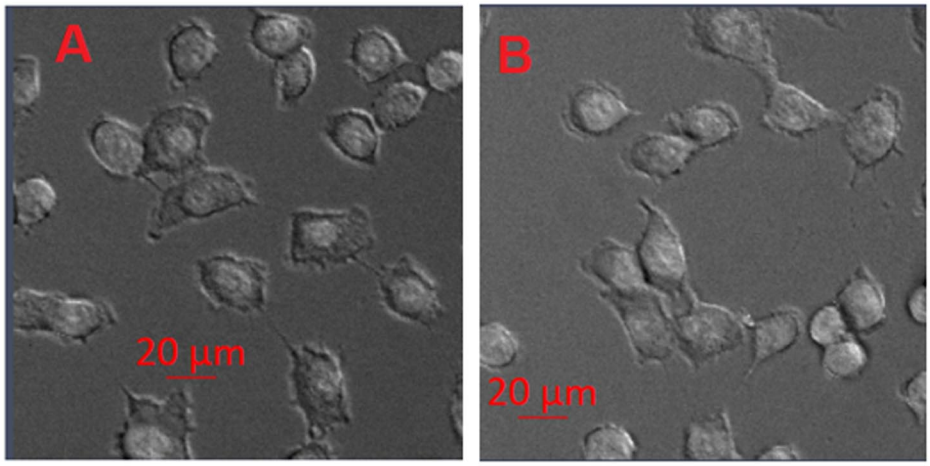
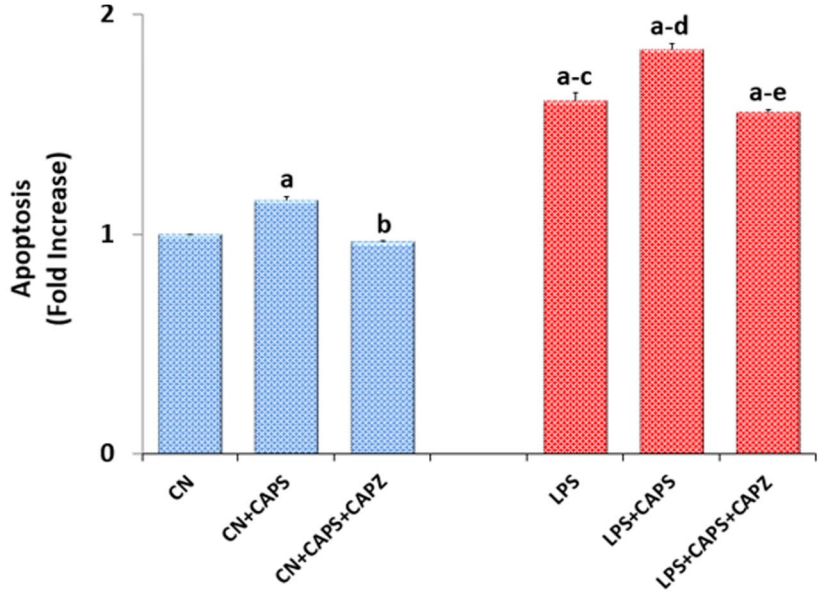
E-ISSN: 2149-7222 (Online)

Journal Cellular Neuroscience and Oxidative Stress





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http://dergipark.org.tr/jcnos

Former name; Cell Membranes and Free Radical Research

Volume 16, Number 3, 2024

Journal of Cellular Neuroscience and Oxidative Stress

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BSN Health Analyses, Innovation, Consultancy, Organization, Industry and Trade Limited Company

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Formerly known as:

Cell Membranes and Free Radical Research (2008 - 2014)

Volume 16, Number 3, 2024

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Volume 16, Number 3, 2024
E-ISSN Number: 2149-7222 (Online)
Indexing: Scopus (Elsevier), CAS (Chemical Abstracts Service), Citation Index Database, EBSCOhost Research Database, Google Scholar

AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A-Ion Channels (Na^+- K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B-Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C-Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D-Gene and Oxidative Stress
(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

READERSHIP

Biophysics	Biochemistry
Biology	Biomedical Engineering
Pharmacology	PhysiologyGenetics
Cardiology	Neurology
Oncology	Psychiatry
Neuroscience	Neuropharmacology

Keywords
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.

RESEARCH ARTICLE

J Cell Neurosci Oxid Stress 2024;16(3): 1229-1236

DOI: 10.37212/jcnos.1609591

Lipopolysaccharide induces apoptosis and oxidative cytotoxicity through stimulation of the TRPV1 channel in retinal pigment epithelium cell line

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Received: 29 December 2024; **Accepted:** 12 January 2025

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List of Abbreviations;

ARPE-19, *adult retinal pigment epithelial-19;* ***Ca^{2+},*** *calcium ion;* ***CAPS,*** *capsaicin;* ***CAPZ,*** *capsazepine;* ***CN,*** *control;* ***DCF,*** *2',7'-dichlorofluorescein;* ***LPS,*** *lipopolysaccharide;* ***ROS,*** *reactive free oxygen radicals;* ***TRPV1,*** *transient receptor potential vanilloid 1;* ***VGCC,*** *voltage-gated Ca^{2+} channels.*

Abstract

Common and vision-threatening inflammatory ocular disorders are major issues on a global scale. The etiology and whole treatment for inflammatory disorders are yet unknown. Numerous cells except human retinal pigment epithelial-19 (ARPE-19) have been shown to be involved in lipopolysaccharide (LPS)-induced free reactive oxygen species (ROS) and apoptosis through TRPV1 cation channel stimulation. I wanted to investigate how TRPV1 affected the oxidative cytotoxicity and apoptosis caused by LPS in ARPE-19.

Two main groups in the ARPE-19 cells were induced as control and LPS (1 µg/ml for twenty-four hours). TRPV1 antagonist (100 µM capsazepine (CAPZ) for 1 hour) blocked TRPV1 in the channel, whereas TRPV1 agonist (10 µM capsaicin (CAPS) for 1 hour) stimulated cells of the main groups.

The incubation of CAPS increased the amounts of apoptosis, caspases (caspase -3, -8, and -9), mitochondrial dysfunction, and ROS in the control and LPS groups, while CAPZ incubation diminished these amounts. However, their amounts were additionally increased in the LPS than in the control. LPS-induced increases of cell viability were diminished in the control and LPS groups by the CAPZ.

In summary, CAPZ treatment through TRPV1 inhibition contributes to the oxidative stress and apoptosis that LPS causes in ARPE-19 cells. TRPV1 inhibition by CAPZ may be a viable treatment option for oxidative retinal damage induced by LPS.

Keywords: Apoptosis; Inflammation; Retina oxidative injury; TRPV1 channel.

Introduction

Common inflammatory eye diseases include retinopathies, macular degeneration, and uveitis (Chistyakov et al., 2024; Zhang et al., 2024). The genesis and progression of retinal diseases and vision loss are caused by immune suppression and ocular inflammatory

activation (Murakami et al., 2020). Anti-inflammatory treatments have recently been utilized to treat intensive eye inflammation that is resistant to immunomodulator medications (Srejovic et al., 2024). It is still unclear what causes inflammatory disorders and how to fully treat them. In order to cure inflammatory diseases, finding an efficient treatment is therefore a crucial and urgent topic. Since the pathophysiology of many inflammatory diseases is significantly influenced by this layer, human retinal pigment epithelial-19 (ARPE-19) cells have been employed in investigations in recent years (Özkaya et al., 2021; Daldal and Nazıroğlu 2022; Tang and Liu 2024).

LPS is a substance found in the outer membrane of gram-negative bacteria that is produced when the bacterial cell is disturbed (Caroff and Karibian 2013). One of the main causes of the development and spread of inflammatory ocular diseases is LPS-induced inflammation and free reactive oxygen species (ROS) (Ozal et al. 2018; Chistyakov et al., 2024; Zhang et al., 2024). A full-blown inflammation reaction is triggered by the buildup of free radicals and oxidized lipoproteins in the retina, which causes chronic oxidative damage and exacerbates it (Ahmed et al., 2023). Through calcium channel activation, particularly stimulation of the melastatin 2 channel (TRPM2) of transient receptor potential (TRP), LPS also exerts Ca^{2+} influx stimulator effects on these cells (Hayashi et al., 2012; Saddala et al., 2020). Even though they were controlled by blocking TRPM2 and voltage-gated calcium channels (VGCC), the characteristics ultimately lead to an increase in mitochondrial dysfunction, which in turn induces an upregulation of the production of ROS and the induction of apoptosis (Saddala et al., 2020; Özkaya et al., 2021; Daldal and Nazıroğlu 2022). Thus, increased Ca^{2+} influx carried by LPS is crucial for the production of ROS in ARPE-19 (Özkaya et al., 2021; Daldal and Nazıroğlu 2022).

Numerous TRP channels have been found in the retina, and studies have shown how important they are for pathophysiology of vision and other retinal functioning (Souza Monteiro de Araújoet al., 2020; Özkaya et al., 2021; Daldal and Nazıroğlu 2022). A Ca^{2+}-permeable cation channel of TRP is vanilloid 1 (TRPV1) channel. Although capsazepine (CAPZ) is an antagonist of TRPV1, capsaicin (CAPS) is an agonist of TRPV1 (Caterina et al., 1997). Additionally, nitrogen radicals and oxidative stress activate TRPV1 (Chuang and Lin 2009; Leonelli et al., 2013). Results from studies on the function of TRPV1 in retinal injury models have been inconsistent, most likely as a result of the various insult types used. On the other hand, it has been observed that TRPV1 activation in retinal explants promotes cell death, whereas its suppression protects retinal ganglion cells from a hydrostatic pressure injury (Sappington et al. 2009). TRPV1 activation is necessary for ROS-induced retinal cell damage (Chuang and Lin 2009; Leonelli et al., 2013). TRPV1 contributes significantly to the death of the mouse retina caused by ischemia/reperfusion through the increases of ROS, caspase-3, and apoptosis (Souza Monteiro de Araújoet al., 2020). On the other hand, its suppression produced a protective effect against ROS, mitochondrial dysfunction, and apoptosis in the inflammatory cells caused by LPS (Güzel and Akpınar 2021). Therefore, CAPZ therapy may have a protective effect versus LPS-induced ROS and apoptotic damage in ARPE-19 via inhibiting TRPV1.

I'm not aware of any research on how CAPZ protects ARPE-19 cells against LPS-induced oxidative stress and retinal apoptosis by inhibiting TRPV1. In this study, I aimed to find out how TRPV1 exacerbated LPS-induced oxidative cell death in ARPE-19 cells, while its suppression prevents oxidative cell death in cells.

Material and Methods

Cell lines

The research on retinal disease and LPS primarily employed human ARPE-19 cells (Özkaya et al., 2021; Daldal and Nazıroğlu 2022). The cells were also expressing the natural TRPV1 channel (Cordeiro et al., 2010). The current study used ARPE-19 cells for two reasons. We obtained a gift of the ARPE-19 cell line from Dr. Suat Erdoğan, Trakya University, Edirne, Türkiye. The cells were kept in a sterile cell culture environment (95% air and 5% CO_2). They were also cultivated in a medium mixture consisting of 45%/45% Ham's F12/DMEM mixture, 10% fetal bovine serum, and 1% antibiotic mixture (Daldal and Nazıroğlu 2022).

Study groups

The retina cells in the 25 cm^2 flasks with $1x10^7$ cells were divided into two essential groups as control (CN), and LPS. TRPV1 antagonist (100 μM CAPZ for 1 hour) blocked TRPV1 in the channel, whereas TRPV1 agonist (10 μM CAPS for 1 hour) stimulated cells of the main groups (Figure 1). The cells of CN group were cultured for

24 hours without receiving any LPS treatments. For twenty-four hours, the cells in the LPS groups were exposed to 1 µg/ml LPS (Cat #L2880-100MG, LPS from Escherichia coli O55:B5, Sigma-Aldrich) (Saddala et al., 2020; Daldal and Nazıroğlu 2022).

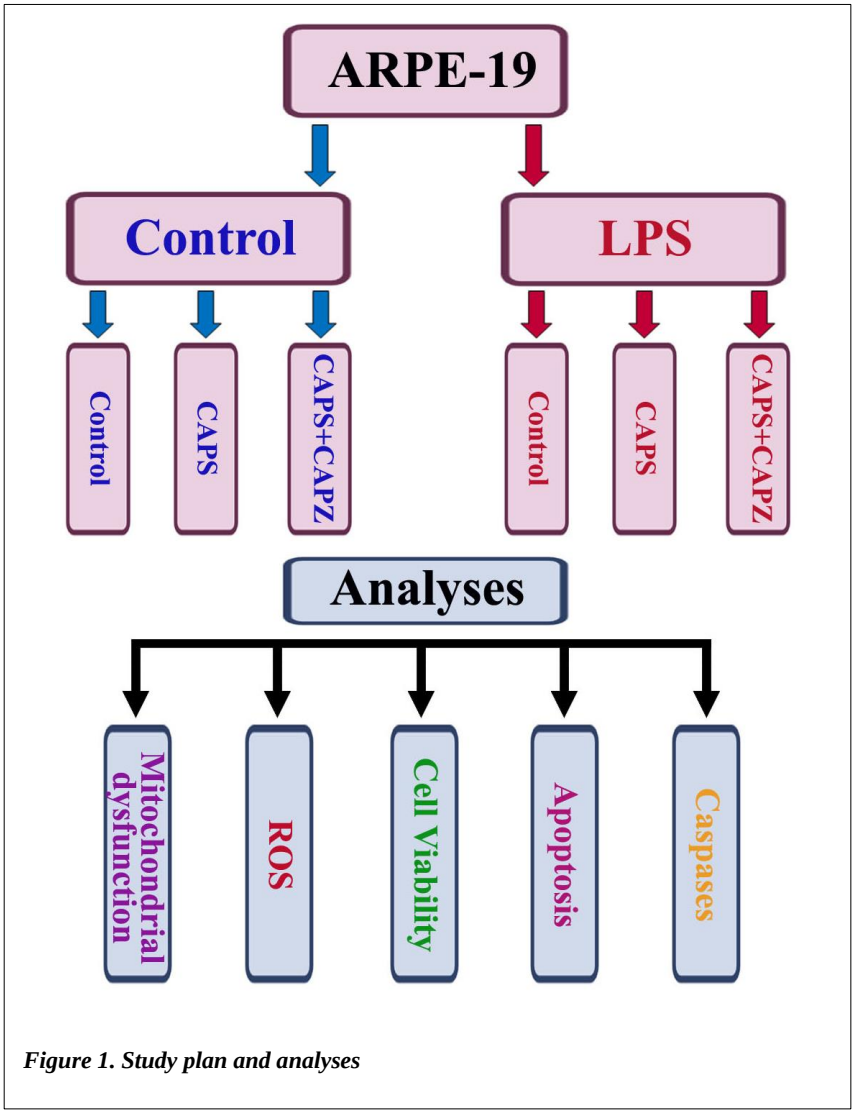

Figure 1. Study plan and analyses

Assays of cell viability and apoptosis

The MTT technique was used to assess the viability of cells. An Infinite PRO 200 model automated microplate reader (Tecan Inc., Groedig, Austria) used to measure the absorbance changes of MTT at 490 and 650 nm (Ertuğrul et al., 2023).

I performed the apoptosis experiment using a commercial kit called ApoPercentage. It was purchased from Biocolor Ltd. in Northern Ireland. Using the Infinite PRO 200, the absorbance change upon intracellular dye release was quantified (Ertuğrul et al., 2023).

In order to carry out the apoptosis and cell viability studies, I performed three analyses (n = 3). The results for the caspases were expressed as fold increase (experiment/control) changes.

Evaluations of caspases

The evaluations for caspases (-3, -8, and -9) activities in the cells were carried out using a previously published methodology with only minor modifications (Ertuğrul et al., 2023). In order to evaluate the cleavage of the caspase-3 (Ac-DEVD-AMC), caspase-8 (Ac-LEHD-AMC), and caspase-9 (Ac-IETD-AFC) substrates, the Infinite PRO 200 microplate reader was utilized (Bachem, Heidelberg, Germany). The wavelengths for their excitation and emission were set at 340 and 460 nm, respectively. The results for the caspases were expressed as fold increase (experiment/control) changes.

Assays for ROS and mitochondrial dysfunction

Using DCFH-DA (2',7'-dichlorodihydrofluorescein (DCF) diacetate) (Cat # D399, Thermo Fisher Sci.), the generation of ROS in the ARPE-19 was measured. The cells were treated for 15 to 20 minutes at 37 °C and in the dark with 1 µM non-fluorescence DCFH-DA. Oxidation of DCFH-DA in the cytosol results in the production of fluorescent DCF (Vaglienti et al., 2022). Changes in DCF fluorescence were measured using the Infinite PRO 200 microplate reader.

JC-1 is a cationic carbocyanine dye that is delivered to mitochondria. According to the manufacturer (ThermoFischer Scientific), the JC-1 probe (Cat #: T3168) is an orange-fluorescent J-aggregate that manifests when membrane potentials in living cells exhibit hyperpolarization, indicating mitochondrial dysfunction. The cells were cultivated for microplate studies in a black 96-well plate. The ARPE-19 was kept at 37 °C for 15 to 20 minutes after the addition of the JC-1 dye (4 µM) in DMEM/Ham's F12 mix. One BPS wash was then used to

get rid of the medium. To measure JC-1 florescence intensity, the ratio, or 578/F485, between the fluorescence intensity obtained by excitation/emission of 578/599 nm and the fluorescence intensity obtained by excitation/emission of 485/535 nm was calculated. In the analysis using the automatic microplate reader, the JC-1 data from the orange color were presented as a fold increase (experiment/control).

Statistical analysis

The data was presented as mean ± standard deviation (SD) for two major groups and six subgroups. Following completion of the Tukey's post hoc test, a one-way analysis variance (ANOVA) was used by the SPSS software (22.0). The p-value was deemed statistically significant if it was less than 0.05.

Results

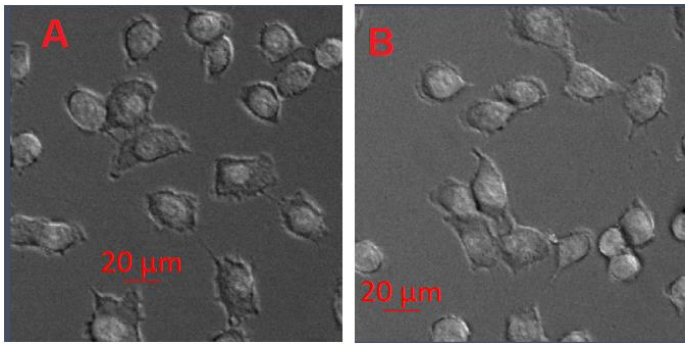

Figure 2. ARPE-19 cells of control (A) and LPS (B) groups. *(Objective 20x)*

The treatment of CAPZ attenuated LPS-induced cell viability, apoptosis, and caspase changes

Black/white images of ARPE-19 were shown in the Figure 2, and there were no morphological changes in the cells.

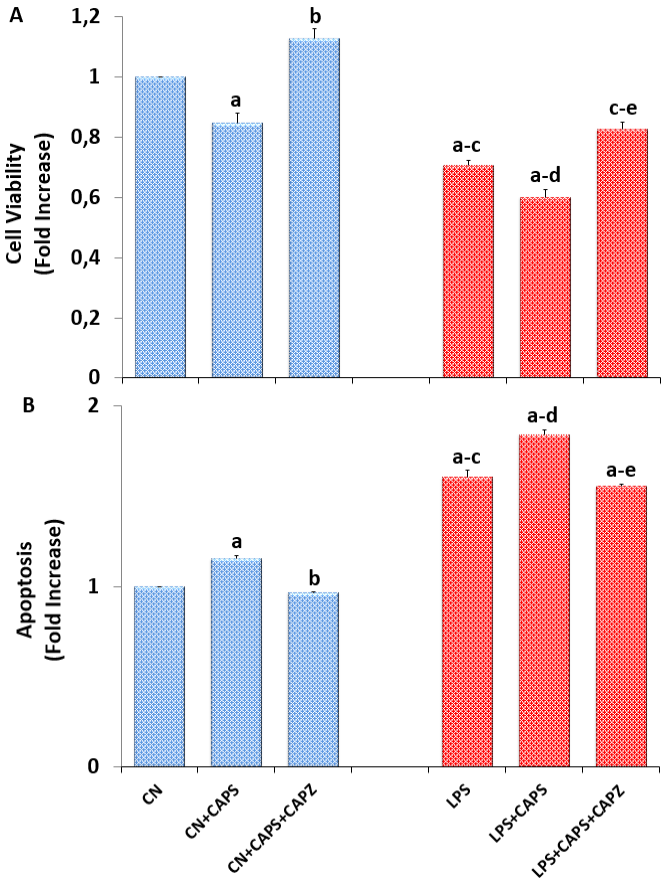

Figure 3. TRPV1 antagonist (CAPZ) therapy reduced the LPS-induced alterations in cell viability and apoptosis in ARPE-19. *(n=3 and mean ± SD). The TRPV1 in the cells was stimulated in the cells of control (CN) and LPS by CAPS (10 µM for 1 hour), while CAPZ (100 µM for 1 hour) inhibited it. (**A**) The MTT was used in the plate reader to identify the changes in cell viability. (**B**) A commercial kit was used to measure the amount of apoptosis in the cells in the plate reader. ($^{a}p < 0.05$ vs CN; $^{b}p < 0.05$ vs CN+CAPS; $^{c}p < 0.05$ vs CN+CAPS+CAPZ; $^{d}p < 0.05$ vs LPS; $^{e}p < 0.05$ vs LPS+CAPS).*

LPS decreases retinal cell viability and triggers TRP alterations such as TRPA1 and TRPM2, which raise apoptosis and caspase levels. However, by blocking the channels in the retina cells, TRP channel blocker therapies reduced the alterations (Souza Monteiro de Araújo et al., 2020; Özkaya et al., 2021; Daldal and Nazıroğlu 2022). Retinal ganglion cell death brought on by pressure was lessened by TRPV1 inhibition (Sappington et al., 2015). CAPS therapy did not alter cell viability to influence cell survival, and reports of CAPS in retinal cells are inconsistent (Freitas et al. 2019). CAPZ has not, however, been shown to enhance cell survival in the ARPE-19 and reduce caspase and apoptosis levels by inhibiting TRPV1.

In order to further understand how CAPZ protected ARPE-19 cells, I looked into how it increased cell viability while decreasing caspase levels and LPS-induced apoptosis.

The apoptosis levels were higher in the groups of CN + CAPS and LPS + CAPS than in the groups of CN and LPS ($p < 0.05$) (Figure 3B), but the cell viability levels were lower in the CN + CAPS and LPS + CAPS groups than in the CNT and LPS groups ($p < 0.05$) (Figure 3A). In contrast, the LPS groups exhibited decreased cell viability and higher levels of apoptosis than the CN group. In comparison to the LPS + CAPS group, the LPS + CAPS + CAPZ group displayed lower levels of apoptosis and cell viability ($p < 0.05$). Thus, by suppressing TRPV1, I discovered that CAPZ treatment enhanced ARPE-19 viability and reduced the LPS-induced rise in apoptosis.

The CN + CAPS and LPS + CAPS groups exhibited increased levels of caspase -3 (Figure 4A), -8 (Figure 4B), and -9 (Figure 4C) activity compared to the CNT and LPS groups ($p < 0.05$). In contrast, the CN group had low caspase activities (caspases -3, -8, and -9) than the LPS groups. In comparison to the LPS + CAPS group, the LPS + CAPS + CAPZ exhibited a decrease in caspases -3, -8, and -9 ($p < 0.05$). Thus, by suppressing TRPV1 in ARPE-19 cells, I found that CAPZ treatment reduced the rise in caspases -3, -8, and -9 induced by LPS.

The treatment of CAPZ attenuated LPS-induced increased of mitochondrial dysfunction and ROS

Incubation with LPS increased the mean values of JC-1 (Fig. 5A) and DCFH-DA (Fig. 5B) in the ARPE-19 ($p < 0.05$). CAPZ incubations decreased the impact of LPS by preventing ROS production and mitochondrial dysfunction in the cells ($p < 0.05$). Although CAPZ incubations reduced the upregulations in ROS production and mitochondrial dysfunction, they were still linked to the rise in caspases and apoptosis following LPS incubation. The results of ROS and mitochondrial dysfunction therefore further illustrated how LPS affects oxidative stress in ARPE-19 that is induced on by TRPV1 stimulation.

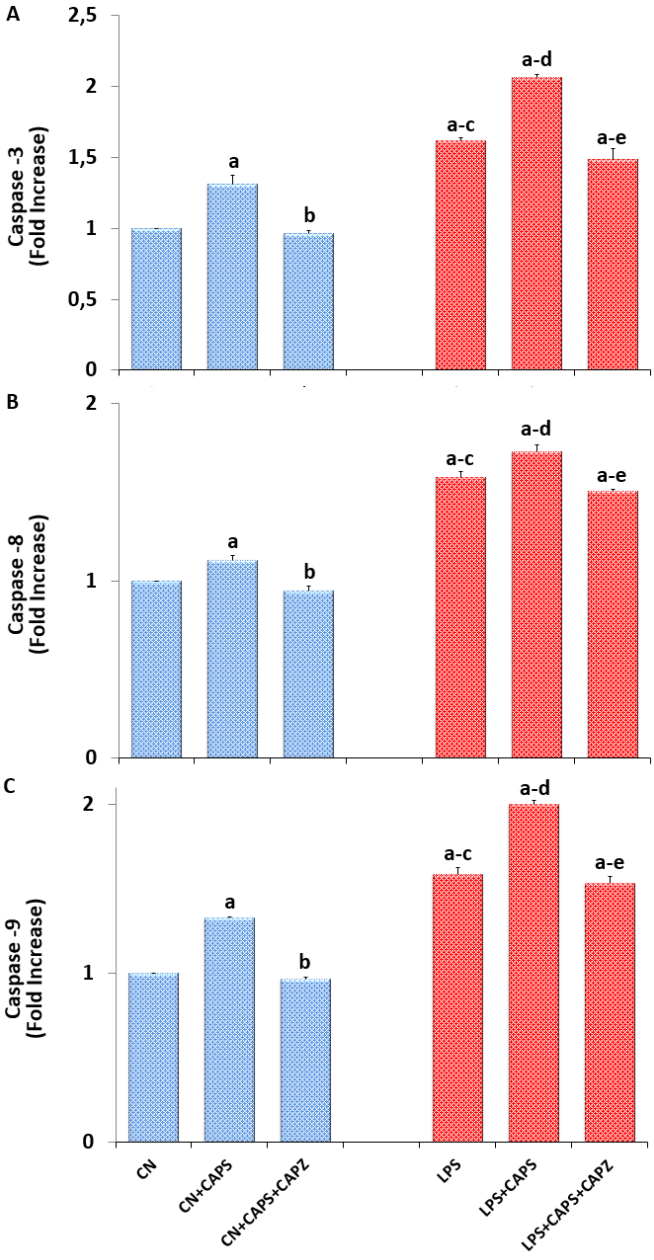

Figure 4. CAPZ reduced the LPS-caused upregulations of caspases. *(n=3 and mean ± SD). CAPS (10 μM for 1 hour) increased TRPV1 in the cells of control (CN) and LPS, while CAPZ (100 μM for 1 hour) inhibited it. Caspase substrates were used in the plate reader for the assays of caspase -3 (**A**), caspase -8 (**B**), and caspase 9 (**C**) activities. ($^{a}p < 0.05$ vs CN; $^{b}p < 0.05$ vs CN+CAPS; $^{c}p < 0.05$ vs CN+CAPS+CAPZ; $^{d}p < 0.05$ vs LPS; $^{e}p < 0.05$ vs LPS+CAPS).*

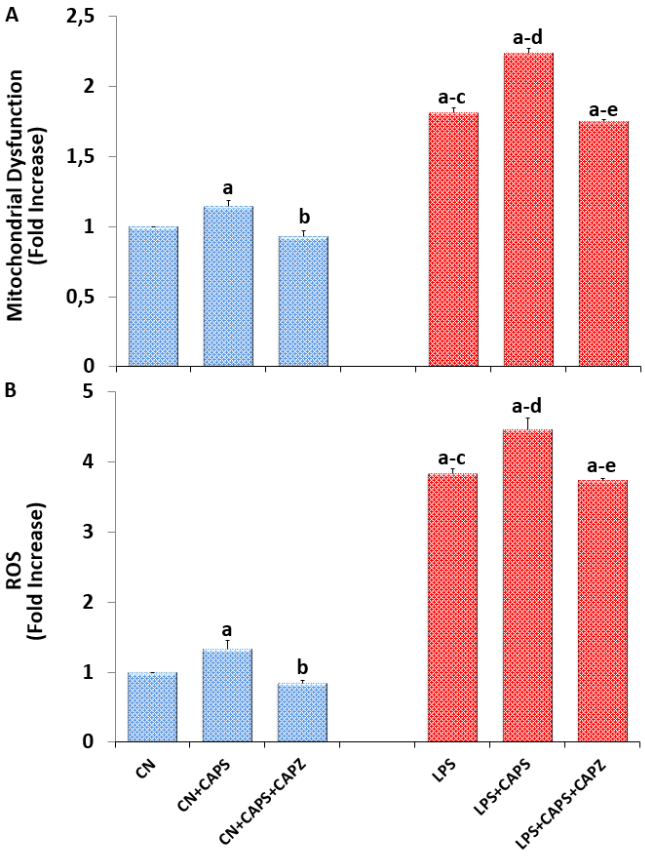

Figure 5. CAPZ reduced the LPS-induced increases of mitochondrial dysfunction and ROS. *(n=3 and mean ± SD). CAPS (10 μM for 1 hour) increased TRPV1 in the cells of control (CN) and LPS, while CAPZ (100 μM for 1 hour) inhibited it. Mitochondrial dysfunction was assayed in the plate reader by using JC-1 stain (**A**), although ROS was assayed by using the DCFH-DA stain (**B**). ($^{a}p < 0.05$ vs CN; $^{b}p < 0.05$ vs CN+CAPS; $^{c}p < 0.05$ vs CN+CAPS+CAPZ; $^{d}p < 0.05$ vs LPS; $^{e}p < 0.05$ vs LPS+CAPS).*

Discussion

Central vision loss is primarily caused by inflammatory eye conditions like age-related macular degeneration, retinopathies, and uveitis (Chistyakov et al., 2024; Zhang et al., 2024). Worldwide, inflammatory eye diseases impact a vast number of individuals, and in the years to come, their prevalence will rise (Zhang et al., 2024). Retinal oxidative stress caused by inflammation (LPS) is a primary cause of the development and spread of inflammatory eye disorders (Ahmed et al., 2023). The accumulation of ROS caused by excessive Ca^{2+}-influx stimulates TRPA1, TRPM2, and VGCC in the retina, causing oxidative damage and apoptosis. This triggers a full-blown inflammatory response, which exacerbates oxidative damage and thus feeds the vicious cycle (Souza Monteiro de Araújo et al., 2020; Hu et al. 2021; Özkaya et al., 2021; Daldal and Nazıroğlu 2022). TRPV1 channels have not yet been found to play a role in oxidative stress and apoptosis in ARPE-19. Therefore, I investigated the impact of inflammatory (LPS) and TRPV1 agonist (CAPS) stimulations on the ROS and apoptotic values in ARPE-19 cells. It was examined how the protective effect of TRPV1 inhibition (CAPZ) affected the cell values.

In the present research, I explored the protective effect of TRPV1 channel blocker (CAPZ) on LPS-induced oxidative injury and apoptosis, a classic model representative of inflammatory human eye diseases. I demonstrated that CAPZ treatment attenuated the levels of LPS-induced apoptosis and ROS. Caspase analysis showed increased apoptosis through the increases of caspase -3, -8, and -9 in the ARPE-19 of CAPS-treated LPS. Moreover, CAPZ was proved to be able to mitochondrial dysfunction/ ROS balance by suppressing the oxidative stress.

LPS-induced excessive Ca^{2+} influx through the stimulation cation channels, including the TRPV1 induces stimulation of phagocytic cell such as neutrophils and microglia (Güzel and Akpınar 2021). The excessive Ca^{2+} influx-induced ROS and apoptosis are main actors for retina injury and blindness (Meléndez García et al., 2016). LPS-caused increase of ROS induces retina injury via the activation several factors, including the TRP channels such as TRPA1 and TRPM2 activations (Souza Monteiro de Araújo et al., 2020; Özkaya et al., 2021; Daldal and Nazıroğlu 2022). In turn, the LPS-induced excessive Ca^{2+} influxes via the increase of mitochondrial membrane dysfunction cause excessive ROS generations. Hence, the ROS generations also play a vital role in LPS-mediated retinal injury (Wang et al., 2017; Zhang et al., 2021). An upregulation in ROS accumulation under inflammatory (LPS) conditions leads to retinal apoptosis via the activation of the TRP channels such as TRPA1 and TRPM2 activations (Souza Monteiro de Araújo et al., 2020; Özkaya et al., 2021; Daldal and Nazıroğlu 2022). The treatment of nimodipine via the inhibition of VGCC and CAPZ through the inhibition of TRPV1 modulate the LPS-induced oxidative human periodontal ligament stem cells, ARPE-19, and experimental animal retina injury and death (Hu et al., 2021; Yu et al., 2024). CAPS induced inflammatory oxidative injury in blood cells and neurons (Güzel and Akpınar 2021; Laorob et al., 2024), although conflicting information is also present (Bok et al., 2018). In the current study, the modulator action of CAPZ through

the modulation of TRPV1 was tested on the CAPS and LPS-caused oxidative toxicity and apoptosis in the ARPE-19. I observed that the LPS-caused increase of oxidative retinal changes through the modulation of TRPV1 was decreased in the cells.

The inside of ARPE-19 contains the inactive forms of caspases such as caspase-3, -8, and -9. (Han et al., 2018; Daldal and Nazıroğlu 2022). The rise in mitochondrial dysfunction, however, stimulates inactive caspase activations. Apoptosis follows the activation of caspases. The increase in TRPV1 stimulation-mediated excess Ca^{2+} entry into mitochondria was the primary cause of an elevation of mitochondrial dysfunction, which has an immediate impact on the pathways governing the viability of human monocytes and neurons. (Güzel and Akpınar 2021; Kievit et al., 2022). But through downregulating TRPV1 stimulation, CAPZ therapy reduced the amounts of apoptosis and active caspases in the neuronal cell and human monocyte (Güzel and Akpınar 2021; Kievit et al., 2022). In the present investigation, I observed that LPS incubation reduced ARPE-19 viability, but it also increased apoptosis, caspase -3, -8, and -9. Cytosolic Ca^{2+} concentration modulation by reduction of mitochondrial dysfunction reduces apoptosis, caspase-3, caspase-8, and caspase-9. According to Han et al. (2023), there are contradictory data in the literature on the protective effect of CAPS through TRPV1 stimulation on ROS, mitochondrial dysfunction, and LPS-induced apoptosis in kidney cells. The effect of CAPZ on ROS, caspases, and apoptosis appears to be cell-specific.

In conclusion, by downregulating TRPV1 stimulation-induced retinal damage, CAPZ incubation via TRPV1 attenuation protected ARPE-19 cells from LPS-mediated oxidant mediators and apoptotic mediators. Even though CAPZ therapy reduces LPS-induced ROS-mediated ARPE-19 damage, it is still possible that excessive apoptotic and oxidant mediators mediated by TRPV1 are a contributing factor to LPS-induced oxidative retinal damage. Due to its restricted funding, the current study has certain limitations. Initially, I was unable to conduct Ca^{2+} and electrophysiological analyses in the cells that were being studied. In addition to CAPZ, I was unable to test the antioxidants that block the TRPV1 channel on the cell values. Future research will use cell lines and mice to assess the limitations.

Acknowledgements

Muhammet Şahin, a technician, assisted with plate reader analyses, for which the authors are grateful.

Author contributions

Alper Ertuğrul designed and planned the study, prepared the figures and pictures, edited the text, and carried out cell culture and laser confocal microscope studies.

Funding

Financial funding for this study was provided by BSN Health, Analyses, Innov., Consult., Org., Agricul., Trade Ltd. (Göller Bölgesi Teknokenti, Isparta, Türkiye) (Project No: 2023-08).

Declarations

Competing Interests No.

Ethical Approve No data of animals and human.

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