Journal of The Cardiovascular Thoracic Anaesthesia and Intensive Care Society

GKDA Derg 2025;31(4):174-182
Doi: 10.14744/GKDAD.2025.46873

EXPERIMENTAL RESEARCH

The Effects of Dexmedetomidine on Injury Induced by Oxygen and Glucose Deprivation/Reperfusion in a Neuroblastoma Cell Line of Mouse Origin

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ABSTRACT

Objectives: The aim of this study was to investigate the preliminary effects of different doses of dexmedetomidine (DEX) on cell viability and neurite growth, as well as the effects of pre- or post-treatment with DEX on oxygen and glucose deprivation/reoxygenation (OGD/R)-induced apoptosis and inflammation in mouse neuroblastoma (NB2a) cells.

Methods: The effects of DEX on cell viability and toxicity were explored using the MTT and NNT methods at DEX concentrations of 0.1, 1, 3, 10, 30, 45, and 60 µM. To investigate the effects of DEX (10 µM) on OGD/R-induced apoptosis and inflammation, four experimental groups were established: Control group (C), in which cells did not undergo the OGD/R procedure; OGD/R group, in which cells were not treated with DEX; DEX+OGD/R group, in which cells were treated with DEX (10 µM) before the OGD/R procedure; and OGD/R+DEX group, in which cells were treated with DEX (10 µM) after the OGD/R procedure. Data were analyzed using one-way ANOVA (post hoc test: Tukey-b) and the Kruskal–Wallis test. Data were presented as mean±SD. P<0.05 was considered statistically significant.

Results: Dexmedetomidine increased cell viability at all concentrations (0.1–45 µM) except 60 µM. H-scores for eNOS and TGFβ antibodies in the DEX+OGD/R and OGD/R+DEX groups were significantly decreased compared with the OGD/R group following treatment with DEX 10 µM (p<0.001). The apoptotic index in the DEX+OGD/R and OGD/R+DEX groups was also significantly decreased compared with the OGD/R group (p<0.001).

Conclusion: Dexmedetomidine increased cell viability at almost all concentrations (0.1–45 µM) except 60 µM. Treatment with DEX 10 µM, either before or after injury, reduced apoptosis induced by OGD/R injury in NB2a cells. Pre-injury treatment with DEX also decreased both iNOS and eNOS antibody expression. The neuroprotective effects of DEX against OGD/R injury may be attributed to its antiapoptotic and antioxidative effects.

Keywords: Apopitosis; dexmedetomidine; IL-6; NB2a; OGD/R

Please cite this article as: "Öztürk T, Darıverenli E, Türköz Uluer E, Önal T, Tatlı DG, Vural K. The Effects of Dexmedetomidine on Injury Induced by Oxygen and Glucose Deprivation/Reperfusion in a Neuroblastoma Cell Line of Mouse Origin. GKDA Derg 2025;31(4):174-182".

Introduction

Dexmedetomidine (DEX) is a powerful and highly specific alpha-2 adrenergic receptor agonist that acts centrally to produce sympatholytic, sedative, and analgesic effects. These receptors are widespread in human brain cells.[1] The sedative effect of DEX is related to the concentration of the drug in the blood. At plasma concentrations between 0.2 and 0.3 ng/mL, rousable sedation occurs, whereas at concentrations above 1.9 ng/mL, unarousable

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"The effects of dexmedetomidine on oxygen-glucose deficiency/reoxygenation-induced damage in mouse neuroblastoma cells" study was presented orally at the 37th European Association of Cardiothoracic Anaesthesiology and Intensive Care Annual Congress (37th EACTAIC Annual Congress 2022, Naples, Italy) and was published only as an abstract in the Journal of Cardiothoracic and Vascular Anesthesia, 36 (2022), S1–S18, S15 (Appendix 3. Printed abstract manuscript).

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Submitted: December 12, 2025 **Revised:** December 22, 2023 **Accepted:** December 23, 2023 **Available Online:** December 29, 2025

The Cardiovascular Thoracic Anaesthesia and Intensive Care - Available online at www.gkdaybd.org

sedation can occur.[2] Animal and experimental studies have demonstrated the neuroprotective effects of DEX.[3–11] Meta-analyses including randomized controlled trials have shown that dexmedetomidine decreases the incidence of postoperative neurological adverse effects in intensive care units.[12–17]

Ischemia/reperfusion injury and inflammatory responses lead to neurological and cognitive problems.[18,19] The pathophysiology of I/R injury involves calcium accumulation, free oxygen radicals, inflammation-triggered signaling pathways, and apoptosis.[20,21]

The aim of this study was to investigate the preliminary effects of different doses of dexmedetomidine (DEX) on cell viability and neurite growth, as well as the effects of pre- or post-treatment with DEX on oxygen and glucose deprivation/reoxygenation (OGD/R)-induced apoptosis and inflammation in mouse neuroblastoma (NB2a) cells.

Methods

This study, conducted in the Cell Culture Laboratory of the Department of Pharmacology, Faculty of Medicine, Manisa Celal Bayar University, Manisa, Türkiye, was approved by the Ethics Committee of Health Sciences, Faculty of Medicine, Manisa Celal Bayar University (date: May 21, 2021; approval No: 20.478.486/808) and was conducted in accordance with the principles of the Declaration of Helsinki.

Preparation of Neuroblastoma Cell Culture

The neuroblastoma cell line (NB2a, 89121404) was purchased from the European Collection of Authenticated Cell Cultures. The NB2a cell line was cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM High Glucose, Capricorn) supplemented with 5% fetal bovine serum (FBS), 5% horse serum, 100 IU/mL penicillin, 100 IU/mL streptomycin (Capricorn), and 200 mM L-glutamine (K0282, Biochrom, Berlin, Germany) at 37°C with 5% CO_2. The procedures for cell passaging, freezing, and cell counting were performed according to the routine of our laboratory.[22]

Establishment of OGD/R Model *in Vitro*

After being washed with high-glucose-containing DMEM and incubated in a normoxic incubator for 4 hours, mouse neuroblastoma cells were subjected to oxygen and glucose deprivation by replacing the normal cell culture medium with glucose-free DMEM (Sartorius) and incubating them in a hypoxic chamber (Billups-Rothberg Inc., Del Mar, CA, USA; Forma Scientific, USA) containing humidified 95% N2 and 5% CO_2 at a flow rate of 10 L/min for 4 hours at 37°C. Subsequently, the cells were incubated under normoxic conditions at 37°C for 4 hours.

Preparation of Dexmedetomidine Concentrations

For the analysis of the determined concentrations of dexmedetomidine (Sedadomid® 200 mcg/2 mL, Koçak Farma İlaç ve Kimya Sanayi A.Ş., Tekirdağ) used in the study, NB2a cells were seeded into 24-well plates, and after 24 hours of incubation, fresh media containing different concentrations of dexmedetomidine were applied to the cells. The concentrations at which DEX would be applied to the NB2a cell line were determined by literature review and preliminary studies conducted prior to the study.[2,3] After 24 hours of incubation, the morphologies in the 24-well plates were examined under an inverted microscope, and DEX was applied to the designated wells at concentrations of 0.1, 1, 10, 30, 45, and 60 µM. Applications were performed in triplicate for each experimental and control group. Incubation continued for 24 hours.

Experimental Groups

The experimental design consisted of four groups according to the aim of the study:

Group C: Control cells (oxygen- and glucose-containing medium; OG) were washed with high-glucose-containing DMEM and incubated in a normoxic incubator for 4 hours.

Group OGD/R: Oxygen and glucose deprivation and reoxygenation (OGD/R) model.

Group DEX+OGD/R: Cells were treated with 10 µM DEX for 3 hours before OGD/R and then exposed to OGD/R.

Group OGD/R+DEX: Cells were treated with 10 µM DEX for 3 hours during the reperfusion period after OGD.

MTT Analysis (Cytotoxicity, Cell Viability Test)

Pure DMEM high-glucose medium was used as a negative control, and cells without DEX were used as a positive control. Neuroblastoma cell morphology and the effects of DEX were observed under a phase-contrast microscope at 10× magnification (Olympus, model IX73, Japan).

NB2a cells were seeded into 96-well culture plates at a density of 1×10^4 cells per well. After incubation at 37°C and 5% CO_2 for 24 hours, cells were exposed to increasing concentrations of DEX (0.1, 1, 10, 30, 45, and 60 µM) for 24 and 48 hours. Following incubation, the culture medium was removed, and 100 µL of fresh medium containing 10% MTT (M6494, Invitrogen, USA) was added to each well. The plates were incubated for 4 hours. Subsequently, the MTT-containing medium was removed, and 100 µL of dimethyl sulfoxide (DMSO; Santa Cruz, USA) was added to each well. Plates were kept at room temperature for 10 minutes. The IC50 concentrations of DEX were determined by measuring absorbance at 570 nm using a BioTek ELx800 device.

Measurement of Neurite Growth

To measure neurite growth, NB2a cells were seeded into 24-well culture plates at a density of 15,000 cells/mL in proliferation medium. After 24 hours, cells were induced to differentiate and form neurites at different concentrations of DEX (1, 10, 30, 45, and 60 µM) using the following procedure: the culture medium was replaced with serum-free medium containing 0.5 mM dibutyryl cyclic AMP. Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) at room temperature for 30 minutes, stained with 0.6% Coomassie Blue (prepared in 10% [v/v] acetic acid, 10% [v/v] methanol, and 80% [v/v] PBS), and washed with PBS. Three blinded observers photographed the samples using an Olympus BX-40 light microscope (Olympus, Tokyo, Japan) equipped with a video camera (JVC-TK-C 601, Tokyo, Japan). Approximately 10 different fields containing about 10 cells each were selected for the drug and control groups. Total neurite length was measured in pixels using image analysis software and expressed as the average neurite length per cell.

Immunocytochemical Analysis

Immunocytochemical analysis was performed on NB2a cells. Cells were seeded onto 24-well culture plates containing 12 mm sterile round coverslips at a density of 5×10^4 cells/well and incubated at 37°C with 5% CO_2 for 24 hours. After treatments, the culture medium was removed, and cells were fixed with 4% paraformaldehyde for 30 minutes at room temperature. Endogenous peroxidase activity was inhibited using 3% hydrogen peroxide for 10 minutes. Cells were permeabilized with 0.1% Triton X-100 for 10 minutes on ice. After blocking for 10 minutes, primary antibodies against eNOS, iNOS, IL-6, and TGF-β were applied and incubated overnight at 4°C. The following day, a biotin–streptavidin horseradish peroxidase secondary antibody was applied for 10 minutes at room temperature. Diaminobenzidine was used for visualization. All washes were performed three times for 5 minutes with PBS. Negative control staining was used to confirm specificity. Hematoxylin was used for counterstaining, and samples were mounted. Immunoreactivity was evaluated using the semi-quantitative H-score method, with staining intensity graded as no staining (0), weak (1), moderate (2), or strong (3). Five random fields per group were analyzed, and the H-score was calculated as H-score=ΣPi(i+1), where Pi represents the percentage of positively stained cells at each intensity (i). Immunocytochemical results were expressed as mean±standard deviation.[22]

Terminal Deoxynucleotidyl Transferase-Biotin Nick End Labeling (TUNEL) Assay For Apoptosis

After treatments, apoptotic cells were detected using the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Millipore, S7101). Cells were fixed in 4% paraformaldehyde for 30 minutes, followed by incubation for 15 minutes at 4°C. After three washes with PBS, cells were treated with 20 µg/mL proteinase K diluted in PBS (1/500) for 15 minutes at 37°C. Following additional PBS washes and treatment with 3% hydrogen peroxide for 5 minutes at room temperature, cells were incubated with equilibration buffer for 5 minutes and then with TdT enzyme for 1 hour at 37°C. Samples were incubated with stop/wash buffer for 10 minutes at room temperature. After treatment with anti-digoxigenin peroxidase conjugate and PBS washing, cells were stained and counterstained with Mayer's hematoxylin. TUNEL-positive cells were examined and photographed using a Leica DM6000B microscope with a DC490 digital camera. The apoptotic index was defined as the ratio of TUNEL-positive cells to the total number of cells in selected areas. The percentage of apoptotic cells was also evaluated by a blinded observer and graded from 0 to ≥5 based on apoptotic cell percentage.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8 software. Data were presented as mean±standard error, and comparisons between groups were analyzed using one-way ANOVA followed by the Tukey B post hoc test. A value of $p<0.05$ was considered statistically significant.

Results

In this study, the effect of DEX on OGD/R injury was investigated in mouse neuroblastoma (NB2a) cells. For this purpose, the effects of different concentrations of DEX on cell viability and neurite outgrowth in an OG-containing medium were first examined. NB2a cells were cultured in high-glucose DMEM in flasks. Passaging was performed when cells reached 80–90% confluency. Observation under an inverted microscope showed that the cells proliferated as a monolayer and exhibited neuronal cell morphology.

Except for the 60µM concentration, dexmedetomidine increased cell viability at all tested concentrations (0.1–45µM) compared with the control group ($p<0.01$, Fig. 1). The inhibitory effects of different concentrations of DEX on the percentage of neurite growth were not significant compared with the control group in NB2a cells ($p>0.05$, Fig. 2).

Immunoreactivity results were evaluated in groups treated with DEX at a concentration of 10µM either before or after OGD/R.

eNOS Immunoreactivity

Immunocytochemical staining of eNOS was evaluated using the H-score method (Fig. 3a). eNOS immunoreactivity was similar in the control and OGD/R groups, with no significant difference between these

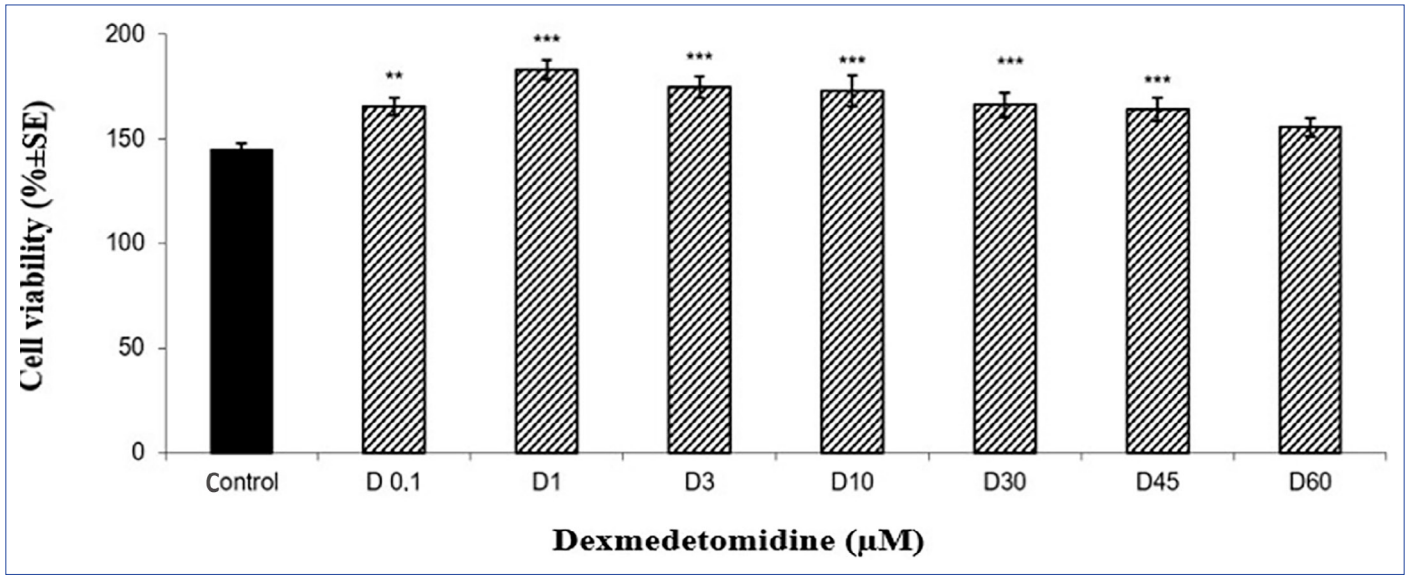

Figure 1. The effects of DEX at concentrations of 0.1-60 uM on cell viability and proliferation compared to the Control Group in NB2a cells.
***: p<0.01; **: p<0.03 according to Control Group. SE: Standart error.

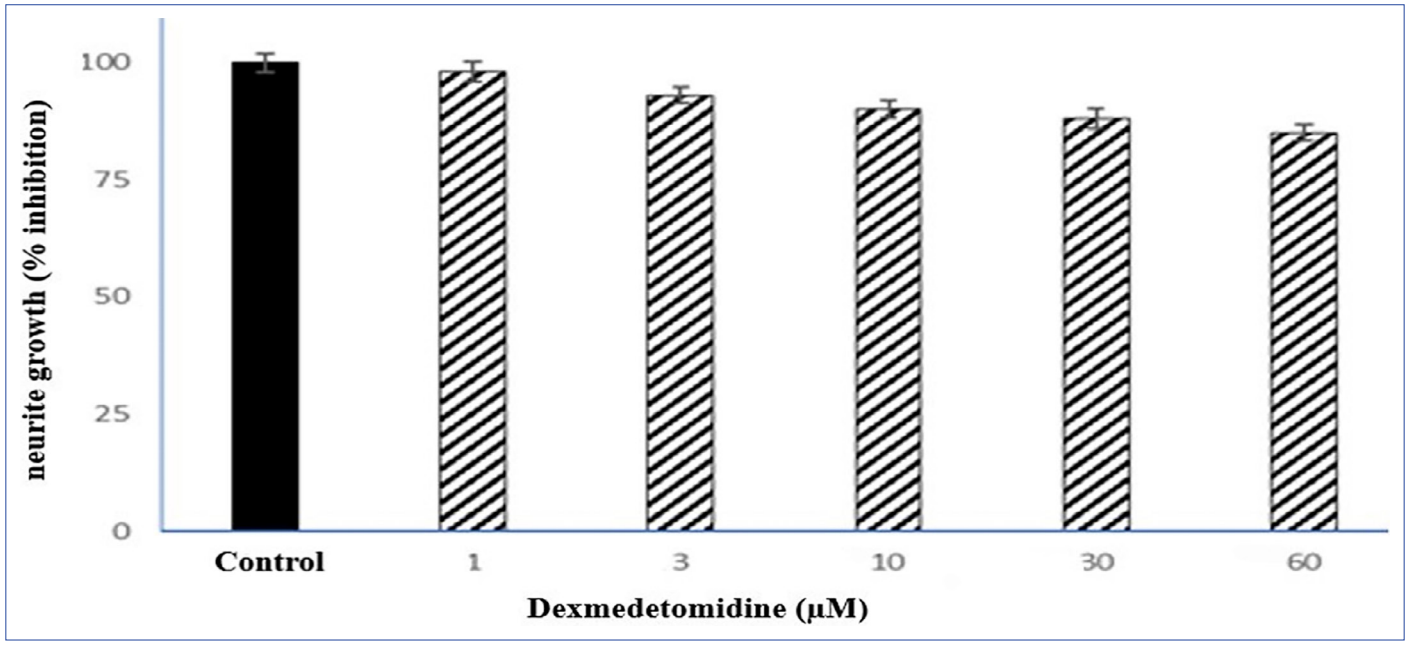

Figure 2. The effect of different concentrations of dexmedetomidine (DEX) on the percentage of neurite inhibition compared to the Control Group in nöroblastoma NB2a cells (p>0.05).

groups. eNOS immunoreactivity decreased significantly in the DEX+OGD/R and OGD/R+DEX groups compared with the OGD/R group (p<0.05). eNOS immunoreactivity was similar between the DEX+OGD/R and OGD/R+DEX groups, with no significant difference (p>0.05).

iNOS Immunoreactivity

Immunocytochemical staining of iNOS was evaluated using the H-score method (Fig. 3b). iNOS immunoreactivity was similar in the control and OGD/R groups, with no significant difference between these groups. iNOS immunoreactivity decreased significantly in the DEX+OGD/R group compared with the OGD/R group. In contrast, iNOS immunoreactivity in the OGD/R+DEX group was similar to that in the OGD/R group, with no significant difference (p>0.05). iNOS immunoreactivity was significantly lower in the DEX+OGD/R group than in the OGD/R+DEX group (p<0.05).

IL-6 Immunoreactivity

Immunocytochemical staining of IL-6 was evaluated using the H-score method (Fig. 3c). IL-6 immunoreactivity increased significantly in the OGD/R group compared with

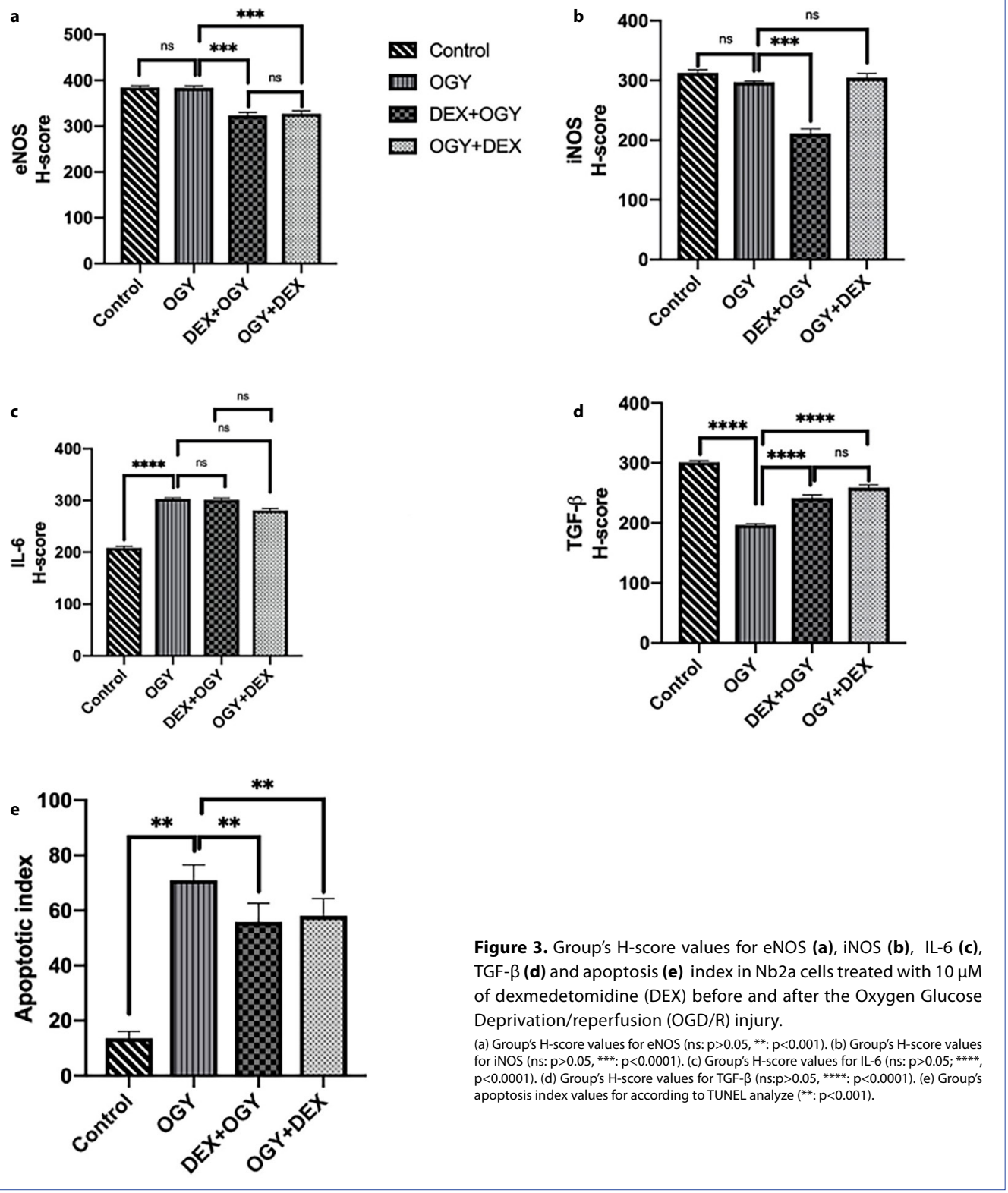

Figure 3. Group's H-score values for eNOS **(a)**, iNOS **(b)**, IL-6 **(c)**, TGF-β **(d)** and apoptosis **(e)** index in Nb2a cells treated with 10 μM of dexmedetomidine (DEX) before and after the Oxygen Glucose Deprivation/reperfusion (OGD/R) injury.

(a) Group's H-score values for eNOS (ns: p>0.05, **: p<0.001). (b) Group's H-score values for iNOS (ns: p>0.05, ***: p<0.0001). (c) Group's H-score values for IL-6 (ns: p>0.05; ****, p<0.0001). (d) Group's H-score values for TGF-β (ns:p>0.05, ****: p<0.0001). (e) Group's apoptosis index values for according to TUNEL analyze (**: p<0.001).

the control group (p<0.0001). There were no significant differences between the DEX+OGD/R and OGD/R+DEX groups compared with the OGD/R group (p>0.05). No significant difference was observed between the DEX+OGD/R and OGD/R+DEX groups (p>0.05).

TGF-β Immunoreactivity

Immunocytochemical staining of TGF-β was evaluated using the H-score method (Fig. 3d). TGF-β immunoreactivity was significantly higher in the control group compared

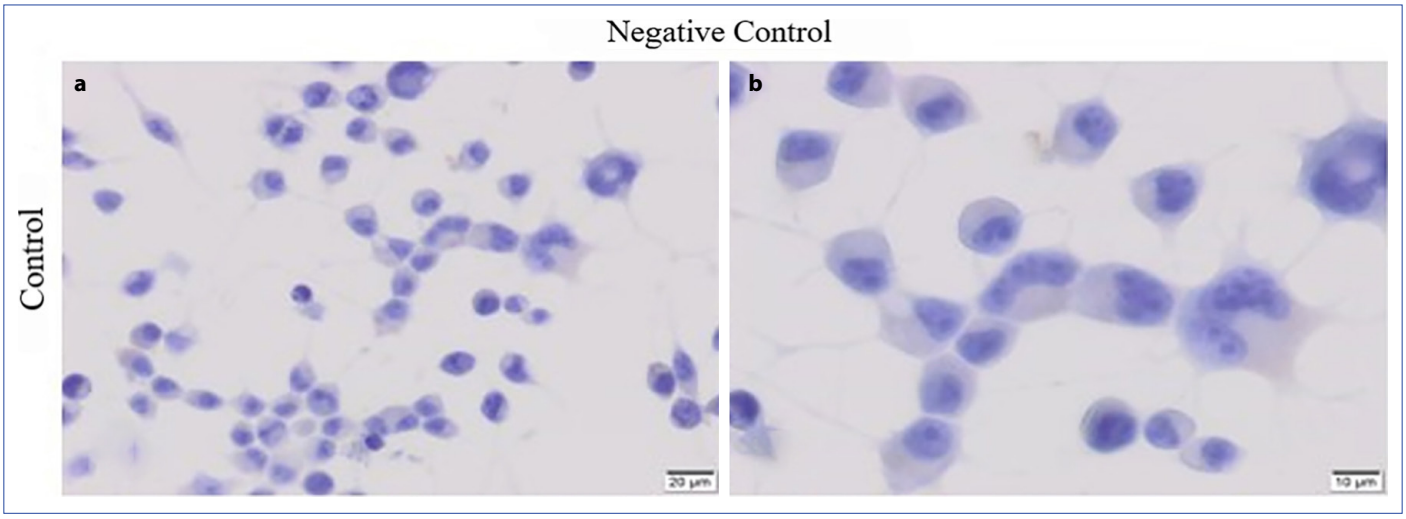

Figure 4. The immunocytochemical staining of negative controls in the NB2a cell line. No immunoreactivity was observed. **(a)** Lower magnification. (10X). **(b)** Higher magnification (40X)).

with the OGD/R group. TGF-β immunoreactivity was also significantly increased in the DEX+OGD/R and OGD/R+DEX groups compared with the OGD/R group ($p<0.0001$). There was no significant difference between the DEX+OGD/R and OGD/R+DEX groups ($p>0.05$).

Evaluation of Apoptosis

The TUNEL method was used to determine the number of apoptotic cells before and after DEX treatment. The number of TUNEL-positive cells increased significantly in the OGD/R group ($p<0.001$). Both pre-treatment and post-treatment with DEX significantly reduced the number of TUNEL-positive cells ($p<0.001$, Fig. 3e).

No immunoreactivity was observed in the immunocytochemical staining of negative controls in the NB2a cell line (Fig. 4).

Photographs of immunocytochemical stainings (eNOS, iNOS, IL-6, TGF-β, and TUNEL) were not provided.

Discussion

In this study, we investigated the effect of DEX on OGD/R-induced damage in mouse neuroblastoma (NB2a) cells. For this purpose, the effects of different concentrations of DEX on neurite outgrowth and cell viability in an OG-containing medium were first examined. All concentrations of DEX, except 60μM, increased cell viability. No significant inhibitory effect on neurite outgrowth was observed at any DEX concentration. Subsequently, DEX 10μM was evaluated in an OGD/R damage model in the cell line. Administration of DEX 10μM both before and after OGD/R reduced apoptosis caused by OGD/R injury. Pre-treatment with DEX 10μM statistically reduced both iNOS and eNOS levels.

In this study, when mouse NB2a neuroblastoma cells were treated with DEX at concentrations of 0.1, 1, 3, 10, 30, and 45μM in an OG-containing medium for 3 hours, cell viability (MTT) was significantly increased compared with the DEX-free control group. No change was observed at a concentration of 60μM. In contrast, Huang et al.[10] reported that when DEX at doses of 0.1, 1.0, 2.0, 5.0, and 10.0μM was applied for 48 hours, a much longer duration than in our study, in human SK-N-SH cells, no change in cell viability was observed compared with the DEX-free control group. However, 20.0μM DEX, a concentration twice as high as that used in our study, significantly inhibited cell viability. These findings indicate that the effects of DEX on cell viability may vary depending on the cell line, concentration, and duration of exposure.

It has also been reported that DEX prevents the inhibitory effects of OGD/R on cell viability and proliferation in different cell lines.[9–11] In an OGD medium, the viability and proliferation of mouse NB2a cells are significantly inhibited. As the concentration of DEX administered after OGD/R-induced injury increased (50, 100, and 500ng/mL), the inhibitory effects of OGD/R on cell viability and proliferation were more effectively reversed.[9] In contrast, treatment with dexmedetomidine (0.01–10μM) after OGD/R-induced injury did not cause a statistically significant change in cell viability. However, when DEX (1μM) was applied simultaneously with OGD/R, cell viability increased by approximately 68%.[11] Additionally, as the duration of OGD lengthened, the percentage of viable cells decreased in rat C6 glioma cells. During a 2–3-hour OGD period, the percentage of viable cells decreased from 81% to 49%.[11] On the other hand, in human SK-N-SH cells treated with 10μM DEX for 24–48 hours before OGD-induced injury, the inhibitory effects of OGD on cell viability, as assessed by

the CCK-8 assay, were prevented.[10] In the present study, cell viability and neurite inhibition in NB2a cells under the OGD/R model were not evaluated, which constitutes a limitation of this study. However, these changes were assessed under OG (oxygen+high glucose) conditions at different DEX concentrations.

In our study, administration of DEX at a concentration of 10μM, which was determined to be closest to the median ED50,[23] either before or after OGD/R reduced apoptosis induced by OGD/R. Similarly, Wang and Zu studied an OGD/R model in mouse N2A cell cultures using 100 and 500ng/mL DEX, concentrations lower than those used in our study. They administered DEX after OGD/R and reported, consistent with our findings, that DEX inhibited apoptosis caused by I/R injury by activating the p38 MAPK/ERK signaling pathway, downregulating TLR4, increasing the Bcl-2/Bax ratio, and inhibiting caspase-3 activity.[9] In another *in vitro* study, DEX was reported to protect rat C6 glioma cells against OGD/R-induced injury by activating the I2 imidazoline receptor–phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B (I2 imidazoline receptor–PI3K/AKT) pathway and upregulating hypoxia-inducible factor-1α, vascular endothelial growth factor, and RTP801 expression.[11] While DEX activates I2 receptors, it also activates the PI3K/AKT pathway, thereby inhibiting apoptosis caused by OGD/R-induced injury. I2 receptors protect neurons from I/R damage, whereas the PI3K/AKT pathway is involved in regulating fundamental cellular responses such as proliferation, viability, and apoptosis.[11]

In the present *in vitro* model, the anti-inflammatory effects of DEX were observed only partially. IL-6 levels were significantly increased following OGD/R injury; however, treatment with 10μM DEX, either before or after OGD/R, did not alter IL-6 levels.

Anti-inflammatory effects and underlying mechanisms of DEX have been extensively studied in the literature. DEX has been reported to exert anti-inflammatory effects by inactivating toll-like receptor (TLR)-4/nuclear factor kappa B (NF-κB) signaling pathways in temporary global cerebral I/R damage and retinal I/R models.[3,4] Toll-like receptor-4 is one of the thirteen known TLRs in mammals and is involved in the activation of NF-κB. NF-κB is a member of the transcription factor family and regulates the transcription of many inflammatory genes, including TNF-α, IL-6, IL-1β, iNOS, and COX-2. Toll-like receptors are widely distributed in nature and are activated as part of the innate immune response to pathogens, cytokines, and stressors. Inhibition of TLR-4 activation in brain ischemia has been shown to exert a neuroprotective effect.[4] Similar anti-inflammatory mechanisms of DEX have also been reported in other rat brain ischemia–reperfusion models and *in vivo* focal cerebral I/R models.[5–7]

Dexmedetomidine is a potent alpha-2 adrenergic receptor agonist. Activation of these receptors initiates intracellular signaling mechanisms that suppress increases in TNF-α and IL-1β. These effects of DEX were reversed by the alpha-2 antagonist atipamezole. However, the increase in the anti-inflammatory cytokine IL-10 induced by ischemic damage could not be prevented by DEX, suggesting that IL-10 regulation may involve mechanisms independent of alpha-2 receptors.[6]

In another focal cerebral I/R damage model, miR-214 expression, which has been shown to regulate Rho-associated kinase 1 (ROCK1) in osteosarcoma cells, was investigated. Administration of DEX during ischemia reduced the brain infarct area, decreased apoptosis in hippocampal neurons, and increased miR-214 expression in rats. Increased miR-214 negatively regulated ROCK1 activity and inhibited NF-κB activation following cerebral I/R. Ultimately, DEX treatment attenuated inflammation.[24] Conversely, it has also been reported that the neuroprotective effects of DEX are independent of the mammalian target of rapamycin (mTOR) pathway. In a focal cerebral I/R model, DEX inhibited neuronal autophagy via upregulation of hypoxia-inducible factor-1α, thereby protecting the mouse brain against I/R-induced injury.[25]

Dexmedetomidine has been shown to exert neuroprotective effects in oxidative stress and I/R models by regulating microRNAs, which are potent regulators of gene expression, thereby reducing apoptosis, inflammation, and oxidative stress. In the present study, treatment with DEX 10μM before injury reduced oxidative stress, as evidenced by decreased iNOS and eNOS immunoreactivity. In an oxidative stress model induced by H_2O_2, DNA damage and cell death occur in PC12 rat pheochromocytoma cells. Dexmedetomidine has been demonstrated to reduce apoptosis, inflammation, and oxidative stress and to promote cell viability by inhibiting miR-134; upregulating miR-381; regulating the miR-199a/HIF-1α axis; and activating miR-206, miR-19a, miR-145, and miR-17-5p in various cell lines, including pheochromocytoma PC12 cells, SK-N-SH cells, rat neurons and astrocytes, and neural stem cells.[10,11,26–31] Dexmedetomidine also exhibited neuroprotective effects by significantly increasing cell viability in an H_2O_2-induced oxidative stress model in human SK-N-SH cells and by negatively regulating Annexin A1 through miR-206 activation. Additionally, DEX reduced malondialdehyde, lactate dehydrogenase, and reactive oxygen species levels.[28] Dexmedetomidine has also been shown to modulate the SNHG11/miR-324-3p/VEGFA axis in a rat cerebral I/R model and in a neuron injury model induced by OGD/R in SH-SY5Y cells. In ischemic stroke, microRNA-434-3p binds to SNHG11, leading to upregulation of SNHG11 and

reduced cell viability. Dexmedetomidine was shown to reverse the apoptotic effects mediated by SNHG11.[32]

Limitations

First, cell viability and neurite outgrowth were evaluated as preliminary dose studies only in OG (high-glucose)-containing medium. Therefore, the effects of different concentrations of DEX on cell viability, proliferation, and neurite outgrowth under low-glucose OG conditions could not be assessed. Second, this study was conducted using a mouse NB2a cell line rather than a human neuroblastoma cell line. Selection of an appropriate cell line is critical when establishing *in vitro* models, as molecular and physiological characteristics vary between species. Although mouse neuroblastoma cell lines are widely used in the literature, human neuroblastoma cell lines may be preferable because of species compatibility.

Conclusion

Dexmedetomidine increased cell viability at almost all concentrations (0.1–45μM), except 60μM. Treatment with DEX 10μM, either before or after injury, reduced apoptosis induced by OGD/R injury in NB2a cells. Pre-injury treatment with DEX 10μM also decreased both iNOS and eNOS immunoreactivity. The neuroprotective effects of DEX against OGD/R injury may be attributed to its antiapoptotic and antioxidative properties, and administration of DEX before injury may be more effective in terms of antioxidative effects.

Disclosures

Ethics Committee Approval: The study was approved by the Manisa Celal Bayar University Faculty of Medicine Health Sciences Ethics Committee (no: 20.478.486/808, date: 21/05/2021).

Informed Consent: Informed consent was obtained from all participants.

Conflict of Interest Statement: The authors declare that there is no conflict of interest.

Funding: The study was supported by Scientific Research Project Coordination Unit of Manisa Celal Bayar University (BAP 2019/146). Title in Project: "Dexmedetomidin' in fare nöroblastoma (nb2a) hücre kültüründe oksijen-glukoz yoksunluluğu/reoksijenizasyon hasarı üzerine etkileri"

Use of AI for Writing Assistance: No AI technologies utilized.

Authorship Contributions: Concept – T.Ö., K.V.; Design – T.Ö., K.V.; Supervision – K.V., T.Ö., E.T.U.; Materials – T.Ö., E.D., D.G.T.; Data collection and/or processing – E.D., T.Ö., D.G.T., T.Önal, K.V.; Analysis and/or interpretation – T.Ö., K.V., E.T.U., E.D.; Literature search – T.Ö., K.V., E.D., T.Önal; Writing – T.Ö., K.V., T.Önal; Critical review – K.V., T.Ö.

Peer-review: Externally peer-reviewed.

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