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The possibilities of using the effects of ozone therapy in neurology

Ján Masan[1,2,3], Miron Sramka[2], Daria Rabarova[3]

1 University of St. Cyril and Methodius in Trnava, Slovak Republic
2 St. Elizabeth University of Health Care and Social Work in Bratislava, Slovak Republic
3 Trnava University in Trnava, University Hospital Trnava, Slovak Republic

Correspondence to: h.Doc. MUDr. Ján Mašán, PhD.
St. Elizabeth University of Health Care and Social Work in Bratislava, Slovak Republic
e-mail: masanjan@gmail.com

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Abstract

OBJECTIVES: The beneficial effects of ozone therapy consist mainly of the promotion of blood circulation: peripheral and central ischemia, immunomodulatory effect, energy boost, regenerative and reparative properties, and correction of chronic oxidative stress. Ozone therapy increases interest in new neuroprotective strategies that may represent therapeutic targets for minimizing the effects of oxidative stress.

METHODS: The overview examines the latest literature in neurological pathologies treated with ozone therapy as well as our own experience with ozone therapy. The effectiveness of treatments is connected to the ability of ozone therapy to reactivate the antioxidant system to address oxidative stress for chronic neurodegenerative diseases, strokes, and other pathologies. Application options include large and small autohemotherapy, intramuscular application, intra-articular, intradiscal, paravertebral and epidural, non-invasive rectal, transdermal, mucosal, or ozonated oils and ointments. The combination of different types of ozone therapy stimulates the benefits of the effects of ozone.

RESULTS: Clinical studies on O_2-O_3 therapy have been shown to be efficient in the treatment of neurological degenerative disorders, multiple sclerosis, cardiovascular, peripheral vascular, orthopedic, gastrointestinal and genitourinary pathologies, fibromyalgia, skin diseases/wound healing, diabetes/ulcers, infectious diseases, and lung diseases, including the pandemic disease caused by the COVID-19 coronavirus.

CONCLUSION: Ozone therapy is a relatively fast administration of ozone gas. When the correct dose is administered, no side effects occur. Further clinical and experimental studies will be needed to determine the optimal administration schedule and to evaluate the combination of ozone therapy with other therapies to increase the effectiveness of treatment.

REVIEW ARTICLE

Abbreviations:

O_3	- ozone
GSH	- glutathione
NADPH	- nicotinamide adenine dinucleotide
(SOD)	- superoxide dismutase
PUFA	- unsaturated fatty acids
LOP	- lipid ozonation products
ARE	- antioxidant response elements
ATP	- adenosine triphosphate
ROS	- reactive oxygen species
DNA	- deoxyribonucleic acid
NADH	- nicotinamide adenine dinucleotide

INTRODUCTION

Van Mauren was first to discover the distinctive odor of O_3 in 1785 (Altman 2007). However, its first identification as a distinct chemical compound was made by Schönbein in Basel in 1840. In 1896, Nikola Tesla patented a generator for producing ozone. The use of ozone became normal practice after the studies of Dr. H.H. Wolff. In 1915, during World War I, ozone was used to treat war wounds. Following Bocci's studies, ozone therapy has been incorporated into the treatment of chronic inflammatory diseases in the orthopedic field. When used in appropriate doses, it has been shown to be effective in inducing well-tolerated oxidative stress. Ozone is a metastable substance and must be generated on-site. Contraindication of O_3 therapy is lung inhalation, activating factors triggering an inflammatory response (Bocci 2006; Pryor *et al.* 2019). Jacobs (1982) carefully examined all the possible negative effects of ozone therapy. Despite the famous "toxicity" of ozone, it appears that the incidence is only 0.0007%, one of the lowest in medicine. Four deaths due to direct IV injection of the gas were included in his data but, since 1982, other deaths due to malpractice have occurred, of which at least three were in Italy (Bocci 2010).

It is currently applied in a wide range of medical fields: surgery, orthopedics, neurology, oncology, dermatology, cardiology, psychiatry, radiology, rheumatology, gynecology, gastroenterology, angiology, dentistry, etc.

The effects of medical ozone as a substance can be attributed to those defined by hormesis (from the Greek word hormáein = to set in motion), i.e. referring to the hypothesis regarding the beneficial effects of low doses. At the same time, it triggers a response with high doses, thus increasing the body's resistance.

Induction of the response to stress by short low doses usually protects the body for a longer period of time and against other possible types of doses (Rattan *et al.* 2009). The administration of O_3 therapy varies based on treatment goals and treatment focus. Ozone therapy combines a mixture of oxygen (O_2)-O_3 with a diverse therapeutic range (10–80 μg/ml of gas per ml of blood) (Bocci 2006). Human blood contains a large number of antioxidants such as uric acid, ascorbic acid, cysteine, glutathione, albumin, certain chelating proteins such as albumin, and enzymes such as catalase, the redox system of GSH, NADPH (nicotinamide adenine dinucleotide), and superoxide dismutase (SOD) (Bocci *et al.* 2005).

Like any other gas, ozone dissolves upon contact with body fluids according to Henry's Law in relation to temperature, pressure, and ozone concentration. It reacts immediately as soon as it is dissolved: O_3 + biomolecules → O_2 + O_2 + energy. Atomic oxygen behaves as a reactive atom. The fundamental ROS (reactive oxygen species) molecule is hydrogen peroxide H_2O_2, which is a non-radical oxidant able to act as an ozone Messenger responsible for eliciting several biological and therapeutic effects. In physiological amounts, they act as regulators of signal transduction and represent important mediators of host defense and immune responses (Dattilo *et al.* 2015).

The reaction of O_3 with water causes the formation of one mole of hydrogen peroxide (H_2O_2) and two moles of lipid oxidation products with polyunsaturated fatty acids (PUFA), forming a mixture of lipid ozonation products (LOP) (Barone *et al.* 2015) including lipoperoxyl radicals (Inal *et al.* 2011). Moderate oxidative stress caused by O_3 increases the activation of the transcription factor of the pathway-mediated nuclear factor-related erythroid factor 2 (Nrf2) (Bocci *et al.* 2015, Re *et al.* 2014), which is responsible for activating the transcription of antioxidant response elements (ARE). After their induction, the concentration of antioxidant enzymes increases in response to the transient oxidative stress O_3 (Bocci *et al.* 2015).

POTENTIAL APPLICATION OF OZONE THERAPY

Vascular and Haematological Modulation. The production of antioxidant enzymes affects the whole body (Gonzalez *et al.* 2004; Valacchi 2000). O_3 is a stimulator of O_2 transmembrane flow. Increased intracellular O_2 levels secondarily form the mitochondrial respiratory chain (Madej *et al.* 2007). In red blood cells, O_3 increases phosphofructokinase activity, thereby increasing the rate of glycolysis. Increased glycolytic rate increases ATP and 2.3-diphosphoglycerate (2.3-DPG) in the cell. Following a long treatment cycle of the elderly, Bocci *et al.* 2011 noted a significant increase in the 2.3-diphosphoglycerate level in oxyhaemoglobin. As a result of the Bohr effect, its dissociation curve shifts to the right, making it easier to transfer oxygen. Under physiological conditions, the endothelium regulates vascular tone (Molinari *et al.* 2017). Ozonized blood increases the release of prostacyclin (PGI2) and angiopoietins, both important factors in improving ischemic vasculopathy (Fernández *et al.* 2008; Elvis *et al.* 2011).

The correction of chronic oxidative stress via the increase of antioxidant enzymes can increase erythroblast differentiation. This leads to a progressive increase in erythrocytes and preconditions them

to having resilience towards oxidative stress, to an increase of erythrocytes with improved metabolic properties, as well as ensuring that young erythrocytes contain more G6PDH than older cells generated prior to the treatment, which is a type of "super-gifted erythrocytes" capable of correcting hypoxia in vascular diseases (Chang *et al.* 2005). An improvement in blood circulation and oxygen supply to ischemic tissues has been recorded, leading to an increased supply of O_2 to hypoxic tissues (Brigelius-Flohé *et al.* 2011).

Activation of the Immune System. O_3 has been shown to react with antioxidants and alter peroxidation compounds. H_2O_2 has been shown to act as a regulatory step in signal transduction by diffusing into immune cells and by facilitating a myriad of immune responses (Gulmen *et al.* 2013; Caliskan *et al.* 2011). An increase in interferon, tumor necrosis factor, and interleukin (IL)-2 was observed. IL-2 increases were initiated by immune response mechanisms (Elvis 2011). In addition, H_2O_2 activates nuclear factor-kappa B (NF-κB) and transforms growth factor-beta (TGF-β), thus increasing the immunoactive release of cytokines and tissue refurbishment.

After each restarted therapy, a small percentage of immune cells are activated and these cells release cytokines into the micro-environment, activating neighboring cells and, as a result, slowly enhancing immune responses. Only submicromolar concentrations can reach all organs, particularly bone marrow, liver, central nervous system, endocrine glands, etc., where they act as signaling molecules of an ongoing acute oxidative stress (Mancuso *et al.* 1997). These molecules can elicit the upregulation of antioxidant enzymes. The induction of HO-1 has been described as one of the most important antioxidant defense and protection enzymes. Throughout the treatments, LOP acts as an acute oxidative stressor in the bone marrow micro-environments and thus activates the release of metalloproteinases, of which particularly MP-9 may favor the detachment of staminal cells. These cells, once in the blood circulation, may be attracted to sites where a previous injury has taken place (Mancuso *et al.* 2008).

Lahodny (2021) collected ten blood samples with 70 μg/ml of ozone and reinfusion from a patient as part of a session to implement the therapeutic concept. This therapy generated a significant activation of stem cells, and subsequent rapid healing of wounds and inflammation were documented in patients (Rowen 2018). Thanks to this therapy, a several-year defect was cured in a mere 14 days (Mašán 2018).

Ozone therapy has a neuroimmunomodulatory effect, it activates the psychosomatic system, thus allowing the release of the growth hormone ACTH-cortisol, neurotonic hormones, and neurotransmitters. We clarified why patients report a feeling of euphoria and wellness during therapy: the disappearance of asthenia and depression, a reduction of pessimism syndrome, associated with a lack of side effects, represent positive results (Fernández *et al.* 2008; Mancuso 2017).

CLINICAL APPLICATION OF OZONE HERAPY IN NEUROLOGY

The first beneficial effects of ozone in the treatment of neurological disorders refer to the treatment of headaches and facial pain associated with pathological changes in the optic thalamus. Ozone is used to treat allodynia, neuropathic pain, and hyperalgesia (Kal *et al.* 2017; Hu *et al.* 2018).

Neurodegenerative diseases – Parkinson's disease, Alzheimer's and Wilson's disease, senile and vascular dementia, amyotrophic lateral sclerosis, optic nerve dysfunction, bilateral sensorineural hearing loss, and maculopathy, Huntington's disease, cognitive and movement disorders of the elderly who experience common effects of oxidative stress (Aso *et al.* 2012). The process of aging is characterized by the loss of homeostasis, leading to pathologic formation of reactive oxygen species (ROS), mitochondrial dysfunction, and metabolic unbalance (Dugger *et al.* 2017). These pathophenotypes determine abnormal aggregation of specific proteins (Yanar *et al.* 2020), given the connection between excessive ROS accumulation and impairment in the proteostasis network. A natural bioactive molecule with an antioxidant property such as ozone (O_3) can be indicated as a potential new strategy to delay neurodegeneration. This hypothesis is based on the evidence regarding the interaction between O_3 and Nrf2 (Galie *et al.* 2018; Siniscalco *et al.* 2018; Re *et al.* 2014; Vaillant *et al.* 2013). Molecular mechanisms **are** related to antioxidant/anti-apoptotic/pro-autophagy processes targeted by O_3 administration via an Nrf2 biological pathway.

It is best to implement ozone therapy (O_2-O_3) in an early phase before the potential development of a neurodegenerative pathology (Scassellati *et al.* 2020). The O_2 availability affects the expression of different hypoxia-inducible factors (HIFs) and plays the role of a cellular adapter to hypoxia (Curro *et al.* 2018; Zhang *et al.* 2014; Re *et al.* 2014), leading to the activation of trophic proteins and, consequently, to specific biological processes, including erythropoiesis and angiogenesis (Zhou *et al.* 2019).

Antioxidant Property of Ozone (O_3): Oxidative stress is a condition where ROS production exceeds the cellular antioxidant defense system, leading to an imbalance between the two systems, and this may contribute to neuronal damage. It has implications on the pathogenesis and progression of neurodegenerative diseases (Singh *et al.* 2019).

Oxidative damage may impair the cells in their structure and function as a result of the reduced activity of mitochondria. The damage is not confined to the brain but is also evident in peripheral cells and tissues, which require a high-energy source such as

the heart, muscles, brain, or liver. To function properly, neurons rely on the mitochondria, which produce the energy required for most of the cellular processes, such as neurotransmitter synthesis, including synaptic plasticity.

Mitochondrial dysfunctions cause an increase in ROS for lowered oxidative capacity and antioxidant defense, resulting in increased oxidative damage to protein and lipids, decreased ATP production, and accumulation of DNA damage. Moreover, mitochondrial bioenergetic dysfunction and the release of pro-apoptotic mitochondrial proteins into the cytoplasm initiate a variety of cell death pathways (Garcia-Escudero *et al.* 2013; Reutzel *et al.* 2020).

Ozone therapy stimulates the Krebs cycle by enhancing the oxidative carboxylation of pyruvate and stimulating the production of adenosine triphosphate (ATP) (Guven *et al.* 2008). It causes a significant reduction of nicotinamide adenine dinucleotide (NADH), an increase of the coenzyme A levels to fuel the Krebs cycle, and oxidizes cytochrome C (Brigelius-Flohe *et al.* 2011; Elvis *et al.* 2011).

Ozone (O_3)-influenced pro-oxidative and antioxidant defense biomarkers and their role in aging processes and neurodegenerative disorders (ND). Twenty-nine biomarkers implicated in oxidative stress, in endogenous antioxidant, and in vitagene systems have been identified. These biomarkers have been studied and found modulated after O_3 therapy performed in *in vivo* (human and animal models) samples. Neurodegenerative disorders are characterized by progressive loss of cognitive and behavioral deterioration (Scassellati *et al.* 2020).

Molecular Mechanisms Involving Ozone Therapy and Their Biological Relevance in Neuroprotection of Nrf2, and the Vitagene Network. Oxidative stress is a condition where ROS and nitrogen (RNS) production exceeds the cellular antioxidant defense system, leading to an imbalance between the two systems and this may contribute to neuronal damage and abnormal neurotransmission. ROS and RNS are also major factors in cellular senescence that lead to an increase in the number of senescent cells in tissues on a large scale (Liguori *et al.* 2018). Cellular senescence is a physiological mechanism that stops cellular proliferation in response to damage that occurs during replication. Senescent cells acquire an irreversible senescence-associated secretory phenotype (SASP), involving secretion of soluble factors (interleukins, chemokines, and growth factors), degradative enzymes such as matrix metalloproteases (MMPs), and insoluble proteins/extracellular matrix (ECM) components.

When O_3 is administrated, it dissolves immediately in the plasma/serum and reacts with PUFA (polyunsaturated fatty acids), leading to the formation of the two fundamental messengers: hydrogen peroxide (H_2O_2) as a ROS and 4-hydroxynonenal (4HNE) as a lipid oxidation product (LOP) (Bocci *et al.* 1998).

LOPs diffuse into all cells and inform them of minimal oxidative stress. After the oxidative/electrophilic stress challenge (Ishii *et al.* 2004), other aldehydes (Levonen *et al.* 2004) induced by O_3 (Galie *et al.* 2018, Siniscalco *et al.* 2018, Re *et al.* 2014, Vaillant *et al.* 2013) inhibit the Nrf2 conjugation, provoking the nuclear accumulation of Nrf2. This leads to the decreased expression of pro-inflammatory cytokines.

Another mechanism involves casein kinase 2 (CK2), another regulator of Nrf2 activity through its phosphorylation. It has been demonstrated that O_3 influences CK2 levels together with Nrf2 phosphorylation, reducing oxidative stress and pro-inflammatory cytokines in multiple sclerosis patients (Delgado-Roche *et al.* 2017). Similarly, O_3 inhibits oxidative stress through inhibition of the mitogen-activated protein kinase phosphatase (MAPK) 1 signaling pathway (Wang *et al.* 2018a).

Oxidative stress is one of the major drivers of protein misfolding that, accumulating and aggregating as insoluble inclusions, can determine neurodegeneration (Hohn *et al.* 2020; Knowles *et al.* 2014). It is known that Nfr2 promotes the clearance of oxidized or otherwise damaged proteins through the autophagy mechanism (Tang *et al.* 2019), Interestingly, O_3 can also modulate the degradation protein systems, *not only* via the Nrf2 pathway but also via activation of the AMP-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) signaling pathway, as demonstrated (Zhao *et al.* 2018).

O_3 can protect against the overproduction of nitric oxide (NO) when NO is a toxic oxidant. NO can rapidly react with other free radicals such as O_2 – the highly reactive oxidant peroxynitrite (ONOO) - and other RNS, which in turn damage the biomolecules (e.g. lipids, protein, DNA/RNA), playing a key role in chronic inflammation and neurodegeneration (Massaad, 2011; Toda *et al.* 2009), It has been demonstrated that O_3 downregulates inducible nitric oxide synthase (iNOS), which generates NO (Manoto *et al.* 2018; Smith *et al.* 2017) *via* NF-κB signaling.

CO acts as an inhibitor of another important pathway, NF-κB (nuclear factor kappa B subunit 1) signaling, which leads to the decreased expression of pro-inflammatory cytokines, while bilirubin also acts as an important lipophilic antioxidant. Furthermore, HO-1 directly inhibits pro-inflammatory cytokines and activates anti-inflammatory cytokines, leading to a balancing of the inflammatory process (Ahmed *et al.* 2017). Our research group confirmed that mild ozonization, tested on in vitro systems, induced modulation of genes including HO-1 (Scassellati *et al.* 2017),

In addition, Nrf2 also regulates the constitutive and inducible expression of antioxidants including, but not limited to, Superoxide Dismutases (SOD), Glutathione Peroxidase (GSH-Px), Glutathione-S-Transferase (GST), Catalase (CAT), and NADPH quinone oxidoreductase 1 (NQO1), phase II enzymes

of drug metabolism and HSP (Galie *et al.* 2018, Bocci *et al.* 2015, Pedruzzi *et al.* 2012).

In this context, Nrf2 is considered a hormetic-like pathway (Calabrese *et al.* 2010). It has widely been reported that the activation of Nrf2 by several different mechanisms (calorie restriction, physical exercise, polyphenols) can be a way to improve health due to its transcriptional modulation on the vitagene network. Nfr2 is strongly implicated in aging processes (Zhang *et al.* 2015; Schmidlin *et al.* 2019; Silva-Palacios *et al.* 2018). These conditions share common mechanisms, and the results represent a first attempt to structure Nrf2 as a common therapeutic medicine approach. Research on the antioxidant activities of O_3 correlated with the interaction with Nrf2 (Galie *et al.* 2018; Siniscalco *et al.* 2018; Re *et al.* 2014; Vaillant *et al.* 2013). Different antioxidants can combat many associated pathologies, including neurodegenerative disorders (Leri *et al.* 2020; Calabrese 2020). The mechanisms of the positive effects of O_3 are attributed not only to up-regulation of cellular antioxidant enzyme activity, but also to the activation of the immune and anti-inflammatory systems, modulation of NPRL3 inflammasome, action on the proteasome, enhancement in the release of growth factors from platelets, improvement in blood circulation and O_2 delivery to damaged tissues, and enhancement of general metabolism (Scassellati *et al.* 2020).

The gradual escalation of the ozone dose enhances cerebral blood flow, improves metabolism, and corrects chronic oxidative stress. Neuronal cells may reactivate the synthesis of antioxidant enzymes, which is crucial to normalize the redox state and avoid cell death. The local induction of haeme oxygenase-1 played a critical role in reducing oxidative damage. The enzyme caused the local release of CO and bilirubin that acts as a potent antioxidant of peroxynitrite (Clavo *et al.* 2004). The trace amounts can pass through the blood-brain barrier to reach the sites of neurodegeneration and upregulate the cellular synthesis of antioxidant enzymes, which is a crucial step towards readjusting the impaired cell redox system.

Neurodegenerative disorders affect approximately 50 million people globally and have a tremendous and increasingly negative social-economic impact on families and society. A better understanding of degenerative events and the effects of ozone therapy during the early stage of the disease may be able to slow down the demise of critical populations of neurons and thus provide patients with a better quality of life through ozone therapy. The use of ozone in the treatment of various diseases can have a therapeutic effect, provided that the correct dose is administered at the right time interval, and depending on the antioxidant capacity of the tissue exposed, as well as ensuring the concentration is used within a non-toxic range. The versatility of ozone therapy is due to the cascade of ozone-derived compounds able to act on several targets leading to a multifactorial correction of various pathological conditions. Ozone therapy can improve well-being and delay the negative effects of aging. The aging process is essentially linked to the balance of oxidants and antioxidants, advanced glycation end products (AGE), the role of genes and the immune system, the importance of telomeres and telomerase, hormones, nutrition, environmental factors, and certain other factors. Stem cell therapy, virtual reality rehabilitation, electromagnetic fields, and ozone therapy are among the therapeutic approaches in the treatment of neurodegenerative disorders (Mitrečić *et al.* 2020; Sramka *et al.* 2020a, Sramka *et al.* 2020b).

Ozone Autohaemotherapy Induces Cerebral Metabolic Changes in Multiple Sclerosis Patients.

The oxygenated hemoglobin concentration is increased and the chronic oxidative stress level typical of MS sufferers is reduced (Molinari *et al.* 2014). Ozone has an effect on biomarkers of oxidative stress and inflammation, it significantly improves the activity of antioxidant enzymes and increases the reduced cellular glutathione levels, and demonstrates antioxidant and anti-inflammatory effects.

Ozone promotes Nrf2 phosphorylation, reducing oxidative stress and pro-inflammatory cytokines in multiple sclerosis patients (Delgado-Rosche *et al.* 2017).

Mechanisms of vascular pathophysiology in multiple sclerosis patients treated with ozone therapy demonstrate revascularization and regeneration of the blood-brain barrier as a result of immunoreactive glial cells coming into contact with blood vessel walls during ozone therapy (Ameli *et al.* 2019; Biomed 2019). The cerebrovascular system enhanced by ozone autohemotherapy in multiple sclerosis patients increases brain metabolism and helps them recover from the lower activity levels that predominate in MS patients (Molinari *et al.* 2017). At both primary and chronic stages of MS, antioxidant therapy is an active approach to disease progression. O_3 therapy increases total tissue-oxygen levels in patients with multiple sclerosis. Ozone therapy is a therapeutic alternative for patients with multiple sclerosis (Molinari *et al.* 2014; Simonetti *et al.* 2014).

Ozone therapy applied in acute ischemic stroke increases blood oxygen saturation, improves blood circulation, activates erythrocyte metabolism, improves tissue oxygenation and oxygen supply, restores cell function, promotes oxygen metabolism, and induces thrombolysis by hydrogen peroxide (H2O2) formation (Qiu *et al.* 2021). Ozone was administered daily via rectal inflation (at an ozone concentration of 40 mg/l and 200 ml) for 15 days. In the clinical phase, an improvement in the quality of life was recorded in 80% of patients treated with ozone therapy Qiu *et al.* 2021). Ozone therapy has certain therapeutic benefits for ischemic stroke patients. Jing Qiu, Hui-sheng Chen (2016) reported that ozonated autohemotherapy substantially improved neurological function in stroke patients.

Neurological Deficits Treated with Ozone Therapy. Trigeminal neuralgia, postherpetic neuralgia, meningitis,

cervical myelopathy, demyelinating of nerves. Acoustic neuroma, muscular neuropathy, and parkinsonism have shown satisfactory improvements (Kakkad 2018).

The treatment of trigeminal neuralgia with percutaneous ozone and injections into the Gasserian ganglion has been shown to have positive long-term effects on pain (An *et al.* 2018; Gao *et al.* 2020).

The treatment of phantom limb pain with ozone injection into the nerve root in the stump was met with positive results (Li *et al.* 2020).

Ozone therapy in patients with pain secondary to chemotherapy-induced peripheral neuropathy with cancer of the colon and rectum treated with oxaliplatin and rectal insufflation sessions of O_3/O_2 as part of the ongoing **randomized controlled trial (O3NPIQ) (O3NPIQ)** 2020 (Clavo *et al.* 2019). Chemotherapy-induced peripheral neuropathy decreases the quality of life of patients and can lead to a decrease in and interruption of chemotherapy treatment. Potential pathophysiological mechanisms involved in chemotherapy include chronic oxidative stress and consequent increases in free radicals and pro-inflammatory cytokines. Several antioxidant-based therapies have been tested. On the other hand, ozone therapy can elicit an adaptive antioxidant and anti-inflammatory response that could prove to be useful (Clavo *et al.* 2021).

Ozone therapy in cerebral ischemia and hypometabolism in meningioma treated by stereotactic radiosurgery. Following ozone autohemotransfusion treatment, there was an improvement in brain perfusion and metabolism, as demonstrated by SPECT and PET scans (Clavo *et al.* 2011). Rectal ozone therapy showed positive effects of improving neurorehabilitation in children with infratentorial ependymoma (Mašán & Golská 2017).

Ozone therapy of resistant meningitis in infants – a mixture of ozone gas with pure oxygen was used to treat resistant meningitis in infants with hydrocephalus and the infection was cured (Dahhan 2015).

Endolumbal ozone therapy in the treatment of patients with a complicated spinal injury in the acute period improved neurological symptoms (Yuldashev *et al.* 2020).

In the treatment of lumbar discopathy, ozone therapy is used with verified clinical benefits in the treatment of lumbar disc damage. Ozone application methods can be administered paravertebrally, juxtaforaminally, intradiscally (De Oliveira – Magalhaes *et al.* 2012; Mašán 2017; Li *et al.* 2020; Yuldashev *et al.* 2020).

CONCLUSION

Ozone therapy is a biological treatment method with a wide range of applications in medicine. The versatility of ozone therapy is due to the cascade of ozone-derived compounds able to act on several targets leading to a multifactorial correction of pathological conditions. O_3 therapy induces moderate oxidative stress when interacting with lipids, increases endogenous production of antioxidants, local perfusion, and oxygen delivery, as well as enhances immune responses. Oxidative stress occurs when there is an imbalance between free radical formation and antioxidant defense and is associated with damage to lipids, proteins, and nucleic acids. Ozone is being examined as a master regulator of multiple cytoprotective responses, as a key actor across a wide range of diseases, and as a therapeutic objective for aging and aging-associated disorders. It provides scientific evidence for the application of oxygen-ozone (O_2-O_3) in the treatment of neurological diseases. Oxidative stress is currently thought to play a significant role in the development of inflammatory diseases, ischemic diseases, hypertension, Alzheimer's disease, Parkinson's disease, muscular dystrophy, and many others.

The versatility of ozone therapy is due to the cascade of ozone-derived compounds able to act on several targets leading to a multifactorial correction of various pathological conditions, as well as cardiovascular, peripheral vascular, neurological, orthopedic conditions, skin diseases, wound healing, diabetes, and lung diseases, including the pandemic disease caused by the COVID-19 coronavirus. Ozone therapy also promotes tissue perfusion, immunomodulatory effect, energy effect of the body, and has regenerative and reparative properties. It appears to be a potentially effective treatment method without no side effects when a correct dose is administered. Further clinical and experimental studies will be needed to determine the optimal administration schedule and to evaluate the potential combination of ozone therapy with other therapies to increase the effectiveness of treatment.

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