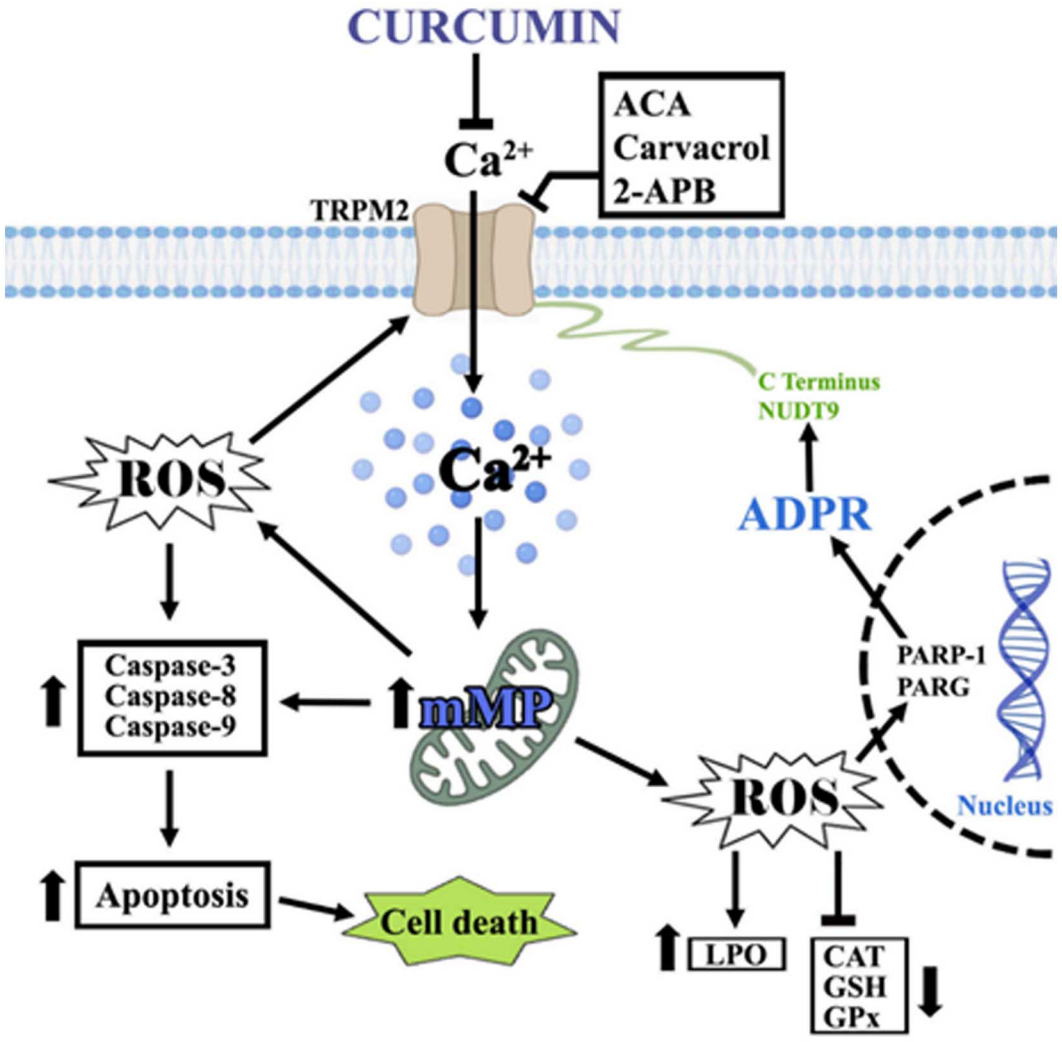
E-ISSN: 2149-7222 (Online)

Journal Cellular Neuroscience and Oxidative Stress



OPEN ACCESS and NO PUBLICATION FEE

http://dergipark.gov.tr/jcnos

Former name; Cell Membranes and Free Radical Research

Editor in Chief
Prof.Dr. Mustafa NAZIROĞLU

Volume 14, Number 3, 2022

Journal of Cellular Neuroscience and Oxidative Stress

http://dergipark.gov.tr/jcnos

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

http://www.bsnsaglik.com.tr/

info@bsnsaglik.com.tr

Formerly known as:

Cell Membranes and Free Radical Research (2008 - 2014)

Volume 14, Number 3, 2022

[CONTENTS]

Volume 14, Number 3, 2022
E-ISSN Number: 2149-7222 (Online)
Indexing: Scopus (Elsevier), CAS (Chemical Abstracts Service), Citation Index Database, EBSCOhost Research Database, Google Scholar, Index Copernicus,

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 2022;14(3): 1095-1104.

Ketamine attenuates hypoxia-induced cell death and oxidative toxicity via inhibition of the TRPM2 channel in neuronal cells

Haci Ömer OSMANLIOĞLU

Department of Anesthesiology and Reanimation, Faculty of Medicine, Suleyman Demirel University, Isparta, Türkiye.

Received: 1 November 2022; **Accepted:** 22 December 2022

***Address for correspondence:**

Dr. Haci Ömer OSMANLIOĞLU,
Department of Anesthesiology and Reanimation,
Faculty of Medicine, Suleyman Demirel University, Isparta, Türkiye.
Tel: +902462112073
E-mail: omerosmanlioglu@gmail.com

List of Abbreviations;

***ADPR**, ADP-ribose; **BF**, bright field; **Ca^{2+},** calcium ion; **CNT,** control; **LSM-800,** confocal laser scanning microscope; **DCFH-DA,** 2',7'-dichlorodihydrofluorescein diacetate; **DCF,** 2',7'-dichlorofluorescein; **$[Ca^{2+}]_i$,** intracellular free calcium; **HYP,** hypoxia; **iROS,** intracellular free reactive oxygen radicals; **$\Delta\Psi m$,** mitochondrial membrane potential; **KET,** ketamine; **mROS,** mitochondrial free reactive oxygen radicals; **TRP,** transient receptor potential; **TRPM2,** transient receptor potential melastatin 2; **VGCC,** voltage gated Ca^{2+} channels; **2APB,** 2-aminoethoxydiphenyl borate*

Abstract

Ketamine (KET) is a pediatric anesthetic agent, and it acts antioxidant action via the inhibition of Ca^{2+} influx and N-methyl-D-aspartate (NMDA) receptors, apoptosis, intracellular (iROS), and mitochondrial reactive oxygen species (mROS) productions. Hypoxia (HYP)-induced oxidative stress activates the TRPM2 channel, although 2-aminoethoxydiphenyl borate (2APB) inhibits it. The treatment of KET inhibits HYP-induced oxidative stress and apoptosis in neuronal cells, although conflicting information is also present. We aimed to the modulator role of KET on the HYP-mediated oxidative cytotoxicity and apoptosis in the SH-SY5Y neuronal cells via modulating the TRPM2 signaling pathways.

We induced five primary groups in the SH-SY5Y cells: Control, KET (0.3 mM for 24h), HYP ($CoCl_2$ and 200 μM for 24h), HYP+KET, and HYP+2APB.

The amounts of apoptosis, cell death (propidium iodide positive cell number), oxidants (mROS and iROS), and cytosolic free Ca^{2+} were increased via TRPM2 stimulation by the incubation of HYP, although their amounts were diminished by KET and 2APB.

In conclusion, the treatment of KET attenuated the HYP-induced oxidative stress and neuronal death levels via TRPM2 inhibition in the SH-SY5Y neuronal cells. The KET may be considered as a potential therapeutic way to HYP-induced oxidative neuronal injury.

Keywords: Hypoxia; Ketamine; Neuronal injury; Oxidative stress; TRPM2 channel.

Introduction

Oxidative stress is induced by the generations of intracellular (iROS) and mitochondrial (mROS) reactive oxygen species (Halliwell 1992). The excessive generations of iROS and mROS induce injury in the main structures of cells such as nucleic acid, lipids, and proteins (Halliwell 1992). The iROS and mROS such as superoxide radical and hydroxyl radical are produced by several physiological and pathophysiological conditions (Nazıroğlu 2007). A main pathophysiological function of ischemia is hypoxia (HYP) in the brain and neurons. In addition, the ischemia-induced HYP causes further injury via the excessive Ca^{2+} influx and oxidative stress after the induction of reperfusion (Kumar et al. 2014). The brain and neurons are very sensitive to the HYP-induced iROS and mROS, because they have low antioxidant levels but have high amounts of polyunsaturated fatty acids and oxygen consumption.

Ischemia-induced hypoxic brain injury is induced by complications such as shock, cardiac arrest, cardiopulmonary bypass or accidents during operation and anesthesia, and it is a main cause of death and disability in adults (Pereira et al. 2019; Kounis et al. 2020). The main target of treating ischemia injury is to modulate blood flow to the injured region within the limited therapeutic time windows. However, the treatments cause further injury via the reperfusion induction (Zhao et al. 2008). The results of the recent studies suggested that anesthetic drugs induced neuroprotective action on HYP via decreasing the iROS and mROS levels (Burchell et al. 2013; Zhang et al. 2023). Ketamine (KET) is a pediatric anesthetic agent, and it acts antioxidant action via the inhibition of apoptosis, iROS, and mROS productions (Gascoigne et al. 2022), although high dose of KET has oxidant and apoptotic actions (Gu and Kanungo 2021). KET modulates Ca^{2+} influx via an antagonist action of N-methyl-D-aspartate (NMDA) receptors (Gascoigne et al. 2022). The HYP-induced oxidative stress in human umbilical vein endothelial cells and zebrafish embryos was decreased by the treatment of KET (Zhou et al. 2020; Gu and Kanungo 2021). However, the molecular pathway of KET on the HYP induction in the neuronal cell has not been clarified yet.

A member of and transient receptor potential (TRP) superfamily is melastatin 2 (TRPM2), and it is activated by H_2O_2 and ADP-ribose (Perraud et al. 2001; Hara et al. 2002; Nazıroğlu and Lückhoff 2008), although it is inhibited by antagonists, including the 2-aminoethoxydiphenyl borate (2APB) (Nazıroğlu 2007). Cobalt chloride ($CoCl_2$)-induced chemical HYP is a valuable model, because it stabilizes HYP inducible factors 1α and 2α under normoxic conditions (Muñoz-Sánchez and Chánez-Cárdenas 2019). The induction of HYP evokes the Ca^{2+} influx processes via the activation of transient receptor potential (TRP) melastatin 2 (TRPM2) by the excessive generation of iROS and mROS (Akyuva and Nazıroğlu 2020; Armağan and Nazıroğlu 2021; Yıldızhan and Nazıroğlu 2023). As a result of the accumulations of intracellular free calcium ions ($[Ca^{2+}]_i$) in the mitochondria of neuronal cells caused by the stimulation of the TRPM2 channel, the mitochondrial membrane potential ($\Delta\Psi m$), mROS, iROS, and cell death are all increased (Yıldızhan and Nazıroğlu 2020; Akyuva et al. 2021; Çınar 2022). Therefore, the increased TRPM2-mediated Ca^{2+} influx induced by $CoCl_2$-mediated HYP is a major factor in the emergence of mitochondrial oxidative stress and death in the neuronal cells (Akyuva et al. 2021). Although the suppression of TRPM2 by the use of KET results in protective action against oxidative neuronal injury, TRPM2-mediated cell death contributes to oxidative neuronal death (Demirdaş et al. 2017). However, conflicting information on the TRPM2 and KET in kidney cells is also present (Bracke et al. 2022).

To my knowledge, the protective action of KET on the HYP-induced oxidative neuronal death in SH-SY5Y cells has not been investigated yet. In the current investigation, I aimed to determine how KET modulated HYP-caused oxidative cell death in the SH-SY5Y neuronal cells.

Material and Methods

Cell lines

SH-SY5Y cells were mostly used in the experiments of KET (Ulbrich et al. 2016; Chen et al. 2020). In the SH-SY5Y cells, the natural presence of TRPM2 was indicated by the results of recent studies (Chen et al. 2013; Akyuva and Nazıroğlu 2020). For two reasons, the SH-SY5Y cells were used in the current study (ATTC, Germany). The cells were kept in a cell culture condition (95% air and 5% CO_2), and they were cultured in a medium mixture (45% DMEM, 45% DMEM Ham's F12 medium, 10% fetal bovine serum, and 1% penicillin/streptomycin) (Akyuva and Nazıroğlu 2020).

Study groups

Five main groups of SH-SY5Y cells were induced

using the $1x10^6$ cells in 25T flasks: control (CNT), KET, HYP, HYP+KET, and HYP+2APB. Cells of CNT groups were kept in the cell culture conditions without treatments for 24h. The cells in the KET and HYP+KET groups were incubated by KET (300 μM) for 24h (Demirdaş et al. 2017). The cells in the HYP groups were treated with $CoCl_2$ (200 μM) for 24h (Akyuva and Nazıroğlu 2020). The cells in the HYP+KET and HYP+2APB channel blocker groups were additionally incubated with KET (0.3 mM for 24h) and 2APB (100 μM for 2h) after the $CoCl_2$ (200 μM for 24h) incubation, respectively.

In the dishes with bottom glass (Mattek Corporation, Ashland, MA, USA), the cells were cultured for the laser confocal microscope analyses (LSM-800) coupled with an Axio Observer.Z1/7 microscope (Zeiss, Oberkochen, Germany) and a Plan-Apochromat 40x1.3 oil objective (Zeiss).

The determination of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$)

For the determination of LPS-induced $[Ca^{2+}]_i$ concentration changes in the microglia cells, we used a fluorescence dye (Fluo 3⁄AM) under the argon laser stimulation in the LSM-800 at 488 nm (Daldal and Nazıroğlu 2022). The SH-SY5Y cells in the cell culture medium of 35-mm plates were incubated with 1 μM Fluo 3/AM (Cat #ab145254, Abcam, Istanbul, Turkiye) at dark and 37 ℃ for 45 min. The TRPM2 channel in the cells were stimulated by H_2O_2 (1 mM), although it was inhibited by 100 μM 2APB. The changes in fluorescence intensity were expressed using the arbitrary unit (a.u.).

Cell death analyses

Hoechst 33342 (8.1 μM) and PI (1.5 μM) were incubated in the cell culture medium for 15-20 minutes while they were in the dark and at 37 °C. The red (PI) and blue (Hoechst 33342) images were recorded in the LSM-800 and Axio Observer.Z1/7 inverted microscope with an objective (Plan-Apochromat 40x/1.3 Oil DIC-UV). The PI-positive (death) SH-SY5Y cell number was manually counted after measuring the fluorescence intensity of Hoechst 33342 and PI using the ZEN program. The black/white bright field (BF) images of the cells were recorded in in the Axiocam 702 camera. (Yıldızhan and Nazıroğlu 2020).

mROS and iROS production assays

MitoSOX Red fluorogenic (mSOX) dye (Cat # M36008, Thermo Fisher Sci.) is a superoxide indicator in mitochondria of live cells, and it was used to assay the generation of mROS. The generation of iROS was assayed in the SH-SY5Y cells by using the 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) (Cat # D399, Thermo Fisher Sci.). The cells were incubated with 1 μM non-fluorescence DCFH-DA and mSOX for 15-20 min at dark and 37 °C. In the cytosol, the DCFH-DA is converted to fluorescent 2',7'-dichlorofluorescein (DCF) by oxidation (Vaglienti et al. 2022). The images of DCF and mSOX were captured in the LSM-800 with the objective (40x/1.3 Oil DIC-UV) at 504/525 nm (Ext/Ems) for DCF and at 576/598 nm (Ext/Ems) for mSOX. The fluorescence change of each treatment (n=9-12) was determined in the captured green (DCF) and red (mSOX) images by using the ZEN software, and the results were presented as arbitrary unit (a.u.) (Yıldızhan and Nazıroğlu 2020).

Mitochondrial membrane potential (ΔΨm) assay

The changes of ΔΨm were evaluated in the SH-SY5Y cells by using the probes of JC-1 (Cat # sc-364116, Santa Cruz Chemical, Heidelberg, Germany) and DHR123 (Cat # sc-203027, Santa Cruz Chemical). The cells in the dishes were incubated with 5 μM JC-1 and 4 μM DHR123 for 15-20 min at dark and 37 °C (Yıldızhan and Nazıroğlu 2020). The non-fluorescent DHR123 is converted to the Rh123 in the cytosol of cell by the iROS such as peroxide and peroxynitrite. The JC-1 (orange) and Rh123 (green) fluorescence intensities were measured in the captured images of LSCM/800 at 593/595 nm (Ext/Ems) (Perelman et al. 2012). The fluorescence change of each treatment (n=9-12) was determined in the images by using the ZEN software. The fluorescent data of JC-1 were shown as a.u.

Statistical analysis

The data of five groups were presented as mean ± standard deviation (SD). In order to determine whether statistical significance existed, the SPSS program (25.0) utilized the one-way analysis of variance (ANOVA). When an ANOVA revealed that there were differences between groups, the Student's T test was used to look at significant differences. A p value ($p \leq 0.05$) was used to determine statistical significance.

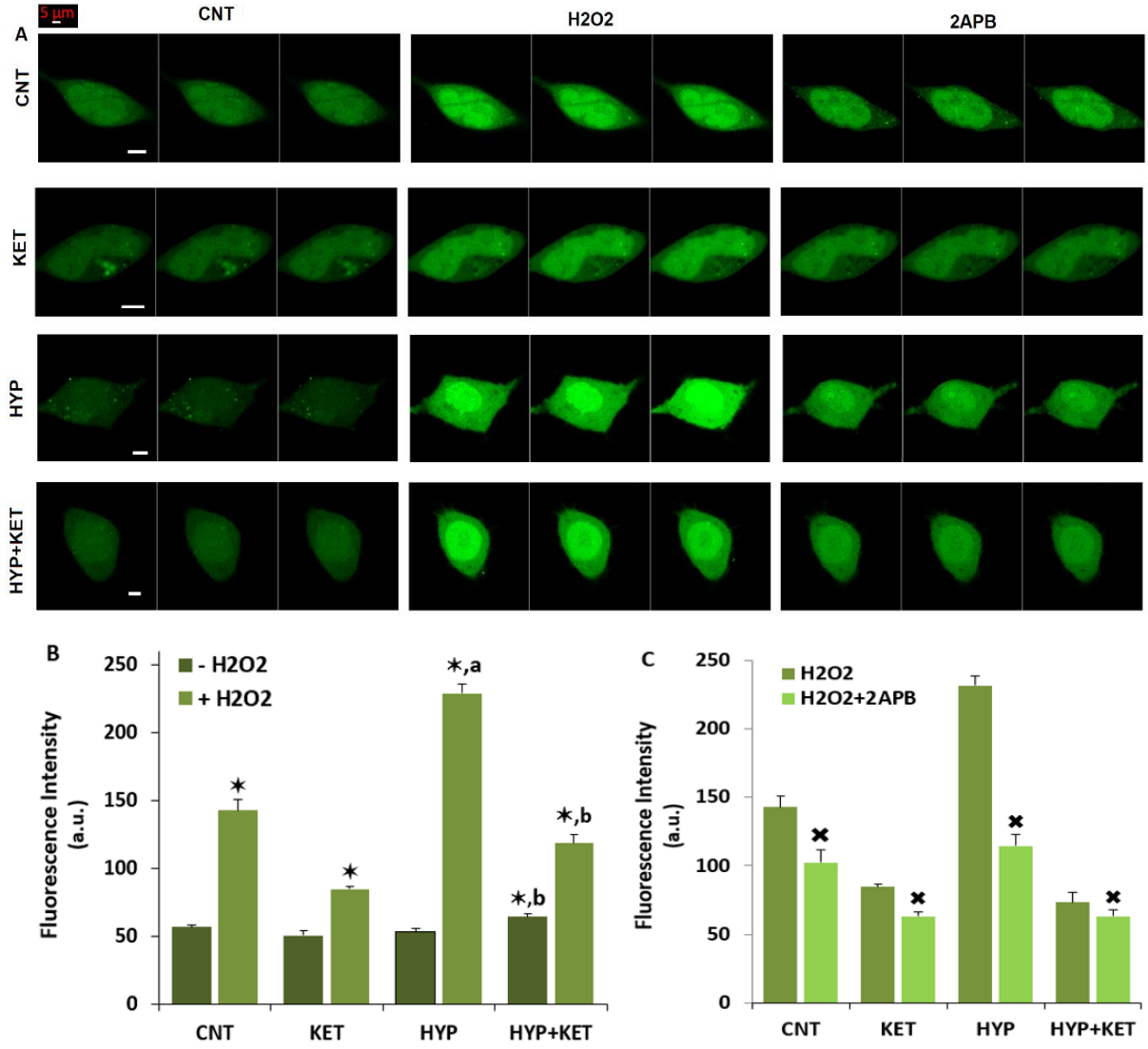


Fig. 1. The incubation of KET of (0.3 mM for 24h) modulated HYP (200 µM $CoCl_2$ for 24h)-caused the increase of $[Ca^{2+}]_i$ in the SH-SY5Y cells. *(Mean ± SD). The SH-SY5Y cells were stained with Fluo 3/AM (1 µM for 45min). After washing the cells with extracellular buffer, they were stimulated with H_2O_2 (1 mM for 5 min) and then they were inhibited by 2APB (100 µM for 5 min) in the LSM-800 with 40x oil objective.* ***A.*** *The representative images of the Fluo 3/AM in the four groups [control (CNT), KET, HYP, and HYP+KET.* ***B*** *and* ***C****. The mean fluorescence intensity changes as arbitrary unit (a.u.) in the four groups after the H_2O_2 and 2APB treatments, respectively. The scale bar in the images was kept as 5 µm. One represented image of each figure was selected from 10-15 SH-SY5Y of 10 independent experiments for each condition. ($^{a}p \leq 0.05$ vs the groups of CNT and KET. $^{b}p \leq 0.05$ vs the groups of HYP. $^{*}p \leq 0.05$ vs without H_2O_2 stimulation (- H_2O_2 group). $^{\times}p \leq 0.05$ vs H_2O_2 stimulation (+ H_2O_2 group).*

Results

The treatment of KET attenuated HYP-induced TRPM2 stimulation in the SH-SY5Y cells

Through the activation of cation channels, particularly the TRPM2, the HYP causes an increase in $[Ca^{2+}]_i$ and oxidative stress levels in the SH-SY5Y cells (Akyuva and Nazıroğlu 2020; Armağan and Nazıroğlu 2021), whereas the KET treatment acted Ca^{2+} channel blocker and antioxidant actions via the inhibition of the TRPM2 channel in dorsal root ganglion (DRG) neurons and human umbilical vein endothelial cells (Demirdaş et al. 2017; Zhou et al 2020). The HYP-caused TRPM2 stimulation is blocked in the neuronal cells by the treatment of antioxidant (Chen et al. 2013; Yıldızhan and Nazıroğlu 2023), although there is no report of KET on the TRPM2 stimulation-mediated increase of $[Ca^{2+}]_i$ concentration in

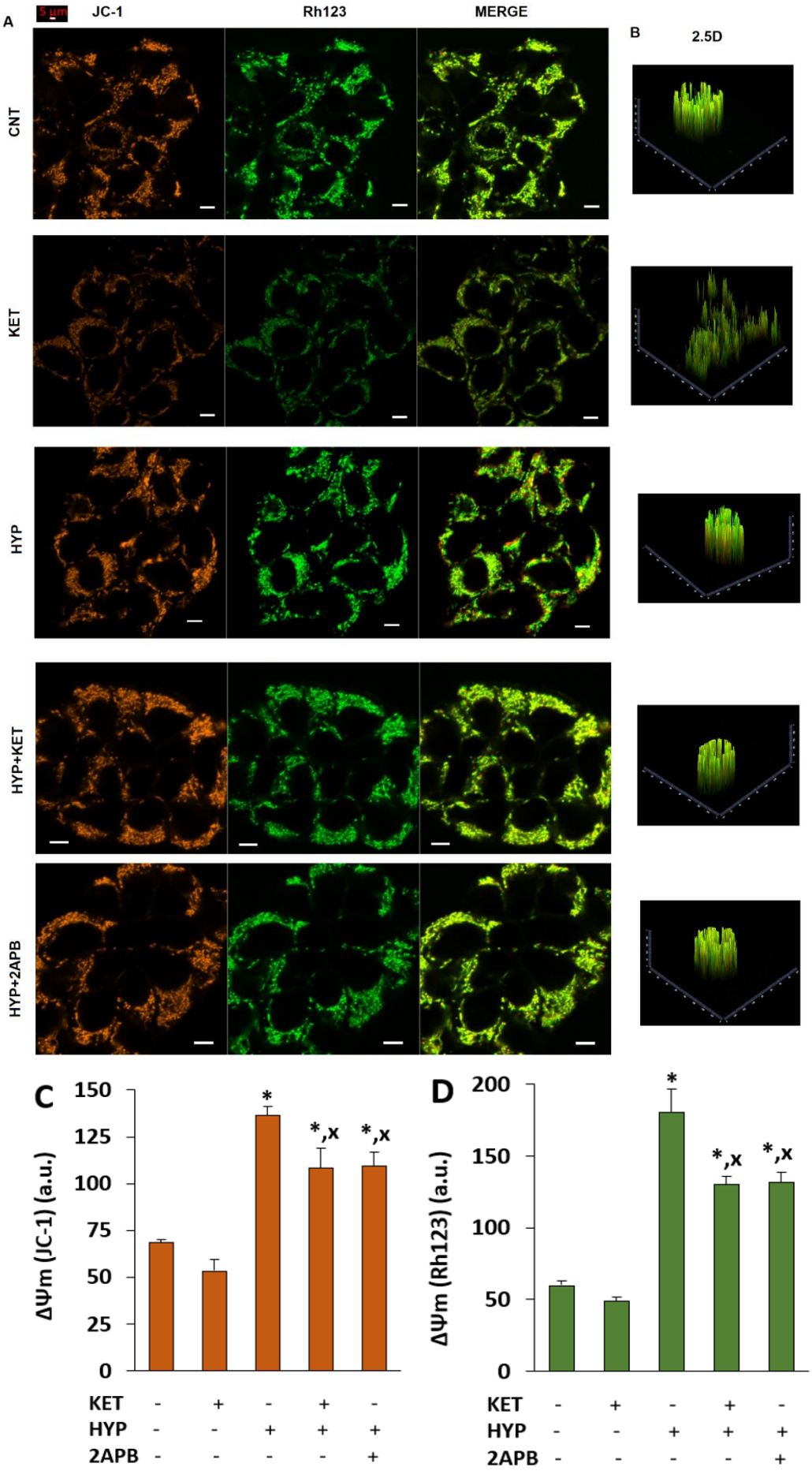


Fig. 2. The incubation of KET of (0.3 mM for 24h) modulated HYP (200 µM $CoCl_2$ for 24h)-caused the increase of mitochondrial membrane potential (ΔΨm) in the SH-SY5Y cells. *(Mean ± SD). The JC-1 and Rh123 are fluorescent stains of ΔΨm.* ***The*** *SH-SY5Y cells were stained with JC-1 and DHR123 (5 µM for 15-20 min). After washing the cells with extracellular buffer, their orange (JC-1) and green (Rh123) images were captured in the LSM-800 with 40x oil objective.* ***A.*** *The JC-1, Rh123, and their merge images in the five groups [control (CNT), KET, HYP, HYP+KET, and HYP+2APB).* ***B****. 2.5D images of JC-1 of Rh123.* ***C****. and* ***D.*** *The mean fluorescence intensity changes of JC-1 and Rh123 as arbitrary unit (a.u.) in the five groups, respectively. The scale bar in the images was kept as 5 µm. (*$^{*}p \leq 0.05$ *vs the groups of CNT and KET.* $^{x}p \leq 0.05$ *vs the group of HYP).*

the SH-SY5Y cells. Hence, we investigated the protective role of KET on the increase of HYP-induced $[Ca^{2+}]_i$ increase via the TRPM2 activation in the SH-SY5Y cells.

The green images of fluorescence dye (Fluo 3/AM) in four groups (CNT, KET, HYP, and HTYP+KET) are shown in the Fig. 1A, whereas the mean values of fluorescence intensities were shown by columns after the stimulation of H_2O_2 (Fig. 1B) and 2APB (Fig. 1C). The Fluo 3/AM intensity changes were increased in the HYP groups as compared to the CNT and KET groups ($p \leq 0.05$) (Fig. 1B). However, the changes were decreased in the HYP+KET group as compared to the HYP group only ($p \leq 0.05$). Hence, we observed that the HYP-mediated increase of $[Ca^{2+}]_i$ via the TRPM2 activation was decreased in the SH-SY5Y by the treatment of KET.

HYP-caused productions of mROS, ΔΨm, and iROS productions were decreased in the SH-SY5Y cells by the incubations of KET and TRPM2 antagonist (2APB)

The orange (JC-1), green (Rh123), merge (Fig. 2A), and 2.5D (Fig. 2B) images were captured in the LSM-800 laser scan confocal microscope. The images of red (mROS) and green (iROS), (Fig. 3A), and 2.5D (Fig. 3B) were recorded in the LSCM/800 by using a ZEN program. The mean fluorescence intensity changes of JC-1 (Fig. 2C), Rh123 (Fig. 2D), mROS (Fig. 3C), and iROS (Fig. 3D) were upregulated in the cells by the effect of $CoCl_2$ treatment ($p \leq 0.05$). However, KET and TRPM2 channel blocker (2APB) incubations reduced the effect of HYP via inhibition of mROS, ΔΨm, and iROS generations in the SH-SY5Y ($p \leq 0.05$).

Whereas the mROS, ΔΨm, and iROS production increases were downregulated by the KET and 2APB treatments, they still correlated with the rise in $[Ca^{2+}]_i$ after $CoCl_2$ incubation. Thus, the findings of mROS, ΔΨm, and iROS further demonstrated the impact of KET and $CoCl_2$ on oxidative stress in SH-SY5Y caused by TRPM2 stimulation.

The upregulation of HYP-induced cell death (PI positive cell number) was diminished by the KET treatments

We recorded bright filed (BF) (Fig. 4A) and color (Fig. 4B) images in the CNT, KET, HYP, HYP+KET, and HYP+2APB groups of SH-SY5Y cells. The images of red (PI), blue (Hoechst) (Fig. 4B), and their 2.5D merge (Fig. 4C), and bright field (BF) (Fig. 4A) were saved in the LSCM/800 by using the ZEN program. In the PI/Hoechst images, the PI positive (death rate) SH-SY5Y numbers in the group of HYP was higher compared to the CNT and KET ($p \leq 0.05$), whereas the numbers were significantly ($p \leq 0.05$) lower in the HYP+KET and HYP+2APB compared to the HYP only (Fig. 3D). The current findings supported TRPM2 stimulation-mediated SH-SY5Y cell death caused by HYP. Nevertheless, the KET incubation induced a protective effect against the SH-SY5Y cell death number increase caused by HYP.

Discussion

A state of insufficient oxygen supply in neuronal cells is HYP. An essential cause for the high incidence rate of death and morbidity related to anesthesia is HYP (Han et al. 2020). Ischemia-reperfusion-caused HYP induces neuronal injury via the activation several factors, including the TRPM2 activation (Akyuva and Nazıroğlu 2020). In turn, the HYP-induced excessive Ca^{2+} influxes via the increase of mitochondrial membrane depolarization cause excessive iROS and mROS generations. Hence, the iROS and mROS generations also play a vital role in HYP-mediated neuronal inflammation and injury (Burchell et al. 2013; Armağan and Nazıroğlu 2021). An upregulation in iROS and mROS accumulation under hypoxic conditions leads to neuronal apoptosis and death via the activation of TRPM2 (Yıldızhan and Nazıroğlu 2023). The treatment of KET via the inhibition of TRPM2 modulates the oxidative neuronal injury and death, although conflicting information is also present (Zhao et al. 2008; Demirdaş et al. 2017; Chen et al. 2020; Zhou et al. 2020). In the current study, the protective action of KET via the modulation of TRPM2 was tested on the oxidative injury and death in the SH-SY5Y neuronal cells. I observed that the HYP-mediated increase of oxidative neuronal injury via the inhibition of TRPM2 was decreased in the neuronal cells.

The TRPM2 channel is activated by oxidative stress (Hara et al. 2002; Nazıroğlu and Lückhoff 2008). HYP is characterized by the increase of iROS and mROS, although the treatment of KET decreases HYP-induced oxidative stress (Zhou et al. 2020). KET is an antagonist of glutamate receptors, including the NMDA receptors (Gascoigne et al. 2022). It was reported that the treatment of KET also modulated the oxidative stress via the inhibition of TRPM2 (Demirdaş et al. 2017). It is reported to be an antioxidant anesthetic that suppresses oxidative stress, cellular dysfunction, and apoptosis (Demirdaş et al. 2017). In the

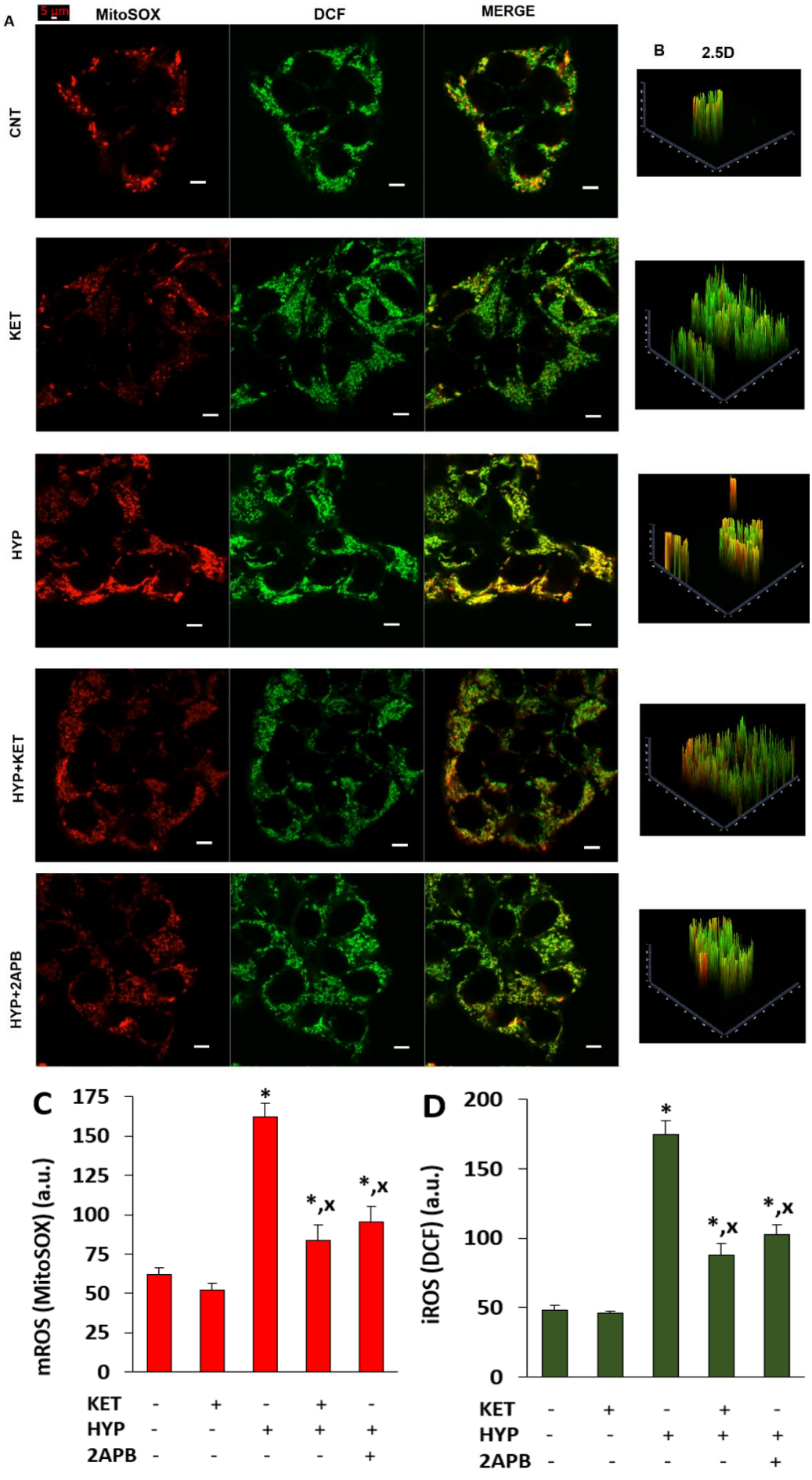
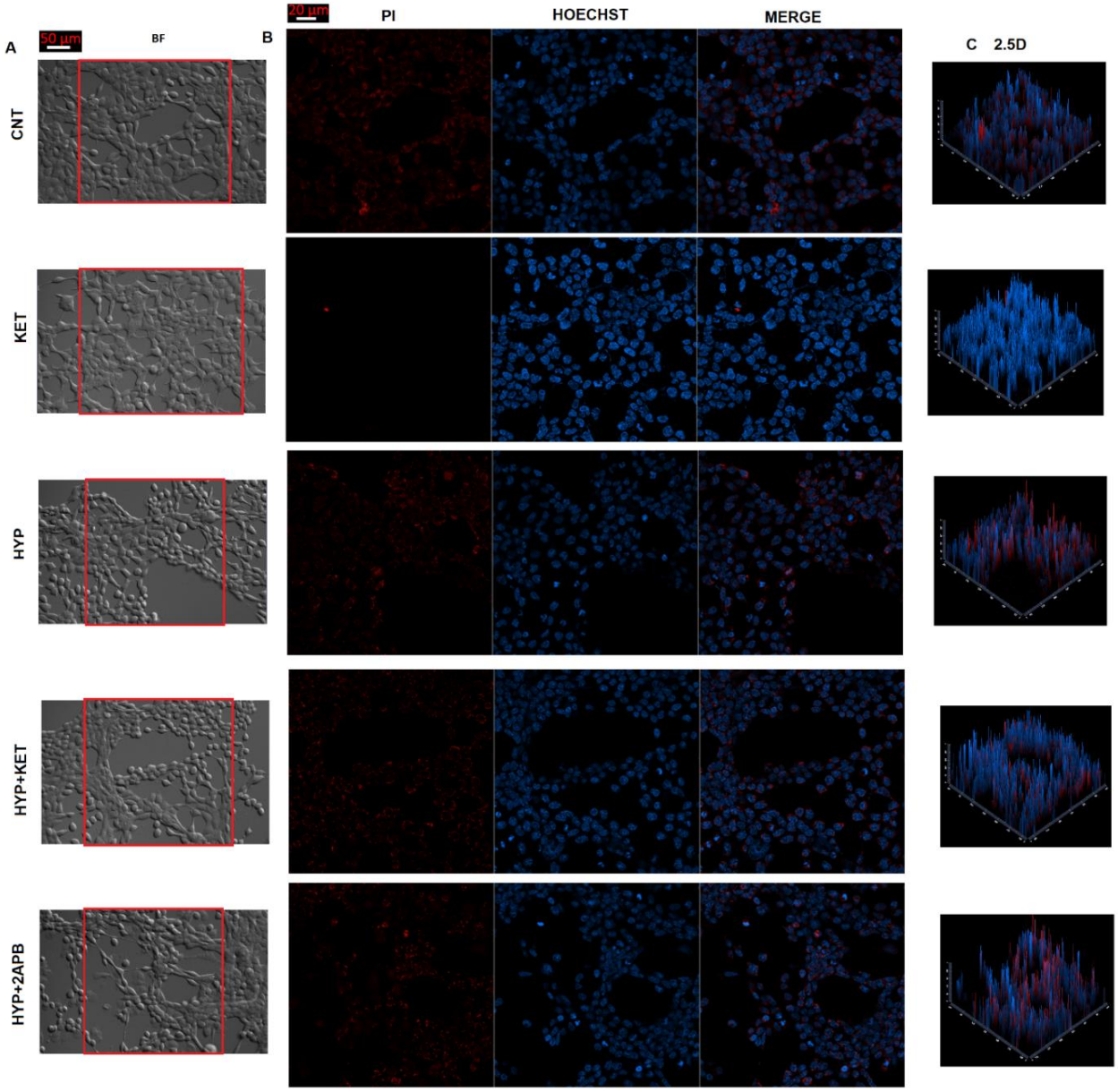


Fig. 3. The incubation of KET of (0.3 mM for 24h) modulated HYP (200 µM $CoCl_2$ for 24h)-induced the increase of mROS (MitoSOX) and iROS (DCF) in the SH-SY5Y cells. *(Mean ± SD). The MitoSOX and DCF are fluorescent stains of* ***mROS and iROS, respectively****. The SH-SY5Y cells were stained with MitoSOX and DCFH-DA (2 µM for 15-20min). After washing the cells with extracellular buffer, their red (MitoSOX) and green (DCF) images were captured in the microscope of LSM-800 with 40x oil objective.* ***A.*** *The MitoSOX, DCF, and their merge images in the five groups [control (CNT), KET, HYP, HYP+KET, and HYP+2APB).* ***B.*** *2.5D images of MitoSOX of DCF.* ***C.*** *and* ***D.*** *The mean fluorescence intensity changes (a.u.) of MitoSOX and DCF in the five groups, respectively. The scale bar: 5 µm. (*$p \leq 0.05$ vs the groups of CNT and KET. $^{x}p \leq 0.05$ vs the group of HYP).*



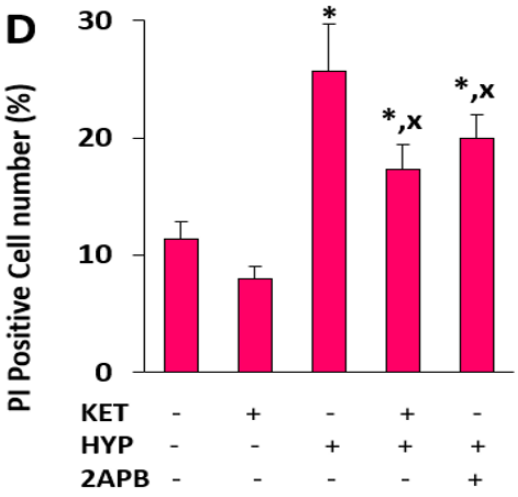


Fig. 4. The incubation of KET of (0.3 mM for 24h) modulated HYP (200 µM $CoCl_2$ for 24h)-induced the increase of PI positive SH-SY5Y cell number. *(Mean ± SD). The SH-SY5Y cells were stained with PI (8 µM for 15-20min) and Hoechst (5 µM for 15-20min). After washing the cells with extracellular buffer, their bright filed (BF) (**A**), red (PI), blue (Hoechst), merge (**B**), and 2.5D (**C**) images were captured in the microscope of LSM-800 with 40x oil objective. **D.** The mean percentage changes of PI positive cell number in the five groups, respectively. (*$p \leq 0.05$ vs the groups of CNT and KET. $^{x}p \leq 0.05$ vs the group of HYP).*

traumatic brain injury-induced mice, KET was found to elicit neuroprotective actions by inhibiting iROS (Liang et al. 2018). Additionally, the HYP-induced apoptotic pathways in fetal ovine kidneys was decreased by the treatment of KET (Chang et al. 2016). However, few studies on the antioxidative effect of ketamine on vascular endothelial cells exist. My findings indicated that KET decreased hypoxia-induced Ca^{2+} influx, iROS, and mROS via the downregulation of TRPM2 in the in SH-SY5Y. In accordance with the present data, KET has been reported to inhibit various L-type Ca^{2+} currents (Luo et al. 2015). Hara et al (1998) reported that KET dose-dependently blocked Ca^{2+} currents in guinea pig ventricular myocytes.

In hypoxia, the generations of iROS and mROS in neuronal cells are increased by the increase of mitochondrial Ca^{2+} (Wu et al. 2017; Chang et al. 2019). The TRPM2 stimulator actions of HYP were reported in the SH-SY5Y cells (Akyuva et al. 2021; Nazıroğlu 2022; Yıldızhan and Nazıroğlu 2023). In turn, the actions cause the increases of ΔΨm, resulting the upregulations of the oxidant (iROS and mROS) and cell death markers (Akyuva et al. 2021; Nazıroğlu 2022; Yıldızhan and Nazıroğlu 2023). However, the inhibition of TRPM2 (excessive Ca^{2+} influx) decreases the iROS and mROS generations, and cell death (PI positive cell number) in the neuronal cells, including the SH-SY5Y (Sha'fie 2022; Yıldızhan and Nazıroğlu 2020; Akyuva et al. 2021). According to the available data, HYP-induced TRPM2 activation (via overload Ca^{2+} influx) caused the upregulation of ΔΨm in SH-SY5Y, which in turn caused the upregulation of iROS, mROS, and cell death (PI positive cell number).

As a result of the downregulation of TRPM2 stimulation-induced neuronal injury, SH-SY5Y cells were protected against the HYP-mediated cell death and oxidative mediators by the incubation of KET through the attenuation of TRPM2. Although KET treatment reduces HYP-induced iROS and mROS-mediated neuronal injury and death, the activation of TRPM2-mediated excessive Ca^{2+} influx, neuronal death, and oxidant mediators may be considered as a potential source of HYP-induced oxidative injury.

Acknowledgements

The authors wish to thanks technician Hulusi Gül (BSN Health, Isparta, Turkiye) for helping laser confocal microscope analyses. The cell culture and laser confocal microscope analyses in the present study were performed in the BSN Health, Analyses, Innov., Consult., Org., Agricul., Trade Ltd, Isparta, Turkiye by H.Ö.O.

Author contributions

HÖO conceived and designed the study, and he also prepared figures and images, and he revised the manuscript and her performed laser confocal microscope analyses and cell culture.

Funding

This study was carried out with financial support from BSN Health, Analyses, Innov., Consult., Org., Agricul., Trade Ltd, Göller Bölgesi Teknokenti, Isparta, Turkey (Project No: 2019-04). The owner of the project is Dr. Haci Ömer Osmanlıoğlu.

Declarations

Competing Interests No relevant financial or non-financial interests to disclose.

Ethical Approve No data of human, live vertebrates, and higher invertebrates.

ORCID

Haci Ömer Osmanlıoğlu 0000-0002-8622-6072

References

Armağan HH, Nazıroğlu M. (2021). Curcumin attenuates hypoxia-Induced oxidative neurotoxicity, apoptosis, calcium, and zinc ion influxes in a neuronal cell line: Involvement of TRPM2 channel. Neurotox Res. 39(3):618-633. https://doi.org/10.1007/s12640-020-00314-w.

Bracke F, Frieling H, Leffler A. (2022) Antidepressants Are Poor Inhibitors of Heat-Evoked Ion Currents Mediated by TRPM2. Pharmacology. 107(9-10):472-479. https://doi.org/10.1159/000524934.

Burchell SR, Dixon BJ, Tang J, Zhang JH. (2013) Isoflurane provides neuroprotection in neonatal hypoxic ischemic brain injury. J Investig Med. 61(7):1078-1083. https://doi.org/10.2310/JIM.0b013e3182a07921.

Chang EI, Zárate MA, Rabaglino MB, Richards EM, Keller-Wood M, Wood CE. (2016) Ketamine suppresses hypoxia-induced inflammatory responses in the late-gestation ovine fetal kidney cortex. J Physiol. 594(5):1295-310. https://doi.org/10.1113/JP271066.

Chang JC, Lien CF, Lee WS, Chang HR, Hsu YC, Luo YP, Jeng JR, Hsieh JC, Yang KT. (2019) Intermittent Hypoxia Prevents Myocardial Mitochondrial Ca^{2+} Overload and Cell Death during Ischemia/Reperfusion: The Role of Reactive Oxygen Species. Cells. 8(6):564. https://doi.org/10.3390/cells8060564.

Chen SJ, Zhang W, Tong Q, Conrad K, Hirschler-Laszkiewicz I, Bayerl M, Kim JK, Cheung JY, Miller BA. (2013) Role of TRPM2 in cell

proliferation and susceptibility to oxidative stress. Am J Physiol Cell Physiol. 304(6):C548-60. https://doi.org/10.1152/ajpcell.00069.2012.

Chen Y, Yang Z, Wei L, Wang J, Xuan W, Wang Y, Li J, Ke Z, Li Y. (2020) Yes-associated protein protects and rescues SH-SY5Y cells from ketamine-induced apoptosis. Mol Med Rep. 22(3):2342-2350. https://doi.org/10.3892/mmr.2020.11328.

Demirdaş A, Nazıroğlu M, Övey IS. (2017) Short-Term Ketamine Treatment Decreases Oxidative Stress Without Influencing TRPM2 and TRPV1 Channel Gating in the Hippocampus and Dorsal Root Ganglion of Rats. Cell Mol Neurobiol. 37(1):133-144. https://doi.org/10.1007/s10571-016-0353-4.

Gascoigne DA, Minhaj MM, Aksenov DP. (2022) Neonatal Anesthesia and Oxidative Stress. Antioxidants (Basel). 11(4):787. https://doi.org/10.3390/antiox11040787.

Gu Q, Kanungo J. (2021) Effect of ketamine on gene expression in zebrafish embryos. J Appl Toxicol. 41(12):2083-2089. https://doi.org/10.1002/jat.4199.

Halliwell B. (1992) Reactive oxygen species and the central nervous system. J Neurochem. 59(5):1609-23. https://doi.org/10.1111/j.1471-4159.1992.tb10990.x.

Han Y, Zhang W, Liu J, Song Y, Liu T, Li Z, et al. (2020) Metabolomic and Lipidomic Profiling of Preoperative CSF in Elderly Hip Fracture Patients With Postoperative Delirium. Front Aging Neurosci. 12:570210. https://doi.org/10.3389/fnagi.2020.570210.

Hara Y, Chugun A, Nakaya H, Kondo H. Tonic block of the sodium and calcium currents by ketamine in isolated guinea pig ventricular myocytes. J Vet Med Sci 1998; 60: 479–83.

Hara Y, Wakamori M, Ishii M, Maeno E, Nishida M, Yoshida T, et al. (2002) LTRPC2 Ca2+-permeable channel activated by changes in redox status confers susceptibility to cell death. Mol Cell. 9(1):163-173. https://doi.org/10.1016/S1097-2765(01)00438-5.

Kounis NG, Koniari I, Tzanis G, Soufras GD, Velissaris D, Hahalis G. (2020) Anaphylaxis-induced atrial fibrillation and anesthesia: Pathophysiologic and therapeutic considerations. Ann Card Anaesth. 23(1):1-6. https://doi.org/10.4103/aca.ACA_100_19.

Kumar VS, Gopalakrishnan A, Naziroğlu M, Rajanikant GK. (2014) Calcium ion--the key player in cerebral ischemia. Curr Med Chem. 21(18):2065-2075. https://doi.org/10.2174/09298673216613122820424 6.

Liang J, Wu S, Xie W, He H. (2018) Ketamine ameliorates oxidative stress-induced apoptosis in experimental traumatic brain injury via the Nrf2 pathway. Drug Des Devel Ther. 12:845-853. https://doi.org/10.2147/DDDT.S160046.

Luo AT, Cao ZZ, Xiang Y, Zhang S, Qian CP, Fu C, Zhang PH, Ma JH. (2015) Ketamine attenuates the Na+-dependent Ca2+ overload in rabbit ventricular myocytes in vitro by inhibiting late Na+ and L-type Ca2+ currents. Acta Pharmacol Sin. 36(11):1327-1336. https://doi.org/10.1038/aps.2015.75.

Muñoz-Sánchez J, Chánez-Cárdenas ME. (2019) The use of cobalt chloride as a chemical hypoxia model. J Appl Toxicol. 39(4):556-570. https://doi.org/10.1002/jat.3749.

Nazıroğlu M, Lückhoff A. (2008) Effects of antioxidants on calcium influx through TRPM2 channels in transfected cells activated by hydrogen peroxide. J Neurol Sci. 270(1-2):152-158. https://doi.org/10.1016/j.jns.2008.03.003.

Nazıroğlu M. (2007) New molecular mechanisms on the activation of TRPM2 channels by oxidative stress and ADP-ribose. Neurochem Res. 32(11):1990-2001. https://doi.org/10.1007/s11064-007-9386-x.

Pereira JEG, El Dib R, Braz LG, Escudero J, Hayes J, Johnston BC. (2019) N-acetylcysteine use among patients undergoing cardiac surgery: A systematic review and meta-analysis of randomized trials. PLoS One. 14(5):e0213862. https://doi.org/10.1371/journal.pone.0213862.

Perelman A, Wachtel C, Cohen M, Haupt S, Shapiro H, Tzur A. (2012) JC-1: alternative excitation wavelengths facilitate mitochondrial membrane potential cytometry. Cell Death Dis. 3(11):e430. https://doi.org/10.1038/cddis.2012.171.

Perraud AL, Fleig A, Dunn CA, Bagley LA, Launay P, Schmitz C, et al. (2001). ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. Nature. 411(6837):595-9.

Sha'fie MSA, Rathakrishnan S, Hazanol IN, et al. (2020) Ethanol Induces Microglial Cell Death via the NOX/ROS/PARP/TRPM2 Signalling Pathway. Antioxidants (Basel). 9(12):1253. https://doi.org/10.3390/antiox9121253.

Ulbrich F, Eisert L, Buerkle H, Goebel U, Schallner N. (2016) Propofol, but not ketamine or midazolam, exerts neuroprotection after ischaemic injury by inhibition of Toll-like receptor 4 and nuclear factor kappa-light-chain-enhancer of activated B-cell signalling: A combined in vitro and animal study. Eur J Anaesthesiol. 33(9):670-80. https://doi.org/10.1097/EJA.0000000000000449.

Vaglienti MV, Subirada PV, Barcelona PF, Bonacci G, Sanchez MC. 2022. Quantification of Reactive Oxygen Species Using 2',7'-Dichlorofluorescein Diacetate Probe and Flow-Cytometry in Müller Glial Cells. J Vis Exp. (183). https://doi.org/10.3791/63337.

Wu QF, Qian C, Zhao N, Dong Q, Li J, Wang BB, Chen L, Yu L, Han B, Du YM, Liao YH. (2017) Activation of transient receptor potential vanilloid 4 involves in hypoxia/reoxygenation injury in cardiomyocytes. Cell Death Dis. 8(5):e2828. https://doi.org/10.1038/cddis.2017.227.

Yıldızhan K, Nazıroğlu M. (2020) Glutathione Depletion and Parkinsonian Neurotoxin MPP^+-Induced TRPM2 Channel Activation Play Central Roles in Oxidative Cytotoxicity and Inflammation in Microglia. Mol Neurobiol. 57(8): 3508-3525. https://doi.org/10.1007/s12035-020-01974-7.

Yıldızhan K, Nazıroğlu M. (2023) NMDA Receptor Activation Stimulates Hypoxia-Induced TRPM2 Channel Activation, Mitochondrial Oxidative Stress, and Apoptosis in Neuronal Cell Line: Modular Role of Memantine. Brain Res. 1803:148232. https://doi.org/10.1016/j.brainres.2023.148232.

Zhang X, Wang C, Xu H, Cai S, Liu K, Li S, Chen L, Shen S, Gu X, Tang J, Xia Z, Hu Z, Ma X, Zhang L. (2023) Propofol inhibits myocardial injury induced by microvesicles derived from hypoxia-reoxygenated endothelial cells via lncCCT4-2/CCT4 signaling. Biol Res. 56(1):20. https://doi.org/10.1186/s40659-023-00428-3.

Zhao J, Wu HW, Chen YJ, Tian HP, Li LX, Han X, Guo J. (2008) Protein phosphatase 2A-negative regulation of the protective signaling pathway of Ca2+/CaM-dependent ERK activation in cerebral ischemia. J Neurosci Res. 86(12):2733-2745.

Zhou X, Liu J, Yang S, Su Y, Meng Z, Hu Y. (2020) Ketamine ameliorates hypoxia-induced endothelial injury in human umbilical vein endothelial cells. Clinics (Sao Paulo). 75:e1865. https://doi.org/10.6061/clinics/2020/e1865.