidant treatment ameliorates respiratory syncytial virus-induced disease and lung inflammation. *Am J Respir Crit Care Med* 2006; 174: 1361-9.
- [23] Kim TB, Moon KA, Lee KY, et al. Chlamydia pneumoniae triggers release of CCL20 and vascular endothelial growth factor from human bronchial epithelial cells through enhanced intracellular oxidative stress and MAPK activation. *J Clin Immunol* 2009; 29: 629-36.
- [24] Holguin F, Fitzpatrick A. Obesity, asthma, and oxidative stress. *J Appl Physiol* 2010; 108: 754-9.
- [25] Chen E, Miller GE. Stress and inflammation in exacerbations of asthma. *Brain Behav Immun* 2007; 21: 993-9.
- [26] Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. *Lancet* 2010; 376: 826-34.
- [27] Jackson DJ, Johnston SL. The role of viruses in acute exacerbations of asthma. *J Allergy Clin Immunol* 2010; 125: 1178-87.

- [28] Stohs SJ. The role of free radicals in toxicity and disease. *J Basic Clin Physiol Pharmacol* 1995; 6: 205-28.
- [29] Davies KJ. Oxidative stress: the paradox of aerobic life. *Biochem Soc Symp* 1995; 61: 1-31.
- [30] Dozor AJ. The role of oxidative stress in the pathogenesis and treatment of asthma. *Ann N Y Acad Sci* 2010; 1203: 133-7.
- [31] Rahman I, Yang SR, Biswas SK. Current concepts of redox signaling in the lungs. *Antioxid Redox Signal* 2006; 8: 681-9.
- [32] Adler V, Yin Z, Tew KD, Ronai Z. Role of redox potential and reactive oxygen species in stress signaling. *Oncogene* 1999; 18: 6104-11.
- [33] Wright JG, Christman JW. The role of nuclear factor kappa B in the pathogenesis of pulmonary diseases: implications for therapy. *Am J Respir Med* 2003; 2: 211-9.
- [34] Srivastava SK, Ramana KV. Focus on molecules: nuclear factor-kappaB. *Exp Eye Res* 2009; 88: 2-3.
- [35] Anssen-Heininger YM, Poynter ME, Aesif SW, *et al.* Nuclear factor kappaB, airway epithelium, and asthma: avenues for redox control. *Proc Am Thorac Soc* 2009; 6: 249-55.
- [36] Edwards MR, Bartlett NW, Clarke D, *et al.* Targeting the NF-kappaB pathway in asthma and chronic obstructive pulmonary disease. *Pharmacol Ther* 2009; 121: 1-13.
- [37] Wong ET, Tergaonkar V. Roles of NF-kappaB in health and disease: mechanisms and therapeutic potential. *Clin Sci (Lond)* 2009; 116: 451-65.
- [38] Humbert M, Tonnel AB. Anti IgE antibodies for the treatment of difficult asthma. *Rev Mal Respir* 2005; 22: 983-90.
- [39] Flood-Page P, Swenson C, Faierman I, *et al.* A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. *Am J Respir Crit Care Med* 2007; 176: 1067-71.
- [40] Leckie M, Brinke JA, Khan J, *et al.* Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyperresponsiveness, and the late asthmatic response. *Lancet* 2000; 356: 2144-8.
- [41] Nair P, Pizzichini MM, Kjarsgaard M, *et al.* Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009; 360: 985-93.
- [42] Cheng G, Arima M, Honda K, *et al.* Anti-interleukin-9 antibody treatment inhibits airway inflammation and hyper-reactivity in mouse asthma model. *Am J Respir Crit Care Med* 2002; 166: 409-16.
- [43] Mitchell J, Dimov V, Townley RG. IL-13 and the IL-13 receptor as therapeutic targets for asthma and allergic disease. *Curr Opin Investig Drugs* 2010; 11: 527-34.
- [44] Yang G, Li L, Volk A, *et al.* Therapeutic dosing with anti-interleukin-13 monoclonal antibody inhibits asthma progression in mice. *J Pharmacol Exp Ther* 2005; 313: 8-15.
- [45] Martin PL, Fisher D, Glass W, *et al.* Preclinical safety and pharmacology of an anti-human interleukin-13 monoclonal antibody in normal macaques and in macaques with allergic asthma. *Int J Toxicol* 2008; 27: 351-8.
- [46] Ma Y, HayGlass KT, Becker AB, *et al.* Novel recombinant interleukin-13 peptide-based vaccine reduces airway allergic inflammatory responses in mice. *Am J Respir Crit Care Med* 2007; 176: 439-45.
- [47] Fujisawa T. Role of oxygen radicals on bronchial asthma. *Curr Drug Targets Inflamm Allergy* 2005; 4: 505-9.
- [48] Henricks PA, Nijkamp FP. Reactive oxygen species as mediators in asthma. *Pulm Pharmacol Ther* 2001; 14: 409-20.
- [49] Sugiura H, Ichinose M. Oxidative and nitrate stress in bronchial asthma. *Antioxid Redox Signal* 2008; 10: 785-97.
- [50] Park SJ, Lee YC. Antioxidants as novel agents for asthma. *Mini Rev Med Chem* 2006; 6: 235-40.
- [51] Schwartz J, Weiss ST. Relationship between dietary vitamin C intake and pulmonary function in the First National Health and Nutrition Examination Survey (NHANES I). *Am J Clin Nutr* 1994; 59: 110-4.
- [52] Martinez-Losa M, Cortijo J, Juan G, *et al.* Inhibitory effects of N-acetylcysteine on the functional responses of human eosinophils *in vitro*. *Clin Exp Allergy* 2007; 37: 714-22.
- [53] Aliyali M, Poorhasan Amiri A, Sharifpoor A, Zalli F. Effects of N-acetylcysteine on asthma exacerbation. *Iran J Allergy Asthma Immunol* 2010; 9: 103-9.
- [54] Reinero CR, Lee-Fowler TM, Dodam JR, Cohn LA, DeClue AE, Guntur VP. Endotracheal nebulization of N-acetylcysteine increases airway resistance in cats with experimental asthma. *J Feline Med Surg* 2011; 13: 69-73.
- [55] Duan W, Chan JH, Wong CH, Leung BP, Wong WS. Antiinflammatory effects of mitogen-activated protein kinase inhibitor U0126 in an asthma mouse model. *J Immunol* 2004; 172: 7053-9.
- [56] Duan W, Aguinaldo Datiles AM, Leung BP, Vlahos CJ, Wong WS. An anti-inflammatory role for a phosphoinositide 3-kinase inhibitor LY294002 in a mouse asthma model. *Int. Immunopharmacol* 2005; 5: 495-502.
- [57] Bao Z, Lim S, Liao W, *et al.* Glycogen synthase kinase-3b inhibition attenuates asthma in mice. *Am J Respir Crit Care Med* 2007; 176: 431-8.
- [58] Duan W, Chan JH, McKay K, *et al.* Inhaled p38a mitogen-activated protein kinase antisense oligonucleotide attenuates asthma in mice. *Am J Respir Crit Care Med* 2005 171: 571-8.
- [59] Chue SC, Seow CJ, Duan W, Yeo KS, Koh AH, Wong WS. Inhibitor of p42/44 mitogen-activated protein kinase, but not p38 MAPK, attenuated antigen challenge of guinea pig airways *in vitro*. *Int Immunopharmacol* 2004; 4: 1089-98.
- [60] Henderson WR, Chi EY Jr, Teo JL, Nguyen C, Kahn M. A small molecule inhibitor of redox-regulated NF-kB and activator protein-1 transcription blocks allergic airway inflammation in a mouse asthma model. *J Immunol* 2002; 169: 5294-9.
- [61] Desmet C, Gosset P, Pajak B, *et al.* Selective blockade of NF-kB activity in airway immune cells inhibits the effector phase of experimental asthma. *J Immunol* 2004; 173: 5766-75.
- [62] Srivastava SK, Ramana KV, Bhatnagar A. Role of aldose reductase and oxidative damage in diabetes and the consequent potential for therapeutic options. *Endocr Rev* 2005; 26: 380-92.
- [63] Srivastava S, Chandra A, Bhatnagar A, *et al.* Lipid peroxidation product, 4-hydroxynonenal and its conjugate with GSH are excellent substrates of bovine lens aldose reductase. *Biochem Biophys Res Commun* 1995; 217: 741-6.
- [64] Srivastava S, Watowich SJ, Petrash JM, *et al.* Structural and kinetic determinants of aldehyde reduction by aldose reductase. *Biochemistry* 1999; 38: 42-54.
- [65] Hsu DK, Guo Y, Peifley KA, *et al.* Differential control of murine aldose reductase and fibroblast growth factor (FGF)-regulated-1 gene expression in NIH 3T3 cells by FGF-1 treatment and hyperosmotic stress. *Biochem J* 1997; 328: 593-8.
- [66] Donohue PJ, Alberts GF, Hampton BS, *et al.* A delayed-early gene activated by fibroblast growth factor-1 encodes a protein related to aldose reductase. *J Biol Chem* 1994; 269: 8604-9.
- [67] Jacquin-Becker C, Labourdette G. Regulation of aldose reductase expression in rat astrocytes in culture. *Glia* 1997; 20: 135-44.
- [68] Laeng P, Bouillon P, Taupenot L, *et al.* Long-term induction of an aldose reductase protein by basic fibroblast growth factor in rat astrocytes *in vitro*. *Electrophoresis* 1995; 16: 1240-50.
- [69] Ruef J, Liu SQ, Bode C, *et al.* Involvement of aldose reductase in vascular smooth muscle cell growth and lesion formation after arterial injury. *Arterioscler Thromb Vasc Biol* 2000; 20: 1745-52.
- [70] Spycher SE, Tabataba-Vakili S, O'Donnell VB, *et al.* Aldose reductase induction: a novel response to oxidative stress of smooth muscle cells. *FASEB J* 1997; 11: 181-8.
- [71] Rittner HL, Hafner V, Klimiuk PA, Szveda LI, Goronzy JJ, Weyand CM. Aldose reductase functions as a detoxification system for lipid peroxidation products in vasculitis. *J Clin Invest* 1999; 103: 1007-13.

- [72] Yadav UC, Srivastava SK, Ramana KV. Aldose reductase inhibition prevents endotoxin-induced uveitis in rats. *Invest Ophthalmol Vis Sci* 2007; 48: 4634-42.
- [73] Yadav UC, Ramana KV, Aguilera-Aguirre L, et al. Inhibition of aldose reductase prevents experimental allergic airway inflammation in mice. *PLoS One* 2009; 4: e6535.
- [74] Yadav UC, Naura AS, Aguilera-Aguirre L, et al. Aldose reductase inhibition suppresses the expression of Th2 cytokines and airway inflammation in ovalbumin-induced asthma in mice. *J Immunol* 2009; 183: 4723-32.
- [75] Yadav UC, Aguilera-Aguirre L, Ramana KV, et al. Aldose reductase inhibition prevents metaplasia of airway epithelial cells. *PLoS One* 2010; 5: e14440.
- [76] Ravindranath TM, Mong PY, Ananthakrishnan R, et al. Novel role for aldose reductase in mediating acute inflammatory responses in the lung. *J Immunol* 2009; 183: 8128-37.
- [77] Barth BM, Stewart-Smeets S, Kuhn TB. Proinflammatory cytokines provoke oxidative damage to actin in neuronal cells mediated by Rac1 and NADPH oxidase. *Mol Cell Neurosci* 2009; 41: 274-85.
- [78] Zhao H, Ma JK, Barger MW, et al. Reactive oxygen species- and nitric oxide-mediated lung inflammation and mitochondrial dysfunction in wild-type and iNOS-deficient mice exposed to diesel exhaust particles. *J Toxicol Environ Health* 2009; 72: 560-70.
- [79] Sevanian A, Hochstein P. Mechanisms and consequences of lipid peroxidation in biological systems. *Annu Rev Nutr* 1985; 5: 365-90.
- [80] Keller JN, Mattson MP. Roles of lipid peroxidation in modulation of cellular signaling pathways, cell dysfunction, and death in the nervous system. *Rev Neurosci* 1998; 9: 105-16.
- [81] Calabrese V, Guagliano E, Sapienza M, et al. Redox regulation of cellular stress response in neurodegenerative disorders. *Ital J Biochem* 2006; 55: 263-82.
- [82] Ramana KV, Srivastava SK. Aldose reductase: a novel therapeutic target for inflammatory pathologies. *Int J Biochem Cell Biol* 2010; 42: 17-20.
- [83] Tsukagoshi H, Kawata T, Shimizu Y, et al. 4-Hydroxy-2-nonenal enhances fibronectin production by IMR-90 human lung fibroblasts partly via activation of epidermal growth factor receptor-linked extracellular signal-regulated kinase p44/42 pathway. *Toxicol Appl Pharmacol* 2002; 184: 127-35.
- [84] Dixit BL, Balendiran GK, Watowich SJ, et al. Kinetic and structural characterization of the glutathione-binding site of aldose reductase. *J Biol Chem* 2000; 275: 21587-95.
- [85] Ramana KV, Dixit BL, Srivastava S, et al. Selective recognition of glutathiolated aldehydes by aldose reductase. *Biochemistry* 2000; 39: 12172-80.
- [86] Housset B. Free radicals and respiratory pathology. *C R Seances Soc Biol Fil* 1994; 188: 321-33.
- [87] Rahman I, van Schadewijk AA, Crowther AJ, et al. 4-Hydroxy-2-nonenal, a specific lipid peroxidation product, is elevated in lungs of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2002; 166: 490-5.
- [88] Ramana KV, Bhatnagar A, Srivastava S, et al. Mitogenic responses of vascular smooth muscle cells to lipid peroxidation-derived aldehyde 4-hydroxy-trans-2-nonenal (HNE): role of aldose reductase-catalyzed reduction of the HNE-glutathione conjugates in regulating cell growth. *J Biol Chem* 2006; 281: 17652-60.
- [89] Ramana KV, Fadl AA, Tammali R, et al. Aldose reductase mediates the lipopolysaccharide-induced release of inflammatory mediators in RAW264.7 murine macrophages. *J Biol Chem* 2006; 281: 33019-29.
- [90] Drazen JM. Anti-leukotrienes as novel anti-inflammatory treatments in asthma. *Adv Exp Med Biol* 2002; 507: 217-21.
- [91] Djukanović R, Wilson SJ, Kraft M, et al. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med* 2004; 170: 583-93.
- [92] Hotta N, Toyota T, Matsuoka K, et al. Clinical efficacy of fidarestat, a novel aldose reductase inhibitor, for diabetic peripheral neuropathy: a 52-week multicenter placebo-controlled double-blind parallel group study. *Diabetes Care* 2001; 24: 1776-82.