The Ocular Endothelin System: A Novel Target for the Treatment of Endotoxin-Induced Uveitis With Bosentan

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Purpose. We compared the anti-inflammatory effects of bosentan and dexamethasone in endotoxin-induced uveitis (EIU).

Methods. Endotoxin-induced uveitis was induced by subcutaneous injection of lipopolysaccharide (LPS, 200 μg) in Wistar rats. Rats were divided randomly into 10 groups ($n = 6$). Bosentan at doses of 50 and 100 mg/kg were administered orally 1 hour before and 12 hours after LPS injection, and dexamethasone was administered by intraperitoneally 30 minutes before and 30 minutes after LPS injection at a dose of 1 mg/kg. Data were collected at two time points for each control and treatment; animals were killed at either 3 or 24 hours after LPS injection. Histopathologic evaluation and aqueous humour measurements of TNF-α level were performed, and endothelin-1 (ET-1), inducible nitric oxide synthase (iNOS), and endothelin receptor A and B (EDNRA and B) expression were analyzed.

Results. The group treated with 100 mg/kg bosentan at 24 hours displayed significantly milder uveitis and fewer inflammatory cells compared to LPS-injected animals, and there were similar findings in the dexamethasone-treated group at 24 hours. The TNF-α levels in the dexamethasone treatment group were lower than those in the LPS-induced uveitis control group ($P < 0.05$); however, there was no difference between the dexamethasone and bosentan treatment groups at 3 and 24 hours after LPS administration. Bosentan treatment at doses of 50 and 100 mg/kg significantly decreased iNOS expression compared to LPS-injected animals ($P < 0.001$). The ET-1 expression was suppressed significantly by bosentan and dexamethasone at 3 and 24 hours after LPS administration ($P < 0.001$). The EDNRA expression in the bosentan treatment groups was statistically significantly lower than that in the LPS-induced uveitis control group at 3 and 24 hours after LPS administration ($P < 0.05$).

Conclusions. Bosentan reduces intraocular inflammation and has similar effects as dexamethasone in a rat model of EIU.

Keywords: endotoxin-induced uveitis, bosentan, TNF-α, iNOS, endothelin-1

Uveitis is an intraocular inflammatory condition that can cause serious complications and permanent vision loss. It primarily affects the uvea, but also can affect surrounding tissues, such as the vitreous, retina, and optic nerve. Infection and autoimmune mechanisms are known to have a role, but the exact pathogenesis remains unclear. Therefore, intraocular inflammation continues to be studied to understand its pathophysiology and develop effective treatments. The eye tissue specific reaction found in uveitis can be caused by abnormal immune response or immune response to autoantigens.[1]

Lipopolysaccharide (LPS) is the main component of the exterior walls of gram-negative bacteria, and is essential for their physical integrity and function. Intraperitoneal injection of LPS causes B lymphocyte, macrophage, and granulocyte activation, resulting in cytokine release. Experimental LPS administration is an established model for studying the etiopathogenesis of idiopathic nonspecific uveitis. This model has applications not only in evaluating the pathophysiology of this condition, but also in investigating new potential treatments.[2]

An acute but transient ocular inflammatory reaction peaks 24 hours after LPS administration. Tumor necrosis factor-α is an important target of interest in the endotoxin-induced uveitis (EIU) model. It has been shown in clinical studies that various cytokines are elevated in the aqueous humour and serum of acute uveitis patients. A clinical presentation comparable to uveitis in humans forms after cytokine injections in experimental animal models.[3–5] Cytokine levels, especially those of

TNF-α, can be used as a marker for monitoring the inflammation and disease progression.

The role of receptors in idiopathic uveitis has been the subject of many studies in recent years.[6–8] Their findings indicate that endothelin receptors have a significant role. Endothelin-1 (ET-1) causes vasoconstriction and elevated levels have been found in systemic pathologies.[9] In clinical and experimental studies, ET-1 receptors have been found in corneal epithelial and endothelial cells, nonpigmented ciliary epithelial cells, ciliary muscle, trabecular meshwork (TM) cells, lens, choroid, RPE, retinal photoreceptor cells, retinal nerve fiber, ganglion cell layer astrocytes, and retinal blood vessels. The wide distribution of ET-1 receptors in ocular tissues suggests the importance of ET-1 in ocular homeostasis.[10–18] An endothelin receptor antagonist recently has been found to be beneficial in the treatment of glaucoma. The ET-1 has been shown to be an indicator of glaucoma and intravitreal injection of ET-1 decreased blood flow to the optic nerve.[19,20] It also has been shown that inhibition of ET-1 corrects ocular blood flow and ocular functions are regained.[21]

The presence of ET-1 has been demonstrated in human and rat eye,[10] and ET-1 is present in the aqueous humour at concentrations several times higher than in plasma, presumably because it is secreted by the ciliary epithelium and not derived from plasma.[22] In a recent study, it was suggested that the endothelin receptor antagonist, bosentan, can effectively control inflammation in a rat model of arthritis.[23] In a previous study, the investigators concluded that ET-A and ET-B have a role in inflammation by increasing endothelial and/or epithelial permeability, and indirectly increasing migration of inflammatory cells into the lung parenchyma.[24] In another study, ET-1 was injected into the anterior chamber of rabbits to induce inflammation; the investigators concluded that the ET-1 concentration in the aqueous humor was significantly higher than in normal controls, and it was shown that ET-1 is an important mediator in ocular inflammatory reactions via arachidonic acid cascade.[25]

The aim of this study was to evaluate the function and importance of endothelin and endothelin receptors in the experimental LPS-induced uveitis model. Another aim was to use histopathologic and biochemical analyses to compare oral bosentan to dexamethasone, which currently is the standard treatment for uveitis, in terms of their effect on inducible nitric oxide synthase (iNOS) and TNF-α expression.

Materials and Methods

Chemicals

Bosentan monohydrate was obtained from Abdi Ibrahim (Istanbul, Turkey), dexamethasone was purchased from OSEL Pharmaceuticals (Istanbul, Turkey), LPS (*E. coli* 055:B5) was obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany), and all other chemicals were purchased from Sigma-Aldrich Chemie GmbH or Merck (Darmstadt, Germany).

Animals

We obtained 60 male Wistar rats weighing between 240 and 280 g from Ataturk University's Experimental Animal Laboratory of Medicinal and Experimental Application and Research Centre for this study. All studies were performed in accordance with National Institutes of Health guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and with the approval of the Ataturk University animal care committee. Rats were housed in standard plastic cages on sawdust bedding in a climate-controlled room at 22°C under a 14:10-hour light-dark cycle. Standard rat feed pellets and tap water were given ad libitum.

Uveitis Induction and Treatments

Rats were divided randomly into 10 groups, each containing six animals (see Table).

Two standard immunosuppressive and anti-inflammatory pharmacologic agents currently used in the treatment of ocular inflammation were compared: dexamethasone and two different doses of bosentan. The LPS (200 μg) was injected subcutaneously to induce uveitis as described previously.[26] Bosentan at doses of 50 (BOS50) and 100 (BOS100) mg/kg were prepared in 0.9% NaCl solution and administered orally; LPS solution was prepared with the same vehicle.[27,28] Dexamethasone was administered by intraperitoneal injection at a dose of 1 mg/kg. Bosentan was given 1 hour before and 12 hours after LPS injection. Dexamethasone was given 30 minutes before LPS, coadministered with LPS, and 30 minutes after LPS injection. The two different doses of bosentan (BOS50 and BOS100) used in the study were each given to its respective group 1 hour before and 12 hours after LPS injection. Rats in the control group were injected intraperitoneally with the vehicle (0.9% NaCl) only. Data were collected at two time points for each control and treatment; animals were killed using 50 mg/kg thiopental at either 3 or 24 hours after LPS injection. The aqueous humour (AqH) was collected from randomly selected eyes immediately by an anterior chamber puncture with a 30-gauge needle under a surgical microscope. Samples were collected after visual microscopic examination of the eyes and were kept at −80°C for further use. The remaining eyes were transferred immediately into 4% paraformaldehyde for immunohistochemical analysis.

Histopathologic Analysis

For histopathologic analysis, enucleated eye specimens were dissected from anterior to posterior and fixed in 10% neutral formalin for 48 hours. Routine tissue processing protocols were performed and tissues were embedded in paraffin; three or five sections with 4- to 5-μm thickness were cut from each specimen and stained with hematoxylin and eosin. Histopathologic findings in the uveal structures and cornea were scored according to the method of Tilton et al.[29] as follows: 0, normal tissue; 1+, dilated iris vessels, and thickened iris stroma with exudate, protein, and/or a few scattered inflammatory cells in the anterior chamber (AC); 2+, infiltration of inflammatory cells into the stroma of the iris and/or ciliary body with a moderate number of inflammatory cells within the AC; 3+, heavy infiltration of inflammatory cells within the iris stroma, ciliary body, and AC; 4+, heavy exudation of cells and dense protein aggregation in the AC, and inflammatory cells deposits on the corneal endothelium. Histopathologic analyses were performed by a single pathologist in a masked manner. Ten groups of eyes were compared histopathologically.

Biochemical Analysis – Aqueous Humour Measurement of Cytokine, TNF-α Level

The aqueous humour samples were allowed to clot, then the sera were separated by centrifugation at 2860*g* for 10 minutes at 4°C and kept at −86°C until they were thawed for the assay. The amount of TNF-α in each sample was determined in duplicate with highly sensitive ELISA kits specifically designed for rats (KRC30011; Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

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TABLE. Defines the Groups

Group	Group Code	Treatment Applied
1	SAL-3h	Control, saline injected, euthanized after 3 h
2	SAL-24h	Control, saline injected, euthanized after 24 h
3	LPS-3h	LPS-induced uveitis control, 200 μg LPS injected subcutaneously, euthanized after 3 h
4	LPS-24h	LPS-induced uveitis control, 200 μg LPS injected subcutaneously, euthanized after 24 h
5	DEX-3h	Dexamethasone treatment + LPS-induced uveitis, 1 mg/kg dexamethasone injected intraperitoneally 30 min before LPS, coadministered with LPS and 30 min following LPS, euthanized 3 h after LPS injection
6	DEX-24h	Dexamethasone treatment + LPS-induced uveitis, 1 mg/kg dexamethasone injected intraperitoneally 30 min before LPS, coadministered with LPS and 30 min following LPS, euthanized 24 h after LPS injection
7	BOS50-3h	Bosentan treatment + LPS-induced uveitis, 50 mg/kg bosentan administrated orally 1 h before LPS, euthanized 3 h after LPS injection
8	BOS50-24h	Bosentan treatment + LPS-induced uveitis, 50 mg/kg bosentan administrated orally 1 h before and 12 h following LPS, euthanized 24 h after LPS injection
9	BOS100-3h	Bosentan treatment + LPS-induced uveitis, 100 mg/kg bosentan administrated orally 1 h before LPS, euthanized 3 h after LPS injection
10	BOS100-24h	Bosentan treatment + LPS-induced uveitis, 100 mg/kg bosentan administrated orally 1 h before and 12 h following LPS, euthanized 24 h after LPS injection

Real Time-PCR Analysis – Total RNA Extraction and cDNA Synthesis

We studied the total eye after removal of conjunctiva. Eye tissue samples (20 mg) were stabilized in RNA Stabilization Reagent (RNAlater; Qiagen, Venlo, The Netherlands), and then disrupted using the TissueLyser II (2 × 2 minutes for eye, 2 × 5 minutes; Qiagen). Total RNA was purified using RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions in Qiaqube (Qiagen). The RNA samples were reverse-transcribed into complementary DNA by High Capacity cDNA Reverse Transcription Kit (TaqMan; Applied Biosystems, Foster City, CA, USA). To a 10 μL sample of total RNA, 2 μL ×10 RT buffer, 0.8 μL ×25 dNTPs mix, 2 μL ×10 RT Random Primers, 1 μL MultiScribe Reverse Transcriptase, and 4.2 μL diethylpyrocarbonate (DEPC)-treated H_2O were added, then incubated in a Veriti 96 Well Thermal Cycler (Applied Biosystems) under the following conditions: 25°C for 10 minutes, 37°C for 120 minutes, and 85°C for 5 minutes. The cDNA concentration and quality were assessed and quantified by using the Epoch Spectrophotometer System and Take3 Plate (BioTek Instruments, Inc., Winooski, VT, USA).

Real-Time Quantitative PCR

The ET-1 and iNOS expression analyses were performed with StepOne Plus Real Time PCR System technology (Applied Biosystems) using cDNA synthesized from eye RNA. The PCR amplification was achieved with TaqMan Gene Expression Assays Rn00561649_m1 for rat iNOS, Rn00561129_m1 for rat END1, and Rn00667869 for rat β-actin (Applied Biosystems), with β-actin expression used as endogenous control. Endothelin-A (EDNRA; forward, 5′-AGA TGC TCA ACA GAA GGA ATG G-3′ and reverse, 5′-CAC AGG GCG AAG ATG ACA AC-3′) and endothelin-B receptor (EDNRB; forward, 5′-CAC CAT CTC TTC TCG GGA CTA A-3′ and reverse, 5′-CGG AGG AAC GCA TCA GAC T-3′) were run using Primer Perfect Probe mix, TaqMan Probe–based technology (Primer Design Ltd., Southampton, UK). For each tissue, the assay was performed in quadruplicate in a 96-well optical plate for both targets and the control (iNOS, EDN1, EDNRA, EDNRB, and β-actin) using 2.5 μL of cDNA (100 ng), 1 μL of TaqMan Gene Expression Assay, 10 μL of TaqMan PCR Master Mix (Applied Biosystems), and 6.5 μL of RNase free water in each 20 μL reaction. The PCR conditions were: 50°C for 2 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. All data are expressed as fold-change in expression compared to the expression in other animal groups, using the $2^{(-\Delta\Delta Ct)}$ method.[30]

Statistical Analyses

Values are expressed as the mean ± SD. The TNF-α results were analyzed by 1-way ANOVA followed by the Duncan test. IBM-SPSS-20 for Windows was used for all statistical analyses. *P* values less than 0.05 were considered significant. Data from histopathologic scoring are represented as mean ± SD. Kruskal-Wallis ANOVA and Bonferroni adjustment were used to calculate differences and variances between groups. *P* values less than 0.05 were considered significant. Statistical analysis for molecular studies was performed according to 1-way ANOVA and the Tukey multiple comparison test. The values represent mean ± SD. $^{***}P < 0.0001$.

RESULTS

Histopathologic Results

Histopathologic scores analyses are shown in Figure 1. No infiltrating cells were detected in the eyes of animals in the control group (Fig. 2A). Conversely, the histological evaluation from the LPS-treated rats revealed signs of severe uveitis with massive neutrophil and monocyte infiltration, predominantly into the iris ciliary body. Hyperemia, perivascular edema, and multifocal hemorrhages were the main findings. The capillaries of the ciliary processes were severely congested (Fig. 2B). The

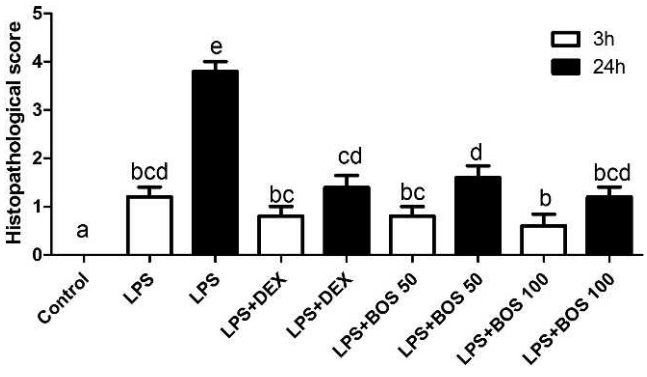

FIGURE 1. Histopathologic scores of each group.

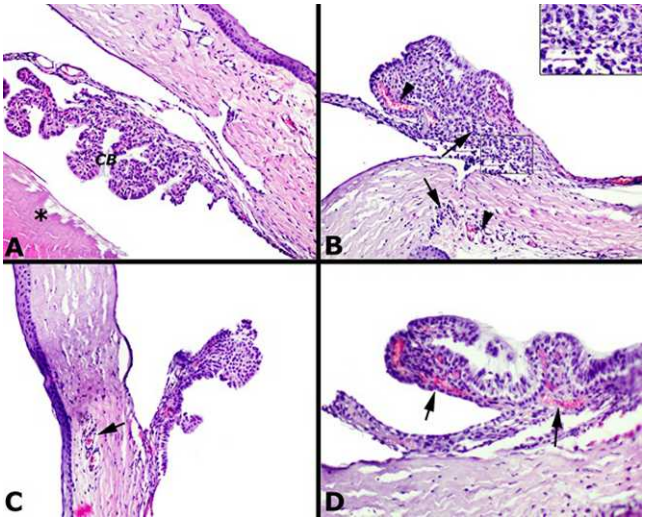

FIGURE 2. (**A**) Histopathologic section of an eye from control group. *Lens. CB, ciliary body. Hematoxylin and eosin, magnification ×200. (**B**) LPS-treated rats revealed signs of severe uveitis with massive neutrophil and monocyte infiltration, predominantly into the iris ciliary body (*arrows*). Hyperemia, perivascular edema. The capillaries of the ciliary processes were congested (*arrowheads*). Hematoxylin and eosin, magnification ×200. (**C**) Treatment with BOS100 24 hours displayed a significantly slighter uveitis and decreased amount of inflammatory cells compared to control animals. These findings were similar in the LPS dexamethasone 24-hour group. Hematoxylin and eosin, magnification ×200. (**D**) In the 3-hour LPS plus dexamethasone, LPS plus BOS50, and LPS plus BOS100 groups, inflammatory changes in uveal tissues and also vascular changes were very slight (*arrows*). Hematoxylin and eosin magnification ×400.

BOS100 24-hour group displayed significantly milder uveitis and fewer inflammatory cells compared to LPS-injected animals (Fig. 2C). There were similar findings in the dexamethasone 24-hour group. In the 3-hour dexamethasone, BOS50, and BOS100 groups, the inflammatory reaction in uveal tissues was very slight, with less prominent vascular changes and milder inflammation (Fig. 2D).

Effect of Bosentan on LPS-Induced Inflammatory TNF-α in Rat AqH

The comparison involving all groups showed the mean level of major proinflammatory cytokine TNF-α was higher in the LPS-induced uveitis control group than in the healthy control group at 3 hours after LPS administration ($P < 0.05$, Fig. 3). The TNF-α levels in the bosentan treatment groups were statistically significantly lower than that in the LPS-induced uveitis control group at 3 hours after LPS administration ($P < 0.05$). The TNF-α levels in the dexamethasone treatment group were lower than that in the LPS-induced uveitis control group ($P < 0.05$); however, there was no difference between the dexamethasone and bosentan treatment groups at 3 hours after LPS administration ($P > 0.05$). A comparison among all study groups revealed that TNF-α levels were highest in the LPS 24-hour group ($P < 0.05$). However, pretreatment with bosentan suppressed the LPS-induced secretion of TNF-α level in the AqH of rat eyes at 24 hours after administration of LPS ($P < 0.05$, Fig. 3). Bosentan administration decreased TNF-α levels all groups.

Effect of Bosentan on Endotoxin-Induced iNOS Expression During Ocular Inflammation

As iNOS is a well-known inflammatory marker, the effect of bosentan on endotoxin-induced activation of iNOS in rat eye

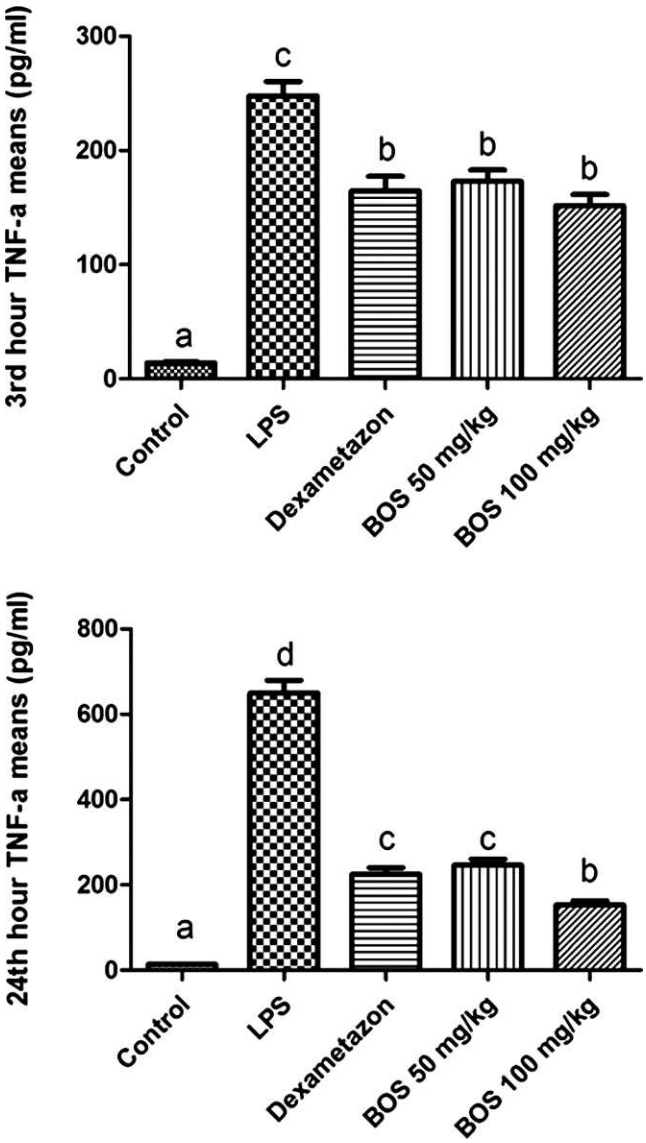

FIGURE 3. Pretreatment with bosentan significantly suppressed TNF-α level in the AqH at 3 and 24 hours.

tissue was investigated. Figure 4 shows that LPS exposure significantly increases iNOS expression compared to the control group at 3 and 24 hours after LPS administration ($P < 0.001$).

Dexamethasone treatment decreased iNOS expression compared to the LPS-induced uveitis control group at 3 and 24 hours after LPS administration. Treatment with bosentan at doses of 50 and 100 mg/kg significantly decreased iNOS expression compared to LPS-treated animals ($P < 0.001$). No significant changes in iNOS expression were found in rat eye tissue taken from the 3-hour dexamethasone or BOS50, or 24-hour BOS100 groups.

Effect of Bosentan on Endothelin Expression in LPS-Induced Uveitis

Endothelin expression was measured in rat eyes by quantitative RT-PCR. The results shown in Figure 5 indicated that expression of endothelin significantly increased in the EIU rat eyes, and this increase was significantly suppressed by

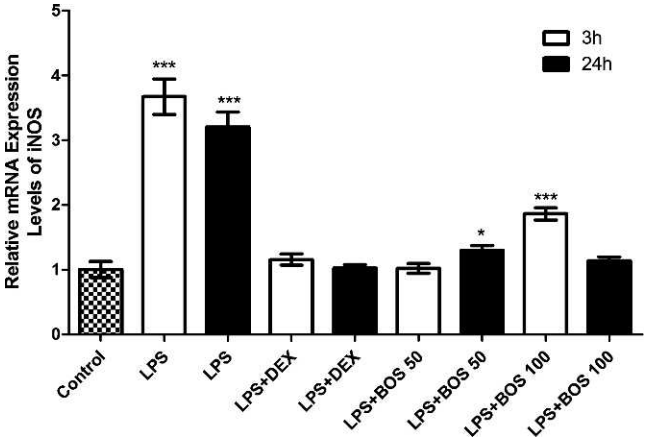

FIGURE 4. Inducible nitric oxide synthase expression in all groups. *$P < 0.05$. ***$P < 0.0001$.

bosentan and dexamethasone at 3 and 24 hours after LPS administration ($P < 0.001$).

Effect of Bosentan on LPS-Induced Inflammatory EDNRA and EDNRB in Rat Eye

The EDNR-A level was significantly higher in the LPS-induced uveitis control group than in the healthy control group at 3 and 24 hours after LPS administration ($P < 0.05$, Fig. 6). The EDNR-A levels in the bosentan treatment groups were statistically significantly lower than that in the LPS-induced uveitis control group at 3 and 24 hours after LPS administration ($P < 0.05$, Fig. 6). Bosentan administration decreased EDNR-A expression all groups. Although there was a difference between EDNR-B levels in all groups, this difference between groups was not significant (Fig. 7).

DISCUSSION

Uveitis is an inflammatory disease that can cause permanent vision loss and poses a significant threat to public health. The etiopathologic factors in uveitis formation and progression have not been established to our knowledge, and, therefore, there is no definitive treatment. Corticosteroids are the primary treatment choice, but steroid use is associated with various complications.[31–34]

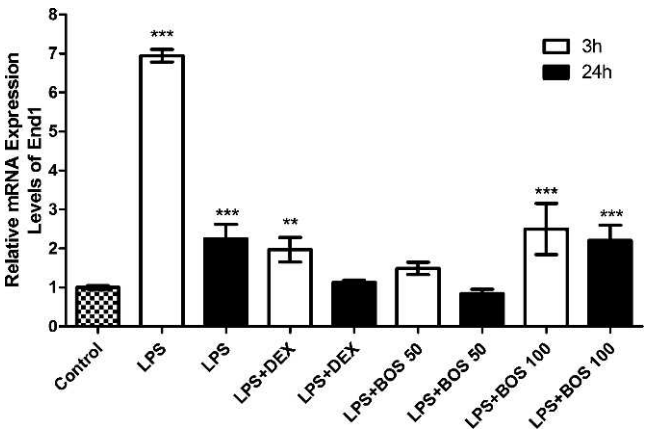

FIGURE 5. Endothelin expression in all groups.

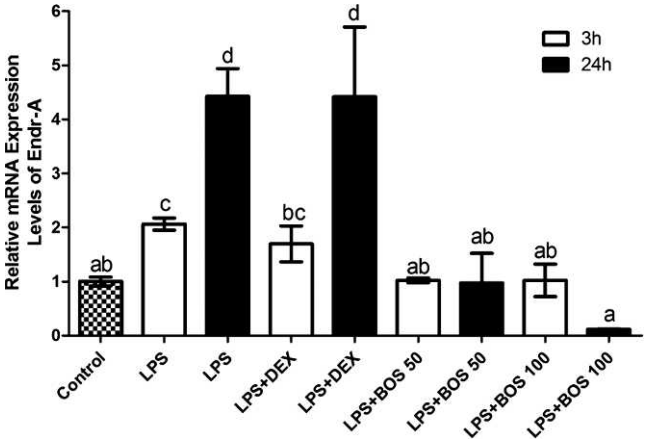

FIGURE 6. Endothelin receptor A (Endr-A) expression in all groups.

Due to its unclear pathogenesis and the lack of definitive treatment options, an increasing number of clinical and experimental studies focus on uveitis.[6,7,35] The current study investigated the possible role of ET-1 in uveitis and the efficacy of bosentan, a nonselective ET-1 receptor antagonist, as a treatment for uveitis. Clinical and experimental studies have shown that ET-1 receptors are present in various ocular tissues, including corneal epithelial and endothelial cells, nonpigmented ciliary epithelial cells, ciliary muscle, TM cells, lens, choroid, RPE, retinal photoreceptor cells, retinal nerve fiber and ganglion cell layer astrocytes, and retinal blood vessels.[11–18] The current study showed elevated ocular ET-1 mRNA expression in the rat model of experimental uveitis. It also was shown that the standard immunosuppressive therapy for uveitis, dexamethasone, decreases ET-1 mRNA expression, which suggests that ET-1 is important in the inflammatory response present in uveitis. In an experimental study, dexamethasone administration was found to significantly suppress ET-1 mRNA expression in sensitized guinea pigs.[36] Furthermore, it was found that administration of ET-1 receptor antagonist lowered ET-1 mRNA expression. The inhibition of these receptors, which likely factor in the development and progression of uveitis, decreases ET-1 mRNA expression. These findings indicate that ET-1 is important in the physiopathology of uveitis and blocking ET-1 receptors ameliorates the immune response, thereby lowering ET-1 mRNA expression. Studies on ET-1 and its receptors indicate their significance in immune diseases. A study by Lehrke et al.[37] showed increased

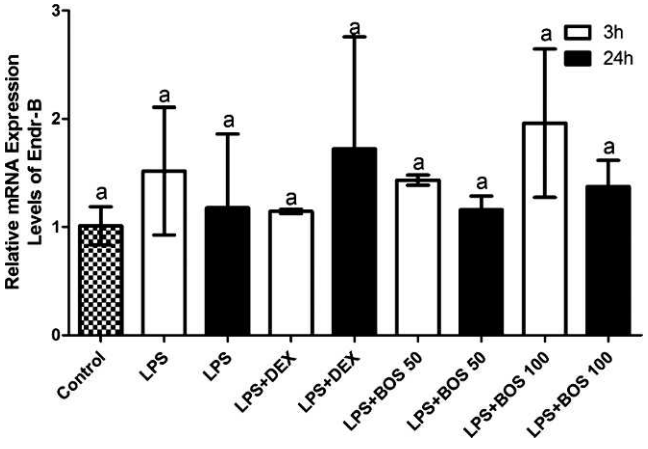

FIGURE 7. Endothelin receptor B (Endr-B) expression in all groups.

expression of ET-1 and its receptors in IgA nephropathy patients. Similar results were found in lupus nephritis.[38] Ergul et al.[39] identified ET-1 as a proinflammatory factor in diabetic conditions, and Saleh et al.[40] demonstrated that endothelin receptor antagonist administration reversed abnormal inflammatory response.

In our study, it was found that anti-inflammatory effects of bosentan are predominantly mediated via the EDNRA. In a recent study it was suggested that daily oral administration of the mixed ET(A) and ET(B) endothelin receptor antagonist bosentan significantly attenuated knee joint swelling and inflammation to an extent that was comparable to dexamethasone, and these investigators concluded that anti-inflammatory effect of bosentan is mediated mainly via EDNRB.[23] Also, another study explained that inhibition of ETB receptors in LPS-stimulated monocytes by bosentan was responded by suppression of PGE2 and increased production of leukotrienes, indicating strong effects in the cyclooxygenase pathway, which is known to control cellular ET transcription. Endothelin-1–mediated induction of PGE2 reflects the importance of ETB-receptor activation in monocytes to induce COX-II metabolites that control ET transcription under physiologic conditions, whereas ETB-receptor antagonism might be necessary for protection of higher concomitant ET levels in different inflammatory diseases.[41] In a manner similar to our study, ET-1 via the ETA receptor was found to induce renal injury, at least in part, by promoting macrophage infiltration, and stimulating production of TGF-β and PGE2.[42] All these findings support the involvement of endothelin in inflammatory conditions.

Tumor necrosis factor-α also was investigated in this study, as elevated levels of TNF-α have been found in the ocular fluid of uveitis patients.[43] Other studies have shown elevated levels of TNF-α in patients with Behçet's disease who developed uveoretinitis.[33,34,44] Consistent with the literature, elevated TNF-α was observed in the LPS-induced uveitis model used in the current study, and dexamethasone and bosentan treatment significantly lowered TNF-α levels. The TNF-α levels were higher at 24 hours compared to 3 hours after LPS administration, though bosentan treatment significantly reduced TNF-α at 24 hours.

In vivo and in vitro studies show that elevated ET-1 leads to increased levels of TNF-α.[45] Other studies conclude that ET-1 receptor antagonists reverse the increase in TNF-α levels caused by elevated levels of ET-1.[46] Another study showed increased cardiac TNF-α levels due to elevated ET-1.[47] Bosentan treatment in the mouse experimental rheumatoid arthritis model lowered TNF-α levels.[48] We have shown previously that bosentan treatment in paracetamol-induced liver toxicity model lowered the elevated TNF-α levels.[49] In our other previous studies, we have shown the beneficial effect of bosentan administration in the treatment of diseases resulting in inflammation.[27,28] Taking these findings into consideration, it can be posited that bosentan treatment lowers the elevated levels of TNF-α in uveitis and ameliorates inflammation.

Histopathologic analysis of ocular tissue at 3 and 24 hours after uveitis induction reveals a time-dependent increase in inflammatory cell infiltration. Bosentan treatment suppressed this infiltration at both time points, likely due to bosentan blocking ET-1 receptors and lowering TNF-α levels.

Nitric oxide (NO) is synthesized by iNOS in phagocytes and TNF-α induces this synthesis.[35] Elevated iNOS activity has been found in several inflammatory diseases.[50–53] Elevated NO levels were found in patients with Behçet's disease who developed uveitis.[54] Treatment with the iNOS inhibitor NG-nitro-L-arginine (L-NAME) inhibited iNOS activity and prevented uveitis development.[55] In the current study, significantly higher iNOS expression levels were found in the ocular tissue of rats at 3 and 24 hours after uveitis induction by LPS, although there was no significant difference in iNOS expression between the time points. Dexamethasone and bosentan treatments reduced iNOS levels.

Increased ET-1 levels during inflammation lead to excessive NO synthesis.[56,57] The NO and ET-1 levels also were shown to be higher in patients with endotoxic shock.[58] Keller et al.[59] demonstrated that bosentan treatment effectively lowers elevated iNOS levels in LPS-induced liver injury.

In conclusion, this study showed that endothelin expression significantly increases in the acute uveitis model and this increase has a significant role in uveitic inflammation. The ET-1 receptor antagonist, bosentan, reverses the increased inflammation and prevents the development of uveitis through two pathways: lowering the levels of proinflammatory cytokine TNF-α and inhibiting the expression of iNOS. These findings indicate that ET-1 should be investigated in more detail in uveitis pathophysiology and suggest that ET-1 receptor antagonists, like bosentan, may be potential targets of therapy development for uveitis.

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