Aldose Reductase Inhibition Prevents Endotoxin-Induced Uveitis in Rats

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PURPOSE. The purpose of the present study was to elucidate the role of the polyol pathway enzyme aldose reductase (AR) in the mediation of ocular inflammation in a rat model of endotoxin-induced uveitis (EIU).

METHODS. EIU was induced by a subcutaneous injection of 200 μg lipopolysaccharide (LPS) in male Lewis rats treated with the AR inhibitor, zopolrestat (25 mg/kg body weight, intraperitoneally) or its carrier. The rats were killed 24 hours after LPS injection, the eyes were enucleated immediately, and aqueous humor (AqH) was determined. The number of infiltrating cells, protein concentration, and levels of nitric oxide (NO), tumor necrosis factor (TNF-α), and prostaglandin E2 (PGE2) in the AqH were determined. Immunohistochemical analysis was performed in paraformaldehyde-fixed eye sections by staining with antibodies against iNOS, COX-2, TNF-α, NF-κB, and AR. The levels of reactive oxygen species (ROS) in rat eye sections were determined by dihydroethidium (hydroethidine) fluorescence staining.

RESULTS. In the EIU rat eye AqH, both the number of infiltrating cells and protein concentrations of the inflammatory markers, TNF-α, NO, and PGE2 were significantly higher than in the control rats, and inhibition of AR by zopolrestat suppressed the LPS-induced increases. The LPS-induced increased expression of AR, TNF-α, iNOS, and COX-2 proteins in the ciliary body, corneal epithelium, and retinal wall was also significantly inhibited by zopolrestat. Furthermore, AR inhibition prevented the LPS-induced increased levels of ROS and activation of NF-κB in the ciliary body, corneal epithelium, and retinal wall of the rat eye. AR inhibition also prevented the LPS-induced activation of NF-κB and expression of COX-2 and iNOS in the human monocyte cell line U-937.

CONCLUSIONS. The results indicate that AR inhibition suppresses the inflammation in EIU by blocking the expression and release of inflammatory markers in ocular tissues, along with the attenuation of NF-κB activation. This finding suggests that AR inhibition could be a novel therapeutic target for the treatment of uveitis and associated ocular inflammation. (Invest Ophthalmol Vis Sci. 2007;48:4634–4642) DOI:10.1167/iovs.07-0485

Uveitis is the major cause of severe visual impairment and has been estimated to account for 5% to 15% of all cases of total blindness in the United States.1,2 It is even more prevalent in developing nations with limited access to healthcare.3 The uvea has good vasculature that nourishes the eye, and inflammation of the uvea can affect ocular functions. Although the cause of uveitis can include autoimmune disorders, infection, or exposure to toxins, in several cases the etiology remains unknown.4 However, the ocular inflammation due to autoimmune diseases and infections is considered the major source.5 Steroids and other drugs that suppress the immune response are currently used to control the inflammation and have many serious side effects, including severely diminishing the patient’s quality of life.6,7 In uveitis, the cytokine levels significantly increase in ocular tissues and initiate distinct intracellular signaling cascades that lead to both acute physiological effects and long-term changes in inflammatory gene expression.8,9 Therefore, elucidation of cytokine signaling is critical for understanding uveitis. Endotoxin-induced uveitis (EIU) is an acute anterior segment intraocular inflammation that can be induced by lipopolysaccharide (LPS) in rodents.9 Although EIU was originally used as a model of anterior uveitis, increasing evidence suggests that it also involves inflammation in the posterior segment of the eye, with recruitment of leukocytes that adhere to the retinal vasculature and infiltrate the vitreous cavity.1,9,10 This phenomenon could serve as a model for certain types of human uveitis, such as those associated with seronegative arthritis, where Gram-negative bacteria may play a major role in the pathogenesis.9 LPS enhances the expression of various inflammatory cytokines and chemokines such as TNF-α, IL-6, MIF, IFN-γ, and MCP-1, as well as the production of PGE2 and NO, resulting in the breakdown of the blood–ocular barrier and in the infiltration of leukocytes and monocytes in ocular tissues which contribute to the development of EIU.9

Various reports show that reactive oxygen species (ROS) are obligatory mediators of inflammation induced by cytokines and chemokines,11,12 which in turn induce intracellular ROS generation by mitochondrial respiratory chain reaction, the arachidonic metabolic reactions, and the membrane-bound peroxide-generating enzyme NADPH oxidase. Further, ROS activate redox-sensitive transcription factors such as NF-κB and AP-1, which play a central and crucial role in inflammation.13,14 Inflammation is probably due to the overexpression of inflammatory cytokines and iNOS and COX-2 enzymes, resulting in increased NO and PGE2.16,17 These local messenger molecules act further in an autocrine and paracrine fashion and amplify ROS effects. The ROS in turn activate various genes involved in cytotoxicity. For example the proinflammatory cytokines TNF-α, IL-1, and IL-6 play an important role at initial stages of cell growth or apoptosis. Among the proinflammatory cytokines, TNF-α is known to be recognized as a central mediator in the pathophysiology of chronic inflammatory bowel conditions, such as Crohn’s disease and ulcerative colitis, that cause increased risk of uveitis.18,19 Recent studies have examined the use of anti-TNF-α therapy to treat uveitis.20–23 However, it is not clear how an inflammation-associated increase in free radicals could cause activation of NF-κB.

Our recent studies suggest that the polyol pathway enzyme aldose reductase (AR; AKR1B1), besides reducing aldo sugars, reduces various lipid aldehydes and their glutathione conju-
gates and is an obligatory mediator of ROS signals. Further, we have shown that inhibition or small interfering (si)RNA ablation of AR prevents the cytokine, growth factor-, and hyperglycemia-induced cytotoxic signals in vascular smooth muscle cells (VSMCs), vascular endothelial cells (VECs), and macrophages. We have also demonstrated that TNF-α and high glucose-induced activation of NF-κB and apoptosis of human lens epithelial cells (HLECs) are significantly prevented by AR inhibition. Further, AR inhibition prevents LPS-induced expression of TNF-α, MMP2, MMP9, and COX-2 in HLECs, which indicates the role of AR in mediating inflammatory signals in lens epithelial cells. However, the role of AR in mediating ocular inflammation leading to uveitis is not known.

In the present study, we therefore investigated the effect of inhibition of AR on the ocular inflammation caused by LPS during EIU in Lewis rats. Inhibition of AR prevented LPS-induced activation of NF-κB and production of inflammatory markers such as NO, PGE2, COX-2, and TNF-α and accumulation of infiltrating cells in various ocular tissues, suggesting possible therapeutic applications of AR inhibitors in ocular inflammation.

Materials and Methods

Materials

RPMI-1640 medium, phosphate-buffered saline (PBS), gentamycin sul fate solution, trypsin/EDTA solution, and fetal bovine serum (FBS) were purchased from Invitrogen-Gibco (Grand Island, NY). Zopolrestat was a gift from Pfizer (New York, NY). Dimethyl sulfoxide (DMSO) was purchased from Fischer Scientific (Pittsburgh, PA); nitrite/nitrate and PGE2 assay kits from Cayman Chemical, Inc. (Ann Arbor, MI); a rat TNF-α ELISA kit from BD Biosciences (San Diego, CA); LPS derived from Escherichia coli from Sigma-Aldrich (St. Louis, MO); antibodies against TNF-α and phospho-p65 (serine 536) from Cell Signaling (Dan vers, MA); iNOS from Cayman Chemicals; and COX-2 and GAPDH from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Polyclonal antibodies against human recombinant AR were made for us by Alpha Diagnostic International (San Antonio, TX). All other reagents were of analytical grade.

Animals and Treatment

Six- to 8-week-old male Lewis rats weighing approximately 150 to 160 g were used in the study (n = 6). All animals were kept in the University of Texas Medical Branch (UTMB) Animal Care Center. All the animal studies were conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. EIU was induced by a subcutaneous injection at two different locations of E. coli LPS (200 μg) dissolved in phosphate-buffered saline (100 μL PBS, pH 7.4). Rats in the AR inhibitor and EU + AR inhibitor groups were injected intraperitoneally with the AR inhibitor zopolrestat (25 mg/kg body weight) dissolved in DMSO 24 hours before and immediately after injection of LPS. Rats in the control group were injected with vehicle (PBS + 20% DMSO).

Infiltrating Cells and Proteins in Aqueous Humor

The rats were euthanatized 3, 6, and 24 hours after LPS injection, and the aqueous humor (AqH) was collected from the eyes immediately by an anterior chamber puncture with a 30-gauge needle under a surgical microscope. For cell counting, the AqH samples were suspended in an equal amount of Trypan-blue solution, and the cells were counted with a hemocytometer under a light microscope (Olympus Optical Ltd., London, UK). The total protein concentration in the AqH samples was measured with a protein assay kit (Bio-Rad, Hercules, CA). The AqH samples were stored in ice water until testing, and the cell counts and total protein concentra-


tions were measured on the day of sample collection. The remaining AqH was stored at −80°C until it was used.

TNF-α, NO, and PGE2 in AqH

The level of TNF-α in the AqH (stored at −80°C) was assessed with a commercially available ELISA kit. The total level of nitrate plus nitrite in the AqH was measured by using a total nitrite colorimetric assay (lactate dehydrogenase [LDH]) kit. PGE2 production was measured by an enzyme immunoassay kit. All assays were performed according to the manufacturers’ instructions.

Histopathological Evaluation

The rats were euthanatized 24 hours after LPS injection, and the eyes were enucleated immediately and stored in 4% paraformaldehyde solution for 48 hours at 4°C. The eyes were washed in ice-cold PBS twice and kept in 70% alcohol at 4°C until they were embedded in paraffin. Sagittal sections (5 μm) were cut and stained with hematoxylin and eosin (H&E). The iris-ciliary body complex, anterior chamber, vitreous, and retina were observed by light microscope.

Immunohistochemical Studies

The paraffin-embedded sections were warmed at 60°C for 1 hour and deparaffinized in xylene, rehydrated by passing through 100%, 95%, 80%, and 70% ethanol, and washed in deionized water. After the peroxidase reaction was blocked with 3% H2O2, the sections were rinsed in PBS twice and incubated with blocking buffer (2% BSA, 0.1% Triton X-100, 2% normal rabbit IgG, and 2% normal goat serum) overnight at 4°C. The sections were incubated with antibodies against TNF-α, iNOS, COX-2, and phospho-p65 antibodies (Ser536), and AR, and for 1 hour at room temperature. They were then stained with peroxidase (LSAB+System HRP, DakoCytomation, Carpinteria, CA). The sections were examined by bright-field light microscopy (EPI-800 microscope; Nikon, Tokyo, Japan) and photographed with a camera (Nikon) fitted to the microscope.

Measurement of ROS

The level of ROS in the rat eye was quantified by dihydroethidium (DHE; Invitrogen-Molecular Probes, Eugene, OR), which gives red fluorescence when oxidized to ethidium in the presence of ROS. Serial sections (5 μM) of paraformaldehyde-fixed rats’ eyes were deparaffinized, rehydrated, and incubated with ROS-sensitive dye (5 μM) for 30 minutes at 37°C followed by acquisition of images with a fluorescence microscope.

Cell Culture and LPS Treatment

U-937, a human monocyte cell line, was obtained from ATCC (Manas sa, VA). The cells were cultured in RPMI-1640 medium supplemented with 2 mM glutamine, 1 mM sodium pyruvate, 25 mM HEPES, antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) and 10% heat-inactivated fetal bovine serum and maintained at 37°C in a humidified incubator containing 95% O2 and 5% CO2. The cells were pre-treated with 10 μM of the AR inhibitor zopolrestat in serum-free medium overnight and subsequently were stimulated with 1 μg/mL LPS from E. coli for 24 hours, unless otherwise stated.

Western Blot Analysis

U-937 cells were washed twice with ice-cold PBS and lysed in ice-cold lysis buffer containing 50 mM HEPES [pH 7.6], 10 mM KCl, 0.5% NP-40, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1:100 dilution of protease inhibitor cocktail (Sigma-Aldrich) for 15 minutes with occasional vortexing at maximum speed at 4°C. The crude lysates were cleared by centrifugation at 12,000g for 10 minutes at 4°C. Aliquots of the lysates were diluted with 2× SDS sample buffer and boiled for 5 minutes. The lysates were separated on 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes.
Transient Transfection and NF-κB-Dependent Secretory Alkaline Phosphatase (SEAP) Expression Assay

To examine NF-κB promoter activity in U-937 cells in response to LPS treatment, U-937 cells (2.5 x 10^6 cells/well in 6-well plate) in RPMI-1640 (with 10% FBS) were transfected with a pNF-κB-SEAP2 construct and pTAL-SEAP control plasmid (BD Biosciences-Clontech, Palo Alto, CA) with a lipophilic transfection reagent (Lipofectamine 2000; Invitrogen, Carlsbad, CA) according to the suppliers’ instructions. The cells were harvested and plated in a 24-well plate in serum-free medium, treated with AR inhibitor for 6 hours, and then stimulated with LPS (1 μg/mL) for 48 hours. The cell culture media were centrifuged at 5000 rpm and the supernatants were stored at −80°C. The media were thawed and used for a chemiluminescent SEAP assay (Great EscAPE SEAP; BD Biosciences-Clontech), used essentially according to the protocol described by the manufacturer, with a 96-well chemiluminescence plate reader. All the controls suggested by the manufacturer were used in the assay.

Statistical Analysis

Data are expressed as the mean ± SD. All the data were analyzed by Student’s t-test (Excel 2003; Microsoft, Redmond, WA). *P < 0.05 was considered statistically significant.

RESULTS

Effect of AR Inhibition on Leukocyte Infiltration and Protein Concentration Induced by EIU in the Rat Eye

To investigate the effect of AR inhibition on LPS-induced infiltration of inflammatory cells such as leukocytes and monocytes into the anterior chamber and AqH of the eye, sagittal sections of rats’ eyes were stained with H&E and examined by bright-field microscope. The EIU-caused infiltration of a large number of cells was significantly inhibited by the AR inhibitor zopolrestat. No significant infiltration of cells was observed in either the carrier- or the zopolrestat-treated groups (data not shown). In the EIU rat eye, a few infiltrating cells were also present in the vitreous chamber (VC), but none was observed in AR inhibitor+EIU or control rats. The accumulation of infiltrating cells in AqH was also confirmed by manually counting the cells in AqH with a hemocytometer (Fig. 1A). As observed in histologic examination, the manual cell counting also demonstrated a significant (>200-fold) EIU-induced increase in the infiltration of the inflammatory cells that was significantly (>80%) decreased by AR inhibitor treatment of the EIU rats (Fig. 1A). In addition, the total protein concentration in the AqH of EIU rat eyes was increased up to 24-fold compared with that in control rat eyes and inhibition of AR reduced it by >60% (Fig. 1B). These results suggest that AR inhibition prevents EIU-induced infiltration of inflammatory cells as well as release of inflammatory proteins in the AqH of rat eyes.

Effect of AR Inhibition on EIU-Induced Inflammatory Markers in AqH

We next examined the effect of AR inhibition on the levels of inflammatory markers (TNF-α, NO, and PGE2) in the AqH of EIU rat eyes. TNF-α was not detectable in the AqH of control animals, whereas in EIU rat eyes, the TNF-α levels were approximately 30 and 190 ng/mL after 6 and 24 hours of EIU induction (Fig. 2). Treatment of rats with zopolrestat followed by EIU significantly (>60%) reduced the TNF-α concentration in AqH during both time points. These results were further confirmed by immunohistochemistry with antibodies against TNF-α. The
EIU rats showed a significant intensity of antibody staining in the iris–ciliary complex and AqH region, whereas AR inhibitor–treated animals showed diminished antibody staining, indicating that AR inhibition prevents accumulation of TNF-α (data not shown). Because EIU-induced acute inflammation is not restricted to the anterior chamber only,9,10 we immunohistochemically examined the levels of TNF-α in the vitreous region as well. The results demonstrated increased levels of TNF-α in the vitreous and retina of LPS-treated rats, compared with control groups. These increases were significantly prevented by the AR inhibitor (data not shown), which suggests an anti-inflammatory role of AR inhibition in EIU. Similarly, the levels of NO and PGE₂ (Figs. 3A, 3B) significantly increased in the AqH of EIU rat eyes compared with the control, and treatment with the AR inhibitor significantly (>70%) reduced their levels. Because NO and PGE₂ are produced by iNOS and COX-2 enzymes, respectively, we immunohistochemically examined the expression of iNOS and COX-2 proteins in the various regions of the eye. The EIU rat eyes showed increased expression of iNOS and COX-2 proteins in the iris–ciliary body complex, the corneal epithelium in the anterior segment, and the retinal wall in the posterior segment (Figs. 3A, 3B). The AR inhibitor significantly prevented the expression of iNOS as well as COX-2 in the ocular tissues.

Effect of the AR Inhibitor on the Expression of AR in EIU Rat Eyes

Since AR is an oxidative stress response protein and increased AR protein levels have been observed in many diseases,31–33 we next examined the AR expression in EIU rat ocular tissues. Immunohistochemical staining of EIU rat eye sections with antibodies against AR showed a strong staining for AR in cells in the iris–ciliary body, corneal epithelial layer, and retina (Fig. 4A) compared with control eyes. However, the AR inhibitor in endotoxin-injected rat ocular tissues significantly inhibited the expression of AR, suggesting that AR inhibition prevents signaling events responsible for its own gene expression.

Effect of AR Inhibition on NF-κB Activity in EIU Rat Eyes

Since redox-sensitive transcription factor NF-κB transcribes various inflammatory marker genes including those of TNF-α, iNOS, COX-2, and AR,33–35 we next examined the effect of AR inhibition on endotoxin-induced activation of NF-κB in the rat eye. The eye sections were immunostained with antibodies against the active subunit of NF-κB (phospho-p65) which is released subsequent to degradation of the inhibitory protein...
Inhibition prevents EIU-induced NF-κB activity in a human monocyte cell line. Further, we tested the activation of NF-κB in the presence of the AR inhibitor, the LPS-induced increase in NF-κB activity in the anterior and posterior chambers of AR inhibitor–treated EIU eyes was significantly decreased.

**Effect of AR Inhibition on Endotoxin-Induced Oxidative Stress**

Since NF-κB is an ROS-sensitive transcription factor and AR inhibition prevents EIU-induced NF-κB activation, we next examined the effect of AR inhibition on ROS generation in the EIU rat eye. As shown in Figure 5, the increased fluorescence corresponding to the increased level of ROS was observed in the iris-ciliary body complex and the corneal epithelium in the anterior segment, and inhibition of AR significantly prevented an LPS-induced increase in ROS. Further, LPS increased the ROS levels in the retinal region of the posterior segment, and the increase was prevented by AR inhibitor.

**Effect of AR Inhibition on LPS-Induced NF-κB-Dependent Inflammatory Protein Expression in a Human Monocyte Cell Line**

The in vivo observations made in EIU were confirmed by in vitro studies in the U-937 human monocyte cell line, as monocytes are one of the major types of infiltrating cells in AqH during EIU. Incubation of U-937 cells with LPS caused a two- and threefold increase in the expression of COX-2 and iNOS proteins, respectively, compared with control cells. However, in the presence of the AR inhibitor, the LPS-induced increase in COX-2 and iNOS proteins in monocytes was significantly prevented (Fig. 6A). Furthermore, we tested the activation of NF-κB in LPS-treated cells with an SEAP reporter assay, and the results showed a ~40% increase in SEAP activity (corresponding to NF-κB activation) compared with the control, and this increase was significantly reduced by AR inhibition (Fig. 6B).

**DISCUSSION**

Despite significant research efforts and advances in diagnosis and therapy, ocular inflammatory diseases, which cover a variety of ocular diseases with various clinical symptoms and pathogenicity, remain a significant cause of visual impairment in humans. The disease may be of infectious or putative autoimmune etiology. Because uveitis frequently leads to severe vision loss and blindness with retinal vasculitis, retinal detachment, and glaucoma, it is important to elucidate the mechanisms that cause ocular inflammation. The present study is the first to demonstrate that the inhibition of the polyol pathway enzyme leads to the suppression of ocular inflammation (uveitis) including leukocyte infiltration and protein leakage in the AqH and the expression of various inflammatory markers in eye tissues.

Oxidative stress–induced ROS generation is the major factor in triggering inflammation and tissue damage during the inflammatory process induced by LPS. The rationale for this study stems from our previous work showing that AR inhibitors can prevent cytokine and chemokine signals downstream from ROS that activate various transcription factors, indicating the involvement of AR in several inflammatory mechanisms responsible for carcinogenesis and sepsis. Our studies have shown that the inhibition of AR prevents production of inflammatory markers such as PGE$_2$, COX-2, TNF-α, IL-6, and NO in murine macrophages stimulated with LPS and in human colon cancer cells stimulated with growth factors. We have also demonstrated that treatment of mouse macrophages or VSMCs with HNE, glutathione (GS-HNE), and glutathione-dihydroxyrononone (GS-DHN) causes cytotoxicity via NF-κB-dependent signaling. AR inhibitors prevent HNE and GS-HNE-induced cytotoxic effects but not those of GS-DHN. These
observations assigned an important role to the AR-catalyzed reduced product GS-DHN in inflammatory signaling and indicate that AR inhibition could be anti-inflammatory. In our most recent study, LPS-induced cytotoxicity in HLECs was mediated by AR and inhibition of AR prevented LPS-induced activation of the redox-sensitive transcription factor NF-kB and production of inflammatory markers such as TNF-α, MMP2, and MMP9. Since the microenvironment in the uveitic eye is characterized by the high expression of inflammatory factors including the cytokines iNOS and COX-2, and their products PGE2 and NO,1–3 inhibition of AR could represent a useful approach for prevention and/or treatment of ocular inflammatory response such as uveitis. In the past several years, many AR inhibitors have been tested for the potential treatment of diabetic complications such as diabetic neuropathy and retinopathy. The AR inhibitor epalrestat is currently marketed in Japan for the treatment of diabetic neuropathy.42 AR inhibitors such as fidarestat, zenarestat, and minalrestat are currently in phase-3 clinical trials. Common limitations to these drugs include critical hepatic and renal toxicity.45 The AR inhibitor used in this study, zopolrestat, is a new one synthesized by Pfizer Inc.44 Zopolrestat has already been tested in phase-3 clinical trials for long-term (several years) use in the treatment of diabetic neuropathy and in phase-2 clinical trials for diabetic cardiomyopathy and nephropathy.40 No major side effects have been observed during the clinical trials. Johnson et al.45 have shown that patients with diabetic neuropathy treated with 500 to 1000 mg zopolrestat daily for 1 year did not show any major toxic effects, and the drug reversed the cardiac abnormalities observed in these patients. Our recent studies suggest the use of AR inhibitors as anti-inflammatory drugs, since they can prevent the generation of NF-kB-dependent inflammatory cytokines and chemokines and their signals.57–59 Ocular inflammation is the major cause of uveitis and related complications.7–9

The ocular inflammation during EIU is characterized by a breakdown of the blood–aqueous barrier with an increase of total protein content in the AqH and cellular infiltration of leukocytes into the anterior chamber of the eye.46 Our results indicate that AR inhibition suppressed the endotoxin-induced ocular inflammation, which is evident from the significantly reduced number of leukocyte infiltration and protein concentrations in the AqH of rat eyes (Fig 1). Similarly, other investigators have shown that treatment with antioxidants such as vitamin E,50–52 pyrroliidine dithiocarbamate,49 and astaxanthin prevents endotoxin-induced ocular inflammation. Therefore the antioxidative property of AR inhibitors could be used for preventing ocular inflammation. Increased protein concentration in the AqH during EIU is caused by the increased expression of various inflammatory markers, which include cytokines, chemokines, PGE2, and NO.51–54 High levels of TNF-α have been associated with a recurrent pattern of uveits.51,54 TNF-α is a pleiotropic cytokine produced by activated macrophages and monocytes during immune response to various infectious agents or other oxidant stimuli and has also been detected in eyes of patients with Behc¸et’s disease.55 Recent studies suggest that administering anti-TNF-α chimeric monoclonal antibodies (infliximab) to patients with acute uveitis and Behc¸et’s disease ameliorates ocular inflammation.22,23,55 The role of TNF-α has been further substantiated by decreased inflammation in TNF-receptor–deficient mice in immune-complex-induced uveitis.56 Several studies have doc-

Figure 5. Inhibition of AR prevented ROS generation in EIU. Serial sections of paraformaldehyde-fixed rats’ eyes were stained with ROS-sensitive dye dihydroethidium (DHE) for 30 minutes at 37°C followed by acquisition of images with a fluorescence microscope. Representative images are shown (n = 4). ARI AR inhibitor; abbreviations in the images are as in Figure 3. Magnification, ×200.
thereby lead to a marked increase in PGE2 production. Our U-937 cells were transiently transfected with the pNF-onc and pNF-iNOS plasmids, which is responsible for the production of PGE2. Inhibition prevents the expression of the COX-2 enzyme, determined by Western blot analysis using specific antibodies. (A) U-937 cells were incubated with 1 μg/mL of LPS for 24 hours. The expression of COX-2 and iNOS proteins was determined by Western blot analysis using specific antibodies. The cells treated without or with zopolrestat (10 μM) were incubated with 1 μg/mL of LPS. After 24 hours, the culture supernatants were assayed for SEAP activity with a chemiluminescence kit, according to the supplier’s instructions. Data are expressed as the mean ± SD (n = 6). **P < 0.01 vs. the control group; ***P < 0.01 vs. the LPS group.

The effect of inhibition of AR on the LPS-induced inflammatory response in human monocytes. (A) Growth-arrested U-937 cells with or without zopolrestat (10 μM each) were incubated with 1 μg/mL of LPS for 24 hours. The expression of COX-2 and iNOS proteins was determined by Western blot analysis using specific antibodies. (B) U-937 cells were transiently transfected with the pNF-iNOS, iNOS, and TNF-α expression by AR inhibition could be the inhibition of LPS-induced NF-κB activation. Indeed, our results suggest that AR inhibition prevents NF-κB activation in rat’s eye tissues (Fig. 4B). We and others have shown that inhibition of AR prevents PKC and NF-κB activation by a variety of stimuli such as TNF-α, FGF, PDGF, angiostatin-H, and high glucose- and hyperglycemia-induced MAPK and JAK2. These findings suggest that AR could be an obligatory mediator of the stress response, including the activation of NF-κB and other ROS-sensitive transcription factors. Although the mechanisms by which AR activates NF-κB remain unclear, we propose that inhibition of AR prevents the events that could lead to the activation of PLC and PKC isozymes, which are activated by LPS, as observed in our in vitro cell culture studies.

In summary, our results provide evidence of an unanticipated role of AR in mediating the NF-κB-dependent inflammatory response during acute inflammatory conditions and provide a novel concept that inhibition of AR could be therapeutically useful in preventing ocular inflammations such as uveitis.

References


