Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

ICAM-1 and E-selectin Levels in the Aqueous Humor and in the Ocular Tissue and Their Correlation With Oxidative Stress Markers in Endotoxin-induced Uveitis

Erdem Doğan
Harran University
Tuğba Göncü Fırat
drtgoncu@hotmail.com

Harran University
Elif Oğuz
Harran University
Sezen Koçarslan
Harran University
Hatice Sezen
Harran University
Fatih Mehmet Adıbelli
Harran University

Research Article

Keywords: E-selectin, ICAM-1, Inflammation, Oxidative stress, Uveitis

Posted Date: July 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10055323/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Purpose

To determine the levels of intercellular adhesion molecule-1 (ICAM-1) and E-selectin in aqueous humor and ocular tissue in a rat model of endotoxin-induced uveitis (EIU) and to investigate their relationship with oxidative stress parameters.

Methods

Endotoxin-induced uveitis was induced in Sprague–Dawley rats by subcutaneous injection of lipopolysaccharide (LPS). Twenty-four hours after LPS administration, aqueous humor and ocular tissue samples were obtained. Inflammatory cell count, protein concentration, and the levels of tumor necrosis factor-a (TNF-a), interleukin (IL)-6, nitric oxide (NO), ICAM-1, and E-selectin were measured in aqueous humor samples. Levels of ICAM-1, and E-selectin and oxidative stress parameters, including total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI), were evaluated in both aqueous humor and ocular tissue. Correlations between oxidative stress parameters and adhesion molecule levels were analyzed.

Results

In the uveitis group, inflammatory cell count, aqueous humor protein concentration, TNF-a, IL-6, NO, and E-selectin levels were significantly increased compared with controls ($p = 0.01$ for all). ICAM-1 concentrations were significantly elevated in ocular tissue, while aqueous humor ICAM-1 levels showed a non-significant increase ($p = 0.004$ and $p = 0.078$ respectively). Oxidative stress markers, including TOS and OSI, were significantly increased in both aqueous humor and ocular tissue samples in the uveitis group ($p = 0.004$ for both). Significant positive correlations were observed between oxidative stress parameters and ICAM-1 and E-selectin levels, particularly in ocular tissue samples ($p = 0.004$ for all).

Conclusions

ICAM-1 and E-selectin expression were increased during endotoxin-induced intraocular inflammation, especially at the tissue level. In addition, oxidative stress parameters were positively associated with adhesion molecule expression. These findings suggest that endothelial adhesion molecules, in conjunction with oxidative stress, may play important roles in inflammatory cell recruitment.

INTRODUCTION

Among all ocular inflammatory diseases, uveitis is a serious vision-threatening disease and one of the leading causes of visual impairment worldwide, accounting for approximately 10–25% of legal

blindness[1, 2]. Although uveitis primarly affects the uveal tissue, adjacent ocular structures such as retina and vitreous may also be involved. Autoimmune diseases, infections and toxins are considered main etiological causes; however, the exact pathogenesis of uveitis remains incompletely understood[2, 3]. Endotoxin-induced uveitis (EIU) in rats is a well-established experimental model that closely resembles human acute anterior uveitis and widely used to investigate the mechanisms underlying intraocular inflammation [4, 5]. EIU is induced by lipopolysaccharide (LPS), a major glycolipid component of the outer membrane of gram-negative bacteria. Following recognition by membrane-bound reveptors, LPS stimulates the production of several proinflammatory mediators including nitric oxide (NO), prostaglandin-E2 (PGE2), tumor necrosis factor-a (TNF-a), interleukin (IL)-1 and IL-6[6–8]. The resulting inflammatory cascade leads to disruption of the blood-ocular barrier and infiltration of inflammatory cells into ocular tissues, contributing to the progression of EIU [6–9]. The leukocyte infiltration and protein extravasation typically reach maximal levels approximately 24 hours after LPS administration[4].

Oxidative stress also plays a significant role in LPS-induced inflammation and contributes to the production of inflammatory mediators as well as tissue injury[10, 11]. Excessive reactive oxygen species production may disturb the balance between the oxidation and antioxidant systems, resulting in further ocular damage[12]. Despite increasing evidence supporting the involvement of oxidative stress in uveitis, its relationship with inflammatory mediators, particularly cellular adhesion molecules, has not been fully elucidated.

Leukocyte migration and accumulation in the anterior segment represents critical events in the inflammatory response during uveitis. Cell-adhesion molecules which include integrins, selectins, cadherins, and members of the immunoglobulin superfamily, play essential roles in leukocyte recruitment and endothelial involvement during inflammation[13–15]. Intercellular adhesion molecule- 1 (ICAM-1), a member of the immunoglobulin superfamily and E-selectin, a member of the selectin family, are expressed on vascular endothelial cell under inflammatory conditions[13, 14]. E-selectin primarily mediates the initial tethering and rolling phases of leukocyte recruitment and is induced by inflammatory cytokines such as TNF-a and IL-6[16, 17]. ICAM-1 is involved in firm leukocyte adhesion and transendothelial migration and is upregulated by cytokines including TNF-a and IL-1β [18]. Increased expression of these adhesion molecules reflects endothelial activation and blood–ocular barrier dysfunction and may be enhanced by oxidative stress. Although inflammatory cytokines and oxidative stress have been extensively investigated in EIU, the relationship between oxidative stress parameters and endothelial adhesion molecules, particularly ICAM-1 and E-selectin, remains insufficiently characterized.

Therefore, in the present study, we aimed to evaluate the level of proinflamatory cytokines, ICAM-1, and E-selectin in both the aqueous humor and ocular tissue tissue in an experimental EIU model. In addition, we investigated the oxidative stress parameters and their correlations with ICAM-1 and E-selectin levels during EIU-associated inflammation.

MATERIALS AND METHODS

Chemicals:

LPS derived from Salmonella typhimurium was purchased from Sigma-Aldrich (St. Louis, MO, US). Rat TNF-a, IL-6, NO, E-selectin and ICAM-1 enzyme-linked immunosorbent assay (ELISA) kits were obtained from Ray Biotech Inc. (Norcross, GA, USA), Diaclone (Besançon cedex, France), Cayman Chemical Inc. (Ann Arbor, MI, USA) and Shanghai YEHUA Biological Technology (Shanghai, China) respectively.

Animals and Experimental protocol:

This experimental study was conducted using 12 healthy adult Sprague-Dawley rats weighing 240–275 g. The animals were obtained from Dollvet Animal Laboratory (Sanliurfa, Turkey), where all experimental procedure were performed. The study protocol adhered to the National Institutes of Health guidelines and the ARVO (Association for Research in Vision and Ophthalmology) Statement for the Use of Animals in Ophthalmic and Vision Research. The ethical approval was obtained from the Dollvet Animal Care and Use Committee (Approval number: 2014/52). The animals were housed under standard laboratory conditions with a 12-hour light/12-hour dark cycle at a controlled room temperature of 24 ± 3 ^{o}C for one week before the experiments. Food and water were provided ad libitum throughout the study period.

EIU was induced by a single subcutaneous injection of LPS (200 µg) dissolved in 0,1 ml sterile saline as previously described[4].

After the acclimatization period, the rats were randomly divided into two groups.

Group 1 (Control group, n = 6): received subcutaneous injection of vehicle (0.9% NaCl, 0.1ml).

Group 2 (Uveitis group, n = 6): received subcutaneous LPS injection (200 µg/0.1ml).

Twenty-four hours after LPS administration, all animals were anaesthetised with intramuscular injection of ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/kg), and aqueous humour sampling and enucleation were performed immediately. The absence of corneal and pedal withdrawal reflexes before any procedure confirmed adequate depth of anaesthesia. After the sampling procedures animals were euthanised by an intraperitoneal overdose of anaesthetic (sodium pentobarbital, 150 mg/kg). Animals were maintained on deep general anaesthesia during the entire euthanasia procedure and did not regain consciousness at any point. The aqueous humor samples were collected from both eyes by anterior chamber puncture using a 30-gauge needle under a surgical microscope. One eye from each animal was fixed with 10% buffered formalin for histopathological evaluation, while the fellow eye was stored at −80°C for biochemical analysis.

Biochemical measurements:

For inflammatory cell counting, the aqueous humor samples were mixed with an equal volume of Türk staining solution (Merck, Darmstadt, Germany). Cell counts were determined using a hemocytometer under light microscopy, and the mean value of four randomly selected fields was recorded for each sample. Total protein concentration was measured using the Lowry method[19]. The remaining aqueous humor samples were centrifuged at 3000 x g for 10 minutes at 10–18°C and stored at -80°C until analysis. The concentration of TNF-a, NO, IL-6, ICAM-1, and E-selectin in aqueous humor samples were determined using a commercially available rat ELISA kits according to the manufacturers' instructions.

Analysis of oxidative stress markers in aqueous humor and ocular tissue

The crystalline lenses were removed from enucleated eyes because of their high glutathione content[20]. Ocular tissues were weighed, mechanically fragmented into small pieces, and transferred into glass tubes. Subsequently, 1 ml of 140 mM KCl solution per gram of tissue was added, and tissues were homogenized using a motor-driven homogenizer. The homogenates were centrifuged at 2800 × g for 10 min at 4°C [21]. The obtained supernatants were used for oxidative stress analyses.

Oxidative stress parameters in aqueous humor and ocular tissue, including total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI), were evaluated as previously described [22].

Total antioxidant status:

TAS levels were measured spectrophotometrically using commercially available essay kits (Rel® Assay Diagnostics, Gaziantep, Türkiye). Results for aqueous humor were expressed as mmol Trolox equivalent/L whereas.ocular tissue results were expressed as mmol Trolox equivalent/g protein.

Total oxidant status:

TOS levels were measured spectrophotometrically using commercial essay kits (Rel® Assay Diagnostics, Gaziantep, Türkiye). Results were expressed as micromolar hydrogen peroxide equivalent per liter (μmol H2O2 equivalent/L) for aqueous humor samples and as μmol H2O2 equivalent/g protein for ocular tissue samples.

Oxidative stress index:

OSI was calculated as the percentage ratio of TOS to TAS levels and expressed as arbitrary Units (AU).

Statistical analyses

Data were expressed as means±standard deviation (SD). Differences between groups were analyzed using the Mann-Whitney U test. Correlations between parameters were evaluated using Pearson's correlation analysis. Statistical analyses were performed using SPSS software for Windows (Version 18.0; SPSS Inc., Chicago, IL, USA).A p-value < 0.05 was considered statistically significant.

RESULTS

Cellular infiltration in the aqueous humor:

The number of infiltrating cells in the aqueous humor was markedly increased in the uveitis group 24 hours after LPS administration compared with the control group (946 ± 1125 cells/μL vs. 6 ± 16 cells/μL p = 0.003) (Fig. 1A).

Protein concentration in the aqueous humor:

Aqueous humor protein concentration was significantly elevated higher in the uveitis group compared with controls (11.0 ± 3.1 mg/dL vs. 1.5 ± 1.5 mg/dL, p = 0.002) (Fig. 1B).

The TNF-a, NO, and IL-6 levels in the aqueous humor:

The concentrations of TNF-a, NO, and IL-6 in the aqueous humor were significantly higher in the uveitis group than in the control group

TNF-a levels increased from 48.4 ± 0.8 ng/mL in controls to 71.0 ± 22.3 ng/Ml in the uveitis group (*p* = 0.004) (Fig. 1C). NO concentrations were elevated from 0.14 ± 0.0 Mm to 0.29 ± 0.1 μM following LPS administration (*p* = 0.004) (Fig. 1D). Similarly, IL-6 concentrations increased significantly in the uveitis group compared with controls (121.6 ± 3.0 pg/mL vs. 108.9 ± 7.9 pg/mL, p = 0.009) (Fig. 1E).

ICAM-1 and E-selectin levels in the aqueous humor:

Aqueous humor ICAM-1 concentration was higher in the uveitis group than in controls (8.4 ± 1.2 ng/mL vs. 5.9 ± 1.7 ng/Ml); however. this difference did not reach statistical significance (*p* = 0.078) (Fig. 2A).

In contrast, aqueous humor E-selectin concentrations were significantly increased following LPS administration (122.9 ± 3.6 ng/L vs. 111.6 ± 5.2 ng/ml pg/mL, *p* = 0.004) (Fig. 2B).

ICAM-1 and E-selectin levels in ocular tissue:

Ocular tissue ICAM-1 levels were significantly elevated in the uveitis group compared with controls (147.2 ± 28.6 ng/mL vs. 89.7 ± 6.8 ng/mL, p = 0.004) (Fig. 3A). Similarly, ocular tissue E-selectin concentrations were significantly higher in the uveitis group than in the control group (264.7 ± 22.2 ng/mL vs. 156.5 ± 25.0 ng/mL, p = 0.004) (Fig. 3B).

Oxidative stress markers in the aqueous humor:

The mean TAS, TOS, and OSI values in aqueous humor were significantly increased in the uveitis group compared with controls. In the control group, TAS, TOS, and OSI values were 0.02 ± 0.01 mmol Trolox equivalent/L, 1.9 ± 0.1 μmol H_2O_2 equivalent/L, and 8.9 ± 2.1 AU, respectively. In the uveitis group, these values increased to 0.03 ± 0.01 mmol Trolox equivalent/L, 2.2 ± 0.2 μmol H_2O_2 equivalent/L, and 14.5 ± 0.3 AU, respectively ($p = 0.008$, $p = 0.016$, and $p = 0.003$, respectively) (Fig. 4A).

Oxidative stress markers in ocular tissue:

In ocular tissue samples, mean TOS and OSI values were significantly higher in the uveitis group than in controls ($p = 0.004$ for both). TAS levels were higher in the uveitis group; however, the difference was not statistically significant ($p = 0.137$).

Control group TAS, TOS, and OSI values were 0.5 ± 0.1 mmol Trolox equivalent/g protein, 5.7 ± 1.1 μmol H_2O_2 equivalent/g protein, and 0.9 ± 0.2 AU, respectively, whereas corresponding values in the uveitis group were 0.8 ± 0.2 mmol Trolox equivalent/g protein, 10.8 ± 2.1 μmol H_2O_2 equivalent/g protein, and 1.5 ± 0.2 AU, respectively (Fig. 4B).

Correlation analysis:

Significant positive correlations were observed between aqueous humor ICAM-1 and E-selectin concentrations and their corresponding ocular tissue levels ($p < 0.05$). Aqueous humor TAS levels demonstrated positive correlations with ocular tissue ICAM-1 and E-selectin concentrations, whereas no significant correlations were detected with aqueous humor ICAM-1 or E-selectin levels. Aqueous humor TOS levels were positively correlated with ocular tissue ICAM-1 concentrations ($p < 0.01$). In addition, aqueous humor OSI values showed significant positive correlations with aqueous humor E-selectin as well as ocular tissue ICAM-1 and E-selectin levels ($p < 0.01$). Ocular tissue TAS levels were positively correlated with aqueous humor ICAM-1, aqueous humor E-selectin, and ocular tissue E-selectin concentrations; however, no significant correlation was identified with ocular tissue ICAM-1 levels. Furthermore, ocular tissue TOS and OSI values demonstrated positive correlations with all aqueous humor and ocular tissue ICAM-1 and E-selectin concentrations ($p < 0.05$).

DISCUSSION

Uveitis is an important cause of visual impairment and blindness due to its complications such as cystoid macular edema, glaucoma, and proliferative vitreoretinopathy[23]. Considerable research has focused on understanding the inflammatory mechanisms involved in uveitis pathogenesis and identifying more targeted therapeutic approaches. EIU is a well established experimental model of acute uveal inflamation and is widely used to investigate inflammatory pathways in uveitis[4, 5]. In the present study, we evaluated the expression of ICAM-1 and E-selectin in both aqueous humor and ocular tissueina rat model of EIU. Consistent with previous studies, the inflammatory cell count, aqueous protein concentration, and proinflamatory cytokine levels, including TNF-a, NO and IL-6, were significantly increased following LPS administration[6–8, 24]. The cell count and protein concentration in the aqueous

humor are useful indicators for assessing the integrity of the blood-ocular barrier 24 hours after the LPS injection[5].

Leukocyte recruitment represents a critical step in the inflammatory response during EIU. In this process, circulating neutrophils and monocytes initially interact with vascular endothelial cells through Rolling, adhesion, and transmigration mechanisms regulated by cellular adhesion molecules. Selectins are primarily involved in leokocyte rolling, whereas ICAM-1 plays a major role in firm leokocyte adhesion and transendothelial migration[25, 26]. Previous studies have demonstrated that ICAM-1 expression is constitutively present at low levels in retinal pigment epithelium and endothelial cells but is markedly upregulated under inflammatory conditions[27]. Experimental studies have shown that ICAM-1 expression on endothelial cells increases significantly between 16 and 24 hours after inflammatory stimulation and remains elevated in the presence of proinflammatory cytokines[28]. In the present study, ICAM-1 concentrations in ocular tissue were significantly higher in the uveitis group 24 hours after LPS administration compared with the control group. Although aqueous humor ICAM-1 concentrations were also elevated in the uveitis group, the difference did not reach statistical significance. Consistent with previous studies, our findings demonstrated increased ICAM-1 expression in EIU; however, the increase was more pronounced in ocular tissue than in aqueous humor in the current study[25, 29–32]. Similar experimental studies have shown that ICAM-1 expression is markedly upregulated in the ciliary in EIU[25, 30]. ICAM-1 has been recognized as a key mediator of leukocyte infiltration in EIU, particularly in neutrophil adhesion to vascular endothelium. Previous studies reported that neutralizing antibodies against ICAM-1 significantly reduced leukocyte adhesion within iris vessels during intraocular inflammation[30].

E-selectin is another important endothelial adhesion molecule involved in the early stages of leukocyte recruitment. In vitro studies demonstrated E-selectin expression peaks approximately 6–8 hours after TNF-a stimulation and remains elevated for at least 24 hours[33]. Similarly, a previous EIU study demonstrated increased E-selectin expression in iris vessels several hours after LPS administration, with sustained expression persisting at 24 hours[34]. In the present study, both aqueous humor and ocular tissue E-selectin levels were significantly elevated in the uveitis group compared with controls. Although E-selectin has been implicated in the inflammatory process of uveitis, its precise role remains unclear, and the available literature contains conflicting findings. In vivo studies have demonstrated progressive upregulation of P-selectin and E-selectin on the retinal vascular endothelium during the development of experimental autoimmune uveitis[35]. In an experimental EIU model, Suzuma et al. demonstrated that blockade of P-selectin and E-selectin reduced leukocyte infiltration into ocular tissues. Furthermore, delayed administration of anti-E-selectin antibodies 6 or 12 hours after LPS injection significantly suppressed cellular infiltration in EIU. The authors proposed that E-selectin becomes fully active several hours after LPS administration and that inhibition of E-selectin after the onset of inflammation may reduce leukocyte recruitment[34]. In contrast, Whitcup et al. reported that monoclonal antibodies against E-selectin alone did not significantly suppress ocular inflammation[36]. It has been proposed that

selectins predominantly mediate the early phase of leukocyte recruitment, whereas adhesion molecules such as ICAM-1 and VCAM-1 become increasingly important during sustained inflammation and continuous leukocyte transmigration[13].

An important finding of the present study was that ICAM-1 and E-selectin concentrations were substantially higher in ocular tissue than in aqueous humor samples. This finding is biologically plausible because these molecules are primarily expressed on vascular endothelial cells within the iris, ciliary body, and retinal vasculature. Nevertheless, considerable levels were also identified in aqueous humor, particularly for E-selectin.

Previous investigations of adhesion molecules in uveitis have mainly focused on experimental autoimmune uveitis models characterized predominantly by retinal vascular inflammation[31, 32, 35, 37]. Similar to these studies, increased ICAM-1 and E-selectin expression has also been demonstrated in iris and ciliary body vessels during EIU [25, 34, 38, 39]. In EIU, acute anterior segment inflammation typically peaks approximately 24 hours after LPS injection, corresponding to maximal neutrophil infiltration into the iris and ciliary body[24, 38, 39]. Previous studies reported that inflammatory cell infiltration correlates closely with E-selectin mRNA expression in ocular tissues[34].

Oxidative stress is considered an important contributor to inflammatory signaling and tissue injury during uveitis. Increased reactive oxygen species production may induce endothelial dysfunction, disrupt the blood–ocular barrier, and promote leukocyte migration into ocular tissues[40]. Previous studies demonstrated elevated oxidative stress biomarkers in EIU, supporting the close interaction between oxidative stress and inflammation[5]. In the present study, we investigated the relationship between oxidative stress parameters and adhesion molecule expression. Significant positive correlations were observed between ocular tissue oxidative stress markers and ICAM-1 and E-selectin levels. In contrast, correlations involving aqueous humor oxidative stress parameters were less consistent. These findings may suggest that oxidative stress-related endothelial activation is more pronounced at the tissue level than in aqueous humor during acute EIU.

Increasing evidence has suggested Oxidative stress biomarkers are shown to be elevated in EIU, suggesting that inflammation and oxidative stress contribute to its pathogenesis which is shows that EIU is a good model to study inflammatory and oxidative damage mechanisms of uveitis[5]. Oxidative stress is a critical signaling to the progression of inflammatory response and the increased reactive oxygen species causes endothelial dysfunction and tissue injury[40]. The disturbed endothelial cells are lead to promotion of passage of inflammatory cells and inflammatory molecules[40]. In our study, we have evaluated correlation of the concentration cellular adhesion molecules and oxidative stress parameters. Significant positive correlations were observed between oxidative stress parameters and adhesion molecule levels, particularly in ocular tissue samples. Ocular tissue TOS and OSI values demonstrated positive correlations with both ICAM-1 and E-selectin concentrations in aqueous humor and ocular tissue. These findings suggest that oxidative stress may contribute to endothelial activation

and adhesion molecule expression during intraocular inflammation. The stronger correlations observed at the tissue level compared with aqueous humor may indicate that oxidative stress-related inflammatory changes are more pronounced within ocular tissues during EIU.

The present study has several limitations. First, the sample size was relatively small. Second, we evaluated the inflammatory response at only a single time point, 24 hours after LPS administration, which may not fully reflect the dynamic changes in adhesion molecule expression during different stages of inflammation. Third, the molecular signaling pathways involved in ICAM-1 and E-selectin upregulation were not investigated. In addition, immunohistochemical analyses were not performed to determine the localization of adhesion molecule expression within specific ocular tissues. Despite these limitations, our study quantitatively evaluated ICAM-1 and E-selectin levels separately in aqueous humor and ocular tissue and investigated their relationship with oxidative stress parameters. Further experimental studies with larger sample sizes and more detailed molecular analyses are needed to better clarify the roles of adhesion molecules in intraocular inflammation.

In conclusion, the present study demonstrated increased E-selectin expression in both ocular tissue and aqueous humor, as well as increased ICAM-1 expression in ocular tissue, during endotoxin-induced intraocular inflammation in rats. In addition, oxidative stress parameters were positively associated with adhesion molecule expression, particularly at the tissue level. These findings suggest that endothelial adhesion molecules, together with oxidative stress, may play important roles in inflammatory cell recruitment and tissue injury during uveitis and may represent potential therapeutic targets in ocular inflammatory diseases.

Abbreviations

EIU; endotoxin-induced uveitis

LPS; lipopolysaccharide

TNF; tumor necrosis factor-a

IL; interleukin

NO; nitric oxide

ICAM; intercellular adhesion molecule

TAS; total antioxidant status

TOS; total oxidant status

OSI; oxidative stress index

PGE-2; prostaglandin-E2

ELISA; enzyme-linked immunosorbent assay

SD; standard deviation

Declarations

Authors' Contributions:

Doğan E, Funding, Data curation; Formal analysis, Investigation, Writing original draft; **Göncü Fırat T**, Funding, Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Writing original draft, review & editing; **Oğuz E**, Conceptualization, Methodology, Investigation, review & editing; **Koçarslan S**, Methodology, Formal analysis, review & editing; **Sezen H**, Methodology, Formal analysis, review & editing; **Adıbelli FM**, Formal analysis, Data curation, review & editing.

Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the first author (Doğan E) and the corresponding author (Göncü Fırat T) upon request. The data are not publicly available because the study was conducted as part of a residency thesis in the Department of Ophthalmology.

Consent for publication: Not Applicable

Foundation: This work was supported by grants from the Harran University Scientific Research Council (HUBAK-14119).

Conflicts of interest: The authors report no conflicts of interest. **Doğan E**, None; **Göncü Fırat T**, None; **Oğuz E**, None; **Koçarslan S**, None; **Sezen H**, None; **Adıbelli FM**, None. The authors alone are responsible for the content and writing of the paper.

Research involving Human Participants and/or Animals: This study was carried out using 12 healthy adult Sprague-Dawley rats. The study was performed in accordance with National Institutes of Health guidelines and the ARVO (Association for Research in Vision and Ophthalmology) Statement for the Use of Animals in Ophthalmic and Vision Research. The study was also approved by Dollvet Animal Care and Use Committee (Approval number: 2014/52). The animals were allowed to acclimate under standard laboratory conditions.

Acknowledgements: Not applicable

References

1. Dandona L, Dandona R, John RK, McCarty CA, Rao GN. Population based assessment of uveitis in an urban population in southern India. Br J Ophthalmol. 2000;84(7):706–9.
2. Nussenblatt RB. The natural history of uveitis. Int Ophthalmol. 1990;14(5–6):303–8.

3. Gupta R, Murray PI. Chronic non-infectious uveitis in the elderly: epidemiology, pathophysiology and management. Drugs Aging. 2006;23(7):535–58.
4. Rosenbaum JT, McDevitt HO, Guss RB, Egbert PR. Endotoxin-induced uveitis in rats as a model for human disease. Nature. 1980;286(5773):611–3.
5. Kubota S, Kurihara T, Mochimaru H, Satofuka S, Noda K, Ozawa Y, Oike Y, Ishida S, Tsubota K. Prevention of ocular inflammation in endotoxin-induced uveitis with resveratrol by inhibiting oxidative damage and nuclear factor–B activation. Invest Ophthalmol Vis Sci. 2009;50(7):3512–9.
6. De Vos AF, van Haren MA, Verhagen C, Hoekzema R, Kijlstra A. Kinetics of intraocular tumor necrosis factor and interleukin-6 in endotoxin-induced uveitis in the rat. Invest Ophthalmol Vis Sci. 1994;35(3):1100–6.
7. De Vos AF, Hoekzema R, Kijlstra A. Cytokines and uveitis, a review. Curr Eye Res. 1992;11(6):581–97.
8. Jacquemin E, de Kozak Y, Thillaye B, Courtois Y, Goureau O. Expression of inducible nitric oxide synthase in the eye from endotoxin-induced uveitis rats. Invest Ophthalmol Vis Sci. 1996;37(6):1187–96.
9. Park J, Kim JT, Lee SJ, Kim JC. The anti-inflammatory effects of angiogenin in an endotoxin induced uveitis in rats. Int J Mol Sci. 2020;21(2):413.
10. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408(6809):239–47.
11. Satici A, Guzey M, Gurler B, Vural H, Gurkan T. Malondialdehyde and antioxidant enzyme levels in the aqueous humor of rabbits in endotoxin-induced uveitis. Eur J Ophthalmol. 2003;13(9–10):779–83.
12. Hsu SM, Yang CH, Teng YT, Tsai HY, Lin CY, Lin CJ, Shieh CC, Chen SH. Suppression of the Reactive Oxygen Response Alleviates Experimental Autoimmune Uveitis in Mice. Int J Mol Sci. 2020;21(9):3261.
13. Chen YH, Lightman S, Eskandarpour M, Calder VL. Adhesion Molecule Targeted Therapy for Non-Infectious Uveitis. Int J Mol Sci. 2022;23(1):503.
14. Osborn L. Leukocyte adhesion to endothelium in inflammation. Cell. 1990;62(1):3–6.
15. Harjunpää H, Llort Asens M, Guenther C, Fagerholm SC. Cell Adhesion Molecules and Their Roles and Regulation in the Immune and Tumor Microenvironment. Front Immunol. 2019;10:1078.
16. Walz G, Aruffo A, Kolanus W, Bevilacqua M, Seed B. Recognition by ELAM-1 of the sialyl-Lex determinant on myeloid and tumor cells. Science. 1990;250(4984):1132–5.
17. Phillips ML, Nudelman E, Gaeta FC, Perez M, Singhal AK, Hakomori S, Paulson JC. ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, siayl-LeX. Science. 1990;250(4984):1130–2.
18. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. Blood. 1994;84(7):2068–101.
19. Lowry R, Rosebrough OH, Farr NL, Randall AL. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265–75.
20. Mérida S, Sancho-Tello M, Navea A, Almansa I, Muriach M, Bosch-Morell F. An anti-interleukin-2 receptor drug attenuates T- helper 1 lymphocytes-mediated inflammation in an acute model of

endotoxin-induced uveitis. PLoS ONE. 2014;9(3):e90216.

21. Rabus M, Demirbağ R, Sezen Y, Konukoğlu O, Yildiz A, Erel Ö, Zeybek R, Yakut C. Plasma and tissue oxidative stress index in patients with rheumatic and degenerative heart valve disease. Turk Kardiyol Dern Ars. 2008;36(8):536–40.
22. Cakmak A, Soker M, Koc A, Aksoy N. Prolidase activity and oxidative status in patients with thalassemia major. J Clin Lab Anal. 2010;24(1):6–11.
23. Durrani OM, Tehrani NN, Marr JE, Moradi P, Stavrou P, Murray PI. Degree, duration, and causes of visual loss in uveitis. Br J Ophthalmol. 2004;88(9):1159–62.
24. de Vos AF, Klaren VN, Kijlstra A. Expression of multiple cytokines and IL-1RA in the uvea and retina during endotoxin-induced uveitis in the rat. Invest Ophthalmol Vis Sci. 1994;35(11):3873–83.
25. Becker MD, Garman K, Whitcup SM, Planck SR, Rosenbaum JT. Inhibition of leukocyte sticking and infiltration, but not rolling, by antibodies to ICAM-1 and LFA-1 in murine endotoxin-induced uveitis. Invest Ophthalmol Vis Sci. 2001;42(11):2563–6.
26. Sigal A, Bleijs DA, Grabovsky V, van Vliet SJ, Dwir O, Figdor CG, van Kooyk Y, Alon R. The LFA-1 integrin supports rolling adhesions on ICAM-1 under physiological shear flow in a permissive cellular environment. J Immunol. 2000;165(1):442–52.
27. Devine L, Lightman SL, Greenwood J. Role of LFA-1, ICAM-1, VLA-4 and VCAM-1 in lymphocyte migration across retinal pigment epithelial monolayers in vitro. Immunology. 1996;88(3):456–62.
28. Pober JS, Gimbrone MA Jr, Lapierre LA, Mendrick DL, Fiers W, Rothlein R, Springer TA. Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. J Immunol. 1986;137(6):1893–6.
29. Du Y, Zhou L, Wen Z, Feng L, Zhang S, Zhang T. Slit2 suppresses endotoxin-induced uveitis by inhibiting the PI3K/Akt/IKK/NF-κB pathway. Scand J Immunol. 2023;98(6):e13319.
30. Kanagawa T, Matsuda S, Mikawa Y, Kogiso M, Nagasawa H, Himeno K, Hashimoto Y, Mimura Y. Role of ICAM-1 and LFA-1 in endotoxin-induced uveitis in mice. Jpn J Ophthalmol. 1996;40(2):174–80.
31. Whitcup SM, DeBarge LR, Caspi RR, Harning R, Nussenblatt RB, Chan CC. Monoclonal antibodies against ICAM-1 (CD54) and LFA-1 (CD11a/CD18) inhibit experimental autoimmune uveitis. Clin Immunol Immunopathol. 1993;67(2):143–50.
32. Dewispelaere R, Lipski D, Foucart V, Bruyns C, Frère A, Caspers L, Willermain F. ICAM-1 and VCAM-1 are differentially expressed on blood-retinal barrier cells during experimental autoimmune uveitis. Exp Eye Res. 2015;137:94–102.
33. Kluger MS, Johnson DR, Pober JS. Mechanism of sustained E-selectin expression in cultured human dermal microvascular endothelial cells. J Immunol. 1997;158(2):887–96.
34. Suzuma I, Mandai M, Suzuma K, Ishida K, Tojo SJ, Honda Y. Contribution of E-selectin to cellular infiltration during endotoxin-induced uveitis. Invest Ophthalmol Vis Sci. 1998;39(9):1620–30.
35. Xu H, Forrester JV, Liversidge J, Crane IJ. Leukocyte trafficking in experimental autoimmune uveitis: breakdown of blood-retinal barrier and upregulation of cellular adhesion molecules. Invest

Ophthalmol Vis Sci. 2003;44(1):226–34.

36. Whitcup SM, Kozhich AT, Lobanoff M, Wolitzky BA, Chan CC. Blocking both E-selectin and P-selectin inhibits endotoxin-induced leukocyte infiltration into the eye. Clin Immunol Immunopathol. 1997;83(1):45–52.
37. Kerr EC, Copland DA, Dick AD, Nicholson LB. The dynamics of leukocyte infiltration in experimental autoimmune uveoretinitis. Prog Retin Eye Res. 2008;27(5):527–35.
38. Tuaillon N, Shen DF, Berger RB, Lu B, Rollins BJ, Chan CC. MCP-1 expression in endotoxin-induced uveitis. Invest Ophthalmol Vis Sci. 2002;43(5):1493–8.
39. Whitcup SM, DeBarge LR, Rosen H, Nussenblatt RB, Chan CC. Monoclonal antibody against CD11b/CD18 inhibits endotoxin-induced uveitis. Invest Ophthalmol Vis Sci. 1993;34(3):673–81.
40. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal. 2014;20(7):1126–67.

Figures

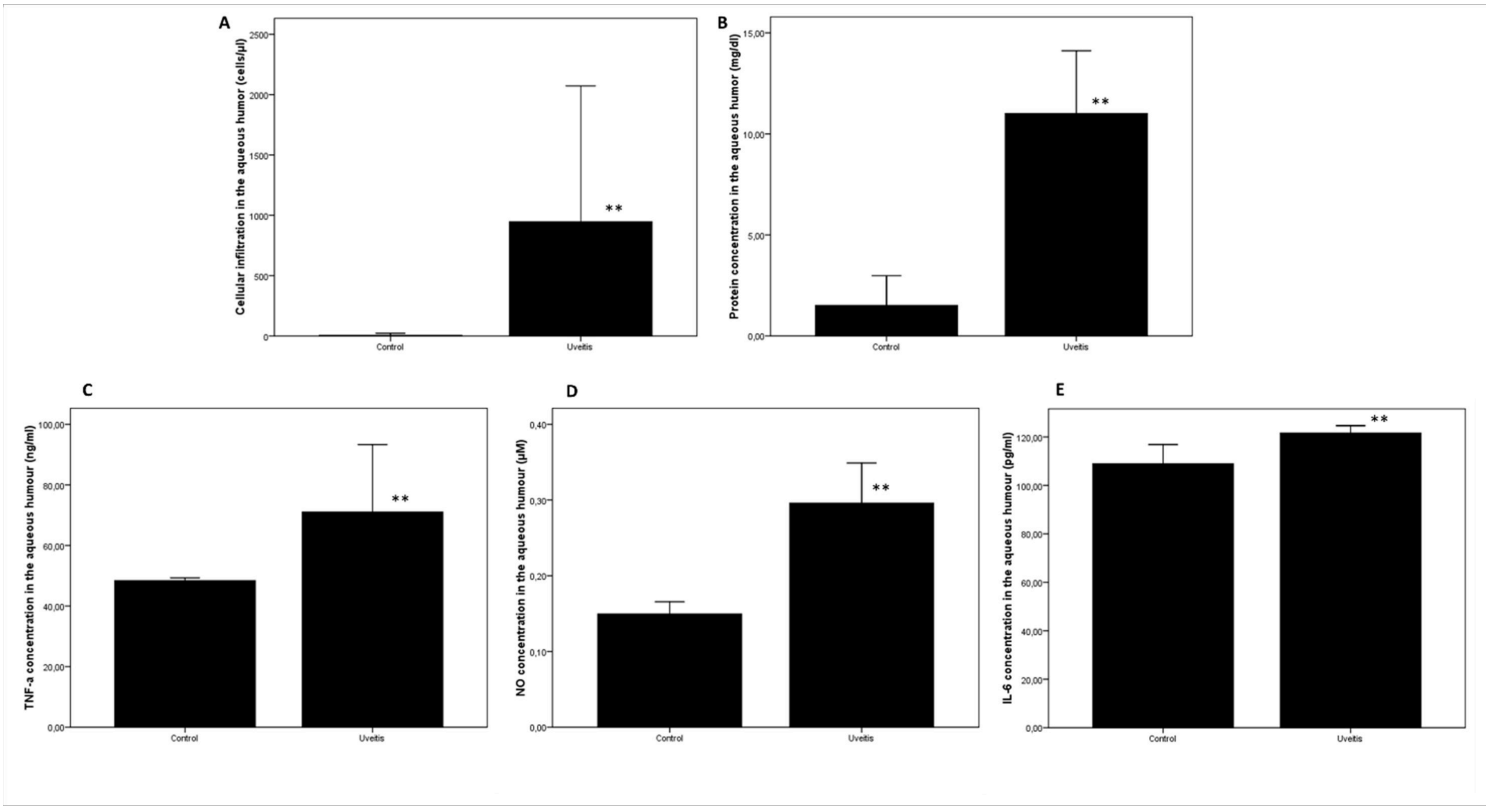


Figure 1

The cellular infiltration, protein concentration and proinflamatory cytokine concentrations in the aqueous humor. In uveitis group, the infiltration of cells **(A)** protein exudation **(B)** TNF-a **(C),** NO **(D)** and IL-6 **(E)** concentrations in the aqueous humor were elevated significantly when compared with control group. * $p<0.05$ and ** $p<0.01$ significantly different from the control group. All data were shown as means±SD

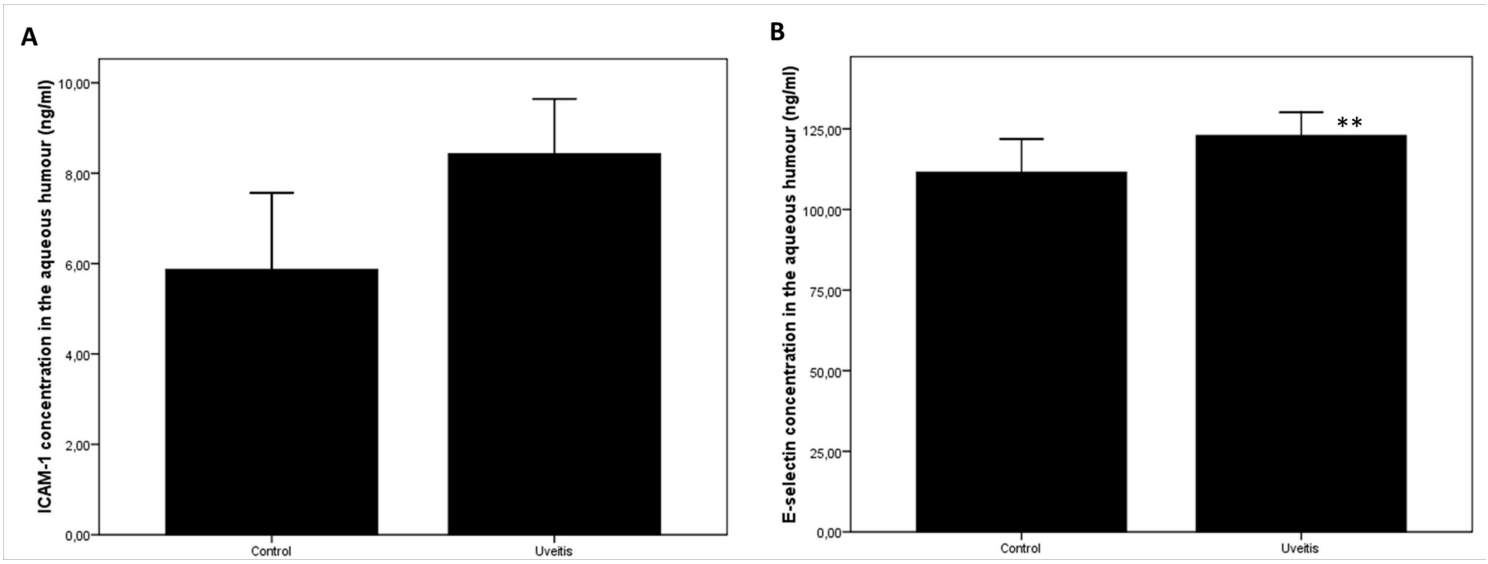


Figure 2

The ICAM-1 and E-selectin concentrations in the aqueous humour. In the uveitis group, the ICAM-1 **(A)** concentration increased however, it was not statistically significant and E-selectin **(B)** concentration increased significantly when compared with control group. * $p<0.05$ and ** $p<0.01$ significantly different from the control group. All data were shown as means±SD

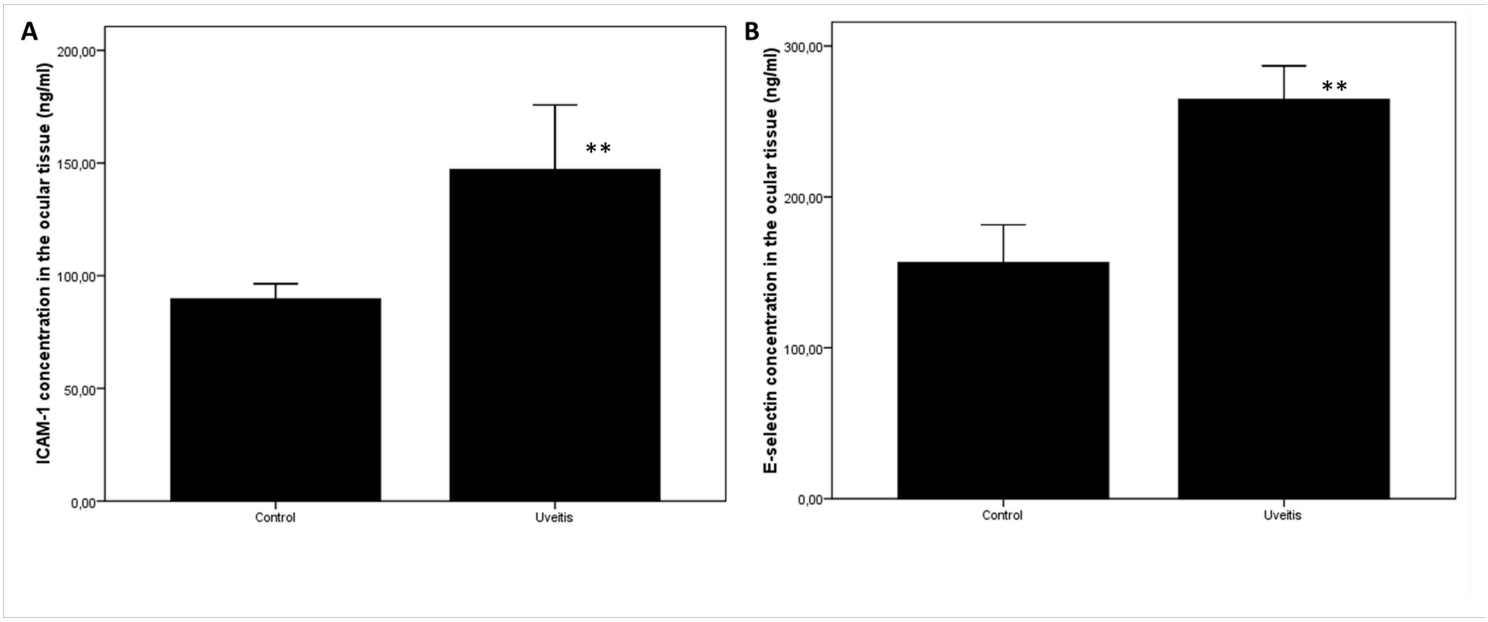


Figure 3

The ICAM-1 and E-selectin concentrations in the ocular tissue. In the uveitis group, both of the ICAM-1 **(A)** and E-selectin **(B)** concentrations increased significantly when compared with control group. * $p<0.05$ and ** $p<0.01$ significantly different from the control group. All data were shown as means±SD.

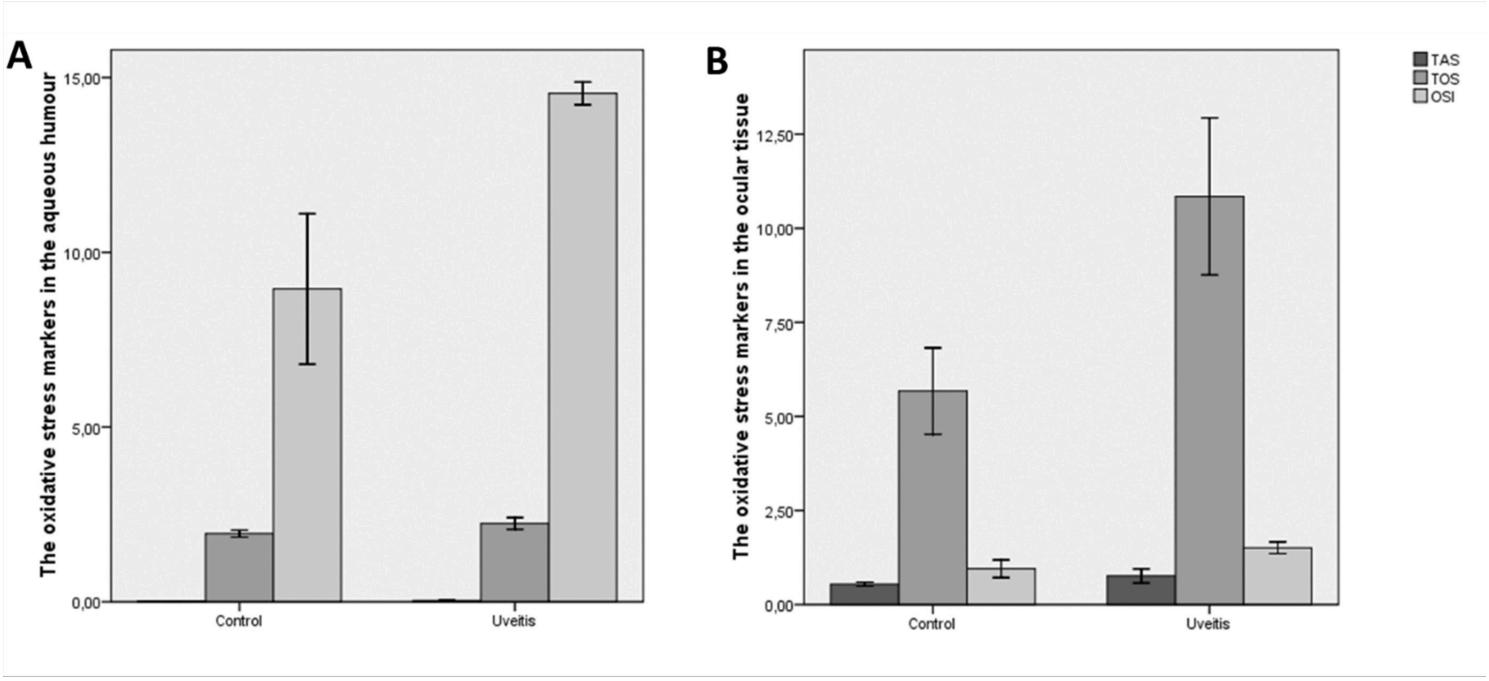


Figure 4

The oxidative stress status in the aqueous humor and in the ocular tissue. In the uveitis group, the aqueous humor TAS, TOS and OSI were significantly higher compared with the control **(A)** The ocular tissue TOS and OSI were significantly higher in the uveitis group compared with the control whereas the ocular tissue TAS did not differ significantly **(B).** All data were shown as means±SD.