Biologia
https://doi.org/10.1007/s11756-021-00911-w

ORIGINAL ARTICLE

The effect of medical ozone on oxidative stress and neuroinflammation in the early stage after experimental status epilepticus

Turgay Cokyaman[1] · Ozlem Oztopuz[2] · Ozlem Coskun[2] · Basak Buyuk[3] · Hasan A Kiraz[4] · Sait Elmas[5]

Received: 13 June 2021 / Accepted: 8 September 2021
© Institute of Molecular Biology, Slovak Academy of Sciences 2021

Abstract

Preservation of the brain is important to reducing recurrent seizures and other neurological sequelae after status epilepticus (SE). Medical ozone ($MedO_3$) shows antioxidant, anti-inflammatory and anti-apoptotic properties in the human body. In this study, it was aimed to understand the neuroprotective potential of $MedO_3$ in the acute period after SE. Mature rats of Wistar Albino were used for the study. Group design O_3 + SE: SE induced after $MedO_3$, O_3: $MedO_3$ alone was given, SE: SE only induced, SE + O_3; $MedO_3$ given after SE, C: control and S: sham control group. SE was induced with lithium-pilocarpine experimental model and evaluated on the Racine scale. Peripheral blood samples and brain tissue samples were taken before decapitation. Histopathological evaluation of the hippocampus of the rats given medical O_3 before and after SE were studied. The highest peripheral blood oxidative stress index (OSI) was found in SE group. The OSI level in O_3 + SE and SE + O_3 was significantly higher than SE/C/S. Gene expressions of TNF-α and IL-1β mRNA were significantly higher in SE compared to other groups. Histopathologically; eosinophilia, cellular shrinkage and interstitial oedema were detected in the most severe SE and to a lesser extent in O_3 + SE/ SE + O_3. $MedO_3$ reduced SE-induced oxidative damage, neuroinflammation and neuronal injury and contributed positively to neuroprotection.

Keywords Status epilepticus · Ozone · Pilocarpine · Lithium · Rats · Neuroprotection

Abbreviations

SE	status epilepticus	Li-Pi	lithium-pilocarpine
$MedO_3$	medical ozone	(q)PCR	(quantitative) real-time polymerase chain reaction
OSI	oxidant stress index	TAC	total antioxidant capacity
TNF-α	tumour necrosis factor- alpha	TOC	total oxidant capacity
IL-1β	interleukine-1beta	H&E	hematoxylin-eosin

✉ Turgay Cokyaman
wolf_6079@hotmail.com

Ozlem Oztopuz
ozlemoztopuz@yahoo.com.tr

Ozlem Coskun
ozlemcd38@hotmail.com

Basak Buyuk
drbasakbuyuk@hotmail.com

Hasan A Kiraz
hakiraz@comu.edu.tr

Sait Elmas
saitelmas@comu.edu.tr

[1] Department of Pediatrics, Division of Pediatric Neurology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

[2] Faculty of Medicine, Department of Biophysics, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

[3] Faculty of Medicine, Department of Histology and Embryology, İzmir Demokrasi University, İzmir, Turkey

[4] Faculty of Medicine Anesthesiology and Reanimation, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

[5] Experimental Research Application and Research Center, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

Published online: 25 October 2021

Group 1	O_3 + SE
Group 2	O_3
Group 3	SE
Group 4	SE+ O_3
Group 5-control group	C
Group 6-sham control group	(S)

Introduction

Status epilepticus (SE) is defined as a single seizure or short seizure series without return to interictal baseline continuing for durations of 30 min or longer. Due to the mortality risk (1.9–40%), it is a life-threatening emergency medical situation requiring cessation in all age groups (Basavappa and Ellory 1996; Chen et al. 2007; Clavo et al. 2019). It causes co-morbidities such as recurrent seizures, motor deficits, intellectual disability and behavioural problems in the chronic period (Clavo et al. 2011). Antiepileptic medications are used for SE treatment; however, immune modulators, ketogenic diet, hypothermia, vagal nerve stimulation, electroconvulsive treatment and surgery come to the agenda for refractory cases (Clifford et al. 1987). However, in spite of all these medical treatment choices, it is not possible to fully resolve all neurologic sequelae occurring in the chronic period.

Ozone (O_3) is a molecule comprising 3 oxygen atoms with cyclic structure which can oxidize organic compounds (Colton 2009). First used on humans in World War I, O_3 has been used in complementary medicine applications for treatment of ischaemic, inflammatory and infectious diseases for nearly a century (Di Filippo et al. 2015; Eastman et al. 2019). O_3 is not used pure in medical use. In medical use, it is injected into body cavities or circulatory system in the form of a certain amount of O_2/ O_3 mixture (Elvis and Ekta 2011). Medical O_3 ($MedO_3$) treatment is reported to strengthen the antioxidant system and protect against cellular damage and apoptosis, to show anti-inflammatory efficacy by regulating the immune response, and to display neuroprotective effects by increasing blood flow in carotid and central cerebral arteries and oxygenation of hypoxic tissues (Fellin 2009; Fountain 2000; Gastaut 1970).

In the early period after SE, it is important to prevent oxidative injury in the brain, neuroinflammation and erroneous synaptic reorganization in order to reduce chronic neurologic sequelae. Therefore, neuroprotection is still an important medical problem faced in the early period after SE. In our study, we aimed to understanding of the antioxidant and neuroprotective potential of $MedO_3$ acute period after SE. Peripheral blood oxidant stress index (OSI) and brain tissue tumour necrosis factor-alpha(TNF-α) and interleukine-1β(IL-1β) gene expressions and hippocampal histopathological changes of the rats treated with $MedO_3$ before and after SE were evaluated.

Materials and methods

For the research, 42 mature female Wistar Albino rats weighing 250–300 g appropriate for working were used. The rats were obtained from Çanakkale Onsekiz Mart University Experimental Research and Application Center and kept in special wire cages with appropriate feeding in the same location. Rooms had 45–50% humidity, 22 ± 2 °C controlled temperature, 12-h light and 12-h dark circadian rhythms (light 08:00–20:00, dark 20:00–08:00), with free feeding with standard commercial pellet feed and municipal drinking water. Rats were randomly divided into 6 groups with equal numbers (n = 7).

Design of study groups

Group 1 (O_3 + SE): Before inducing SE, O_3 administration was performed at 0th and 12th, 24th, 36th, 48th hours by intraperitoneal injection (IP) Two hours after the final O_3 administration, SE was induced with lithium-pilocarpine (Li-Pi). The study was ended in the 24th hour after SE. Group 2 (O_3): O_3 administration was performed IP at 0th and 12th, 24th, 36th, 48th hours and the study ended 24 h after final administration. Group 3 (SE): in this group, only SE was induced, and the study was ended in the 24th hour after SE. Group 4 (SE+ O_3): After inducing SE, O_3 administration was performed IP at 0th, 12th, 24th, 36th and 48th hours. The study was ended in the 24th hour after final O_3 administration. Group 5 (C): control group, normal nourishment was given. Group 6 (S): sham control group, after 2 mL of saline injection IP at 0th, 12th, 36th and 48th hours, rats were taken to normal care. Decapitation of rats was done 24 h after the last dose of drug (Li-Pi), $MedO_3$ or saline.

Medical ozone administration

For O_3 administration, 1 mg/kg/dose (50 μg/mL, 5 mL) IP was given within 1 min with a single-use25–35 G injector with internal section and piston resistant to O_3 and not containing latex material. With the aim of producing the O_3/O_2 mixture for O_3 administration, an O_3 generator (Device name and model: Turkozone Blue S Medical Ozone device, ozone oxygen mixture interval: 5–0.05% O_3/95–99.95% O_2, Device serial No: BG-19144427046, Ozon Sağlık Hizmetleri Ltd. Şti. İstanbul, Turkey) was used.

Status epilepticus induction

To reduce the mortality rate in the experimental model to a minimum, initial Pi dose was 200 mg/kg IP. Rats without Racine stage 4/5 seizure activity in spite of 200 mg/kg/dose injection had additional 100 mg/kg dose Pi administered (Glien et al. 2001). Animals were exposed to SE for 60 min

after reaching Racine stage 4/5 seizure activity. At the end of 60 min, 5 mg/kg/dose diazepam was administered IP to rats, whose SE did not stop spontaneously (Gordon and Martinez 2010; Honchar et al. 1983). Animals that were kept alive for 24 h after the termination of SE were decapitated. No animal death occurred during the experimental protocol period.

To increase Pi sensitivity and reduce lethal side effects, 24 h before administration 127 mg/kg/dose Li in saline was administered subcutaneously (SC)(Kal et al. 2017). Additionally, 30 min before SE induction, 1–5 mg/kg/dose atropine sulfate was administered SC to reduce peripheral cholinomimetic effects linked to Pi (Kim et al. 2012).

Drugs

Pi (hydrochloride) and Li (chloride) were purchased from Sigma Aldrich. These chemicals were dissolved in saline and injected IP. Diazepam and atropine were used as commercial solutions and injected IP.

Racine scale

Stage1. Tics of face and around mouth, fur standing up.
Stage2. Head-shaking movements
Stage3. Clonus of front extremities
Stage4. Seizure with clonus of front extremities
Stage5. Seizure and then falling with clonus of extremities (generalized motor convulsions) (Kim et al. 2012).

Measurement of peripheral blood oxidative stress index

Blood samples were centrifuged for 10 min at 4000 rpm. They were stored at −80 °C until analysis. Serum total antioxidant capacity (TAC) and total oxidant capacity (TOC) levels were measured spectrophotometrically with commercial kits (Rel Assay Diagnostics). TOC/TAC ratio was used to calculate the oxidative stress index (OSI), a marker of the degree of oxidative stress, was expressed as arbitrary unit (AU).

Ribonucleic acid isolation and quantitative real-time polymerase chain reaction analysis

Messenger ribonucleic acid (mRNA) expression was assessed in quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was isolated from 10 to 30 mg hippocampus tissue using QIAamp RNA clone (Ambion PureLink RNA MiniKit) according to the manufacturer's protocol. RNA quality and amount were determined according to 260/280 absorbance rate using NanoDrop ND-1000 spectrophotometer. Reverse transcription was performed with a complementary deoxyribonucleic acid (cDNA) synthesis kit (High-Capacity cDNA Reverse Transcription Kit). PCR conditions were 2 min at 50 °C, 10 min at 90 °C, 15 s at 95 °C for 40 cycles denaturation and one minute at 60 °C for extension. The synthesized cDNA samples were used for qRT-PCR. Gene expression levels were analyzed with qRT-PCR using Applied Biosystems ™ TaqMan® Gene Expression (Thermo Fisher Scientific, USA) (ABI Stepone). Beta-actin was used for normalization of genes. Gene expression measurement was determined using Ct values and level changes were assessed with the $2^{-(\Delta\Delta CT)}$ method. For TNF-α, IL-1β and β-actin, the primer ID numbers were Rn01525859_g1, Rn00580432_m1 and Rn00667869_m1, respectively (Thermo Fisher).

Histopathological evaluation

Hippocampal samples obtained at the end of the experiment were quickly put into 10% neutral buffered formalin. After 48 h of fixation, the samples were passed through increasing alcohol series and dehydration was applied. The tissues, which were then made transparent by xylene changes, were kept in liquid paraffin at a temperature of 600C in three exchanges of paraffin. In the last step, the tissues were embedded in paraffin blocks. Five micron sections taken from paraffin blocks were stained with a routine hematoxylin-eosin(H&E) staining protocol and evaluated for histopathological damage. The evaluation was made separately in terms of interstitial oedema, cellular shrinkage and eosinophilia parameters in the hippocampus regions as follows;

Grade 0: no damage
Grade 1: minimal damage
Grade 2: moderate damage
Grade 3: severe damage.

Statistical analysis

SPSS (Social Sciences Statistics Packages, SPSS for Windows, Version 18.0, Chicago, IC, USA) was used for data analysis. All data were evaluated with Kolmogorov-Smirnov test and Shapiro-Wilk test for determining normal distribution. Parametric tests were performed for biochemical tests and gene expression, and were compared by One-Way ANOVA test, followed by the post-hoc Tukey's test. Non-parametric tests were performed for histopathological examinations, Kruskal Wallis test was performed and then Dum-Bonferroni correction was performed for binary group comparisons. A p value below 0.05 was considered statistically significant. Gene expression levels were evaluated by the $2^{-\Delta\Delta Ct}$ method [ΔΔCt = (Ct Target gene-Ct reference gene)].

Results

Peripheral blood OSI levels (Table 1)

The lowest OSI level was identified in the O_3 (0.20 ± 0.01) and there were no difference from C and S groups (0.25 ± 0.02 and 0.22 ± 0.01). The OSI levels were found to be 0.63 ± 0.01 in O_3 + SE and 0.79 ± 0.07 in SE + O_3, and these results were significantly higher compared to O_3/C/S ($p < 0.05$). The highest OSI level was identified in SE (1.42 ± 0.17) and this result was significantly higher compared to all other groups ($p < 0.001$, Table 1).

Proinflammatory cytokine gene expressions in hippocampus tissue

Tumour necrosis factor-alpha(TNF-α) gene expression levels measured in rat hippocampus tissue are presented in Fig. 1. It has been shown to higher TNF-α expression in SE than in all other groups. This difference was found to be quite significant in post-hoc analysis ($p < 0.001$). Apart from SE, the TNF-α gene expression in the other groups were similar, and no difference was identified.

The highest Interleukin-1 beta (IL-1β) gene expression was detected in SE and was significantly higher than all other groups ($p < 0.05$). This gene expression level was approximately 3 times higher than O_3 and 2 times higher than SE+ O_3. There was no significant difference between all other groups except SE. Here, it is notable that the IL-1β gene expression in SE+ O_3 was identified to have similar levels to the C/S groups.

Histopathologic results

When examining H&E stained hippocampal sections, eosinophilia and cellular shrinkage was found to be high by significant levels in SE compared to the C and S (p .002). Similarly, interstitial oedema was higher in SE compared to C and S (p .026). Additionally, in SE + O_3 there was more interstitial oedema compared to C and S (p .038, Table 2, Fig. 2). Apart from interstitial edema, other histologic findings in O_3 + SE and SE + O_3 were found to be at similar levels to the control groups, showing MedO_3 reduces histologic changes linked to SE (Fig. 2).

Discussion

As is known, in the epileptogenesis process in the early period after SE, non-neuronal mechanisms play a role apart from neuronal injury and synaptic remodelling mechanisms. The best-known non-neuronal mechanism is via reactive oxygen species (ROS)(Kim et al. 2013). Minutes after the onset of seizures in SE, in the hyperdynamic phase, increasing metabolic needs are provided by increased systemic blood pressure and cerebral blood flow. In the exhaustion phase, which begins with the end of the seizures, the required metabolic needs cannot be met due to the decreased cerebral blood flow. This situation causes mainly oxidative stress, neuroinflammation, ischaemic cell death and disruption of the blood-brain barrier (Koca et al. 2010; Lemos and Cavalheiro 1995).

ROS are produced during cellular reactions as by-products of mitochondria and oxidative metabolism (Librizzi et al. 2012). ROS form because of 1-electron reduction of molecular oxygen in superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-). These very reactive molecules

Table 1 Blood total antioxidant capacity (TAC), total oxidant capacity (TOC) and oxidant stress index (OSI) levels

GROUPS (n=7)	TAC (μmol Torolox Equiv/L) (M ±SE)	TOC (μmol H_2O_2 Equiv/L) (M ±SE)	OSI (Arbitratry Unit) (M ±SE)
O_3 +SE	2.06±0.01	13.08±0.20	0.63±0.01[a,b]
O_3	2.04±0.05	4.17±0.18	0.20±0.01[a,c]
SE	1.88±0.05	26.71±3.23	1.42±0.17[a]
SE+O_3	2.10±0.06	16.60±1.41	0.79±0.07[a,b]
C	2.13±0.08	5.27±0.44	0.25±0.02[a,c]
S	2.30±0.29	4.95±0.27	0.22±0.01[a,c]
P	0.332	**<0.001**	**<0.001**

All results are presented as mean ± standard error (m ± SE) for seven rats in each group. One way ANOVA test was used for comparison between groups and results were evaluated according to Post-hoc Tukey's test

[a] The highest OSI level was detected in SE, and this difference was statistically significant from all the remaining groups ($p < 0.001$)

[b] OSI levels in O_3 + SE and SE+ O_3 were similar ($p > 0.05$), but significantly higher than O_3/ C/ S($p < 0.05$)

[c] The lowest OSI levels were detected in the O_3 and C / S groups and there was no difference between them ($p > 0.05$)

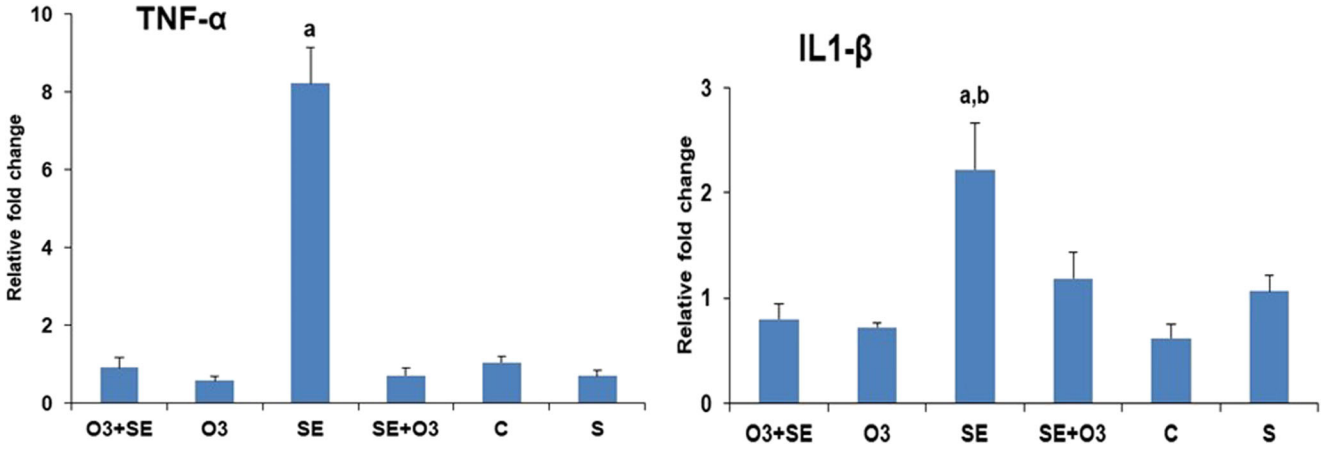


Fig. 1 The data were presented as $2^{-(\Delta\Delta CT)}$ relative expression after the mRNA levels were normalized with β-actin. The statistical significance was calculated by one-way Anova analysis of variance with Tukey's post-hoc test ($n = 7$). The error bars represent the standard errors of the means, and the a and b indicate P values (a: compared to O_3 + SE/O_3/SE + O_3/C/S, $P \leq 0.001$ b: compared to SE + O_3/S $P \leq 0.05$;) calculated by comparison of the different genes at each time point

oxidize lipids, nucleic acid and proteins damaging cell membranes, DNA, enzymes, receptors and ion channels at cellular level (Logroscino et al. 2005). For the continuation of neuro-astroglial homeostasis, the emerging ROS must be balanced with endogenous antioxidant enzymes (like catalase, superoxide dismutase and glutathione reductase) and molecules (like glutathione, ascorbic acid and tocopherol) (Maroso et al. 2011). Disruption of this balance in favour of oxidation,

Table 2 Demonstration of histopathological change scores between experimental groups

Groups	Inflammatory cell filtration	Cellular shrinkage	Eosinophilia
O_3 +SE			
m±SD	1.2±1.0	1.2±0.4	1.4±0.5
median (min-max)	1.0 (0.0–3.0)	1.0 (1.0–2.0)	1.0 (1.0–2.0)
O_3			
m±SD	1.4±1.5	0.6±0.5	1.4±0.5
median (min-max)	1.0 (0.0–3.0)	1.0 (0.0–1.0)	1.0 (1.0–2.0)
SE			
m±SD	2.8±0.4	2.2±0.4[c]	2.6±0.5[c]
median (min-max)	3.0 (2.0–3.0)	2.0 (2.0–3.0)	3.0 (2.0–3.0)
SE+O_3			
m±SD	2.6±0.5	1.3±0.5	1.1±0.7
median (min-max)	3.0 (2.0–3.0)	1.0 (1.0–2.0)	1.0 (0.0–2.0)
C			
m±SD	0.0±0.0[a]	0.0±0.0	0.0±0.0
median (min-max)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
S			
m±SD	0.0±0.0[b]	0.0±0.0	0.0±0.0
median (min-max)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
P	0.002	0.000	0.000

All results are presented as mean ± standard deviation (m ± SD) and median (min-max) for seven rats in each group. Kruskal wallis was used for the statistical evaluation of histopathological results. Dum-Bonferroni correction was made for pairwise group comparisons. There were statistically significant differences between the groups in terms of Interstitial edema, Cellular shrinkage and eosinophilia

[a] compared to SE and SE + O_3

[b] compared to compared to SE and SE + O_3 ($p < 0.05$)

[c] compared to C and S ($p = 0.002$)

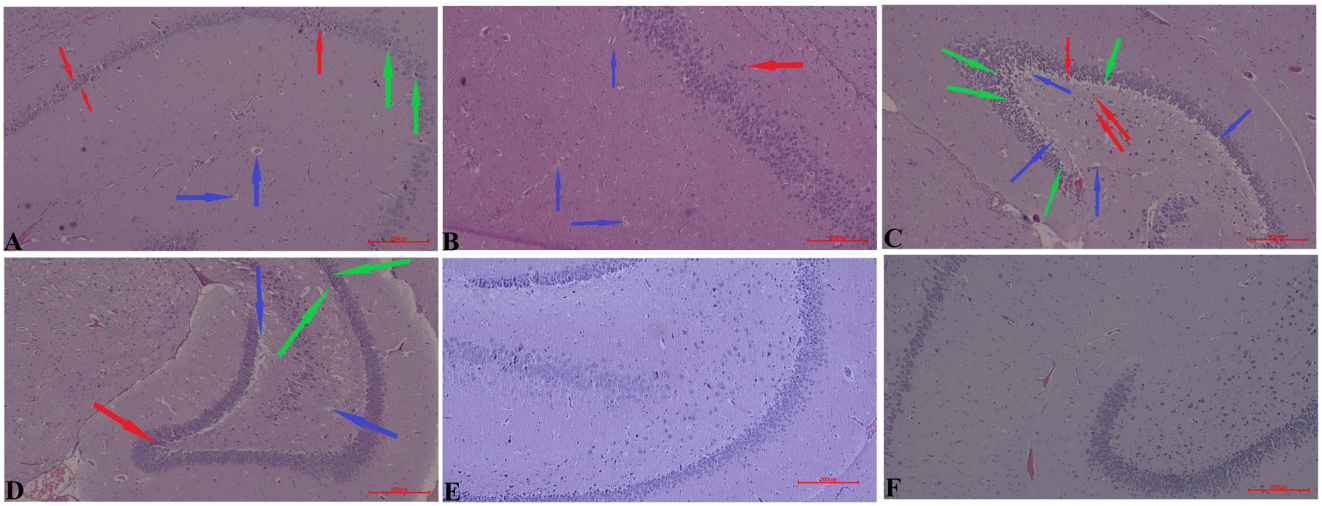


Fig. 2 Hematoxylin-eosin (H&E) staining of histological sections of the hippocampus belonging to Group O_3 + SE, O_3, SE, SE + O_3, C, S can be seen in sections A, B, C, D, E and F, respectively. Interstitial edema, eosinophilia, and cellular shrinkage were evaluated ion the sections and graded. Blue arrows marked interstitial edema, red arrows marked eosinophilia, and green arrows marked cellular shrinkage areas on the H-E stained sections (Magnification ×10). Interstitial edema, cellular shrinkage and eosinophilia scores of the groups were evaluated with the Kruskal Wallis test. Dunn-Bonferroni test was performed for post-hoc multigroup comparisons. Statistically significant ($p < .05$) values are shown in yellow

unfortunately positive contributes to the epileptogenesis process. In our study, peripheral blood OSI was assessed for oxidative injury caused by SE and the highest OSI value was identified in SE group ($p < .001$, Table 1). This finding is consistent with available knowledge showing SE disrupts the oxidant/antioxidant balance and causes oxidative injury in the brain.

Oxidative stress causes inflammation and injury in tissues by means of COX2, PGE2 and cytokines via nuclear transcriptional factor kappa (NFkB). On the other hand, it activates another nuclear transcriptional factor, nuclear factor-erythroid 2-related factor 2 (Nrf2)(McNamara et al., 2006). Nrf2 increases the production of many antioxidant enzymes such as catalase, superoxide dismutase and glutathione reductase by activating the transcription of antioxidant response elements. It is claimed that $MedO_3$ activates Nrf2 by creating moderate oxidative stress in tissues and its antioxidant feature is through this mechanism (Pasantes-Morales and Tuz 2006). In our study, OSI values were found to be lower in O_3 + SE and SE + O_3 groups treated with $MedO_3$ compared to SE group. In fact, this finding may indicate that $MedO_3$ reduces the oxidative damage products that occur in peripheral blood in the early post-SE. Additionally, the lack of difference in OSI between O_3 and C/S groups shows that $MedO_3$ administration was given at appropriate dose and O_3-sourced oxidation imbalance was not induced.

In experimental epilepsy models, it has been reported that the inflammatory process is triggered by proinflammatory cytokines such as TNF-α and IL-1β in the brain regions where epileptic activity originates (Pearson-Smith and Patel 2017). For example, proinflammatory cytokines have been shown to be upregulated very soon after epileptic seizures induced by bicuculline (Racine 1972). With the induction of SE, TNF-α is rapidly released into the brain parenchyma (Sagai and Bocci 2011). TNF-α causes to appear vasogenic oedema and nitric oxide derivatives after SE, especially by affecting the permeability of endothelial cells (Sánchez Fernández et al. 2019).

IL-1β is excessively activated and expressed in neurons and glial cells from the early stages of experimental seizures (Shin et al. 2011). IL-1β activates neuronal sphingomyelinase and Src kinase through its receptor IL-1R1. As a result of this event, NR2B subunit of NMDA receptor is activated and neuronal Ca^{+2} influx increases (Shorvon et al., 2019). Induction of neuronal slow inward currents causes neuronal hypersynchronization and hyperexcitability, leading to spontaneous seizures (Sun et al. 2019). In addition, IL-1β contributes to neuronal hyperexcitability by decreasing glutamate reuptake in astrocytes and increasing glutamate release from glial cells via TNF-α(Terrone et al. 2019).

Proinflammatory cytokines like TNF-α and IL-1β are known to contribute to seizure generation and epileptogenesis (Vasquez et al. 2019). Nowadays, the increasing expression of these cytokines after epileptic seizures has been an undeniable fact that has been supported by many experimental studies (Verhoog et al. 2020; Viviani et al. 2003). In our study, the TNF-α and IL-1β gene expressions in rat hippocampus tissue were found to be high in the SE compared to all other groups (respectively $p < .001$ and $p < .05$, Fig. 1). It was noteworthy that TNF-α and IL-1β gene expressions were found at lower levels in the O_3 and SE+ O_3 groups compared to SE group. Because this finding may indicate that $MedO_3$ has an anti-inflammatory

effect in the hippocampus tissue. Based on this finding, we can claim that the anti-neuroinflammatory effect in the early post-SE period is associated with TNF-α and IL-1β.

Complex pathophysiological, immunological and biochemical events are triggered in the brain very soon after SE or prolonged seizures. In the minutes to hours or days after SE, activation of plasma membrane receptors results in changes in the intracellular signal transduction pathways which are involved in the maintenance of vital cellular functions (Walker 2018; Wang et al., 2014).

High amounts of ATP are consumed in the brain during SE. Due to the decreased amount of ATP in the postictal period, neuronal membrane functions are impaired. Na accumulation and oedema occur for the neuron because of the Na-K ATPase pump not working properly. In addition, enzyme functions and protein synthesis are impaired as a result of intracellular Ca dysregulation. As a result of all these events, neuronal damage, oxidative stress and inflammation are triggered Wang et al. 2020; Yamanel et al. 2011). In our study, satisfactory findings were found about the neuronal antioxidant and anti-inflammatory properties of $MedO_3$. Abnormal histopathologic findings (oedema, eosinophilia, and cellular shrinkage) were identified in the SE, while the O_3 + SE and SE + O_3 groups had similar findings to the C and S groups. This finding indicates that $MedO_3$ reduces histopathologic injury forming in the brain in the acute period after SE and ensures neuroprotection.

Limitations

The Pi dose recommended by Lemos and Cavalheiro (1995) was used to create SE in rats. Visually, the seizures of the rats were evaluated according to whether they reached raccine stage 4/5. The time to reach stage 4/5 seizures could not be recorded in the whole experimental model and in the rats given medical ozone before SE.

ROS measured from peripheral blood does not exactly reflect the level of neuronal oxidative stress. This may seem like an important limitation, but it is striking that we found lower levels of OSI in MedO3 applied SE groups in our study. Similar to oxidative stress, gene expression levels measured in brain tissue are not specific to the neuron also. Because there are small amounts of vascular and lymphatic structures in brain tissue samples. However, the specificity of gene expressions to the neuron is higher compared to OSI measured peripherally. In addition, couldn't be shown a direct neuronal pathway associated with MedO3 is an important limitation for this study. However, it is clear that more literature information is needed to reveal strong hypotheses and studies on this subject.

Conclusion

$MedO_3$ administration reduced oxidative and inflammatory injury to brain tissue the early post-SE stage. MedO3 has an antioxidant and anti-inflammatory effect at the neuronal level and contributes positively to neuroprotection in the acute phase after SE. $MedO_3$ may be put on the agenda as an additional therapeutic option in the early period after SE to reduce neurological sequelae that may occur in the chronic period.

Funding The financial funding of this study was supported by Çanakkale Onsekiz Mart University Scientific Research Projects Coordination Unit (Project ID: 2697). All researchers thank the Scientific Research Projects Coordination Unit.

Declarations

Ethical approval Ethical approval was obtained for the study from Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee for the study on 03.08.2018 with the file number 2018/1800109498. All the procedures performed in this study were carried in accordance with the 1964 Helsinki Declaration standards.

Conflict of interest There have no conflict of interest between authors.

References

Basavappa S, Ellory JC (1996) The role of swelling-induced anion channels during neuronal volume regulation. Mol Neurobiol 13(2):137–153. https://doi.org/10.1007/BF02740638

Chen JWY, Naylor DE, Wasterlain CG (2007) Advances in the pathophysiology of status epilepticus. Acta Neurol Scand Suppl 186:7–15. https://doi.org/10.1111/j.1600-0404.2007.00803.x

Clavo B, Rodriguez-Esparragon F, Rodriguez-Abreu D et al (2019) Modulation of oxidative stress by ozone therapy in the prevention and treatment of chemotherapy-induced toxicity: review and prospects. Antioxidants (Basel) 8(12):588. https://doi.org/10.3390/antiox8120588

Clavo B, Suarez G, Aguilar Y et al (2011) Brain ischemia and hypometabolism treated by ozone therapy. Forsch Komplementmed 18(5):283–287. https://doi.org/10.1159/000333795

Clifford DB, Olney JW, Maniotis A, Collins RC, Zorumski CF (1987) The functional anatomy and pathology of lithium-pilocarpine and high-dose pilocarpine seizures. Neuroscience 23(3):953–968. https://doi.org/10.1016/0306-4522(87)90171-0

Colton CA (2009) Heterogeneity of microglial activation in the innate immune response in the brain. J NeuroImmune Pharmacol 4(4):399–418. https://doi.org/10.1007/s11481-009-9164-4

Di Filippo C, Trotta MC, Maisto R et al (2015) Daily oxygen/O3 treatment reduces muscular fatigue and improves cardiac performance in rats subjected to prolonged high intensity physical exercise. Oxidative Med Cell Longev 2015:190640. https://doi.org/10.1155/2015/190640

Eastman CL, D'Ambrosio R, Ganesh T (2019) Modulating neuroinflammation and oxidative stress to prevent epilepsy and improve outcomes after traumatic brain injury. Neuropharmacology 172:107907. https://doi.org/10.1016/j.neuropharm.2019.107907

Elvis AM, Ekta JS (2011) Ozone therapy: a clinical review. J Nat Sci Biol Med 2(1):66–70. https://doi.org/10.4103/0976-9668.82319

Fellin T (2009) Communication between neurons and astrocytes: relevance to the modulation of synaptic and network activity. J Neurochem 108(3):533–544. https://doi.org/10.1111/j.1471-4159.2008.05830.x

Fountain NB (2000) Status epilepticus: risk factors and complications. Epilepsia 41(Suppl 2):S23–S30. https://doi.org/10.1111/j.1528-1157.2000.tb01521.x

Gastaut H (1970) Clinical and electroencephalographical classification of epileptic seizures. Epilepsia 11(1):102–113. https://doi.org/10.1111/j.1528-1157.1970.tb03871.x

Glien M, Brandt C, Potschka H, Voigt H, Ebert U, Löscher W (2001) Repeated low-dose treatment of rats with pilocarpine: low mortality but high proportion of rats developing epilepsy. Epilepsy Res 46(2):111–119. https://doi.org/10.1016/S0920-1211(01)00272-8

Gordon S, Martinez FO (2010) Alternative activation of macrophages: mechanism and functions. Immunity 32(5):593–604. https://doi.org/10.1016/j.immuni.2010.05.007

Honchar MP, Olney JW, Sherman WR (1983) Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats. Science 220(4594):323–325. https://doi.org/10.1126/science.6301005

Kal A, Kal O, Akillioglu I, Celik E, Yilmaz M, Gonul S, Solmaz M, Onal O (2017) The protective effect of prophylactic ozone administration against retinal ischemia-reperfusion injury. Cutan Ocul Toxicol 36(1):39–47. https://doi.org/10.3109/15569527.2016.1156120

Kim J-E, Ryu HJ, Choi SY, Kang TC (2012) Tumor necrosis factor-α-mediated threonine 435 phosphorylation of p65 nuclear factor-κB subunit in endothelial cells induces vasogenic edema and neutrophil infiltration in the rat piriform cortex following status epilepticus. J Neuroinflammation 9:6. https://doi.org/10.1186/1742-2094-9-6

Kim JE, Ryu HJ, Kang TC (2013) Status epilepticus induces vasogenic edema via tumor necrosis factor-α/ endothelin-1-mediated two different pathways. PLoS One 8(9):e74458. https://doi.org/10.1371/journal.pone.0074458

Koca K, Yurttaş Y, Yıldız C, Caycı T, Uysal B, Korkmaz A (2010) Effect of hyperbaric oxygen and ozone preconditioning on oxidative/nitrosative stress induced by tourniquet ischemia/reperfusion in rat skeletal muscle. Acta Orthop Traumatol Turc 44(6):476–483. https://doi.org/10.3944/AOTT.2010.2327

Lemos T, Cavalheiro EA (1995) Suppression of pilocarpine-induced status epilepticus and the late development of epilepsy in rats. Exp Brain Res 102(3):423–428. https://doi.org/10.1007/BF00230647

Librizzi L, Noè F, Vezzani A, de Curtis M, Ravizza T (2012)Seizure-induced brain-borne inflammation sustains seizure recurrence and blood-brain barrier damage. Ann Neurol 72(1):82–90. https://doi.org/10.1002/ana.23567

Logroscino G, Hesdorffer DC, Cascino G et al (2005) Mortality after a first episode of status epilepticus in the United States and Europe. Epilepsia 46(11):46–48. https://doi.org/10.1111/j.1528-1167.2005.00409.x

Maroso M, Balosso S, Ravizza T, Iori V, Ian Wright C, French J, Vezzani A (2011)Interleukin-1β biosynthesis inhibition reduces acute seizures and drug resistant chronic epileptic activity in mice. Neurotherapeutics 8(2):304–315. https://doi.org/10.1007/s13311-011-0039-z

McNamara JO, Huang YZ, Leonard AS (2006) Molecular signaling mechanisms underlying epileptogenesis. Sci STKE 2006(356):re12. https://doi.org/10.1126/stke.3562006re12

Pasantes-Morales H, Tuz K (2006) Volume changes in neurons: hyperexcitability and neuronal death. Contrib Nephrol 152:221–240. https://doi.org/10.1159/000096326

Pearson-Smith JN, Patel M (2017) Metabolic dysfunction and oxidative stress in epilepsy. Int J Mol Sci 18(11):2365. https://doi.org/10.3390/ijms18112365

Racine RJ (1972) Modification of seizure activity by electrical stimulation II Motor seizure. Electroencephalogr Clin Neurophysiol 32(3):281–294. https://doi.org/10.1016/0013-4694(72)90177-0

Sagai M, Bocci V (2011) Mechanisms of action involved in ozone therapy: is healing induced via a mild oxidative stress? Med Gas Res 1:29. https://doi.org/10.1186/2045-9912-1-29

Sánchez Fernández I, Goodkin HP, Scott RC (2019) Pathophysiology of convulsive status epilepticus. Seizure 68:16–21. https://doi.org/10.1016/j.seizure.2018.08.002

Shin EJ, Jeong JH, Chung YH et al (2011) Role of oxidative stress in epileptic seizures. Neurochem Int 59(2):122–137. https://doi.org/10.1016/j.neuint.2011.03.025

Shorvon S, Trinka E, Walker M (2019) The seventh London-Innsbruck colloquium on status epilepticus and acute seizures. Epilepsy Behav 101(Pt B):106532. https://doi.org/10.1016/j.yebeh.2019.106532

Sun Y, Ma J, Li D et al (2019)Interleukin-10 inhibits interleukin-1β production and inflammasome activation of microglia in epileptic seizures. J Neuroinflammation 16(1):66. https://doi.org/10.1186/s12974-019-1452-1

Terrone G, Frigerio F, Balosso S, Ravizza T, Vezzani A (2019) Inflammation and reactive oxygen species in status epilepticus: biomarkers and implications for therapy. Epilepsy Behav 101(Pt B):106275. https://doi.org/10.1016/j.yebeh.2019.04.028

Vasquez A, Farias-Moeller R, Tatum W (2019) Pediatric refractory and super-refractory status epilepticus. Seizure 68:62–71. https://doi.org/10.1016/j.seizure.2018.05.012

Verhoog QP, Holtman L, Aronica E, van Vliet EA (2020) Astrocytes as guardians of neuronal excitability: mechanisms underlying epileptogenesis. Front Neurol 11:591690. https://doi.org/10.3389/fneur.2020.591690

Viviani B, Bartesaghi S, Gardoni F et al (2003)Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. J Neurosci 23(25):8692–8700. https://doi.org/10.1523/JNEUROSCI.23-25-08692.2003

Walker MC (2018) Pathophysiology of status epilepticus. Neurosci Lett 667:84–91. https://doi.org/10.1016/j.neulet.2016.12.044

Wang L, Chen H, Liu XH, Chen ZY, Weng XD, Qiu T (2014) The protective effect of ozone oxidative preconditioning against hypoxia/reoxygenation injury in rat kidney cells. Ren Fail 36(9):1449–1454. https://doi.org/10.3109/0886022X.2014.950934

Wang M, Deng X, Xie Y, Chen Y (2020) Astaxanthin attenuates neuroinflammation in status epilepticus rats by regulating the ATP-P2X7R signal. Drug Des Devel Ther 14:1651–1662. https://doi.org/10.2147/DDDT.S249162

Yamanel L, Kaldirim U, Oztas Y et al (2011) Ozone therapy and hyperbaric oxygen treatment in lung injury in septic rats. Int J Med Sci 8(1):48–55. https://doi.org/10.7150/ijms.8.48

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.