



Review

Ozone: a natural bioactive molecule with antioxidant property as potential new strategy in aging and in neurodegenerative disorders

Catia Scassellati^{a,1,*}, Antonio Carlo Galoforo^{b,c,1}, Cristian Bonvicini^d, Ciro Esposito^{e,f,g}, Giovanni Ricevuti^{h,i,j}

^a Biological Psychiatry Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy

^b Oxygen-Ozone Therapy Scientific Society (SIOOT), Gorle, Italy

^c University of Pavia, Pavia, Italy

^d Molecular Markers Laboratory, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy

^e Department of Internal Medicine and Therapeutics, University of Pavia, Italy

^f Nephrology and dialysis unit, ICS S. Maugeri SPA SB Hospital, Pavia, Italy

^g High School in Geriatrics, University of Pavia, Italy

^h Department of Drug Sciences, University of Pavia, Italy

ⁱ P.D. High School in Geriatrics, University of Pavia, Italy

^j St. Camillus Medical University, Rome, Italy



ARTICLE INFO

Keywords:

Neurodegenerative Disorders
nuclear factor (erythroid-derived 2)-like 2 (Nrf2)
Ozone (O₃)
Oxygen-Ozone (O₂-O₃) therapy
antioxidant system
stress oxidative biomarkers

ABSTRACT

Systems medicine is founded on a mechanism-based approach and identifies in this way specific therapeutic targets. This approach has been applied for the transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Nrf2 plays a central role in different pathologies including neurodegenerative disorders (NDs), which are characterized by common pathogenetic features. We here present wide scientific background indicating how a natural bioactive molecule with antioxidant/anti-apoptotic and pro-autophagy properties such as the ozone (O₃) can represent a potential new strategy to delay neurodegeneration. Our hypothesis is based on different evidence demonstrating the interaction between O₃ and Nrf2 system. Through a meta-analytic approach, we found a significant modulation of O₃ on endogenous antioxidant-Nrf2 ($p < 0.00001$, Odd Ratio (OR) = 1.71 95%CI:1.17-2.25) and vitagene-Nrf2 systems ($p < 0.00001$, OR = 1.80 95%CI:1.05-2.55). O₃ activates also immune, anti-inflammatory signalling, proteasome, releases growth factors, improves blood circulation, and has antimicrobial activity, with potential effects on gut microbiota. Thus, we provide a consistent rationale to implement future clinical studies to apply the oxygen-ozone (O₂-O₃) therapy in an early phase of aging decline, when it is still possible to intervene before to potentially develop a more severe neurodegenerative pathology. We suggest that O₃ along with other antioxidants (polyphenols, mushrooms) implicated in the same Nrf2-mechanisms, can show neurogenic potential, providing evidence as new preventive strategies in aging and in NDs.

1. Introduction

Life span has almost doubled in the last century (WHO, 2011, Wyss-Coray, 2016), and consequently aging-specific diseases are becoming prevalent (Moskalev et al., 2017). However, the pathophysiologic mechanisms underlying most of them are still poorly understood and challenges regarding treatments efficacy and costs persist.

Neurodegenerative diseases (NDs, Alzheimer's disease, AD; Parkinson disease, PD; amyotrophic lateral sclerosis, ALS, Huntington Disease,

HD) are the most prevalent cognitive and motor disorders of the elderly. These aging-specific diseases are characterized by the loss of homeostasis during aging, leading to low-grade stress by pathologic formation of Reactive Oxygen Species (ROS), chronic inflammation, mitochondrial dysfunction and metabolic unbalance (Dugger, Dickson, 2017). In addition, these pathophenotypes are determined by abnormal aggregation of specific proteins (Yanar et al., 2020), given the connection between excessive ROS accumulation and impairment in proteostasis network.

* Corresponding author at: Biological Psychiatry Unit, IRCCS Istituto Centro S. Giovanni di Dio Fatebenefratelli, Via Pilastroni 4, Brescia 25123, Italy.

E-mail address: c.scassellati@fatebenefratelli.eu (C. Scassellati).

¹ These authors contributed equally to this work.

<https://doi.org/10.1016/j.arr.2020.101138>

Received 4 May 2020; Received in revised form 14 July 2020; Accepted 4 August 2020

Available online 15 August 2020

1568-1637/© 2020 The Author(s).

Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Despite their distinct causative factors and clinical symptoms, these diseases as well as aging have common pathogenetic features (Aso et al., 2012). This implicates potentiality in the identification of therapeutic targets on a set of disease phenotypes and physiological conditions that are mechanistically linked. Thus, contrary to a hitherto linear approach that considered one disease, one medicine, to date there is a need for a new concept of therapy condensed as “several diseases, one medicine”. In this way, diseases are diagnosed not only by clinical symptoms, but mainly by the underlying molecular signatures (Goh et al., 2007). Based on this network medicine approach, Cuadrado et al., 2018, Cuadrado et al., 2019 reported extensive evidence about the central role playing by nuclear factor erythroid-derived 2-like 2 (Nrf2). Nrf2 is widely known and investigated as a master regulator of multiple cytoprotective responses and as a key molecular node within a cluster of a wide spectrum of diseases, including NDs. Moreover, Nrf2 activation is impaired in aging by the involvement of microRNA (Zhang et al., 2015, Schmidlin et al., 2019, Silva-Palacios et al., 2018). This suggests that Nrf2 could represent a common therapeutic and systems medicine target, for aging and for its related disorders. Nrf2 can transcriptionally modulate the cytoprotective genes belonging to the vitagene network. This network regulates endogenous cellular defense mechanisms, and involves redox sensitive genes such as members of the Heat Shock Proteins (HSP) family (*Heme-Oxygenase HO-1*, *Hsp70*), but also sirtuins and the thioredoxin (Trx)/thioredoxin reductase (TrxR1) system (Calabrese et al., 2010).

Based on this rationale, in this review we present wide scientific background indicating how a natural bioactive molecule with antioxidant property such as the ozone (O_3) can be indicated as a potential new strategy to delay neurodegeneration. This hypothesis is based on the widely demonstrated 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). We first describe the relevant, well known and documented molecular mechanisms related to antioxidant/anti-apoptotic/pro-autophagy processes targeted by the O_3 administration via Nrf2 biological pathway. Secondly, we report a list of the main stress oxidative biomarkers modulated by the O_3 treatment via Nrf2 and that, in turn are strongly involved in NDs pathophysiology as well as in aging mechanisms. Different meta-analyses have been performed to demonstrate the effect in terms of Odds Ratio (OR) of O_3 on endogenous antioxidant-Nrf2 and vitagene-Nrf2 systems.

We thus provide scientific evidence to build a consistent rationale to apply for the first time the Oxygen-Ozone (O_2 - O_3) therapy in an early phase of aging decline, when it is still possible to intervene, before to develop a potential neurodegenerative pathology.

2. The Ozone (O_3) molecule and the Oxygen-Ozone (O_2 - O_3) therapy

O_3 is a triatomic gaseous molecule which has been used as a powerful oxidant in medicine for more than 150 years (Elvis, Ekta, 2011). In nature, O_3 is generated during storms due to the electrical discharges of the rays that react with atmospheric O_2 to produce O_3 . In humans, a revolutionary discovery led to demonstrate that neutrophils isolated from human peripheral blood and coated with antibodies can catalyse the generation of O_3 by a water oxidation pathway, leading to efficient killing of bacteria (Wentworth et al., 2002, Babior et al., 2003, Lerner, Eschenmoser, 2003).

In 1785, Van Mauren was the first identifying the distinctive odor of O_3 . The actual gas was later discovered by the German chemist, Christian Friedrich Schonbein at the University of Basel in Switzerland on March 13th, 1839 when working with a voltaic pile in the presence of O_2 (Altman, 2007). Friederich noticed the emergence of a gas with an electric and pungent smell, and named it ozone, which is derived from the Greek word for smell (Bocci, 2011). O_3 was used as first antiseptic for operating rooms and to disinfect surgical instruments in 1856, and in 1860 the first O_3 water treatment plant was built in Monaco to disinfect water (Altman, 2007). Nikola Tesla patented the first portable O_3

generator in 1896 in the United States. The physicist, Joachim Hansler invented the first reliable O_3 generator, and this was the breakthrough in the use of O_3 for medical applications. This invention is considered the prelude to the ozonated autohemotherapy procedure and served as the basis for O_3 therapy expansion over the last 40 years.

The O_2 - O_3 therapy is a non-invasive, non-pharmacological, no-side effect and low-cost procedure applied in medicine for the treatment of more than 50 pathological processes, whose alterations in endogenous oxidative-antioxidative balance play a crucial role. Different clinical trials evidenced the effectiveness of this therapy in the treatment of degenerative disorders such as multiple sclerosis (Smith et al., 2017, Delgado-Roche et al., 2017, Ameli et al., 2019), but also cardiovascular, peripheral vascular, neurological, orthopaedic, gastrointestinal and genitourinary pathologies (Bocci, 2011, Elvis, Ekta, 2011, Re et al., 2008, Bocci, 2012, Smith et al., 2017, Braidy et al., 2018); fibromyalgia (Moreno-Fernandez et al., 2019, Tirelli et al., 2019); skin diseases/wound healing (Fitzpatrick et al., 2018, Wang, 2018); diabetes/ulcers (Martinez-Sanchez et al., 2005, Guclu et al., 2016, Rosul, Patskan, 2016, Izadi et al., 2019, Ramirez-Acuna et al., 2019); infectious diseases (Smith et al., 2017, Mandhare et al., 2012, Song et al., 2018), including the recent global pandemic disease of coronavirus disease 2019 (COVID-19) (Zheng et al., 2020); dentistry (Isler et al., 2018, Khatri et al., 2015, Srikanth et al., 2013, Azarpazhooh et al., 2009); lung diseases (Hernandez Rosales et al., 2005); osteomyelitis (Bilge et al., 2018). The potential role of O_2 - O_3 as an adjuvant therapy for cancer treatment has been also suggested in *in vitro* and animal studies as well as in isolated clinical reports (Clavo et al., 2018).

At present, we have commenced a randomized double-blind clinical trial with the aim to test the efficacy of this therapy in a cognitive frailty cohort, a grant approved by the Italian Minister of Health (RF-2016-02363298). This pilot study will permit to validate the O_2 - O_3 therapy in an early phase of cognitive decline, when it is still possible to intervene, before to develop a potential neurodegenerative pathology.

To date, the O_2 - O_3 therapy acquires a further prestigious significance, after the medicine Nobel prize for “discovery of how cells sense oxygen” in 2019. Indeed, O_2 is the most vital element required for human life and it is the key to good health; O_3 is O_2 with an extra molecule added. The O_2 availability affects genes expression of different factors (HIFs, Hypoxia Inducible Factors), leading to the activation of trophic proteins (VEGF, Vascular Endothelial Growth Factor; PDGF, Platelet-derived growth factor) and consequently to specific biological processes, including erythropoiesis, angiogenesis and anaerobic glucose metabolism (Zhou et al., 2019). O_3 plays a role of cellular adapter to hypoxia, because it is well known its effects in increasing the levels of VEGF, PDGF, HIF (Curro et al., 2018, Zhang et al., 2014, Re et al., 2010), exactly as the cell does when there is no O_2 available.

3. Focus on the biological activities of the ozone (O_3): antioxidant property

Oxidative stress is a condition where ROS and Nitrogen Species (RNS) production exceeds the cellular antioxidant defence system, leading to the imbalance between the two systems and this may contribute to the neuronal damage and the abnormal neurotransmission. It is widely known its implication in the pathogenesis and progression of NDs (Singh et al., 2019). Brain and mitochondria are the most involved systems due to their high sensitivity to oxidative damage caused by free radicals. Oxidative damage may impair the cells in their structure and function, being cause and effect of a mitochondrial reduced activity. The damage is not confined to the brain but also evident in peripheral cells and tissues.

ROS and RNS are also major factors in cellular senescence that leads to increase 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 damages that occur during replication. Senescent cells acquire an irreversible senescence-associated

secretory phenotype (SASP), involving secretion of soluble factors (interleukins, chemokines, and growth factors), degradative enzymes like matrix metalloproteases (MMPs), and insoluble proteins/extracellular matrix (ECM) components.

Nrf2 is a member of the CNC-basic leucine zipper (CNC-bZIP) family of transcription factors. Under basal condition, Nrf2 binds to its repressor Keap1 (Kelch-like ECH-associated protein 1), an adapter between Nrf2 and Cullin 3 protein, which leads to ubiquitination followed by proteasome degradation. This Keap1-mediated degradation activity requires two reactive cysteine residues (Cys273 and Cys288).

When O_3 is administrated, it dissolves immediately in the plasma/serum and it 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) (Fig. 1). ROS are the early and short-acting messengers, while LOPs are late and long-lasting messengers. LOPs diffuse into all cells and inform them of a minimal oxidative stress. After the oxidative/electrophilic stress challenge (4HNE, (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), modification of the cysteine residues of Keap1 (S-HNE or S-S) inhibits ubiquitin conjugation to Nrf2 by the Keap1 complex (Brigelius-Flohe, Flohe, 2011), provoking the nuclear accumulation of Nrf2. Once in the nucleus, Nrf2 dimerizes and binds to cis-acting DNA AREs (Antioxidant Response Elements) in genes such as *HO-1*, a gene encoding enzyme that catalyses the degradation of heme in carbon monoxide (CO) and free iron, and biliverdin to bilirubin. CO acts as an inhibitor of another important pathway NF- κ B (Nuclear Factor Kappa B Subunit 1) signalling, which leads to the decreased expression of pro-inflammatory cytokines, while bilirubin also acts as an important lipophilic antioxidant. Furthermore, *HO-1* directly inhibits the pro-inflammatory cytokines and activates the anti-inflammatory cytokines, thus leads to balancing of the inflammatory process (Ahmed et al., 2017). Our

research group confirmed that mild ozonisation, tested on *in vitro* systems, induced modulation of genes, including *HO-1* (Scassellati et al., 2017). (Fig. 1).

In addition, Nrf2 regulates also 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), NADPH quinone oxidoreductase 1 (NQO1), phase II enzymes of drug metabolism and HSPs (Galie et al., 2018, Bocci and Valacchi, 2015, Pedruzzi et al., 2012) (Fig. 1).

A further mechanism involves casein kinase 2 (CK2), another regulator of the Nrf2 activity through its phosphorylation. It has been demonstrated that O_3 influenced the 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 signalling pathway (Wang et al., 2018a) (Fig. 1A).

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 Nrf2 promotes the clearance of oxidized or otherwise damaged proteins through the autophagy mechanism (Tang et al., 2019). Interestingly, also O_3 can modulate the degradation protein systems, not only via Nrf2 pathway, but also via activation of the AMP-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) signaling pathway, as demonstrated in Zhao et al. (2018) (Fig. 1B).

O_3 can protect against overproduction of nitric oxide (NO), when NO is a toxic oxidant. NO can rapidly react with other free radicals such as O_2^- to generate highly reactive oxidant peroxynitrite ($ONOO^-$) and other RNS, which in turn damage the biomolecules (e.g., lipids, protein, DNA/RNA), playing thus 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),

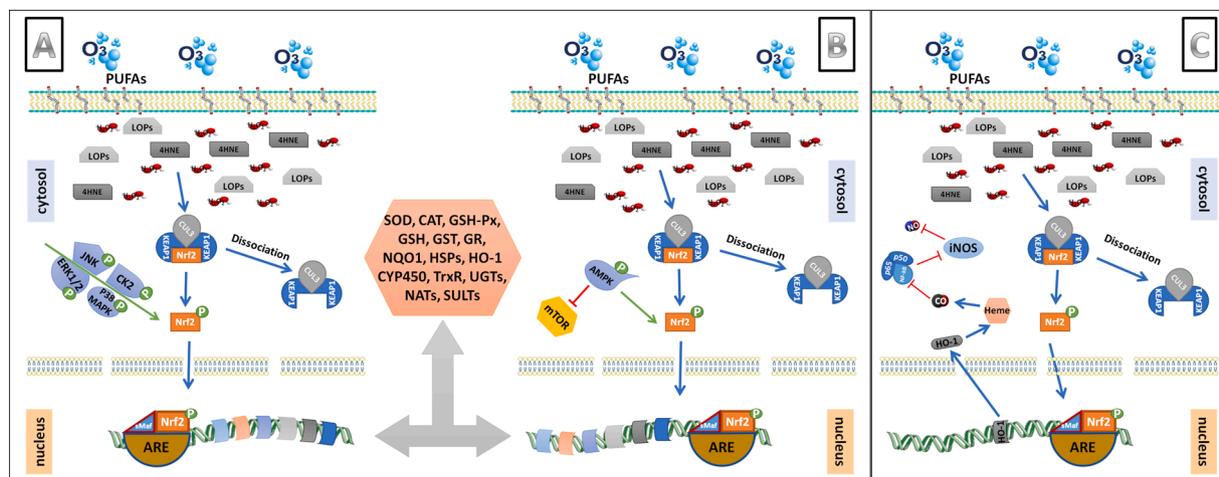


Fig. 1. Molecular mechanisms linked to antioxidant/pro-autophagy activities of ozone (O_3) via Nrf2 signalling.

In the absence of stimuli, Nrf2 (nuclear factor erythroid 2-related factor 2) binds to its repressor Keap1 (kelch-like ECH-associated protein), an adapter between Nrf2 and Cullin 3 (Cul3) protein, which leads to ubiquitination followed by proteasome degradation. When O_3 is administrated, it dissolves immediately and it reacts with PUFA (Poly-Unsaturated Fatty Acids) leading to the formation of fundamental messengers such as hydrogen peroxide (H_2O_2), 4-hydroxynonenal (4HNE) and lipid oxidation products (LOPs). These messengers can influence the modifications of cysteine residues present in Keap1 (S-HNE or S-S) inhibiting ubiquitin conjugation to Nrf2 by the Keap1 complex and provoking the nuclear accumulation of Nrf2. Once in the nucleus, Nrf2 dimerizes and binds to cis-acting DNA AREs (Antioxidant Response Elements) in different genes: *Heme Oxygenase 1 (HO-1)*, *Superoxide dismutases (SOD)*, *Glutathione peroxidase (GSH-Px)*, *Glutathione-S-Transferase (GST)*, *Catalase (CAT)*, *GSH-reductase (GR)*, *NADPH quinone oxidoreductase 1 (NQO1)*, *Heat Shock Proteins (HSPs)*, *Cytochrome P450 monooxygenase (CYP450)*, *Thioredoxin reductase (TrxR)*, *phase II enzymes (UDP-glucuronosyltransferases, UGTs; N-acetyltransferases, NATs, sulfotransferases, SULTs)*.

A) O_3 involves casein kinase 2 (CK2), a regulator of the Nrf2 activity through its phosphorylation, and MAPK (mitogen-activated protein kinase) signalling pathway, that is inhibited with consequent inactivation of oxidative stress and apoptosis by O_3 administration.

B) O_3 modulates the degradation protein systems (autophagy), via activation of the AMP-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) signaling pathway.

C) O_3 downregulates inducible nitric oxide synthase (iNOS), which generates nitric oxide (NO) via NF- κ B (Nuclear Factor Kappa B Subunit 1) pathway. (CO = carbon monoxide).

which generates NO (Manoto et al., 2018, Smith et al., 2017) via NF- κ B signalling (Fig. 1C).

4. Focus on the biological activities of the ozone (O₃): anti-apoptotic mitochondrial property

Mitochondrial dysfunction is one of the main features of the aging process, particularly in organs requiring a high-energy source such as the heart, muscles, brain, or liver. Neurons rely almost exclusively on the mitochondria, which produce the energy required for most of the cellular processes, including synaptic plasticity and neurotransmitter synthesis. Mitochondrial disfunctions cause increase in ROS for lowered oxidative capacity and antioxidant defence, with consequent increased oxidative damage to protein and lipids, decreased ATP production and accumulation of DNA damage (Garcia-Escudero et al., 2013, Reutzel et al., 2020). Moreover, mitochondrial bioenergetic dysfunction and release of pro-apoptotic mitochondrial proteins into the cytosol initiate a variety of cell death pathways.

Nrf2 transcribes several genes not only those implicated in antioxidant expression and energy regulation, but also those involved in mitochondria biogenesis: increases the mitophagy, mitochondrial levels of antioxidant enzymes, and resistance to redox regulated mitochondrial permeability transition pore opening (Holmstrom et al., 2016). Multiple lines of evidence showed that Nrf2 activation is part of the retrograde response aimed at restoring mitochondrial functions after stress insults, and that the impairment of Nrf2 functions is a hallmark of many mitochondrial-related disorders (Shan et al., 2013).

It has been demonstrated that O₃ administration can act on specific mechanisms to promote cell survival and proliferation, blocking the apoptotic processes. In particular, O₃ decreases the expression of

caspses 1-3-9, *HIF α* , *Tumor Necrosis Factor- α (TNF- α)*, *Bcl-2-associated X protein (Bax)*, *poly (ADP-ribose) polymerase 1 (PARP-1)* and *p53* genes (Fig. 2) (Yong et al., 2017, Guclu et al., 2016, Wang et al., 2018a). Bax is located in the mitochondrial membranes and exerts pro-apoptosis effect through the mitochondrial pathway, promoting cytochrome C activation (Mac Nair et al., 2016); p53 and Caspase-3 are executive molecules of apoptosis by blocking cell cycle (Wang et al., 2016). Enzymes such as SOD, CAT, and GSH-Px, can regulate p53, Bax and Bcl-2 (BCL2 Apoptosis Regulator) (Fig. 2).

Moreover, O₃ stimulates the Krebs' cycle in the mitochondria by enhancing the oxidative carboxylation of pyruvate and stimulating the production of adenosine triphosphate (ATP) (Güven et al., 2008). It also 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, Flohe, 2011, Elvis, Ekta, 2011).

O₃ treatment was proven to reduce mitochondrial damage in a rat heart following ischemia-reperfusion (Meng et al., 2017), as well as in a rat brain and cochlea following noise-induced hearing loss (Nasezadeh et al., 2017). Moreover, *in vitro*, O₃ increased the length of the mitochondrial cristae and the content of mitochondrial Hsp70 (Costanzo et al., 2018).

5. Pro-oxidation and antioxidant defence biomarkers influenced by ozone (O₃) and implicated in aging processes and in neurodegenerative disorders (NDs)

5.1. Stress-oxidant biomarkers modulated by the O₃ effect

A list of biomarkers (29 in total) implicated in oxidative stress, in

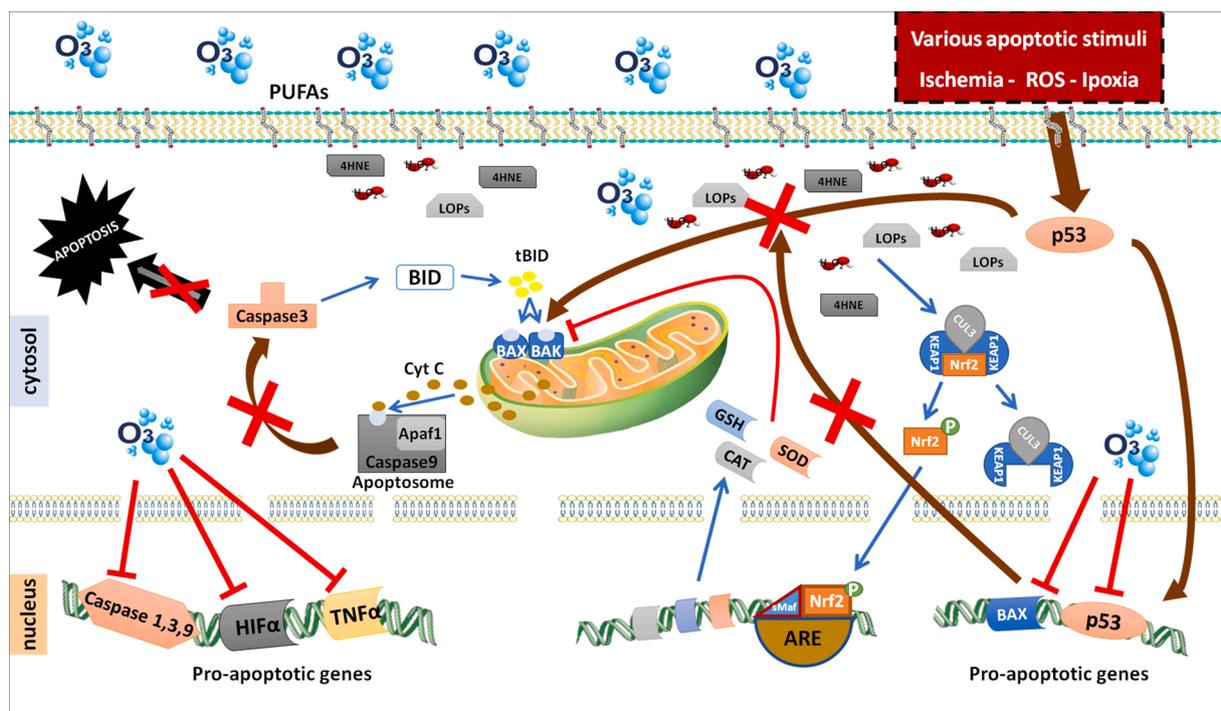


Fig. 2. Molecular mechanisms linked to anti-apoptotic property of ozone (O₃) via pro-apoptotic molecules inactivation.

Various apoptotic stimuli, ischemia, Reactive Oxygen Species, ROS, ipoxia can activate directly p53 that in turn can play a role as transcription factor and activate the expression of pro-apoptotic genes. Among these, Bak (Bcl-2 homologous antagonist/killer) and Bax (Bcl-2-associated X protein) can stimulate in mitochondrial membrane the activation of Cytochrome C that in turn activates Apaf1 (Apoptotic protease activating factor-1) and caspase 9 to close the circle to stimulate the activity of caspase 3. Enzymes such as SOD (Superoxide dismutase), CAT (catalase), and GSH-Px (glutathione peroxidase), can regulate p53, Bax and Bcl-2. O₃ administration decreases the expression of *caspses 1-3-9*, *Hypoxia-inducible factor (HIF α)*, *Tumor Necrosis Factor- α (TNF- α)*, *Bax* and *p53* genes. (BID (BH3-interacting domain death agonist), Nrf2 (Nuclear Factor Erythroid 2-related factor 2), CUL3 (Cullin 3), Keap 1 (Kelch-like ECH-associated Protein), H₂O₂ (Hydrogen Peroxide), 4HNE (4-hydroxynonenal), LOPs (Lipid Oxidation Products), Cyt C (Cytochrome C), PUFAs (Poly-Unsaturated Fatty Acid), AREs (Antioxidant Response Elements)).

Table 1

List of the pro-oxidation and antioxidant defence biomarkers influenced by ozone (O₃) and implicated in neurodegenerative disorders (NDs) as well as in aging processes.

Ozone biomarkers	Name and Function	Involvement in NDs	Involvement in Aging processes
4-HNE	<u>4-Hydroxynonenal</u> : a common aldehyde byproduct of lipid peroxidation during oxidative stress. 4-HNE is highly reactive and primarily produced in the brain via lipid peroxidation of arachidonic acid, a highly abundant omega-6 polyunsaturated fatty acids (PUFA) component of neuronal membranes. HNE may modify the ATP synthase, the final step in the production of ATP from electron transport chain (ETC) inside mitochondria. 4-HNE activates Nrf2 by alkylating thiol groups of cysteine residue in Keap1.	(Moldogazieva et al., 2019, Ayala and Munoz, 2014, Baker et al., 2015)	(Benedetti et al., 2014, Csala et al., 2015)
8-OHdG	<u>8-hydroxydeoxyguanosine (8-Oxo-2'-deoxyguanosine (8-oxo-dG))</u> : oxidized derivative of deoxyguanosine. Its concentrations within a cell are a measurement of oxidative stress (DNA oxidation). Reactive oxygen species (ROS) attack guanine bases in DNA easily and form 8-hydroxydeoxyguanosine, which can bind to thymidine rather than cytosine; thus, the level of 8-OHdG is generally regarded as a biomarker of mutagenesis consequent to oxidative stress.	(Wang et al., 2019c, Nakabeppu et al., 2007, Poulsen et al., 2014, Polidori et al., 1999)	(Mecocci et al., 2018)
AOPP	<u>Advanced Oxidation Protein Products</u> : are a group of oxidatively modified protein products containing dityrosine, pentosidine, and carbonyl-containing products generated by reactive oxygen species (ROS) or formed via myeloperoxidase reaction during oxidative/chlorine stress. They are biomarkers of oxidant-mediated protein damage.	(Wang et al., 2019c, Cristani et al., 2016)	(Maciejczyk et al., 2019, Cakatay et al., 2008, Komosinska-Vashev et al., 2012, Rusanova et al., 2018, Qing et al., 2012, Silva et al., 2015, Muller et al., 2015)
CAT	<u>Catalase</u> : it catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a scavenger enzyme of reactive oxygen species (ROS), protecting the cell from oxidative damage by ROS.	(Feitosa et al., 2018)	(Veal et al., 2018)
FRAP	<u>Ferric Reducing the Ability of Plasma</u> : total antioxidant capacity of plasma.	(Ademowo et al., 2017)	(Muller et al., 2015, Rizvi et al., 2006)
Fructolysine	It is an Amadori adduct of glucose to lysine. It is a precursor of the advanced oxidation protein products, which are induced by oxidative stress, and induces oxidative stress.	-	-
GR	<u>Glutathione reductase (or glutathione-disulfide reductase, GSR)</u> : it catalyses the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell.	(Feitosa et al., 2018, Liu et al., 2004, Rougemont et al., 2002)	(Veal et al., 2018)
GSH	<u>Glutathione</u> : it is antioxidant, capable of preventing damage to important cellular components caused by reactive oxygen species (ROS). It maintains cellular thiol status.	(Mazzetti et al., 2015, Liu et al., 2004, Gu et al., 2015, Rougemont et al., 2002, Oliveira, Laurindo, 2018)	(Maciejczyk et al., 2019, Teskey et al., 2018, Oliveira, Laurindo, 2018)
GSH-Px/GPx	<u>Glutathione peroxidase</u> : it has peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.	(Mazzetti et al., 2015, Gu et al., 2015, Rougemont et al., 2002)	(Maciejczyk et al., 2019, Veal et al., 2018)
GST	<u>Glutathione S-transferase</u> : it is phase II metabolic isozyme, known for the ability to catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification.	(Mazzetti et al., 2015, Gu et al., 2015, Rougemont et al., 2002)	(Veal et al., 2018)
HIF-1 α	<u>Hypoxia-inducible factor (HIF)-1α</u> : is a subunit of a heterodimeric transcription factor hypoxia-inducible factor 1 (HIF-1). It is a basic helix-loop-helix PAS domain containing protein and is considered as the master transcriptional regulator of cellular and developmental response to hypoxia.	(Merelli et al., 2018)	(Yeo, 2019)
HO-1	<u>Heme-Oxygenase-1</u> : it catalyzes the conversion of heme into free iron, carbon monoxide and biliverdin. It possesses two well-characterized isoforms: HO-1 and HO-2. Under brain physiological conditions, the expression of HO-2 is constitutive, abundant and ubiquitous, whereas HO-1 mRNA and protein are restricted to small populations of neurons and neuroglia. HO-1 is an inducible enzyme that has been shown to participate as an essential defensive mechanism for neurons exposed to oxidant challenges, being related to antioxidant defenses in certain neuropathological conditions.	(Facchinetti, 2020)	(Schipper et al., 2019)
HSP70	<u>Heat-Shock Protein 70</u> : it is essential for the folding and repair of damaged proteins. During stressful conditions, such as elevated temperature, it prevents protein aggregation, by facilitating the refolding or elimination of misfolded proteins. These mechanisms serve to promote cell survival conditions that would otherwise result in apoptosis.	(Lackie et al., 2017)	(Martinez de Toda, De la Fuente, 2015)
IMA	<u>Ischemia-modified albumin</u> : it measures ischemia in the blood vessels	(Altunoglu et al., 2015, Can et al., 2013)	-

(continued on next page)

Table 1 (continued)

Ozone biomarkers	Name and Function	Involvement in NDs	Involvement in Aging processes
LPO	<u>Lipid peroxide</u> : is the oxidative degradation of lipids.	(Feitosa et al., 2018, Negre-Salvayre et al., 2010)	(Negre-Salvayre et al., 2010)
MDA	<u>Malondialdehyde</u> : is a marker for oxidative stress. It is a reactive aldehyde produced by lipid peroxidation of polyunsaturated fatty acids.	(Feitosa et al., 2018, Wang et al., 2019c, Ayala and Munoz, 2014)	(Csala et al., 2015, Maciejczyk et al., 2019)
MPO	<u>Myeloperoxidase</u> : is a peroxidase enzyme. It requires heme as a cofactor. It is expressed in neutrophil and monocyte, and is implicated in various stages of inflammatory conditions with the production of a variety of potent oxidants.	(Ray, Katyal, 2016, Maki et al., 2019)	(Son et al., 2005)
Nrf2/CK2	<u>Nuclear factor erythroid 2-related factor 2</u> : is a basic leucine zipper (BZIP) protein that regulates the expression of antioxidant proteins that protect against oxidative damage triggered by injury and inflammation. <u>Casein kinase 2</u> : a serine/threonine-selective protein kinase implicated in cell cycle control, DNA repair, regulation of the circadian rhythm, and other cellular processes. Regulator of the Nrf2 activity through its phosphorylation.	(Perez et al., 2011, Sivandzade et al., 2019)	(Sivandzade et al., 2019)
NO	<u>Nitric Oxide</u> : is an important cellular signaling molecule which is derived from L-arginine by nitric oxide synthase (NOS). It works as a retrograde neurotransmitter in synapses, allows the brain blood flow, and has important roles in intracellular signaling in neurons from the regulation of the neuronal metabolic status to the dendritic spine growth. It is able to perform post-translational modifications in proteins by the S-nitrosylation of the thiol amino acids, which is a physiological mechanism to regulate protein function.	(Hannibal, 2016, Nakamura, Lipton, 2020, Radi, 2018)	(Picon-Pages et al., 2019)
NO-3/NO-2 (NOx)	<u>Nitrate/nitrite</u> : an index of NO production	(Hannibal, 2016, Nakamura, Lipton, 2020, Radi, 2018)	(Picon-Pages et al., 2019)
NOS	<u>Nitric oxide synthase</u> (inducible i II, endothelial e I): it catalyzes the production of nitric oxide (NO) from L-arginine.	(Hannibal, 2016, Nakamura, Lipton, 2020)	(Jung et al., 2012)
PCC/PCO	<u>Protein carbonyl content</u> : catalyses the carboxylation reaction of propionyl CoA in the mitochondrial matrix.	(Chevion et al., 2000, Fedorova et al., 2014)	(Cabiscol et al., 2014, Cakatay et al., 2008)
PP	<u>Protein phosphatase</u> : it is a serine/threonine phosphatase. It has been found to be important in the control of glycogen metabolism, muscle contraction, cell progression, neuronal activities, splicing of RNA, mitosis, cell division, apoptosis, protein synthesis, and regulation of membrane receptors and channels.	(Braithwaite et al., 2012, Clark, Ohlmeyer, 2019)	(Salminen et al., 2016)
SOD	<u>superoxide dismutase</u> : are the first and most important line of scavenger antioxidant enzyme defence systems against ROS and particularly superoxide anion radicals. There are two isoforms of SOD (cytoplasmatic CuZn-SOD or SOD1 and mitochondrial Mn-SOD or SOD2).	(Feitosa et al., 2018, Schaffert, Carter, 2020)	(Maciejczyk et al., 2019, Veal et al., 2018)
TAC	<u>Total antioxidant capacity</u>	(Mota et al., 2019)	(Maciejczyk et al., 2019)
TAS	<u>Total antioxidant status</u>	(Mota et al., 2019)	
TBARS	<u>Thiobarbituric acid reactive substances</u> : byproducts of lipid peroxidation (i.e. as degradation products of fats)	(Vina et al., 2005)	(Muller et al., 2015)
TH	<u>Total Hydroperoxides</u> : indicator of oxidative stress.	(Tarafdar, Pula, 2018)	
TOS	<u>Total oxidant score</u>	(Mota et al., 2019)	

Note: In bold the genes involved in Nrf2 signalling

endogenous antioxidant and vitagene systems are showed in Table 1. These biomarkers have been studied and found modulated after the O₂-O₃ therapy in more of 150 studies performed in different *in vivo* (human and animal models) and *in vitro* samples and conditions. In Table 1, we also reported the relative functions of these biomarkers.

From these 29 biomarkers, we focused, in this section, on those implicated in endogenous antioxidant-Nrf2 pathway (GSH; GSH-Px; glutathione reductase, GR; SOD; CAT; 4HNE; Advanced Oxidation Protein Products, AOPP in bold in Table 1). Where it was possible (available studies), we performed meta-analyses for these biomarkers on human (see supplementary material). The results showed significant increased levels of the SOD-CAT-GSH-Px-GSH-GST-GR after O₃ administration (Fig. 3, Random model, Z = 6.15, p < 0.00001, OR = 1.71 95%CI:1.17-2.25; even after Bonferroni correction 0.05/6 = 0.0083). Similar results were obtained even considering single markers, except for GR (Z = 1.04; p = 0.30) and GSH (Z = 0.80, p = 0.42). GR has been investigated only in two studies, coming from the same authors (Hernandez Rosales et al., 2005). Thus, there are not enough evidence on its single real involvement. Concerning GSH, Diaz-Luis et al., (Díaz-Luis et al., 2018) is the

only study showing a negative effect of O₃. As we followed the criteria for which the data were extracted before and after O₃ treatment (see supplementary material), this study found an increased GSH levels after O₃ administration, only when the authors performed the comparisons with control group of healthy subjects (in a sort of postconditioning). Thus, if we eliminated this study, the results of the single meta-analysis of GSH highlighted its positive increase determined by the O₃ treatment (Z = 2.30; p = 0.02, data not shown).

High heterogeneity in effect size across the studies (P < 0.00001, I² = 97%) was observed in these meta-analyses. This is essentially explained by the presence of different factors: the type of pathology, different concentration of O₃ linked to different administration procedures and duration time treatments, age of the sample (supplementary material Table 1S).

Interestingly, different studies have been performed on aging-specific conditions. A recent work (El-Mehi, Faried, 2020) demonstrated that antioxidant properties of O₃ can ameliorate age-associated structural alterations of the rat cerebral cortex, improving age-related oxidative stress reflected in the histopathological and

immunohistochemical alterations. The authors detected severe structural and cellular neurodegenerative changes in the frontal cortex of the aged rats. O₃ administration produced significant downregulation of tissue Malondialdehyde (MDA), an index of oxidative stress, and upregulation of GSH, SOD and CAT. Similarly, O₃ influenced iNOS, caspase-3, glial fibrillary acidic protein (GFAP), Ki67 and acetylcholinesterase (AChE). These findings indicate reduction not only in oxidative stress, but also in apoptosis (down-regulation caspase-3) and in gliosis (down-regulation GFAP), as well as improving in neurogenesis (upregulation of Ki-67 expression) and in cholinergic plasticity (decrease AChE activity). The authors suggest that O₃ might be useful for improving the age – related cognitive and memory deterioration, by increasing cholinergic communication.

Safwat et al. (Safwat et al., 2014) demonstrated that O₃ showed a beneficial effect on the aging reducing liver and kidney damage through its antioxidant property. O₃ was efficient in elevating the reduced hepatic and renal GSH contents as well as in normalizing hepatic GSH-Px activity of aged rats. Moreover, O₃ succeeded in attenuating the elevated hepatic and renal MDA and protein carbonyls (PC) levels.

Another work (El-Sawalhi et al., 2013) reported that O₃ alleviated age-associated redox state imbalance, as evidenced by reduction of lipid and protein oxidation markers and lessening of lipofuscin deposition. Moreover, O₃ restored GSH levels in brain and heart tissues, and normalized GSH-Px activity in the heart tissue of the aged-rats. O₃ also mitigated age-associated energy failure in the heart and the hippocampus, improved cardiac cytosolic Ca(2+) homeostasis and restored the attenuated Na(+), K(+) -ATPase activity in the hippocampus of these rats.

Similarly, prophylactic administration of O₃ in aged-rats normalized reduced GSH content, adenosine triphosphate/adenosine diphosphate ratio, mitochondrial SOD and complex IV (cytochrome-c oxidase) activities. O₃ improved glutathione redox index (GSHRI), complex I (NADH-ubiquinone oxidoreductase) and mitochondrial mtNOS activities, and attenuated the rise MDA and mitochondrial PC levels (Shehata et al., 2012).

5.2. Stress-oxidant biomarkers implicated in aging mechanisms

Several evidence support the involvement of these biomarkers influenced by the O₃ administration in the mechanisms of aging (Table 1). We prevalently focused on those implicated in the Nrf2 signalling (in bold in Table 1).

It has been reported that the levels of lipid peroxidation products, reactive carbonyl compounds, such as 4HNE, are increased in aging tissues (Csala et al., 2015), and this increase is positively correlated with age. Impaired protein function, manifested as an increase in PC, plays a crucial role in aging processes (Cabiscol et al., 2014). With increase of PC, the spontaneous carbonyl-amino crosslinking and accumulation were mostly irreparable changes associated with aging (Nowotny et al., 2014).

Several findings evidenced altered levels of AOPP in aging (Komoinska-Vassev et al., 2012, Rusanova et al., 2018, Qing et al., 2012, Silva et al., 2015, Muller et al., 2015). A recent work investigated the antioxidant enzymes (GSH-Px, CAT, SOD), nonenzymatic antioxidants (GR), redox status (total antioxidant capacity, TAC, total oxidant status, TOS, oxidative stress index, OSI), and oxidative damage products (AOPP, MDA) in a healthy sample divided according to age: 2-14 (children and adolescents), 25-45 (adults), and 65-85 (elderly people). They demonstrated that salivary and blood antioxidant defense is most effective in adults. Contrarily, a progressive decrease in the efficiency of central antioxidant systems (↓GSH-Px, ↓SOD, ↓GSH, ↓TAC in erythrocytes and plasma vs. adults) was observed in the elderly. Both local and systemic antioxidant systems were less efficient in children and adolescents than in the group of middle-aged people, which indicates age-related immaturity of antioxidant mechanisms. Oxidative damage to proteins (↑AOPP) and lipids (↑MDA) was significantly higher in saliva and

plasma of elderly people in comparison with adults and children/adolescents (Maciejczyk et al., 2019). Similarly, Cakatay et al. (Cakatay et al., 2008) found, in a young, middle-aged and elderly individuals sample, PCO and AOPP levels of the elderly and middle aged individuals higher compared with those of the young.

Although not involved in Nrf2 signaling but influenced by O₃ treatment, the increased oxidative damage to mitochondrial DNA (mitDNA) with the OH8dG (8-hydroxydeoxyguanosine) formation, represents the most common hallmark of the aging brain, marker of oxidative DNA damage. The simultaneous increased oxidation of mtDNA and deficiency of DNA repair could enhance the lesion to mitochondrial genome, potentially causing neuronal damages (Mecocci et al., 2018).

5.3. Stress-oxidant biomarkers implicated in NDs

Several evidence support the implication of the pro-oxidation and antioxidant defence biomarkers influenced by O₃ listed in Table 1 in the aetiopathogenetic mechanisms of NDs. Even for NDs, we prevalently focused on those implicated in the Nrf2 signalling (in bold in Table 1).

5.3.1. Alzheimer's Disease

AD is characterized by progressive loss of cognitive and behavioral deterioration, which leads to the impairment of daily and routine activities. It is one of the most prevalent NDs manifesting 45 million people worldwide. AD is characterized by the deposition of protein aggregates, extracellular amyloid plaques (Aβ), intracellular tau (τ) or neurofibrillary tangles, and loss of synaptic connections in specific regions of brain (Schipper, 2010, Mattson, 2004, Selkoe, 2001). The neuropathological diagnostic feature of AD is the accumulation of neurotoxic Aβ oligomer peptides, which, along with τ protein, mediate neurodegeneration, thus causing neuroinflammation, impairment in synaptic connection, cholinergic denervation, neurotransmitter imbalance, neuronal loss, and dendritic alterations.

Different studies indicate the relationship between Aβ-induced oxidative imbalance and elevated levels of by-products of lipid peroxidation (e.g., 4HNE, MDA), protein oxidation (e.g., carbonyl), and DNA/RNA oxidation (e.g., OH8dG) (Wang et al., 2014c, Zhao and Zhao, 2013, Pratico, 2008, Mecocci et al., 2018). These alterations were observed also in peripheral lymphocytes and lymphocyte mitochondria (for review Mecocci et al., 2018). Higher levels of PC, measured in mitochondria extracted from lymphocytes, have been observed in AD (for review Mecocci et al., 2018).

Decreased levels of antioxidant enzymes like SOD, CAT, GSH, decreased ratio of GSH/GSSG (Glutathione disulfide), and/or impaired expressions or activities of GSH-related enzymes have been observed in blood or brain of AD patients (Singh et al., 2019, Liu et al., 2004, Kim et al., 2006, Oliveira, Laurindo, 2018).

RNS such as NO are also found to have a deleterious effect on neurons. Indeed, RNS elevation has been observed both in astrocytes as well as in neurons in an AD brain (for review Singh et al., 2019). An increase in the expression of neuronal nNOS or NOS-1, cytokine-inducible iNOS or NOS-2, and endothelial eNOS or NOS-3 isozymes has been observed in AD astrocytes. The direct association of iNOS and eNOS with Aβ aggregates indicating towards beta amyloid assisted in the induction of NOS to produce NO, which in turn leads to the formation of 3-nitrotyrosine (NT) (Luth et al., 2002, Luth et al., 2001).

Other findings reported increased levels of CK2 in the hippocampus and temporal cortex of AD patients (Rosenberger et al., 2016) and increased levels in AOPP (Can et al., 2013, Altunoglu et al., 2015), compared to non-demented controls. It has been observed that AD patients showed an increased oxidation of red blood cells GSH, which indicates oxidative stress in peripheral cells, and an increased level of plasma thiobarbituric acid reactive substances (TBARS), which indicates a higher free radical oxidation of plasma unsaturated phospholipids (Vina et al., 2005).

Moreover, HO-1 has been proposed as systemic marker in early

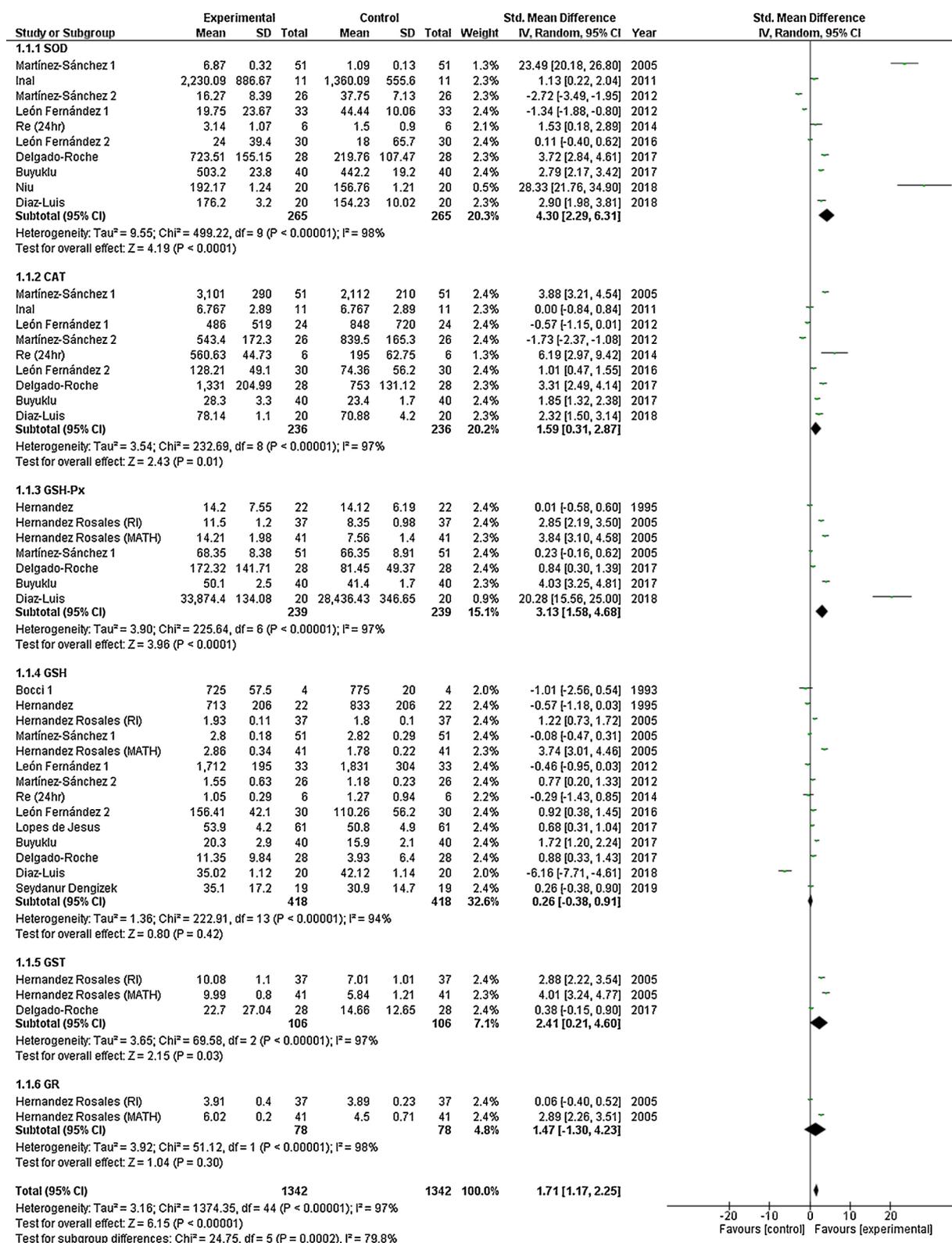


Fig. 3. Forest plot for odds ratio from meta-analysis of the endogenous Nrf2- antioxidant pathway before and after ozone (O₃) treatment. CI, confidence interval; Chi², χ^2 test of goodness of fit; Tau², estimate of the between-study variance in a random-effects meta-analysis. Superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GSH-Px), Glutathione (GSH), Glutathione S-transferase (GST), Glutathione reductase (GR). RI = rectal insufflations; MATH = major autohemotherapy

sporadic AD (Schipper et al., 2000). Indeed, plasma HO-1 protein levels are significantly decreased in patients with probable sporadic AD (Schipper, 2007). The up-regulation of HO-1 in AD brain can be explained because of local oxidative stress. Instead, the mechanism responsible for the downregulation of HO-1 in the blood of AD patients remains unclear, even though the existence of a HO-1 suppressor that inhibits HO-1 mRNA levels in the lymphocytes in AD plasma has been proposed (Maes et al., 2006). However, the results about HO-1 plasma levels in patients with AD are controversial. A study found no changes in the serum level of HO-1 in a big cohort of AD patients, as compared with elderly control subjects, whereas increased levels were observed in PD patients, highlighting different mechanisms involved in the peripheral response to oxidative stress in the two diseases (Mateo et al., 2010). Moreover, another study reports that in plasma of probable AD patients, both HO-1 and biliverdin reductase (BVR) levels were increased because of the enhanced oxidative stress. The authors suggested that plasma BVR status, more than HO-1, can represent a potential biochemical marker for the prediction of AD at the earliest stages of disease (Di Domenico et al., 2012; for review Nitti et al., 2018).

5.3.2. Parkinson's disease (PD)

PD is the second most prevalent neurodegenerative disorder, after AD, which is characterized by the progressive degeneration of the dopaminergic neurons located in the substantia nigra (SN) pars compacta (Spillantini et al., 1998) which affects movement. The main neuropathological hallmark of PD is the presence of intracellular inclusions known as Lewy bodies (LBs) and neurites (LNs) (Forno, 1996); predominantly composed by misfolded and aggregated forms of the presynaptic protein α -synuclein (α Syn; a small protein with 140 amino acids abundant in presynaptic nerve terminals) (Spillantini et al., 1998). α Syn plays a role in synaptic transmission and dopamine levels adjustment. α Syn primarily affect tyrosine hydroxylase phosphorylation and activity and the expression level of dopamine transporter on the cell membrane.

Different evidence supported the involvement of the pro-oxidation and antioxidant defence biomarkers influenced by O_3 listed in Table 1 also with PD (focus on Nrf2). Altered levels of GSH and GSSG, decreased ratio of GSH/GSSG, and/or impaired expressions or activities of GSH-related enzymes have been detected in PD (Liu et al., 2004). TOS and OSI levels were found higher in the PD patients as compared to controls (Mota et al., 2019).

RNS also play major role in nitrosative stress in PD. NO, produced by nNOS or iNOS was found in large quantities in cells, as well as in the extracellular space around dopaminergic neurons (Tieu et al., 2003). It has been observed that in PD brains, NO obstructs various enzymes including complex I and IV of the mitochondrial electron transport chain, hinders the function of proteins by forming S-nitrosothiols, mediates lipid peroxidation, resulting in elevated levels of ROS and brain deteriorating effect. *In situ* hybridization and immunohistochemical studies also established the role of NO in PD *via* postmortem brain tissue analysis, which indicates an elevated level of iNOS and nNOS in basal ganglia structures (Eve et al., 1998, Hunot et al., 1996). ONOO⁻ has been shown to inhibit the presynaptic dopamine transporter, which mediates the uptake of dopamine from the synaptic cleft to stop dopamine signalling, and to refill the dopamine vesicles. Its inactivation will induce a decrease in dopamine delivery (Picon-Pages et al., 2019).

Oxidative damage in nucleic acids is likely to be a major risk factor for PD (Bosco et al., 2006, Puspita et al., 2017). Oxidative DNA lesions, such as 8-oxoguanine (8-oxoG), accumulate in nuclear and mitochondrial genomes during aging, and such accumulation can increase dramatically in these patients (Nakabeppu et al., 2007).

5.3.3. Amyotrophic Lateral Sclerosis (ALS)

Among the various neurodegenerative diseases, ALS is the most common type of motor neuron disease; it is sometimes called Lou Gehrig's disease, after the famous baseball player who had this condition.

ALS is characterized by the progressive degeneration of upper and lower motor neurons in the spinal cord, cortex, and brainstem (Kikuchi et al., 2002). Although for most of the last 2 decades mutation of Cu-Zn SOD1 was the only genetic aberration associated with the onset of familial ALS, recent studies have discovered additional abnormalities associated with the onset of sporadic and non-SOD1 familial ALS. These include a host of RNA/DNA-binding proteins such as the 43-kDa transactive response (TAR) DNA-binding protein (TDP-43) and the fused in sarcoma/translocated in liposarcoma (FUS/TLS). The most common genetic mutation is identified as expanded GGGGCC hexanucleotide repeat in the non-coding region of the *C9orf72* gene located on chromosome 9p21 (Mendez, Sattler, 2015).

Wang et al., (Wang et al., 2019c) reported increased blood levels of 8-OHdG, MDA, and AOPP and decreased GSH and uric acid levels in the peripheral blood of ALS patients. These biomarkers have been found in sporadic ALS patient's urine, cerebrospinal fluid (CSF), blood, and individual tissues.

5.3.4. Huntington Disease (HD)

HD named after George Huntington in 1872, is a fatal and autosomal dominant inherited progressive neurodegenerative disorder, resulting in neuronal degeneration in the striatum followed by deterioration of the cerebral cortex and thalamus. HD is caused by a mutation in the *huntingtin* (*HTT*) gene. It is characterized by an abnormal extension in the cytosine-adenine-guanine (CAG) repeat in this gene, which in turn translates into an abnormally long repeat of polyglutathione in the mutant huntingtin protein. HD is mainly characterized by impaired motor and cognitive traits, personality change, and psychiatric illness (Vonsattel, DiFiglia, 1998).

Lipid peroxidation, DNA damage, and specifically protein carbonylation were found to be more pronounced in HD (Tunee et al., 2011). Dysregulation in cysteine metabolism was observed in HD (Paul et al., 2018). Cysteine plays vital roles in redox homeostasis, being a component of the major antioxidant GSH and a potent antioxidant by itself. In HD patients, decreased GSH levels and increased lipid peroxidation were observed as compared with controls (Oliveira, Laurindo, 2018). In postmortem brain specimens of HD, a twofold increase of OH8dG in mtDNA was found in the parietal and slightly less in the frontal cortex compared to controls (Polidori et al., 1999).

6. Molecular mechanisms involving ozone (O_3), Nrf2 and vitagene network and their biological relevance in neuroprotection

At the core of adaptive responses at the cell and origin of biological organization is the concept of hormesis (Calabrese et al., 2010). Hormesis describes a process that results in ameliorating and improve cellular stress resistance, survival, and longevity in response to sub-lethal levels of stress (Mattson, 2008). Generally, a favorable biological response to low exposure to any stressor is found within the hormetic zone, whereas cell damage occurs at higher doses. The hormetic dose response results from either a direct stimulation or through an overcompensation stimulatory response following disruption in homeostasis (Calabrese and Baldwin, 2000). This theory is, to date a frontier area of neurobiological research, focal to understand and develop new/complementary therapeutic approaches to NDs. In this context, Nrf2 is considered as 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, mushrooms) can be a way to improve life health, due to its transcriptionally modulation on the vitagene network. Calabrese et al. (Calabrese et al., 2010), performed an exhaustive review on this topic, and they described in detail each single element of the vitagene pathway. Members of the Hsp70s are, in their function as molecular chaperones, involved in folding of newly synthesized proteins and

refolding of damaged or misfolded proteins, as well as in assembly and disassembly of protein complexes. Trx, is a major redox control system, consisting of a 12 kDa redox active protein Trx, and a homodimeric seleno-protein called TrxR1. TrxR1 is a flavoprotein that catalyzes the NADPH-dependent reduction of oxidized thioredoxin protein. It is usually located in the cytosol, but it translocates into the nucleus in response to various stimuli associated with oxidative stress, thereby playing a central role in protecting against oxidative stress. Sirtuins are histone deacetylases which, in the presence of NAD⁺ as a cofactor, catalyze the deacetylation reaction of histone substrates and transcriptional regulators. Sirtuins regulate different biological processes, such as apoptosis, cell differentiation, energy transduction, and glucose homeostasis.

Recent reviews support wide evidence on how different nutraceuticals/antioxidants can contrast aging and combat many associated pathologies, including NDs (Leri et al., 2020, Calabrese, 2020). Natural polyphenols (i.e. curcumin, resveratrol, flavonols present in *Ginkgo biloba* extracts, polyphenols present abundantly in the leaves and in the ripening fruits of the olive tree, *Olea europaea*), as well as mushrooms (*Hericium Erinaceus*, *Coriolus versicolor*) can significantly modulate Nrf2 and Nrf2-dependent vitagenes expression, showing neuroprotective action. This can potentially resolve pathologies such as AD, PD and also Meniere's Disease, another degenerative pathology (Amara et al., 2020, Trovato et al., 2016a, Trovato et al., 2016b, Trovato Salinaro et al., 2018, Scuto et al., 2019).

In line with these findings, several studies demonstrated that also O₃ can modulate the vitagenes network expression. Pharmacologically, it acts in a hormetic fashion (Bocci et al., 2011, Calabrese, 2013), according an inverted V shape curve. We researched studies for meta-analyses regarding Nrf2, HO-1, Hsp70, TrxR1 and sirtuins. Whereas no studies were performed between sirtuins, TrxR1 and O₃, the results indicated that O₃ can statistically increase the expression/protein levels of Nrf2, HO-1 and Hsp70 molecules (Fig. 4, Random model, Z = 4.72 p < 0.00001 OR = 1.80 95%CI:1.05-2.55, even after Bonferroni correction 0.05/3 = 0.016). Although our work has been excluded because we performed transcriptomic analyses (Scassellati et al., 2017), we confirmed the increase of the gene encoding HO-1 (*HMOX-1*), after different concentrations of O₃. The high heterogeneity in effect size among the studies (p < 0.0001 I² = 66%) is essentially determined by two factors: different sources of samples (human, cell and animal models) and different methodology (biochemical and western blot analyses, ultrastructural and immunocytochemistry evaluations) (supplementary material Table 1S). Where it was possible, we performed the analysis as homogeneously as possible: in this case, O₃ concentration (20µg/ml) and exposition time (max 24 hr) were constant in all experimental conditions.

Interestingly, a study reported the benefit effect of O₃ on Meniere's disease (Pawlak-Osinska et al., 2004). Moreover, as reported for polyphenols and mushrooms (Hsiao et al., 2016, Ferreira et al., 2018, Oh et al., 2014, Pan et al., 2018, Hasanzadeh et al., 2020, Wang et al., 2019b), O₃ has been found to be involved in β-catenin system (Emon et al., 2017) as well as in NLRP3 (nitrogen permease regulator-like 3) inflammasome (Yu et al., 2017, Wang et al., 2018c).

All these evidence support that, as polyphenols and mushrooms, O₃ acts in the same direction. Induction of vitagenes after their supplementation/administration determines a maintained response to counteract intracellular pro-oxidant status, thus providing neuroprotection.

7. Effect of Ozone Oxidative Preconditioning on Oxidative Stress Injury

Preconditioning is a process whereby an initial low dose of a stressor agent upregulates adaptive mechanisms that enhance resilience against subsequent and acute stressor agents within a time-sensitive window of ~ 10–14 days (Calabrese, 2016). Different studies demonstrated that the supplementation with *Coriolus versicolor* (Ferreiro et al., 2018, Scuto et al., 2019, Trovato Salinaro et al., 2018, Trovato et al., 2016a), and

Hericium Herinaceus (Trovato Salinaro et al., 2018, Trovato et al., 2016b) biomass and polyphenols (Mao et al., 2019) can maintain the response to neutralize intracellular pro-oxidant/neuroinflammatory status, preventing different neurological conditions.

Same behaviour was also widely reported for O₃. The term “ozone oxidative preconditioning” (OzoneOP) was coined when repeated administration of O₃ at nontoxic doses facilitate adaptation to oxidative stress. This occurs through mild immune system activation, enhanced release of growth factors and/or activation of metabolic pathways that help maintain redox balance (increased SOD, GSH activities, decreased peroxidation).

The first studies on OzoneOP were conducted by Barber et al., 1999 (Barber et al., 1999) and Leon OS et al., 1998 (Leon et al., 1998). From 1998-1999 to date, a plethora of investigations on this topic was conducted. In Table 2, we reported 65 findings, of which 55 on OzoneOP, whereas 10 are the studies conducted on postconditioning phenomenon.

We observed that OzoneOP exerts a protective effect on ischemia-reperfusion injury (IRI) in rat models of cochlear, hepatic, intestinal, renal, cardiac, lung and skeletal ischemia through an oxidative preconditioning mechanism that prevents the increase of the endogenous pro-oxidant and stimulates antioxidant mechanisms (Table 2). Some authors also developed an *in vitro* Hypoxia/Reoxygenation (H/R) model to simulate OzoneOP, using normal rat kidney epithelial (NRK-52E) cells. This to eliminate confounding variables linked to animal models (Wang et al., 2014a, Wang et al., 2018a). Interestingly, the results confirmed those obtained in *in vivo* animal model (Table 2).

OzoneOP prevents also other different kind of injury: lipopolysaccharide (LPS) injection, carbon tetrachloride, partial hepatectomy, total body irradiation, methotrexate, intraperitoneal injection of rat fecal material, sepsis, kidney and cardiac transplantation, contrast-induced nephropathy, induction of diabetes, cisplatin-induced nephrotoxicity, contrast-induced nephropathy agent, H₂O₂, doxorubicin, ototoxicity, noise exposure, hypothermia, lipofundin (Table 2).

Different methodological systems have been implemented in these studies. The different authors analysed differences in mRNA gene expression levels as well as protein levels in Western Blot and biochemical analyses. All authors performed morphological, histopathological, immunofluorescence, and immunohistochemistry evaluations, in parallel and in concordance with molecular investigations. Interestingly, in some cases, the effects observed were strongly dose and time-dependent (Table 2).

In some cases (10 in total), the studies have been performed in postconditioning, obtaining the same outcomes. León Fernández et al. (Leon Fernandez et al., 2012) investigated the systemic redox status of patients with low back pain and neck pain, and if O₃ oxidative post-conditioning modified the pathological oxidative stress and protected against oxidative protein damage. In 33 patients with diagnosis of disc hernia (DH), 100% showed a severe oxidative stress. Major changes in SOD, total hydroperoxides, AOPP, fructolysine, and MAD were observed. After O₃ postconditioning, there was a re-establishment of patients' cellular redox balance as well as a decrease in pain in both DH. This demonstrated that O₂-O₃ therapy protected against oxidation of proteins and reduced the pain.

8. Conclusions

According to Cuadrado et al. (2018), Cuadrado et al. (2019), systems medicine identifies a cluster of chronic disease pathophenotypes including NDs in which Nrf2 plays a fundamental role. Similarly, Nfr2 is strongly implicated in aging processes (Zhang et al., 2015, Schmidlin et al., 2019, Silva-Palacios et al., 2018). These condition/diseases share common mechanisms and the results represent a first attempt to structure Nrf2 as a common therapeutic and systems medicine approach.

We here have presented extensively research and strength on the antioxidant activities of O₃ correlated with the interaction with Nrf2 (Galie et al., 2018, Siniscalco et al., 2018, Re et al., 2014, Vaillant et al.,

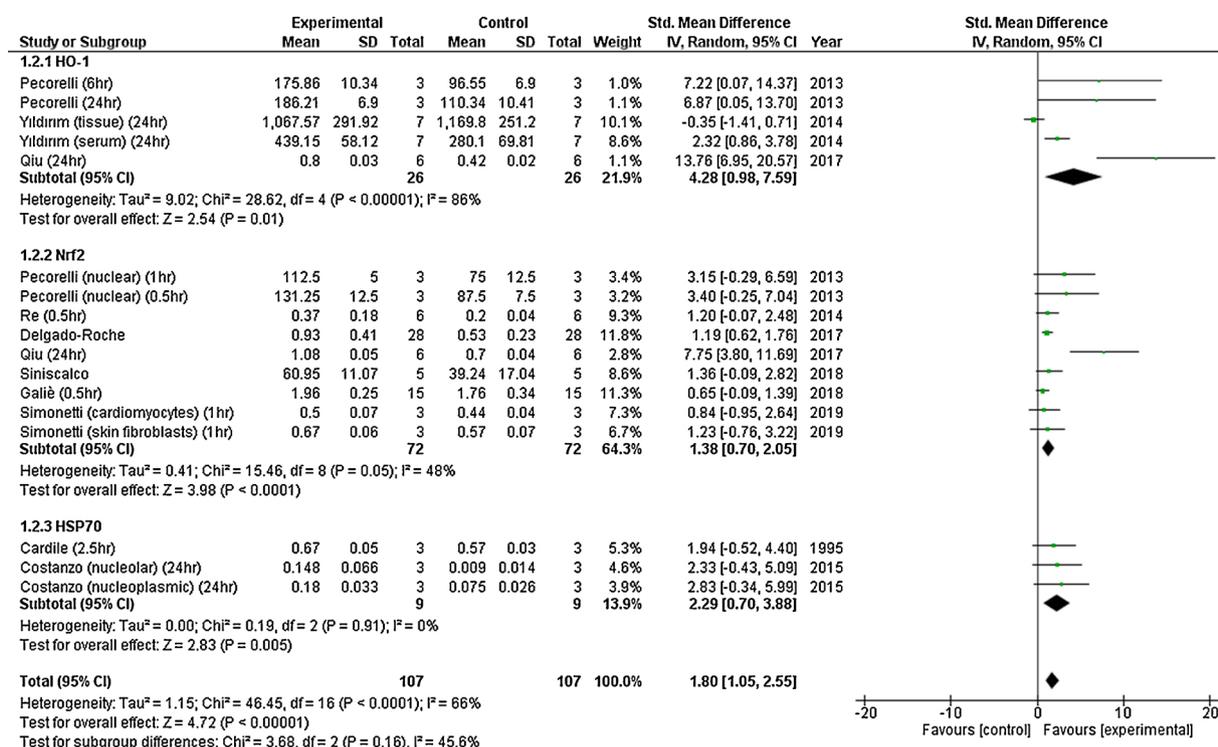


Fig. 4. Forest plot for odds ratio from meta-analysis of the endogenous Nrf2- vitagene pathway before and after ozone (O₃) treatment. CI, confidence interval; Chi², χ^2 test of goodness of fit; Tau², estimate of the between-study variance in a random-effects meta-analysis. Nuclear factor Nrf2, heme-oxygenase (HO-1), heat shock protein (HSP)

2013), along with anti-apoptotic functions by acting on mitochondrial Bax, caspases, p53 and HIF α molecules (Yong et al., 2017, Guclu et al., 2016), pro-autophagy and bioenergetic activities on Krebs's cycle. This paper provides a road map for mechanism-based systems medicine where O₃-Nrf2-vitagene network plays a crucial role in the modulation of the cellular redox balance, in the reduction of the formation of ROS/RNS, in the change of apoptotic and autophagy mechanisms (Vikram et al., 2017). This underlines the evidence to become potential new therapeutic targets for NDs, and at the same time to reduce the aging physiological mechanisms and cognitive decline, potential risk factors to develop more severe neurodegeneration damage.

Challenges regarding treatments efficacy and costs still persist for NDs. Thus, we suggest that O₂-O₃ therapy could represent a useful, safe, no-invasive, no-pharmacological, economical, effective treatment for these neurodegenerative conditions. In the medical setting, this therapy employs a gas mixture of O₂/O₃, obtained from the modification of medical-grade O₂ using certificated O₃ generator device (Bocci, V., 2011). Based on the basic mechanisms of action of O₃ in blood, the therapeutic range of O₃ has been precisely calculated and found to be 10–80 μ g/ml of O₃ in blood (Schwartz-Tapia et al., 2015). O₃ medical preparations are classified into three types: ozonized water, ozonized oil and ozonized gas, whereas different and main routes of application with relative concentrations of O₃ are widely described in Schwartz-Tapia et al., 2015 (Schwartz-Tapia et al., 2015).

The side effects are minimal; the World Federation of Ozone therapy (WFOT) estimates the incidence of complications at 0.0007%. Moreover, the treatment is not only perfectly tolerated but most of patients have reported a feeling of wellness and euphoria throughout the cycle. This fact explains why the compliance of the patients remains excellent throughout the years.

The mechanisms of the positive effects of O₃ 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 proteasome, enhancement in the release of growth factors from platelets, improvement in blood

circulation and O₂ delivery to damaged tissues, and enhancement of general metabolism, along with being a potent bactericide, fungicide and virucidal with potential effect on gut microbiota (for review Scassellati et al., 2020). Consequently, these combinatorial effects could impact on cognitive and neurodegenerative domains, directly or indirectly through the mediation of gut microbiota (Cattaneo et al., 2017). Nrf2-ARE and vitagene network, but also NF- κ B, NFAT (nuclear factor activated T-cells), AP-1 (Activated Protein-1), HIF α are the principal signalling pathways on which O₃ exercises its effects (for review Scassellati et al., 2020). These effects could be sharable with those involved in NDs, where high inflammation and oxidant state, mitochondria dysfunctions, metabolic alterations, and slowdown in regenerative processes and immune system characterize these disorders.

As reported in Smith et al., 2017, to date systems are available and proposed to have a more precise measurement of the redox state of a patient. One system proposes simultaneously measuring different biological markers in the blood such as GSH, GSH-Px, GST, SOD, CAT, conjugated dienes, total hydroperoxides, and TBARS. Using an algorithm, information can be gathered about the total antioxidant activity, total pro-oxidant activity, redox index, and grade of oxidative stress. Thus, systems like this can provide insights to the correct dosage and response to O₃ therapy based on oxidative stress levels seen in the patient.

With the awareness that further studies are needed, this review reports substantial scientific evidence for building a rationale of using the O₂-O₃ therapy to delay aging processes and neurodegeneration, exploiting well documented omni various functions of O₃. This therapy could represent a convenient, inexpensive monodomain intervention, working in absence of side effects that will permit to modulate the oxidant, but also immune, inflammatory, metabolic, microbiota and regenerative processes impaired in NDs.

There is a recent consistent upsurge of interest in complementary medicine, especially dietary supplements and foods functional in delaying the onset of age-associated NDs. O₃ along with other antioxidants (polyphenols, mushrooms) can open new neuroprotective

Table 2

Preconditioning/postconditioning studies of O₃ on endogenous pro-antioxidant mechanisms *in vivo* on animal models and *in vitro* on cells.

Tissues	Dosages	Results	References
	<u>Preconditioning:</u> 0.7 mg/kg, intraperitoneally, 15 applications (once daily), before methotrexate (Mtx) (6 mg/kg).	<u>Reduction:</u> malondialdehyde (MDA). <u>Increase:</u> superoxide dismutase (SOD), glutathione peroxidase GSH-P × . <u>Histologically:</u> ILEUM: less inflammatory cell infiltration and edema, reduction in vacuolated cells in the epithelium; LIVER/ KIDNEY: no significant change, due probably to the cumulative prolonged effect of Mtx on these tissues.	(Kesik et al., 2009)
	<u>Postconditioning :</u> <u>Sprague Dawley rats:</u> 1, 2 mg/kg, rectal insufflations, 15 applications, once a day, ischemia/reperfusion. <u>Renal tubular epithelial cell line, NRK-52E:</u> 20, 30, 40 µg/mL in complete medium, hypoxia–reoxygenation.	<u>IN VIVO:</u> <u>Reduction dose-dependent manner:</u> blood urea nitrogen (BUN), creatinine (Cr), malondialdehyde (MDA), bcl-2-associated X (BAX) and poly (ADP-ribose) polymerase 1 (PARP-1) expression, MAPK signaling pathway. <u>Increase dose-dependent manner:</u> superoxide dismutase (SOD). <u>Histologically:</u> ozone protected the tubular epithelium from swelling and from loss of the brush border. <u>IN VITRO:</u> <u>Reduction dose-dependent manner:</u> MAPK pathways, CREB, c-fos, bcl-2-associated X (BAX) and poly (ADP-ribose) polymerase 1 (PARP-1) expression, apoptosis, malondialdehyde (MDA), phosphorylation of p38, ERK1/2, and JNK. <u>Increase dose-dependent manner:</u> superoxide dismutase (SOD).	(Wang et al., 2018a)
	<u>Postconditioning:</u> <u>Sprague Dawley rats:</u> 2 mg/kg, rectal insufflations, 15 applications, once a day, after ischemia/reperfusion. <u>Renal tubular epithelial cell line, NRK-52E:</u> 20, 30, 40 µg/mL in complete medium, after hypoxia–reoxygenation.	<u>IN VIVO:</u> <u>Reduction:</u> blood urea nitrogen (BUN), creatinine (Cr), malondialdehyde (MDA), caspase 1, caspase 11, interleukin 1β (IL-1β), Interleukin-18 (IL18) expression/protein. <u>Increase:</u> superoxide dismutase (SOD). <u>IN VITRO:</u> <u>Reduction:</u> malondialdehyde (MDA), caspase 1, caspase 11, interleukin 1β (IL-1β), Interleukin-18 (IL-18) expression/protein. <u>Increase:</u> superoxide dismutase (SOD), cell viability. <u>Histologic Examinations, Immunofluorescence Staining:</u> prevented renal damage, reduction in Jablonski grading scale scores, decreased caspase 1.	(Wang et al., 2019a)
	<u>Postconditioning:</u> 0.5 mg/kg, rectal insufflations, after ischemia/reperfusion. A control with Oxygen was used.	<u>Reduction:</u> serum creatinine (Cr), blood urea nitrogen (BUN), myeloperoxidase (MPO), malondialdehyde (MDA), α-smooth muscle actin (α-SMA), transforming growth factor β1 (TGF-β1), phospho-Smad 2 protein. <u>Increase:</u> superoxide dismutase (SOD). <u>Histology:</u> Jablonski scores of histologic appearance in acute tubular necrosis, renal areas of tubulointerstitial fibrosis showed minimal phenomenon. <u>Immunocytochemistry:</u> Myofibroblasts (α-SMA positive) were faintly detected in ozone-treated samples.	(Jiang et al., 2020)
KIDNEY	<u>Preconditioning:</u> 1 mg/kg, rectal insufflations, 15 applications, once a day, before ischemia/reperfusion.	<u>Reduction:</u> α-smooth muscle actin (α-SMA), transforming growth factor-β1 (TGF-β1) expression/protein. <u>Increase:</u> Smad7 expression/protein. <u>Morphological/immunohistochemistry:</u> increase in collagen staining, reduction in α-SMA expression.	(Wang et al., 2014b)
	<u>Postconditioning:</u> 0.5 mg/kg, daily for the 10 days' reperfusion, after ischaemia–reperfusion. A control was performed with Oxygen.	<u>Reduction:</u> serum creatinine (Cr), blood urea nitrogen (BUN), thiobarbituric acid reactive substances (TBARS). <u>Increase:</u> fructosamine, phospholipase A2, superoxide dismutase (SOD). <u>Morphology:</u> minimal alterations.	(Calunga et al., 2009)
	<u>Preconditioning:</u> 1 mg/kg, rectal insufflations, 15 applications, once a day, before the kidney transplantation.	<u>Reduction:</u> serum blood urea nitrogen (BUN), creatinine (Cr), malondialdehyde (MDA), renal allograft cell apoptosis index. <u>Increase:</u> superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), nuclear factor erythroid 2-related factor 2 (Nrf-2), heme oxygenase 1 (HO-1). <u>Morphological/immunohistochemistry:</u> lower levels of damage, less severe renal allograft.	(Qiu et al., 2017)
	<u>Preconditioning:</u> 0.7 mg/kg/d, intraperitoneally, 5 days, before the induction of contrast-induced nephropathy. A control group was with Oxygen.	<u>Reduction:</u> serum blood urea nitrogen (BUN), creatinine (Cr), serum/renal malondialdehyde (MDA), total oxidant status (TOS). <u>Increase:</u> serum/renal nitric acid (NO), total antioxidant status (TAS). <u>Histopathologic evaluation:</u> reduction in degeneration of tubular epithelium, dilatation of Bowman capsule, necrosis in tubular epithelium, vascular congestion.	(Kurtoglu et al., 2015)
	<u>Preconditioning:</u> 1 mg/kg, rectal insufflations, 15 applications, once a day, before ischemia/reperfusion and/or ischemic preconditioning.	<u>Reduction:</u> malondialdehyde (MDA), urea nitrogen (BUN), creatinine (Cr), Jablonski grading scale scores. <u>Increase:</u> serum nitric acid (NO), NO synthase (endothelial, eNOS and inducible, iNOS) expression/protein, glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px). <u>Histological Examination/Immunohistochemistry:</u> improved renal dysfunction, histological damage, renal oxidative stress, increase presence of endothelial, eNOS and inducible, iNOS.	(Chen et al., 2008c)
	<u>Preconditioning:</u> IN VITRO Renal tubular epithelial cell line, NRK-52E, 20, 30, 40 µg/mL in complete medium, before hypoxia/reoxygenation.	<u>Reduction dose-dependent manner:</u> 40 µg/mL apoptosis rate, malondialdehyde (MDA), Lactate dehydrogenase (LDH), bcl-2-associated X (BAX), Bcl2, poly (ADP-ribose) polymerase 1 (PARP-1) expression. <u>Increase dose-dependent manner:</u> superoxide dismutase (SOD). <u>Immunocytochemistry:</u> decrease in cleaved caspase3-positive	(Wang et al., 2014a)

(continued on next page)

Table 2 (continued)

Tissues	Dosages	Results	References
		<u>Reduction:</u> serum blood urea nitrogen (BUN), creatinine (Cr), malondialdehyde (MDA), myeloperoxidase (MPO), Tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), intercellular adhesion molecule (ICAM-1), monocyte chemoattractant protein 1 (MCP-1), Toll-Like Receptor (TLR4), nuclear factor (NF- κ B) expression/protein, caspase-3, bcl-2-associated X (BAX), Bcl2.	(Chen et al., 2008a)
	<u>Preconditioning:</u> 1 mg/kg, rectal insufflations, 15 applications, once a day, before ischemia/reperfusion.		
	<u>Preconditioning:</u> 1 mg/kg, rectal insufflations, 15 applications, once a day, before ischemia/reperfusion.	<u>Morphology:</u> decreased score in Jablonski scale histology grading. <u>Reduction:</u> malondialdehyde (MDA), serum blood urea nitrogen (BUN), creatinine (Cr), tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), intercellular adhesion molecule (ICAM-1), monocyte chemoattractant protein 1 (MCP-1), Toll-Like Receptor (TLR4) and nuclear factor (NF- κ B) expression/protein /immunohistochemical, caspase-3, bcl-2-associated X (BAX), Bcl2. <u>Morphological/Immunohistochemical features:</u> relieved tubular necrosis, medullary haemorrhage, congestion and development of proteinaceous casts, reduction in Jablonski scores.	(Xing et al., 2015)
	<u>Preconditioning:</u> 1 mg/kg, rectal insufflations, 15 treatments, once a day, before ischemia/reperfusion. As control was used also Oxygen.	<u>Reduction:</u> serum blood urea nitrogen (BUN), creatinine (Cr), Jablonski grading scale scores, endothelin-1. <u>Increase:</u> serum nitric oxide (NO), NO synthase (endothelial, eNOS, inducible, iNOS) expression/protein, superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GSH-Px). <u>Morphology:</u> preservation of tissue histology.	(Chen et al., 2008b)
	<u>Postconditioning:</u> 0.5 mg/kg, rectal insufflations, 10 applications, once a day, after ischemia/reperfusion. As control was used also Oxygen.	<u>Histopathological/Morphology:</u> no significant differences for filtration fraction and proteinuria, improvement in glomerular filtrate rate, renal plasma flow, creatinine, less overall histological damage.	(Fernandez Iglesias et al., 2011)
	<u>Preconditioning:</u> 1.1 mg/kg, intraperitoneally, 5 days, before induction of diabetes. Other groups were diabetic rats/insulin.	<u>Reduction:</u> Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Glycosylated hemoglobin (HbA1c), serum blood urea nitrogen (BUN), creatinine (Cr), aldose reductase (AR), malondialdehyde (MDA). <u>Increase:</u> superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT).	(Morsy et al., 2010)
	<u>Preconditioning:</u> 25 mcg/ml, intraperitoneally, 15 days, before methotrexate (20 mg/kg).	<u>Reduction:</u> malondialdehyde (MAD), Myeloperoxidase (MPO), Tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β). <u>Increase:</u> glutathione (GSH). <u>Histopathologically:</u> reduction in degeneration of glomerular structures, glomerular congestion, dilatation of Bowman's space, degeneration of proximal tubuli, degeneration of distal tubuli, tubular basal membrane wrinkling, vascular congestion, interstitial edema, inflammation and cell infiltration.	(Aslaner et al., 2015)
	<u>Preconditioning:</u> 0.36, 0.72, 1.1, 1.8, 2.5 mg/kg, rectal insufflations, 15 applications, before cisplatin-induced nephrotoxicity (6 mg/kg).	<u>Reduction dose-dependent manner:</u> creatinine (Cr) (0.72, 1.1 mg/kg), thiobarbituric acid-reactive substances (TBARS). <u>Increase dose-dependent manner:</u> glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) (0.72, 1.1 mg/kg), catalase (CAT). <u>Histopathological changes:</u> at doses of 1.8 and 2.5 mg/kg, histopathological significant improved changes in renal tissue	(Borrego et al., 2004)
	<u>Preconditioning:</u> 1 mg/kg, intraperitoneally, 6 hours before and 6 hours after contrast-induced nephropathy agent (10 ml/kg), 5 days.	<u>Increase:</u> total antioxidant capacity (TAC), lipocalin (NGAL). No alteration in creatinine. <u>Histopathological alterations:</u> improving in Renal tubular injury, hemorrhage, cast formation.	(Ozturk et al., 2018)
	<u>Preconditioning:</u> Major Ozonated Autohemotherapy in 5 m blood rabbit, before ischemia/reperfusion.	<u>Reduction:</u> interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), white blood cells, neutrophil to lymphocyte ratio (NLR), ischemia-modified albumin (IMA), total oxidant status (TOS), oxidative stress index (OSI). <u>Increase:</u> total antioxidant status (TAS). <u>Histopathological changes:</u> reduced the tubular brush border loss (TBBL), tubular cast (TC), tubular necrosis (TN), intertubular hemorrhage congestion (IHC), dilatation of bowman space (DBS).	(Sancak et al., 2016)
	<u>Preconditioning:</u> 0.5 mg/kg, rectal insufflations, 15 treatments, before ischaemia/reperfusion. Oxygen was used as further control.	<u>Reduction:</u> Phospholipase A, Fructosamine. <u>Increase:</u> p-amino-hippurate (PAH), inulin, superoxide dismutase (SOD). <u>Morphology:</u> increased renal plasma flow (RPF), glomerular filtration rate (GFR).	(Barber et al., 1999)
	<u>Preconditioning:</u> 0.8, 2.4, 4 mg/kg, intraperitoneally, daily for 5 days, with/without sepsis. A control was performed with Oxygen.	<u>Reduction:</u> serum alanine amino transferase (ALT), aspartate amino transferase (AST), creatinine (CRE), thiobarbituric acid reactive substances (TBARS), myeloperoxidase (MPO). <u>Increase:</u> superoxide dismutase (SOD), glutathione peroxidase (GSH-Px).	(Rodriguez et al., 2009)
	<u>Preconditioning:</u> 1 mg/kg, transrectal insufflations, once a day, 15 treatments, before the kidney transplant procedure.	<u>Reduction:</u> blood urea nitrogen (BUN), serum creatinine (Cr) (slightly), Jablonski grade, serum interleukin-6 (IL-6), IL-18, cyclooxygenase-2 (Cox-2), Malonaldehyde (MDA), nuclear factor NF- κ Bp65 and rabbit polyclonal anti-rat antibody (HMGB1) expression/protein. <u>Increase:</u> Superoxide Dismutase (SOD), Glutathione peroxidase (GSH-Px). <u>Morphology:</u> alleviated the morphological damages, attenuated the injury of brush border of proximal renal tubular, restrained the expression level of NF- κ Bp65 in renal tissue, suppressed the expression of HMGB1 in renal tissue.	(Wang et al., 2018b)

(continued on next page)

Table 2 (continued)

Tissues	Dosages	Results	References
	150 mg/kg, intraperitoneally, single dose for 10 days, at the same time <i>Escherichia coli</i> toxin (LPS) (20 mg/kg).	<u>Reduction</u> : lactate dehydrogenase (LDH) (Liver, Kidney, Lungs, Heart). <u>Increase</u> : Succinate Dehydrogenase (SDH) (Lungs, Heart), adenosine triphosphatase (ATPase) (no Kidney), acid phosphatase (AcPase) (Liver, Kidney, Lungs, Heart), β -Glucuronidase (Liver, Kidney, Lungs). <u>Histochemically detected activity of succinate dehydrogenase (SDH)</u> : extinguished enzymatic activity in central parts of the lobule and paralleled by narrowing of zone I (Liver). <u>Histochemically detected activity of lactate dehydrogenase (LDH)</u> : increased activity (hepatocytes, Kupffer cells, Liver). <u>Histochemically detected activity of adenosine triphosphatase (ATPase)</u> : decrease intensity of the reaction for ATPase (Liver). <u>Histochemically detected activity of acid phosphatase (AcPase)</u> : lower decrease in activity (Liver). <u>Histochemically detectable activity of succinate dehydrogenase (SDH)</u> : the reaction in tubular epithelial cells was slightly more pronounced (Kidney). <u>Histochemically detected activity of lactic dehydrogenase (LDH)</u> : less pronounced stimulation of enzyme in principal tubules and other portions of nephrons (Kidney). <u>Histochemically detected activity of adenosine triphosphatase (ATPase)</u> : decreased intensity of the reaction in renal glomeruli and in walls of blood vessels, particularly those of low caliper (Kidney). <u>Histochemically detected activity of acid phosphatase (AcPase)</u> : decreased intensity of the reaction pertained in principal tubuli and collecting ducts (Kidney). <u>Histochemically detected activity of succinate dehydrogenase (SDH)</u> : no more pronounced alterations (Lungs). <u>Histochemically detected activity of lactate dehydrogenase (LDH)</u> : stimulation was less pronounced (Lungs). <u>Histochemically detected activity of adenosine triphosphatase (ATPase)</u> : no changing (Lungs). <u>Histochemically detected activity of acid phosphatase (AcPase)</u> : decreased activity (Lungs).	(Madej et al., 2007)
	<u>Preconditioning</u> : 0.2, 0.4, 1.2 mg/kg intraperitoneally, once daily, for 5 days, before lipopolysaccharide (LPS) injection (30 mg/kg). Dexamethasone (30 mg/kg) used as a reference drug.	<u>Reduction dose-dependent manner</u> : thiobarbituric acid reactive substances (TBARS). <u>Increase dose-dependent manner</u> : glutathione peroxidase (GPx).	(Rodriguez et al., 2011)
	<u>Preconditioning</u> : 0.2, 0.4, 1.2 mg/kg intraperitoneally, once daily, for 5 days, before lipopolysaccharide (LPS) injection (0.1 mg/kg). Dexamethasone (30 mg/kg) used as a reference drug.	<u>Reduction dose-dependent manner</u> : serum Tumor Necrosis Factor (TNF)-alpha, thiobarbituric acid reactive substances (TBARS). <u>Increase dose-dependent manner</u> : glutathion-S transferase (GST), glutathione peroxidase (GSH-Px).	(Zamora et al., 2005)
	<u>Preconditioning</u> : 0.2, 0.4, 1.2 mg/kg, intraperitoneally, 0.2, 0.4 mg/kg, rectal application, once daily for five days, before lipopolysaccharide (LPS) injection (0.1 mg/kg).	<u>Reduction dose-dependent manner</u> : serum Tumor Necrosis Factor (TNF)-alpha.	(Zamora et al., 2004)
	<u>Preconditioning</u> : 50 ug/ml (4.4–5.0 ml), 15 treatments, one per day, before carbon tetrachloride (CCl4). Ozone control groups were: 1. A control was with Oxygen; 2. another control was ozone without CCl4.	<u>Reduction</u> : Aspartic alanine transaminase (AST), phospholipase A, hepatic lipid peroxidation (TBARS, thiobarbituric acid-reactive substances). <u>Increase</u> : cholinesterase (CHEase), superoxide dismutases (SODs), Catalase (CAT), Calcium-dependent (Ca-ATPase), glutathione (GSH), glucose-6-phosphate dehydrogenase (G6PD). <u>Morpho-metric evaluation of the hepatic damage</u> : reduction of the damage area.	(Leon et al., 1998)
LIVER	<u>Preconditioning</u> : 1 mg/kg, rectal insufflations, 15 treatments, one per day, before ischaemia-reperfusion.	<u>Reduction</u> : Aspartic alanine transaminase (AST), serum alanine aminotransferase (ALT), malondialdehyde (MDA) + 4-hydroxyalkenals, nitrite/nitrate (NO ₂ /NO ₃ -). <u>Increase</u> : superoxide dismutase (SOD), total hydroperoxide (TH), glutathione (GSH), Ratio GSH/GSSG.	(Ajameh et al., 2004)
	<u>Preconditioning</u> : 0.7 mg/kg, intraperitoneally, daily five times, before 70% partial hepatectomy.	<u>Reduction</u> : serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), tumor necrosis factor alpha (TNF- α). <u>No alterations</u> : interleukin-6 (IL-6). <u>Histopathological examination</u> : improve in liver weight, mitotic index, proliferating cell nuclear antigen (PCNA) labeling index. <u>Reduction time-dependent manner</u> : serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), tumor necrosis factor alpha (TNF- α), malondialdehyde (MDA). <u>Increase time-dependent manner</u> : superoxide dismutase (SOD).	(Gultekin et al., 2013b)
	<u>Preconditioning</u> : 0.7 mg/kg, intraperitoneally, daily five times, before total body irradiation with a single dose of 6 Gy.	<u>Histopathological examination</u> : reduction in hepatocellular degeneration, inflammation, congestion and dilatation in both sinusoids and central veins; reduced inflammatory cell infiltrate in the lamina propria; regular villous structure, abundant goblet cells in the epithelium; reduced inflammatory cell infiltrate in the lamina propria.	(Gultekin et al., 2013a)
	<u>Preconditioning</u> : 0.5 mg/kg, intraperitoneally, daily five times, before lipopolysaccharide (LPS) injection (20 mg/kg). Ketamine (5 mg/kg) used as a reference drug.	<u>Reduction</u> : Nuclear factor κ B (NF- κ B) staining. <u>Morphology/Immunohistochemistry parameters</u> : intact hepatic architecture, normal liver cell membrane integrity, little inflammatory cell infiltration (low NF- κ B-positive staining).	(Sun, Pei, 2012)

(continued on next page)

Table 2 (continued)

Tissues	Dosages	Results	References
	<u>Preconditioning:</u> 1 mg/kg, rectal insufflations, 15 treatments, one per day, before ischemia/reperfusion. Agonist (2-chloro N6 cyclopentyladenosine, CCPA), Antagonist (8-cyclopentyl-1,3-dipropylxanthine, DPCPX) of A1 subtype receptor.	<u>Reduction:</u> serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), nitric oxide (NO) (nitrite/nitrate (NO ₂ -)/NO ₃ -), adenosine deaminase (ADA), malondialdehyde (MDA), 4-hydroxyalkenals, attenuated GSSG increase, NF-κB (p65 subunit) expression, tumor necrosis factor alpha (TNF-α), heat shock protein-70 (HSP70). <u>Increase:</u> glutathione (GSH). <u>Immunohistochemistry:</u> remarkable preservation of the liver parenchyma architecture, prevention of the inflammatory recruitment.	(Fernández et al., 2008)
	<u>Preconditioning:</u> 1 mg/kg, rectal insufflations, 15 treatments, one per day, before ischemia/reperfusion. Cycloheximide (CHX) to promote protein synthesis inhibition after OzoneOP treatment.	<u>Reduction:</u> serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), 4-hydroxyalkenals. <u>Increase:</u> SOD (MnSOD), glutathione (GSH), GSH/GSSG. <u>Histological lesions:</u> normal morphology of the acinus like sham-operated. <u>Ultrastructural analysis:</u> normal appearance of mitochondrial, rough endoplasmatic reticulum and peroxisome, no alteration on nucleus structure.	(Ajamieh et al., 2005)
	<u>Preconditioning:</u> 1 mg/kg, rectal insufflations, 15 treatments, one per day, before ischemia/reperfusion and/or ischaemic preconditioning. Oxygen was another control comparison.	<u>Reduction:</u> serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), 5'-NT, malondialdehyde (MDA), 4-hydroxyalkenals, calcium, calpain, total Xanthine dehydrogenase (XDH), xanthine oxidase (XO). <u>Increase:</u> total slylfhydryl groups. <u>Improvement in histological parameters:</u> normal morphology of hepatic lobuli.	(Ajamieh et al., 2002)
	<u>Preconditioning:</u> 1 mg/kg, rectal insufflations, 15 treatments, one per day, before carbon tetrachloride (CCl4) (1 ml/kg). An ozone control group was ozone without CCl4.	<u>Reduction:</u> uric acid, lactate, thiobarbituric acid-reactive substances (TBARS). <u>Increase:</u> hepatic glycogen, liver weight (LW)/body weight (BW) ratios, superoxide dismutase (SOD), catalase (CAT). <u>Histopathological findings:</u> the permanence of glycogen deposits in hepatic cells was proved, only a minimal non-parenquimatous cell reaction co-existed around the central vein.	(Candelario-Jalil et al., 2001)
	<u>Preconditioning:</u> 0.7 mg/kg, intraperitoneally, 15 applications (once daily), before methotrexate (Mtx) (6 mg/kg).	<u>Reduction:</u> malondialdehyde (MDA). <u>Increase:</u> superoxide dismutase (SOD), glutathione peroxidase (GSH-Px). <u>Histologically:</u> ILEUM: less inflammatory cell infiltration and edema, reduction in vacuolated cells in the epithelium; LIVER/KIDNEY: no significant change, due probably to the cumulative prolonged effect of Mtx on these tissues.	(Kesik et al., 2009)
	<u>Preconditioning:</u> 10, 30, 50 µg/ml, intraperitoneally, 5 days, before sepsi induced by intraperitoneal injection of rat fecal material (0.5 g per kg of animals weight) extracted from another donor rat. A control group was performed with Oxygen.	<u>Reduction dose-dependent manner in LIVER/LUNG:</u> conjugated dienes (CD), thiobarbituric acid-reactive substances (TBARS), Total pro-oxidant activity. <u>Increase dose-dependent manner:</u> superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), Total antioxidant activity (TAC).	(Guanche et al., 2010)
	<u>Preconditioning:</u> 0.8, 2.4, 4 mg/kg, intraperitoneally, daily for 5 days, with/without sepsis. A control was with Oxygen.	<u>Reduction:</u> serum alanine amino transferase (ALT), aspartate amino transferase (AST), creatinine (CRE), thiobarbituric acid reactive substances (TBARS), myeloperoxidase (MPO). <u>Increase:</u> superoxide dismutase (SOD), glutathione peroxidase (GSH-Px). <u>Reduction:</u> lactate dehydrogenase (LDH) (Liver, Kidney, Lungs, Heart). <u>Increase:</u> Succinate Dehydrogenase (SDH) (Lungs, Heart), adenosine triphosphatase (ATPase) (no Kidney), acid phosphatase (AcPase) (Liver, Kidney, Lungs, Heart), β-Glucuronidase (Liver, Kidney, Lungs). <u>Histochemically detected activity of succinate dehydrogenase (SDH):</u> extinguished enzymatic activity in central parts of the lobule and paralleled by narrowing of zone I (Liver). <u>Histochemically detected activity of lactate dehydrogenase (LDH):</u> increased activity (hepatocytes, Kupffer cells, Liver). <u>Histochemically detected activity of adenosine triphosphatase (ATPase):</u> decrease intensity of the reaction for ATPase (Liver). <u>Histochemically detected activity of acid phosphatase (AcPase):</u> lower decrease in activity (Liver). <u>Histochemically detectable activity of succinate dehydrogenase (SDH):</u> the reaction in tubular epithelial cells was slightly more pronounced (Kidney). <u>Histochemically detected activity of lactic dehydrogenase (LDH):</u> less pronounced stimulation of enzyme in principal tubules and other portions of nephrons (Kidney). <u>Histochemically detected activity of adenosine triphosphatase (ATPase):</u> decreased intensity of the reaction in renal glomeruli and in walls of blood vessels, particularly those of low caliper (Kidney). <u>Histochemically detected activity of acid phosphatase (AcPase):</u> decreased intensity of the reaction pertained in principal tubuli and collecting ducts (Kidney). <u>Histochemically detected activity of succinate dehydrogenase (SDH):</u> no more pronounced alterations (Lungs). <u>Histochemically detected activity of lactate dehydrogenase (LDH):</u> stimulation was less pronounced (Lungs). <u>Histochemically detected activity of adenosine triphosphatase (ATPase):</u> no changing (Lungs).	(Rodriguez et al., 2009)
	150 mg/kg, intraperitoneally, single dose for 10 days, at the same time <i>Escherichia coli</i> toxin (LPS) (20 mg/kg).	<u>Histochemically detected activity of succinate dehydrogenase (SDH):</u> the reaction in tubular epithelial cells was slightly more pronounced (Kidney). <u>Histochemically detected activity of lactic dehydrogenase (LDH):</u> less pronounced stimulation of enzyme in principal tubules and other portions of nephrons (Kidney). <u>Histochemically detected activity of adenosine triphosphatase (ATPase):</u> decreased intensity of the reaction in renal glomeruli and in walls of blood vessels, particularly those of low caliper (Kidney). <u>Histochemically detected activity of acid phosphatase (AcPase):</u> decreased intensity of the reaction pertained in principal tubuli and collecting ducts (Kidney). <u>Histochemically detected activity of succinate dehydrogenase (SDH):</u> no more pronounced alterations (Lungs). <u>Histochemically detected activity of lactate dehydrogenase (LDH):</u> stimulation was less pronounced (Lungs). <u>Histochemically detected activity of adenosine triphosphatase (ATPase):</u> no changing (Lungs).	(Madej et al., 2007)

(continued on next page)

Table 2 (continued)

Tissues	Dosages	Results	References
	<u>Preconditioning</u> : 0.7 mg/kg, intraperitoneally, 5 applications (once daily), before total body irradiation (TBI) (6 Gy).	<u>Histochemically detected activity of acid phosphatase (AcPase)</u> : decreased activity (Lungs). <u>Reduction</u> : malondialdehyde (MDA), serum tumor necrosis factor alpha (TNF- α), interleukin-1 beta (IL-1 β). <u>Increase</u> : superoxide dismutase (SOD). <u>Histopathological evaluation</u> : reduction in alveolar area, interstitial congestion, and alveolar and bronchiolar hemorrhage.	(Bakkal et al., 2013)
	<u>Preconditioning</u> : 100 μ g/kg, intraperitoneally, once daily for 10 days, before ischemia/reperfusion. A control was performed with Oxygen.	<u>Reduction</u> : malondialdehyde (MDA), myeloperoxidase (MPO), inflammasome (NLRP3), apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC), un-cleavable cysteine-requiring aspartate protease-1 (procaspase-1), cysteine-requiring aspartate protease-1 (caspase-1), apoptotic index, interleukin-1 beta (IL-1 β). <u>Increase</u> : transcription factor Nrf2, superoxide dismutase (SOD). <u>Macroscopic and histologic view</u> : dark and edematous tissue, inter alveolar septum, rupturing and alveolar space hemorrhage disappear.	(Wang et al., 2018c)
	<u>Preconditioning</u> : 0.8, 2.4, 4 mg/kg, intraperitoneally, daily for 5 days, with/without sepsis. A control was performed with Oxygen.	<u>Reduction</u> : serum alanine amino transferase (ALT), aspartate amino transferase (AST), creatinine (CRE), thiobarbituric acid reactive substances (TBARS), myeloperoxidase (MPO). <u>Increase</u> : superoxide dismutase (SOD), glutathione peroxidase (GSH-Px). <u>Reduction dose-dependent manner</u> : bcl-2-associated X (BAX), nuclear factor NF- κ B, tumor necrosis factor alpha (TNF- α), Inducible nitric oxide synthase (iNOS), nitrite levels. <u>Increase dose-dependent manner</u> : catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), glutathione (GSH) expression.	(Rodriguez et al., 2009)
	<u>Preconditioning</u> : IN VITRO A549 cell lines, 1, 10, 20, 80 mol/L, before H ₂ O ₂ .	<u>Morphology</u> : recovered the majority of cells from the toxicity, regenerated cell proliferation, prevented 9.6% and 11.0% of cell loss.	(Kucukgul et al., 2016)
LUNG	<u>Preconditioning</u> : 10, 30, 50 μ g/ml, intraperitoneally, 5 days, before sepsis induced by intraperitoneal injection of rat fecal material (0.5 g per kg of animals weight) extracted from another donor rat. A control group was performed with Oxygen.	<u>Reduction dose-dependent manner in LIVER/LUNG</u> : conjugated dienes (CD), thiobarbituric acid-reactive substances (TBARS), Total pro-oxidant activity (TOS). <u>Increase dose-dependent manner</u> : superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), Total antioxidant activity (TAC). <u>Reduction</u> : lactate dehydrogenase (LDH) (Liver, Kidney, Lungs, Heart). <u>Increase</u> : Succinate Dehydrogenase (SDH) (Lungs, Heart), adenosine triphosphatase (ATPase) (no Kidney), acid phosphatase (AcPase) (Liver, Kidney, Lungs, Heart), β -Glucuronidase (Liver, Kidney, Lungs).	(Guanche et al., 2010)
	150 mg/kg, intraperitoneally, single dose for 10 days, at the same time <i>Escherichia coli</i> toxin (LPS) (20 mg/kg).	<u>Histochemically detected activity of succinate dehydrogenase (SDH)</u> : extinguished enzymatic activity in central parts of the lobule and paralleled by narrowing of zone I (Liver). <u>Histochemically detected activity of lactate dehydrogenase (LDH)</u> : increased activity (hepatocytes, Kupffer cells, Liver). <u>Histochemically detected activity of adenosine triphosphatase (ATPase)</u> : decrease intensity of the reaction for ATPase (Liver). <u>Histochemically detected activity of acid phosphatase (AcPase)</u> : lower decrease in activity (Liver). <u>Histochemically detectable activity of succinate dehydrogenase (SDH)</u> : the reaction in tubular epithelial cells was slightly more pronounced (Kidney). <u>Histochemically detected activity of lactic dehydrogenase (LDH)</u> : less pronounced stimulation of enzyme in principal tubules and other portions of nephrons (Kidney). <u>Histochemically detected activity of adenosine triphosphatase (ATPase)</u> : decreased intensity of the reaction in renal glomeruli and in walls of blood vessels, particularly those of low caliber (Kidney). <u>Histochemically detected activity of acid phosphatase (AcPase)</u> : decreased intensity of the reaction pertained in principal tubuli and collecting ducts (Kidney). <u>Histochemically detected activity of succinate dehydrogenase (SDH)</u> : no more pronounced alterations (Lungs). <u>Histochemically detected activity of lactate dehydrogenase (LDH)</u> : stimulation was less pronounced (Lungs). <u>Histochemically detected activity of adenosine triphosphatase (ATPase)</u> : no changing (Lungs). <u>Histochemically detected activity of acid phosphatase (AcPase)</u> : decreased activity (Lungs). <u>Reduction dose-dependent manner</u> : creatine kinase-MB (CK-MB), lactate, myeloperoxidase (MPO), total nitrate/nitrite (NOx), thiobarbituric acid reactive substances (TBARS). <u>Increase dose dependent manner</u> : Myocardial adenosine nucleotides (ATP, ADP, AMP, TAN), glutathione (GSH). <u>Histological examination, ultrastructural analyses</u> : improvement in edema in between muscle fibers, and edema within muscle fibers, good myofibrillar arrangement with only slight edema around	(Madej et al., 2007)
HEART	<u>Preconditioning</u> : rectal insufflations as five applications per week. In a group: 0.3 mg/kg/day in the first week, and 0.5 mg/kg/day in the second week. In another group, 0.6 mg/kg/day in the first week, and 1 mg/kg/day in the second week, before ischemia/reperfusion. A group was performed with Oxygen.		(Ahmed et al., 2012)

(continued on next page)

Table 2 (continued)

Tissues	Dosages	Results	References
	<u>Preconditioning:</u> 100 µg/kg/day, intraperitoneally, once daily, 5 days, before ischemia/reperfusion. A control was performed with Oxygen.	muscle fibers, mild mitochondrial swelling with decreased matrix density and mild disruption of mitochondrial cristae and vesiculation, slight margination of chromatin near nuclear membrane. <u>Reduction:</u> microtubule-associated protein 1 light chain 3 (LC3B1/II), PTEN-induced putative kinase 1 (PINK1), cytochrome c oxidase subunit IV (COX4), Caspase 3, myocardial apoptosis. <u>Increase:</u> nuclear factor (erythroid-derived 2)-like 2 (Nrf2), glutamate-cysteine ligase catalytic subunit (GCLC), glutamate-cysteine ligase modifier subunit (GCLM), superoxide dismutases (SODs) expression. <u>Morphology:</u> mild mitochondrial injury. Validation of: 1. nuclear extracts (TATA-binding protein (TBP) in nuclear extracts), 2. mitochondrial fractions separated from the cytoplasmic fraction (cytochrome c oxidase subunit IV (COX4) detectable).	(Meng et al., 2017)
	<u>Preconditioning:</u> 0.6 mg/kg, rectal insufflations, twice/week for the first 3 months, then once/week till the age of 15 months, in aged rats. A control was performed with Oxygen.	<u>Reduction:</u> malondialdehyde (MDA), protein carbonyls (Pr Co), lipofuscin, cytosolic Ca ²⁺ (heart/hippocampus). <u>Increase:</u> glutathione (GSH), energy status (ATP, ADP) (heart/hippocampus), Na ⁺ , K ⁺ , ATPase (hippocampus).	(El-Sawalhi et al., 2013)
	<u>Preconditioning:</u> 50, 80 mL/kg, single (1x) or repetitive (5x) insufflations, in rat cardiac transplant model.	Prolonged cardiac allograft survival without any adjunctive immunosuppressive therapy, not alternated number of red blood cells, decreased number of thrombocytes, increase of white blood cells, mostly granulocytes.	(Stadlbauer et al., 2008)
	<u>Preconditioning:</u> 0.3 mg/kg, rectal insufflations, once on alternating days for 20 sessions, before doxorubicin (2 mg/kg). The Oxygen group was a further control.	<u>Reduction:</u> pro- brain natriuretic peptide (BNP), malondialdehyde (MDA), advanced oxidation protein products (AOPP). <u>Increase:</u> superoxide dismutase (SOD), catalase (CAT). <u>Morphology:</u> slight damage, normal morphology of cardiac fibres. 90% survival rate, reduced loss of body weight.	(Delgado-Roche et al., 2014)
	150 mg/kg, intraperitoneally, single dose for 10 days, at the same time <i>Escherichia coli</i> toxin (LPS) (20 mg/kg).	<u>Reduction:</u> lactate dehydrogenase (LDH) (Liver, Kidney, Lungs, Heart). <u>Increase:</u> Succinate Dehydrogenase (SDH) (Lungs, Heart), adenosine triphosphatase (ATPase) (no Kidney), acid phosphatase (AcPase) (Liver, Kidney, Lungs, Heart), β-Glucuronidase (Liver, Kidney, Lungs). <u>Histochemically detected activity of succinate dehydrogenase (SDH):</u> extinguished enzymatic activity in central parts of the lobule and paralleled by narrowing of zone I (Liver). <u>Histochemically detected activity of lactate dehydrogenase (LDH):</u> increased activity (hepatocytes, Kupffer cells, Liver). <u>Histochemically detected activity of adenosine triphosphatase (ATPase):</u> decrease intensity of the reaction for ATPase (Liver). <u>Histochemically detected activity of acid phosphatase (AcPase):</u> lower decrease in activity (Liver). <u>Histochemically detectable activity of succinate dehydrogenase (SDH):</u> the reaction in tubular epithelial cells was slightly more pronounced (Kidney). <u>Histochemically detected activity of lactic dehydrogenase (LDH):</u> less pronounced stimulation of enzyme in principal tubules and other portions of nephrons (Kidney). <u>Histochemically detected activity of adenosine triphosphatase (ATPase):</u> decreased intensity of the reaction in renal glomeruli and in walls of blood vessels, particularly those of low caliper (Kidney). <u>Histochemically detected activity of acid phosphatase (AcPase):</u> decreased intensity of the reaction pertained in principal tubuli and collecting ducts (Kidney). <u>Histochemically detected activity of succinate dehydrogenase (SDH):</u> no more pronounced alterations (Lungs). <u>Histochemically detected activity of lactate dehydrogenase (LDH):</u> stimulation was less pronounced (Lungs). <u>Histochemically detected activity of adenosine triphosphatase (ATPase):</u> no changing (Lungs). <u>Histochemically detected activity of acid phosphatase (AcPase):</u> decreased activity (Lungs). <u>Reduction:</u> malondialdehyde (MDA), myeloperoxidase (MPO). <u>Increase:</u> bursting pressure values of anastomosis, Hydroxyproline (HPO), superoxide dismutase (SOD). <u>Histopathological evaluation:</u> improving in anastomotic wound healing, granulation tissue development and histological changes corresponding to the local inflammatory response.	(Madej et al., 2007)
	<u>Preconditioning:</u> 0.7 mg/kg, intraperitoneally, daily five times, before irradiation of 500 cGy.	<u>Reduction time-dependent manner:</u> serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), tumor necrosis factor alpha (TNF-α), malondialdehyde (MDA). <u>Increase:</u> superoxide dismutase (SOD). <u>Histopathological examination:</u> reduction in hepatocellular degeneration, inflammation, congestion and dilatation in both sinusoids and central veins, reduced inflammatory cell infiltrate in	(Tasdovent et al., 2019)
INTESTINE	<u>Preconditioning:</u> 0.7 mg/kg, intraperitoneally, daily five times, before total body irradiation with a single dose of 6 Gy.		(Gultekin, Cakmak et al., 2013)

(continued on next page)

Table 2 (continued)

Tissues	Dosages	Results	References
		the lamina propria, regular villous structure, abundant goblet cells in the epithelium, reduced inflammatory cell infiltrate in the lamina propria.	
	<u>Preconditioning:</u> 0.7 mg/kg, intraperitoneally, 15 applications (once daily), before methotrexate (Mtx) (6 mg/kg).	<u>Reduction:</u> malondialdehyde (MDA). <u>Increase:</u> superoxide dismutase (SOD), glutathione peroxidase (GSH-Px).	(Kesik et al., 2009)
	<u>Postconditioning:</u> 0.7 mg/kg/day, intraperitoneally and intraluminally, laparotomy and/or ischemia/reperfusion.	<u>Histologically:</u> ILEUM: less inflammatory cell infiltration and edema, reduction in vacuolated cells in the epithelium; LIVER/KIDNEY: no significant change, due probably to the cumulative prolonged effect of Mtx on these tissues. <u>Macroscopic Appearance:</u> <u>increase</u> in mucosal weight in jejunum and ileum, bowel weight in jejunum, mucosal DNA and protein in jejunum and ileum, villus height and crypt depth in jejunum and ileum, crypt cell proliferation in jejunum and ileum, p-ERK protein. <u>Reduction:</u> Park's Injury Score in jejunum and ileum, enterocyte apoptosis in jejunum and ileum, caspase 3.	(Haj et al., 2014)
	<u>Preconditioning:</u> 1 mg/kg, intraperitoneally, 7 days, before ischemia/reperfusion.	<u>Reduction:</u> apoptotic index, malondialdehyde (MDA), the total oxidant score (TOS). <u>Increase:</u> superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), total antioxidant capacity (TAC), catalase (CAT).	(Onal et al., 2017)
	<u>Postconditioning:</u> 60 µg/mL, rectal and/or intratympanic, 7 days, after cisplatin-induced ototoxicity (5-mg/kg/day). The rats were tested with distortion product otoacoustic emissions (DPOAE).	<u>Histological evaluation:</u> increased numbers of glial cells in the spiral ganglion, reduced level of vascularization. Statistically significant differences in DPOAE results. <u>Histopathological scoring:</u> decreased stria vascularis damage, decreased inner-outer hair cell damage.	(Koçak et al., 2016)
COCHLEAR	<u>Postconditioning:</u> 30 µg/ml, intravenous, daily administration for 14 days, at the same time with noise exposure.	<u>Reduction:</u> malondialdehyde (MDA), % mitochondrial swelling, mitochondrial membrane potential (MMP), Glutathione disulfide (GSSG), cytochrome c (Brain, cochlear). <u>Increase:</u> glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) (Brain, cochlear), ATP.	(Nasezadeh et al., 2017)
	<u>Preconditioning:</u> 0.7 mg/kg, intraperitoneally; 4 doses, before ischemia.	<u>Histopathological findings:</u> prevents mitochondrial membrane potential (MMP) collapse, mitochondrial swelling, cytochrome c release.	
	<u>Preconditioning:</u> 0.7 mg/kg, intraperitoneally; 6 days, before ischemic period and/or hypothermia.	<u>Reduction:</u> malondialdehyde (MDA), Serum nitrite-nitrate (NOx), Inducible nitric oxide synthase (iNOS) immunostaining. <u>Increase:</u> glutathione peroxidase (GSH-Px), superoxide dismutase (SOD).	(Koca et al., 2010)
SKELETAL	<u>Preconditioning:</u> 0.7 mg/kg, 6 days, before ischemic period and/or hypothermia.	<u>Reduction:</u> malondialdehyde (MDA), interleukin-1β (IL-1β), creatinine kinase (CK), aspartate aminotransferase (AST), K ⁺ , nitric oxide (NO). <u>Increase:</u> glutathione peroxidase (GSH-Px), superoxide dismutase (SOD).	(Ozkan et al., 2015)
	<u>Preconditioning:</u> 50 µg/kg, intraperitoneally, once a day for seven days. Streptozotocin (STZ) (2 ml). A control was performed with Oxygen.	<u>iNOS immunohistochemical staining:</u> mild intensity. <u>Reduction:</u> 4-hydroxynonenal (4-HNE), Poly (ADP-ribose) polymerase-1 (PARP-1), glucagon, glycemia. <u>Increase:</u> nuclear factor Nrf2, glutathione-s-transferase (GST), insulin, leptin.	(Siniscalco et al., 2018)
PANCREAS	<u>Postconditioning:</u> 0.7-mg/kg, intraperitoneally, daily for 3 days, induction of acute necrotizing pancreatitis. A control was performed with Oxygen.	<u>Immunohistochemistry:</u> reduction in tissue degeneration evidenced by the partial restoration of normal cellular population size of islets of Langerhans and absence of islet damage. <u>Immunofluorescence:</u> reduction in cell death, decreased DNA damage. <u>Reduction:</u> serum amylase, neopterin, lipase, aspartate aminotransferase (AST), alanine amino transferase (ALT), γ-Glutamyl transferase (GT), malondialdehyde (MAD). <u>Increase:</u> Alkaline phosphatase (AP), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD).	(Uysal et al., 2010)
	<u>Postconditioning:</u> 80 mg/kg, articular space 3 times/week (3.5 weeks) after PG/PS-induced arthritis. A control was performed with Oxygen.	Increase in weight. Lower number of infected rats. <u>Histopathologic analyses:</u> lower degrees of necrosis and leukocyte infiltration. Improving in the histological injury score. <u>Reduction:</u> TNFα and IL-1β expression/protein, nitric oxide (NO), Fructolysine. <u>Increase:</u> superoxide Dismutase (SOD), catalase (CAT). Ameliorate the joint swelling, decrease of arthritis index.	(Vaillant et al., 2013)
ARTHRTIS	<u>Preconditioning:</u> 1 mg/kg, intraperitoneally, before detorsion for 2 hours.	<u>Histological results:</u> normal morphology.	
TESTICULAR	<u>Preconditioning:</u> 1 mg/kg, intraperitoneally, before detorsion for 2 hours.	<u>Reduction:</u> Ischemia Modified Albumin (IMA), Total Oxidant Status (TOS), Oxidative Stress Index (OSI). <u>Histopathological score:</u> lower.	(Tusat et al., 2017)
OTHER	<u>Preconditioning:</u> 1 mg/kg, rectal insufflations, 15 sessions in 5 weeks, in alternated days, 2 mL/kg of lipofundin. A control group was performed with Oxygen.	<u>Reduction:</u> malondialdehyde (MDA), peroxidation potential (PP), advanced oxidation protein products (AOPP), nitric oxide (NO). <u>Increase:</u> glutathione (GSH). <u>Histopathology:</u> minimal lesions in the aortas, smaller intima/media ratio.	(Delgado-Roche et al., 2013)

strategies, and could represent therapeutic targets to minimize the deleterious consequences associated to oxidative stress, such as in brain aging and NDs.

Authors' Contributions

Catia Scassellati and Antonio Carlo Galoforo contributed equally to this work.

Declaration of Competing Interest

The authors have declared no conflict of interest.

Acknowledgments

This research was supported by grants from the Italian Ministry of Health as Ricerca Corrente and as RF-2016-02363298.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.arr.2020.101138>.

References

- Ademowo, O.S., Dias, H.K.L., Milic, I., Devitt, A., Moran, R., Mulcahy, R., Howard, A.N., Nolan, J.M., Griffiths, H.R., 2017. Phospholipid oxidation and carotenoid supplementation in Alzheimer's disease patients. *Free Radic. Biol. Med.* 108, 77–85.
- Ahmed, L.A., Salem, H.A., Mawsouf, Mohamed N, Attia, Amina S, Agha, A.M., 2012. Cardioprotective effects of ozone oxidative preconditioning in an in vivo model of ischemia/reperfusion injury in rats. *Scandinavian journal of clinical and laboratory investigation* (72), 345–354. *JID* - 0404375.
- Ahmed, S.M., Luo, L., Namani, A., Wang, X.J., Tang, X., 2017. Nrf2 signaling pathway: Pivotal roles in inflammation. *Biochim. Biophys. Acta Mol. Basis Dis.* 1863, 585–597.
- Ajamiéh, H., Merino, N., Candelario-Jalil, E., Menéndez, S., Martínez-Sánchez, G., Re, L., Giuliani, A., Leon, O.S., 2002. Similar protective effect of ischaemic and ozone oxidative preconditionings in liver ischaemia/reperfusion injury. *Pharmacol. Res.* 45, 333–339.
- Ajamiéh, H.H., Berlanga, J., Merino, N., Sanchez, G.M., Carmona, A.M., Cepero, S.M., Giuliani, A., Re, L., Leon, O.S., 2005. Role of protein synthesis in the protection conferred by ozone-oxidative-preconditioning in hepatic ischaemia/reperfusion. *Transpl. Int.* 18, 604–612.
- Ajamiéh, H.H., Menéndez, S., Martínez-Sánchez, G., E Candelario-Jalil, L Re, Giuliani, A., Fernández, O.S.L., 2004. Effects of ozone oxidative preconditioning on nitric oxide generation and cellular redox balance in a rat model of hepatic ischaemia-reperfusion. *Liver international* (24), 55–62.
- Altman, N., 2007. The oxygen prescription : the miracle of oxidative therapies. Healing Arts Press, Rochester, Vt.
- Altunoglu, E., Guntas, G., Erdenen, F., Akkaya, E., Topac, I., Irmak, H., Deric, H., Yavuzer, H., Gelisgen, R., Uzun, H., 2015. Ischemia-modified albumin and advanced oxidation protein products as potential biomarkers of protein oxidation in Alzheimer's disease. *Geriatr. Gerontol. Int.* 15, 872–880.
- Amara, I., Scuto, M., Zappala, A., Ontario, M.L., Petralia, A., Abid-Essefi, S., Maiolino, L., Signorella, A., Trovato Salinaro, A., Calabrese, V., 2020. Hericium Erinaceus Prevents DEHP-Induced Mitochondrial Dysfunction and Apoptosis in PC12 Cells. *Int. J. Mol. Sci.* 21 <https://doi.org/10.3390/ijms21062138>.
- Ameli, J., Banki, A., Khorvash, F., Simonetti, V., Jafari, N.J., Izadi, M., 2019. Mechanisms of pathophysiology of blood vessels in patients with multiple sclerosis treated with ozone therapy: a systematic review. *Acta Biomed.* 90, 213–217.
- Aslaner, A., Cakir, T., Celik, B., Dogan, U., Mayir, B., Akyuz, C., Polat, C., Basturk, A., Soyer, V., Koc, S., Sehirli, A.O., 2015. Does intraperitoneal medical ozone preconditioning and treatment ameliorate the methotrexate induced nephrotoxicity in rats? *Int. J. Clin. Exp. Med.* 8, 13811–13817.
- Aso, E., Lomoio, S., Lopez-Gonzalez, I., Joda, L., Carmona, M., Fernandez-Yague, N., Moreno, J., Juves, S., Pujol, A., Pamplona, R., Portero-Otin, M., Martin, V., Diaz, M., Ferrer, I., 2012. Amyloid generation and dysfunctional immunoproteasome activation with disease progression in animal model of familial Alzheimer's disease. *Brain Pathol.* 22, 636–653.
- Ayala, A., Munoz, M.F., Arguëlles, S., 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid. Med. Cell. Longev.* 2014, 360438.
- Azarpazhooh, A., Limeback, H., Lawrence, H.P., Fillery, E.D., 2009. Evaluating the effect of an ozone delivery system on the reversal of dentin hypersensitivity: a randomized, double-blinded clinical trial. *J. Endod.* 35, 1–9.
- Babior, B.M., Takeuchi, C., Ruedi, J., Gutierrez, A., Wentworth Jr, P., 2003. Investigating antibody-catalyzed ozone generation by human neutrophils. *Proc. Natl. Acad. Sci. U. S. A.* 100, 3031–3034.

- Baker, M.A., Weinberg, A., Hetherington, L., Villaverde, A.I., Velkov, T., Baell, J., Gordon, C.P., 2015. Defining the mechanisms by which the reactive oxygen species by-product, 4-hydroxynonenal, affects human sperm cell function. *Biol. Reprod.* 92, 108.
- Bakkal, B.H., Gultekin, F.A., Guven, B., Turkcü, U.O., Bektas, S., Can, M., 2013. Effect of ozone oxidative preconditioning in preventing early radiation-induced lung injury in rats. *Braz. J. Med. Biol. Res.* 46, 789–796.
- Barber, E., Menendez, S., Leon, O.S., Barber, M.O., Merino, N., Calunga, J.L., Cruz, E., Bocci, V., 1999. Prevention of renal injury after induction of ozone tolerance in rats submitted to warm ischaemia. *Mediators Inflamm.* 8, 37–41.
- Benedetti, E., D'Angelo, B., Cristiano, L., Di Giacomo, E., Fanelli, F., Moreno, S., Cecconi, F., Fidoamore, A., Antonosante, A., Falcone, R., Ippoliti, R., Giordano, A., Cimini, A., 2014. Involvement of peroxisome proliferator-activated receptor beta/delta (PPAR beta/delta) in BDNF signaling during aging and in Alzheimer disease: possible role of 4-hydroxynonenal (4-HNE). *Cell. Cycle* 13, 1335–1344.
- Bilge, A., Ozturk, O., Adali, Y., Ustebay, S., 2018. Could Ozone Treatment be a Promising Alternative for Osteomyelitis? an Experimental Study. *Acta Ortop. Bras.* 26, 67–71.
- Bocci, V., 2012. How a calculated oxidative stress can yield multiple therapeutic effects. *Free Radic. Res.* 46, 1068–1075.
- Bocci, V., 2011. Ozone. A New Medical Drug. Springer, Netherlands.
- Bocci, V., Valacchi, G., 2015. Nrf2 activation as target to implement therapeutic treatments. *Front. Chem.* 3, 4.
- Bocci, V., Valacchi, G., Corradeschi, F., Fanetti, G., 1998. Studies on the biological effects of ozone: 8. Effects on the total antioxidant status and on interleukin-8 production. *Mediators Inflamm.* 7, 313–317.
- Bocci, V.A., Zanardi, I., Travagli, V., 2011. Ozone acting on human blood yields a hormetic dose-response relationship. *J. Transl. Med.* 9, 66-5876-9-66.
- Borrego, A., Zamora, Z.B., Gonzalez, R., Romay, C., Menendez, S., Hernandez, F., Montero, T., Rojas, E., 2004. Protection by ozone preconditioning is mediated by the antioxidant system in cisplatin-induced nephrotoxicity in rats. *Mediators Inflamm.* 13, 13–19.
- Bosco, D.A., Fowler, D.M., Zhang, Q., Nieva, J., Powers, E.T., Wentworth Jr, P., Lerner, R.A., Kelly, J.W., 2006. Elevated levels of oxidized cholesterol metabolites in Lewy body disease brains accelerate alpha-synuclein fibrilization. *Nat. Chem. Biol.* 2, 249–253.
- Braidy, N., Izadi, M., Sureda, A., Jonaidi-Jafari, N., Banki, A., Nabavi, S.F., Nabavi, S.M., 2018. Therapeutic relevance of ozone therapy in degenerative diseases: Focus on diabetes and spinal pain. *J. Cell. Physiol.* 233, 2705–2714.
- Braithwaite, S.P., Stock, J.B., Lombroso, P.J., Nairn, A.C., 2012. Protein phosphatases and Alzheimer's disease. *Prog. Mol. Biol. Transl. Sci.* 106, 343–379.
- Brigelius-Flohe, R., Flohe, L., 2011. Basic principles and emerging concepts in the redox control of transcription factors. *Antioxid. Redox Signal.* 15, 2335–2381.
- Cabiscol, E., Tamarit, J., Ros, J., 2014. Protein carbonylation: proteomics, specificity and relevance to aging. *Mass Spectrom. Rev.* 33, 21–48.
- Cakatay, U., Kayali, R., Uzun, H., 2008. Relation of plasma protein oxidation parameters and paraoxonase activity in the ageing population. *Clin. Exp. Med.* 8, 51–57.
- Calabrese, E.J., 2020. Hormesis and Ginseng: Ginseng Mixtures and Individual Constituents Commonly Display Hormesis Dose Responses, Especially for Neuroprotective Effects. *Molecules* 25. <https://doi.org/10.3390/molecules25112719>.
- Calabrese, E.J., 2016. Preconditioning is hormesis part II: How the conditioning dose mediates protection: Dose optimization within temporal and mechanistic frameworks. *Pharmacol. Res.* 110, 265–275.
- Calabrese, E.J., Baldwin, L.A., 2000. Chemical hormesis: its historical foundations as a biological hypothesis. *Hum. Exp. Toxicol.* 19, 2–31.
- Calabrese, V., Cornelius, C., Dinkova-Kostova, A.T., Calabrese, E.J., Mattson, M.P., 2010. Cellular stress responses, the hormesis paradigm, and vitamins: novel targets for therapeutic intervention in neurodegenerative disorders. *Antioxid. Redox Signal.* 13, 1763–1811.
- Calabrese, E.J., 2013. Hormetic mechanisms. *Crit. Rev. Toxicol.* 43, 580–606.
- Calunga, J.L., Trujillo, Y., Menendez, S., Zamora, Z., Alonso, Y., Merino, N., Montero, T., 2009. Ozone oxidative post-conditioning in acute renal failure. *J. Pharm. Pharmacol.* 61, 221–227.
- Can, M., Varlibas, F., Guven, B., Akhan, O., Yuksel, G.A., 2013. Ischemia modified albumin and plasma oxidative stress markers in Alzheimer's disease. *Eur. Neurol.* 69, 377–380.
- Candelario-Jalil, E., Mohammed-Al-Dalain, S., Fernandez, O.S., Menendez, S., Perez-Davison, G., Merino, N., Sam, S., Ajamiéh, H.H., 2001. Oxidative preconditioning affords protection against carbon tetrachloride-induced glycogen depletion and oxidative stress in rats. *J. Appl. Toxicol.* 21, 297–301.
- Cattaneo, A., Cattane, N., Galluzzi, S., Provasi, S., Lopizzo, N., Festari, C., Ferrari, C., Guerra, U.P., Paghera, B., Muscio, C., Bianchetti, A., Volta, G.D., Turla, M., Cotelli, M.S., Gennuso, M., Prella, A., Zanetti, O., Lussignoli, G., Mirabile, D., Bellandi, D., Gentile, S., Belotti, G., Villani, D., Harach, T., Bolmont, T., Padovani, A., Boccardi, M., Frisoni, G.B., INDIA-FBP Group, 2017. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. *Neurobiol. Aging* 49, 60–68.
- Chen, H., Xing, B., Liu, X., Zhan, B., Zhou, J., Zhu, H., Chen, Z., 2008a. Ozone oxidative preconditioning inhibits inflammation and apoptosis in a rat model of renal ischemia/reperfusion injury. *Eur. J. Pharmacol.* 581, 306–314.
- Chen, H., Xing, B., Liu, X., Zhan, B., Zhou, J., Zhu, H., Chen, Z., 2008b. Ozone oxidative preconditioning protects the rat kidney from reperfusion injury: the role of nitric oxide. *J. Surg. Res.* 149, 287–295.
- Chen, H., Xing, B., Liu, X., Zhan, B., Zhou, J., Zhu, H., Chen, Z., 2008c. Similarities between ozone oxidative preconditioning and ischemic preconditioning in renal ischemia/reperfusion injury. *Arch. Med. Res.* 39, 169–178.

- Chevion, M., Berenshtein, E., Stadtman, E.R., 2000. Human studies related to protein oxidation: protein carbonyl content as a marker of damage. *Free Radic. Res.* 33 (Suppl.), S99–108.
- Clark, A.R., Ohlmeyer, M., 2019. Protein phosphatase 2A as a therapeutic target in inflammation and neurodegeneration. *Pharmacol. Ther.* 201, 181–201.
- Clavo, B., Santana-Rodriguez, N., Llontop, P., Gutierrez, D., Suarez, G., Lopez, L., Rovira, G., Martinez-Sanchez, G., Gonzalez, E., Jorge, I.J., Perera, C., Blanco, J., Rodriguez-Esparragon, F., 2018. Ozone Therapy as Adjuvant for Cancer Treatment: Is Further Research Warranted? *Evid Based. Complement. Alternat. Med.* 2018, 7931849.
- Costanzo, M., Boschi, F., Carton, F., Conti, G., Covi, V., Tabaracci, G., Sbarbati, A., Malatesta, M., 2018. Low ozone concentrations promote adipogenesis in human adipose-derived adult stem cells. *Eur. J. Histochem.* 62 <https://doi.org/10.4081/ejh.2018.2969>.
- Cristani, M., Speciale, A., Saija, A., Gangemi, S., Minciullo, P.L., Cimino, F., 2016. Circulating Advanced Oxidation Protein Products as Oxidative Stress Biomarkers and Progression Mediators in Pathological Conditions Related to Inflammation and Immune Dysregulation. *Curr. Med. Chem.* 23, 3862–3882.
- Csala, M., Kardon, T., Legeza, B., Lizak, B., Mandl, J., Margittai, E., Puskas, F., Szaraz, P., Szelenyi, P., Banhegyi, G., 2015. On the role of 4-hydroxynonenal in health and disease. *Biochim. Biophys. Acta* 1852, 826–838.
- Cuadrado, A., Manda, G., Hassan, A., Alcaraz, M.J., Barbas, C., Daiber, A., Ghezzi, P., Leon, R., Lopez, M.G., Oliva, B., Pajares, M., Rojo, A.I., Robledinos-Anton, N., Valverde, A.M., Guney, E., Schmidt, H.H.H.W., 2018. Transcription Factor NRF2 as a Therapeutic Target for Chronic Diseases: A Systems Medicine Approach. *Pharmacol. Rev.* 70, 348–383.
- Cuadrado, A., Rojo, A.I., Wells, G., Hayes, J.D., Cousin, S.P., Rumsey, W.L., Attucks, O.C., Franklin, S., Levenon, A.L., Kensler, T.W., Dinkova-Kostova, A.T., 2019. Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. *Nat. Rev. Drug Discov.* 18, 295–317.
- Curro, M., Russo, T., Ferlazzo, N., Caccamo, D., Antonuccio, P., Arena, S., Parisi, S., Perrone, P., Ientile, R., Romeo, C., Impellizzeri, P., 2018. Anti-Inflammatory and Tissue Regenerative Effects of Topical Treatment with Ozonated Olive Oil/Vitamin E Acetate in Balanitis Xerotica Obliterans. *Molecules* 23. <https://doi.org/10.3390/molecules23030645>.
- Delgado-Roche, L., Hernandez-Matos, Y., Medina, E.A., Morejon, D.A., Gonzalez, M.R., Martinez-Sanchez, G., 2014. Ozone-Oxidative Preconditioning Prevents Doxorubicin-induced Cardiotoxicity in Sprague-Dawley Rats. *Sultan Qaboos Univ. Med. J.* 14, e342–8.
- Delgado-Roche, L., Martinez-Sanchez, G., Re, L., 2013. Ozone oxidative preconditioning prevents atherosclerosis development in New Zealand White rabbits. *J. Cardiovasc. Pharmacol.* 61, 160–165.
- Delgado-Roche, L., Riera-Romo, M., Mesta, F., Hernandez-Matos, Y., Barrios, J.M., Martinez-Sanchez, G., Al-Dalain, S.M., 2017. Medical ozone promotes Nrf2 phosphorylation reducing oxidative stress and pro-inflammatory cytokines in multiple sclerosis patients. *Eur. J. Pharmacol.* 811, 148–154.
- Di Domenico, F., Barone, E., Mancuso, C., Perluigi, M., Cocciolo, A., Mecocci, P., Butterfield, D.A., Coccia, R., 2012. HO-1/BVR-a system analysis in plasma from probable Alzheimer's disease and mild cognitive impairment subjects: a potential biochemical marker for the prediction of the disease. *J. Alzheimers Dis.* 32, 277–289.
- Díaz-Luis, J., Menéndez-Cepero, S., Macías-Abraham, C., Fariñas-Rodríguez, L., 2018. Systemic Ozone Therapy by Rectal Insufflation for Immunoglobulin A Deficiency. *MEDICC Review* 20, 29–35.
- Dugger, B.N., Dickson, D.W., 2017. Pathology of Neurodegenerative Diseases. *Cold Spring Harb Perspect. Biol.* 9 <https://doi.org/10.1101/cshperspect.a028035>.
- El-Mehi, A.E., Faried, M.A., 2020. Controlled ozone therapy modulates the neurodegenerative changes in the frontal cortex of the aged albino rat. *Ann. Anat.* 227, 151428.
- El-Sawalhi, M.M., Darwish, H.A., Mausouf, M.N., Shaheen, A.A., 2013. Modulation of age-related changes in oxidative stress markers and energy status in the rat heart and hippocampus: a significant role for ozone therapy. *Cell Biochem. Funct.* 31, 518–525.
- Elvis, A., Ekta, J.S., 2011. Ozone therapy: A clinical review.
- Emon, S.T., Uslu, S., Aydinlar, E.I., Irbani, A., Ince, U., Orakdogan, M., Suyen, G.G., 2017. Effects of Ozone on Spinal Cord Recovery via the Wnt/ Β-Catenin Pathway Following Spinal Cord Injury in Rats. *Turk. Neurosurg.* 27, 946–951.
- Eve, D.J., Nisbet, A.P., Kingsbury, A.E., Hewson, E.L., Daniel, S.E., Lees, A.J., Marsden, C. D., Foster, O.J., 1998. Basal ganglia neuronal nitric oxide synthase mRNA expression in Parkinson's disease. *Brain Res. Mol. Brain Res.* 63, 62–71.
- Facchinetti, M.M., 2020. Heme Oxygenase-1. *Antioxidants & Redox Signaling.*
- Fedorova, M., Bollineni, R.C., Hoffmann, R., 2014. Protein carbonylation as a major hallmark of oxidative damage: update of analytical strategies. *Mass Spectrom. Rev.* 33, 79–97.
- Feitosa, C.M., da Silva Oliveira, G.L., do Nascimento Cavalcante, A., Morais Chaves, S.K., Rai, M., 2018. Determination of Parameters of Oxidative Stress in vitro Models of Neurodegenerative Diseases-A Review. *Curr. Clin. Pharmacol.* 13, 100–109.
- Fernandez Iglesias, A., Gonzalez Nunez, L., Calunga Fernandez, J.L., Rodriguez Salgueiro, S., Santos Febles, E., 2011. Ozone preconditioning in renal ischaemia-reperfusion model. Functional and morphological evidences. *Nefrologia* 31, 464–470.
- Ferreiro, E., Pita, I.R., Mota, S.I., Valero, J., Ferreira, N.R., Fernandes, T., Calabrese, V., Fontes-Ribeiro, C.A., Pereira, F.C., Rego, A.C., 2018. Coriolus versicolor biomass increases dendritic arborization of newly-generated neurons in mouse hippocampal dentate gyrus. *Oncotarget* 9, 32929–32942.
- Fitzpatrick, E., Holland, O.J., Vanderlelie, J.J., 2018. Ozone therapy for the treatment of chronic wounds: A systematic review. *Int. Wound. J.* 15, 633–644.
- Forno, L.S., 1996. Neuropathology of Parkinson's disease. *J. Neuropathol. Exp. Neurol.* 55, 259–272.
- Galie, M., Costanzo, M., Nodari, A., Boschi, F., Calderan, L., Mannucci, S., Covi, V., Tabaracci, G., Malatesta, M., 2018. Mild ozonisation activates antioxidant cell response by the Keap1/Nrf2 dependent pathway. *Free Radic. Biol. Med.* 124, 114–121.
- Garcia-Escudero, V., Martin-Maestro, P., Perry, G., Avila, J., 2013. Deconstructing mitochondrial dysfunction in Alzheimer disease. *Oxid. Med. Cell. Longev.* 2013, 162152.
- Goh, K.I., Cusick, M.E., Valle, D., Childs, B., Vidal, M., Barabasi, A.L., 2007. The human disease network. *Proc. Natl. Acad. Sci. U. S. A.* 104, 8685–8690.
- Gu, F., Chauhan, V., Chauhan, A., 2015. Glutathione redox imbalance in brain disorders. *Curr. Opin. Clin. Nutr. Metab. Care* 18, 89–95.
- Guanche, D., Hernandez, F., Zamora, Z., Alonso, Y., 2010. Effect of ozone preconditioning on redox activity in a rat model of septic shock. *Toxicology mechanisms and methods* 20, 466–471.
- Guclu, A., Erken, H.A., Erken, G., Dodurga, Y., Yay, A., Ozcuban, O., Simsek, H., Akcilar, A., Kocak, F.E., 2016. The effects of ozone therapy on caspase pathways, TNF-alpha, and HIF-1alpha in diabetic nephropathy. *Int. Urol. Nephrol.* 48, 441–450.
- Gultekin, F.A., Bakkal, B.H., Guven, B., Tasdoven, I., Bektas, S., Can, M., Comert, M., 2013a. Effects of ozone oxidative preconditioning on radiation-induced organ damage in rats. *J. Radiat. Res.* 54, 36–44.
- Gultekin, F.A., Cakmak, G.K., Turkcu, U.O., Yurdakan, G., Demir, F.E., Comert, M., 2013b. Effects of ozone oxidative preconditioning on liver regeneration after partial hepatectomy in rats. *J. Invest. Surg.* 26, 242–252.
- Guven, A., Gundogdu, G., Sadir, S., Topal, T., Erdogan, E., Korkmaz, A., Surer, I., Ozturk, H., 2008. The efficacy of ozone therapy in experimental caustic esophageal burn. *J. Pediatr. Surg.* 43, 1679–1684.
- Haj, B., Sukhotnik, I., Shaoul, R., Pollak, Y., Coran, A.G., Bitterman, A., Matter, I., 2014. Effect of ozone on intestinal recovery following intestinal ischemia-reperfusion injury in a rat. *Pediatr. Surg. Int.* 30, 181–188.
- Hannibal, L., 2016. Nitric Oxide Homeostasis in Neurodegenerative Diseases. *Curr. Alzheimer Res.* 13, 135–149.
- Hasanzadeh, S., Read, M.L., Bland, A.R., Majeed, M., Jamialahmadi, T., Sahebkar, A., 2020. Curcumin: an inflammasome silencer. *Pharmacol. Res.* 159, 104921.
- Hernandez Rosales, F.A., Calunga Fernandez, J.L., Turrent Figueras, J., Menendez Cepero, S., Montenegro Perdomo, A., 2005. Ozone therapy effects on biomarkers and lung function in asthma. *Arch. Med. Res.* 36, 549–554.
- Hohn, A., Tramutola, A., Cascella, R., 2020. Proteostasis Failure in Neurodegenerative Diseases: Focus on Oxidative Stress. *Oxid. Med. Cell. Longev.* 2020, 5497046.
- Holmstrom, K.M., Kostov, R.V., Dinkova-Kostova, A.T., 2016. The multifaceted role of Nrf2 in mitochondrial function. *Curr. Opin. Toxicol.* 1, 80–91.
- Hsiao, C.M., Wu, Y.S., Nan, F.H., Huang, S.L., Chen, L., Chen, S.N., 2016. Immunomodulator mushroom beta glucan induces Wnt/beta catenin signalling and improves wound recovery in telapia and rat skin: a histopathological study. *Int. Wound. J.* 13, 1116–1128.
- Hunot, S., Boissiere, F., Faucheux, B., Brugg, B., Mouatt-Prigent, A., Agid, Y., Hirsch, E. C., 1996. Nitric oxide synthase and neuronal vulnerability in Parkinson's disease. *Neuroscience* 72, 355–363.
- Ishii, T., Itoh, K., Ruiz, E., Leake, D.S., Unoki, H., Yamamoto, M., Mann, G.E., 2004. Role of Nrf2 in the regulation of CD36 and stress protein expression in murine macrophages: activation by oxidatively modified LDL and 4-hydroxynonenal. *Circ. Res.* 94, 609–616.
- Isler, S.C., Unsal, B., Soysal, F., Ozcan, G., Peker, E., Karaca, I.R., 2018. The effects of ozone therapy as an adjunct to the surgical treatment of peri-implantitis. *J. Periodontol. Implant Sci.* 48, 136–151.
- Izadi, M., Kheirjou, R., Mohammadpour, R., Aliyoldashi, M.H., Moghadam, S.J., Khorvash, F., Jafari, N.J., Shirvani, S., Khalili, N., 2019. Efficacy of comprehensive ozone therapy in diabetic foot ulcer healing. *Diabetes Metab. Syndr.* 13, 822–825.
- Jiang, B., Su, Y., Chen, Q., Dong, L., Zhou, W., Li, H., Wang, Y., 2020. Protective Effects of Ozone Oxidative Postconditioning on Long-term Injury After Renal Ischemia/Reperfusion in Rat. *Transplant. Proc.* 52, 365–372.
- Jung, J., Na, C., Huh, Y., 2012. Alterations in nitric oxide synthase in the aged CNS. *Oxid. Med. Cell. Longev.* 2012, 718976.
- Kesik, V., Uysal, B., Kurt, B., Kismet, E., Koseoglu, V., 2009. Ozone ameliorates methotrexate-induced intestinal injury in rats. *Cancer. Biol. Ther.* 8, 1623–1628.
- Khatri, I., Moger, G., Kumar, N.A., 2015. Evaluation of effect of topical ozone therapy on salivary Candidal carriage in oral candidiasis. *Indian J. Dent. Res.* 26, 158–162.
- Kikuchi, S., Shinpo, K., Ogata, A., Tsuji, S., Takeuchi, M., Makita, Z., Tashiro, K., 2002. Detection of N epsilon-(carboxymethyl)lysine (CML) and non-CML advanced glycation end-products in the anterior horn of amyotrophic lateral sclerosis spinal cord. *Amyotroph Lateral Scler. Other Motor Neuron. Disord.* 3, 63–68.
- Kim, T.S., Pae, C.U., Yoon, S.J., Jang, W.Y., Lee, N.J., Kim, J.J., Lee, S.J., Lee, C., Paik, I. H., Lee, C.U., 2006. Decreased plasma antioxidants in patients with Alzheimer's disease. *Int. J. Geriatr. Psychiatry* 21, 344–348.
- Knowles, T.P., Vendruscolo, M., Dobson, C.M., 2014. The amyloid state and its association with protein misfolding diseases. *Nat. Rev. Mol. Cell Biol.* 15, 384–396.
- Koca, K., Yurttas, Y., Yildiz, C., Cayci, 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, 476–483.
- Koçak, H.E., Taşkın, Ü, Aydın, S., Oktay, M.F., Altınay, S., Çelik, D.S., Yücebaşı, K., Altaş, B., 2016. Effects of ozone (O₃) therapy on cisplatin-induced ototoxicity in rats. *Eur. Arch. Otorhinolaryngol.* 273, 4153–4159.

- Komosinska-Vassev, K., Olczyk, P., Winsz-Szczotka, K., Kuznik-Trocha, K., Klimek, K., Olczyk, K., 2012. Age- and gender-related alteration in plasma advanced oxidation protein products (AOPP) and glycosaminoglycan (GAG) concentrations in physiological ageing. *Clin. Chem. Lab. Med.* 50, 557–563.
- Kucukgul, A., Erdogan, S., Gonenci, R., Ozan, G., 2016. Beneficial effects of nontoxic ozone on H(2)O(2)-induced stress and inflammation". *Biochemistry and cell biology = Biochimie et biologie cellulaire* 94 (6), 577–583. JID - 8606068, [Online].
- Kurtoglu, T., Durmaz, S., Akgullu, C., Gungor, H., Eryilmaz, U., Meteoglu, I., Karul, A., Boga, M., 2015. Ozone preconditioning attenuates contrast-induced nephropathy in rats. *J. Surg. Res.* 195, 604–611.
- Lackie, R.E., Maciejewski, A., Ostapchenko, V.G., Marques-Lopes, J., Choy, W.Y., Duenwald, M.L., Prado, V.F., Prado, M.A.M., 2017. The Hsp70/Hsp90 Chaperone Machinery in Neurodegenerative Diseases. *Front. Neurosci.* 11, 254.
- León Fernández, O.S., Jorge, Ajamieh HH FAU - Berlanga, Berlanga J FAU - Menéndez, Silvia, Menéndez S FAU - Viebahn-Hänsler, Renate, Viebahn-Hänsler R FAU - Re, Lamberto, Re L FAU - Carmona, Anna, M. Carmona, A.M. 2008. Ozone oxidative preconditioning is mediated by A1 adenosine receptors in a rat model of liver ischemia/ reperfusion. *Transplant international : official journal of the European Society for Organ Transplantation JID* – 8908516.
- Leon Fernandez, O.S., Pantoja, M., Diaz Soto, M.T., Dranguet, J., Garcia Insua, M., Viebhan-Hansler, R., Menendez Cepero, S., Calunga Fernandez, J.L., 2012. Ozone oxidative post-conditioning reduces oxidative protein damage in patients with disc hernia. *Neurol. Res.* 34, 59–67.
- Leon, O.S., Menendez, S., Merino, N., Castillo, R., Sam, S., Perez, L., Cruz, E., Bocci, V., 1998. Ozone oxidative preconditioning: a protection against cellular damage by free radicals. *Mediators Inflamm.* 7, 289–294.
- Leri, M., Scuto, M., Ontario, M.L., Calabrese, V., Calabrese, E.J., Bucciantini, M., Stefani, M., 2020. Healthy Effects of Plant Polyphenols: Molecular Mechanisms. *Int. J. Mol. Sci.* 21 <https://doi.org/10.3390/ijms21041250>.
- Lerner, R.A., Eschenmoser, A., 2003. Ozone in biology. *Proc. Natl. Acad. Sci. U. S. A.* 100, 3013–3015.
- Levonen, A.L., Landar, A., Ramachandran, A., Ceaser, E.K., Dickinson, D.A., Zanoni, G., Morrow, J.D., Darley-Usmar, V.M., 2004. Cellular mechanisms of redox cell signalling: role of cysteine modification in controlling antioxidant defences in response to electrophilic lipid oxidation products. *Biochem. J.* 378, 373–382.
- Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., Abete, P., 2018. Oxidative stress, aging, and diseases. *Clin. Interv. Aging* 13, 757–772.
- Liu, H., Wang, H., Shen, S., Hagen, T.M., Liu, R.M., 2004. Glutathione metabolism during aging and in Alzheimer disease. *Ann. N.Y. Acad. Sci.* 1019, 346–349.
- Luth, H.J., Holzer, M., Gartner, U., Staufenbiel, M., Arendt, T., 2001. Expression of endothelial and inducible NOS-isoforms is increased in Alzheimer's disease, in APP23 transgenic mice and after experimental brain lesion in rat: evidence for an induction by amyloid pathology. *Brain Res.* 913, 57–67.
- Luth, H.J., Munch, G., Arendt, T., 2002. Aberrant expression of NOS isoforms in Alzheimer's disease is structurally related to nitrotyrosine formation. *Brain Res.* 953, 135–143.
- Mac Nair, C.E., Schlamp, C.L., Montgomery, A.D., Shestopalov, V.I., Nickells, R.W., 2016. Retinal glial responses to optic nerve crush are attenuated in Bax-deficient mice and modulated by purinergic signaling pathways. *J. Neuroinflammation* 13, 93-016-0558-y.
- Maciejczyk, M., Zalewska, A., Ladny, J.R., 2019. Salivary Antioxidant Barrier, Redox Status, and Oxidative Damage to Proteins and Lipids in Healthy Children, Adults, and the Elderly. *Oxid. Med. Cell. Longev* 2019, 4393460.
- Madej, P., Plewka, A., Madej, J.A., Plewka, D., Mroczka, W., Wilk, K., Dobrosz, Z., 2007. Ozone therapy in induced endotoxemic shock. II. The effect of ozone therapy upon selected histochemical reactions in organs of rats in endotoxemic shock. *Inflammation* 30, 69–86.
- Maes, O.C., Kravitz, S., Mawal, Y., Su, H., Liberman, A., Mehindate, K., Berlin, D., Sahlas, D.J., Chertkow, H.M., Bergman, H., Melmed, C., Schipper, H.M., 2006. Characterization of alpha1-antitrypsin as a heme oxygenase-1 suppressor in Alzheimer plasma. *Neurobiol. Dis.* 24, 89–100.
- Maki, R.A., Holzer, M., Motamedchaboki, K., Malle, E., Masliah, E., Marsche, G., Reynolds, W.F., 2019. Human myeloperoxidase (hMPO) is expressed in neurons in the substantia nigra in Parkinson's disease and in the hMPO-alpha-synuclein-A53T mouse model, correlating with increased nitration and aggregation of alpha-synuclein and exacerbation of motor impairment. *Free Radic. Biol. Med.* 141, 115–140.
- Manoto, S.L., Maepa, M.J., Motaung, S.K., 2018. Medical ozone therapy as a potential treatment modality for regeneration of damaged articular cartilage in osteoarthritis. *Saudi J. Biol. Sci.* 25, 672–679.
- Mao, Z.J., Lin, H., Hou, J.W., Zhou, Q., Wang, Q., Chen, Y.H., 2019. A Meta-Analysis of Resveratrol Protects against Myocardial Ischemia/Reperfusion Injury: Evidence from Small Animal Studies and Insight into Molecular Mechanisms. *Oxid. Med. Cell. Longev* 2019, 5793867.
- Martinez de Toda, I., De la Fuente, M., 2015. The role of Hsp70 in oxi-inflamm-aging and its use as a potential biomarker of lifespan. *Biogerontology* 16, 709–721.
- Martinez-Sanchez, G., Al-Dalain, S.M., Menendez, S., Re, L., Giuliani, A., Candelario-Jalil, E., Alvarez, H., Fernandez-Montequin, J.I., Leon, O.S., 2005. Therapeutic efficacy of ozone in patients with diabetic foot. *Eur. J. Pharmacol.* 523, 151–161.
- Massaad, C.A., 2011. Neuronal and vascular oxidative stress in Alzheimer's disease. *Curr. Neuropharmacol.* 9, 662–673.
- Mateo, I., Infante, J., Sanchez-Juan, P., Garcia-Gorostiaga, I., Rodriguez-Rodriguez, E., Vazquez-Higuera, J.L., Berciano, J., Combarros, O., 2010. Serum heme oxygenase-1 levels are increased in Parkinson's disease but not in Alzheimer's disease. *Acta Neurol. Scand.* 121, 136–138.
- Mattson, M.P., 2008. Hormesis defined. *Ageing Res. Rev.* 7, 1–7.
- Mattson, M.P., 2004. Pathways towards and away from Alzheimer's disease. *Nature* 430, 631–639.
- Mazzetti, A.P., Fiorile, M.C., Primavera, A., Lo Bello, M., 2015. Glutathione transferases and neurodegenerative diseases. *Neurochem. Int.* 82, 10–18.
- Mecucci, P., Boccardi, V., Cecchetti, R., Bastiani, P., Scamosci, M., Ruggiero, C., Baroni, M., 2018. A Long Journey into Aging, Brain Aging, and Alzheimer's Disease Following the Oxidative Stress Tracks. *J. Alzheimers Dis.* 62, 1319–1335.
- Mendez, E.F., Sattler, R., 2015. Biomarker development for C9orf72 repeat expansion in ALS. *Brain Res.* 1607, 26–35.
- Meng, W., Xu, Y., Li, D., Zhu, E., Deng, L., Liu, Z., Zhang, G., Liu, H., 2017. Ozone protects rat heart against ischemia-reperfusion injury: A role for oxidative preconditioning in attenuating mitochondrial injury. *Biomed. Pharmacother.* 88, 1090–1097.
- Merelli, A., Rodriguez, J.C.G., Folch, J., Regueiro, M.R., Camins, A., Lazarowski, A., 2018. Understanding the Role of Hypoxia Inducible Factor During Neurodegeneration for New Therapeutics Opportunities. *Curr. Neuropharmacol.* 16, 1484–1498.
- Moldogazieva, N.T., Mokhosoev, I.M., Mel'nikova, T.I., Porozov, Y.B., Terentiev, A.A., 2019. Oxidative Stress and Advanced Lipoxidation and Glycation End Products (ALEs and AGEs) in Aging and Age-Related Diseases. *Oxid. Med. Cell. Longev* 2019, 3085756.
- Moreno-Fernandez, A., Macias-Garcia, L., Valverde-Moreno, R., Ortiz, T., Fernandez-Rodriguez, A., Molini-Estrada, A., De-Miguel, M., 2019. Autohemotherapy with ozone as a possible effective treatment for Fibromyalgia. *Acta Reumatol. Port.* 44, 244–249.
- Morsy, M.D., Hassan, W.N., Zalal, S.I., 2010. Improvement of renal oxidative stress markers after ozone administration in diabetic nephropathy in rats. *Diabetol. Metab. Syndr.* 2, 29-5996-2-29.
- Moskalev, A., Proshkina, E., Belyi, A., Solovev, I., 2017. Genetics of aging and longevity. *Russian Journal of Genetics: Applied Research* 7, 369–384.
- Mota, A., Hemati-Dinarvand, M., Akbar Taheraghdam, A., Reza Nejabati, H., Ahmadi, R., Ghasemnejad, T., Hasanpour, M., Valilo, M., 2019. Association of Paraoxonase I (PON1) Genotypes with the Activity of PON1 in Patients with Parkinson's Disease. *Acta Neurol. Taiwan.* 28 (3), 66–74.
- Muller, G.C., Gottlieb, M.G., Luz Correa, B., Gomes Filho, I., Moresco, R.N., Bauer, M.E., 2015. The inverted CD4:CD8 ratio is associated with gender-related changes in oxidative stress during aging. *Cell. Immunol.* 296, 149–154.
- Nakabeppu, Y., Tsuchimoto, D., Yamaguchi, H., Sakumi, K., 2007. Oxidative damage in nucleic acids and Parkinson's disease. *J. Neurosci. Res.* 85, 919–934.
- Nakamura, T., Lipton, S.A., 2020. Nitric Oxide-Dependent Protein Post-Translational Modifications Impair Mitochondrial Function and Metabolism to Contribute to Neurodegenerative Diseases. *Antioxid. Redox Signal.* 32, 817–833.
- Nasezadeh, P., Shahi, F., Fridoni, M., Seydi, E., Izadi, M., Salimi, A., 2017. Moderate O3/O2 therapy enhances enzymatic and non-enzymatic antioxidant in brain and cochlear that protects noise-induced hearing loss. *Free Radic. Res.* 51, 828–837.
- Negre-Salvayre, A., Auge, N., Ayala, V., Basaga, H., Boada, J., Brenke, R., Chapple, S., Cohen, G., Feher, J., Grune, T., Lengyel, G., Mann, G.E., Pamplona, R., Poli, G., Portero-Otin, M., Riahi, Y., Salvayre, R., Sasson, S., Serrano, J., Shami, O., Siems, W., Siow, R.C., Wiswedel, I., Zarkovic, K., Zarkovic, N., 2010. Pathological aspects of lipid peroxidation. *Free Radic. Res.* 44, 1125–1171.
- Nitti, M., Piras, S., Brondolo, L., Marinari, U.M., Pronzato, M.A., Furfaro, A.L., 2018. Heme Oxygenase 1 in the Nervous System: Does It Favor Neuronal Cell Survival or Induce Neurodegeneration? *Int. J. Mol. Sci.* 19 <https://doi.org/10.3390/ijms19082260>.
- Nowotny, K., Jung, T., Grune, T., Hohn, A., 2014. Reprint of "accumulation of modified proteins and aggregate formation in aging". *Exp. Gerontol.* 59, 3–12.
- Oh, S., Gwak, J., Park, S., Yang, C.S., 2014. Green tea polyphenol EGCG suppresses Wnt/beta-catenin signaling by promoting GSK-3beta- and PP2A-independent beta-catenin phosphorylation/degradation. *BioFactors* 40, 586–595.
- Oliveira, P.V.S., Laurindo, F.R.M., 2018. Implications of plasma thiol redox in disease. *Clin. Sci. (Lond)* 132, 1257–1280.
- Onal, M., Elsurer, C., Selimoglu, N., Yilmaz, M., Erdogan, E., Bengi Celik, J., Kal, O., Onal, O., 2017. Ozone Prevents Cochlear Damage From Ischemia-Reperfusion Injury in Guinea Pigs. *Artificial organs JID - 7802778* (41), 744–752.
- Ozkan, H., Ekinci, S., Uysal, B., Akyildiz, F., Turkkan, S., Ersen, O., Koca, K., Seven, M., 2015. Evaluation and comparison of the effect of hypothermia and ozone on ischemia-reperfusion injury of skeletal muscle in rats. *J. Surg. Res.* 196, 313–319.
- Ozturk, O., Eroglu, H.A., Ustebay, S., Kuzuco, M., Adali, Y., 2018. An experimental study on the preventive effects of N-acetyl cysteine and ozone treatment against contrast-induced nephropathy. *Acta cirurgica brasileira* 33, 508–517. JID - 9103983.
- Pan, H., Kim, E., Rankin, G.O., Rojanasakul, Y., Tu, Y., Chen, Y.C., 2018. Theaflavin-3, 3'-digallate inhibits ovarian cancer stem cells via suppressing Wnt/beta-Catenin signaling pathway. *J. Funct. Foods* 50, 1–7.
- Paul, B.D., Sbodio, J.L., Snyder, S.H., 2018. Cysteine Metabolism in Neuronal Redox Homeostasis. *Trends Pharmacol. Sci.* 39, 513–524.
- Pawlak-Osinska, K., Kazmierczak, H., Kazmierczak, W., Szpoper, M., 2004. Ozone therapy and pressure-pulse therapy in Meniere's disease. *Int. Tinnitus J.* 10, 54–57.
- Pedrucci, L.M., Stockler-Pinto, M.B., Leite Jr, M., Mafrá, D., 2012. Nrf2-keap1 system versus NF-kappaB: the good and the evil in chronic kidney disease? *Biochimie* 94, 2461–2466.
- Perez, D.I., Gil, C., Martinez, A., 2011. Protein kinases CK1 and CK2 as new targets for neurodegenerative diseases. *Med. Res. Rev.* 31, 924–954.
- Picon-Pages, P., Garcia-Buendia, J., Munoz, F.J., 2019. Functions and dysfunctions of nitric oxide in brain. *Biochim. Biophys. Acta Mol. Basis Dis.* 1865, 1949–1967.

- Polidori, M.C., Mecocci, P., Browne, S.E., Senin, U., Beal, M.F., 1999. Oxidative damage to mitochondrial DNA in Huntington's disease parietal cortex. *Neurosci. Lett.* 272, 53–56.
- Poulsen, H.E., Nadal, L.L., Broedbaek, K., Nielsen, P.E., Weimann, A., 2014. Detection and interpretation of 8-oxodG and 8-oxoGua in urine, plasma and cerebrospinal fluid. *Biochim. Biophys. Acta* 1840, 801–808.
- Pratico, D., 2008. Oxidative stress hypothesis in Alzheimer's disease: a reappraisal. *Trends Pharmacol. Sci.* 29, 609–615.
- Puspita, L., Chung, S.Y., Shim, J.W., 2017. Oxidative stress and cellular pathologies in Parkinson's disease. *Mol. Brain* 10, 53-017-0340-9.
- Qing, Z., Ling-Ling, E., Dong-Sheng, W., Hong-Chen, L., 2012. Relationship of advanced oxidative protein products in human saliva and plasma: age- and gender-related changes and stability during storage. *Free Radic. Res.* 46, 1201–1206.
- Qiu, T., Wang, Z., Liu, X., Chen, H., Zhou, J., Chen, Z., Wang, M., Jiang, G., Wang, L., Yu, G., Zhang, L., Shen, Y., Zhang, L., He, L., Wang, H., Zhang, W., 2017. Effect of ozone oxidative preconditioning on oxidative stress injury in a rat model of kidney transplantation. *Experimental and therapeutic medicine* 13, 1948–1955.
- Radi, R., 2018. Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. *Proc. Natl. Acad. Sci. U. S. A.* 115, 5839–5848.
- Ramirez-Acuna, J.M., Cardenas-Cadena, S.A., Marquez-Salas, P.A., Garza-Veloz, L., Perez-Favila, A., Cid-Baez, M.A., Flores-Morales, V., Martinez-Fierro, M.L., 2019. Diabetic Foot Ulcers: Current Advances in Antimicrobial Therapies and Emerging Treatments. *Antibiotics (Basel)* 8. <https://doi.org/10.3390/antibiotics8040193>.
- Ray, R.S., Katyal, A., 2016. Myeloperoxidase: Bridging the gap in neurodegeneration. *Neurosci. Biobehav. Rev.* 68, 611–620.
- Re, L., Martinez-Sanchez, G., Bordicchia, M., Malcangi, G., Pocognoli, A., Morales-Segura, M.A., Rothchild, J., Rojas, A., 2014. Is ozone pre-conditioning effect linked to Nrf2/EpRE activation pathway in vivo? A preliminary result. *Eur. J. Pharmacol.* 742, 158–162.
- Re, L., Martinez-Sanchez, G., Perez-Davison, G., Siroto, M., 2010. Role of ozone/oxygen in fibroblast growth factor activation. Discovering the facts. *International Journal of Ozone Therapy* 9, 55–58.
- Re, L., Mawsouf, M.N., Menendez, S., Leon, O.S., Sanchez, G.M., Hernandez, F., 2008. Ozone therapy: clinical and basic evidence of its therapeutic potential. *Arch. Med. Res.* 39, 17–26.
- Reutzel, M., Grewal, R., Dilberger, B., Silaidos, C., Joppe, A., Eckert, G.P., 2020. Cerebral Mitochondrial Function and Cognitive Performance during Aging: A Longitudinal Study in NMRI Mice. *Oxidative Medicine and Cellular Longevity* 2020, 4060769.
- Rizvi, S.I., Jha, R., Maurya, P.K., 2006. Erythrocyte plasma membrane redox system in human aging. *Rejuvenation Res.* 9, 470–474.
- Rodriguez, Z.Z., Guanache, D., Alvarez, R.G., Martinez, Y., Alonso, Y., Schulz, S., 2011. Effects of ozone oxidative preconditioning on different hepatic biomarkers of oxidative stress in endotoxic shock in mice. *Toxicol. Mech. Methods* 21, 236–240.
- Rodriguez, Z.Z., Guanache, D., Alvarez, R.G., Rosales, F.H., Alonso, Y., Schulz, S., 2009. Preconditioning with ozone/oxygen mixture induces reversion of some indicators of oxidative stress and prevents organic damage in rats with fecal peritonitis. *Inflamm. Res.* 58, 371–375.
- Rosenberger, A.F., Morrema, T.H., Gerritsen, W.H., van Haastert, E.S., Snkhchyan, H., Hilhorst, R., Rozemuller, A.J., Scheltens, P., van der Vies, S.M., Hoozemans, J.J., 2016. Increased occurrence of protein kinase CK2 in astrocytes in Alzheimer's disease pathology. *J. Neuroinflammation* 13, 4-015-0470-x.
- Rosul, M.V., Patskan, B.M., 2016. Ozone therapy effectiveness in patients with ulcerous lesions due to diabetes mellitus. *Wiad. Lek.* 69, 7–9.
- Rougemont, M., Do, K.Q., Castagne, V., 2002. New model of glutathione deficit during development: Effect on lipid peroxidation in the rat brain. *J. Neurosci. Res.* 70, 774–783.
- Rusanova, I., Diaz-Casado, M.E., Fernandez-Ortiz, M., Aranda-Martinez, P., Guerra-Librero, A., Garcia-Garcia, F.J., Escames, G., Manas, L., Acuna-Castroviejo, D., 2018. Analysis of Plasma MicroRNAs as Predictors and Biomarkers of Aging and Frailty in Humans. *Oxid. Med. Cell. Longev* 2018, 7671850.
- Safwat, M.H., El-Sawalhi, M.M., Mawsouf, M.N., Shaheen, A.A., 2014. Ozone ameliorates age-related oxidative stress changes in rat liver and kidney: effects of pre- and post-ageing administration. *Biochemistry (Mosc)* 79, 450–458.
- Salminen, A., Kaarniranta, K., Kauppinen, A., 2016. Age-related changes in AMPK activation: Role for AMPK phosphatases and inhibitory phosphorylation by upstream signaling pathways. *Ageing Res. Rev.* 28, 15–26.
- Sancak, E.B., Turkon, H., Cukur, S., Erimsah, S., Akbas, A., Gulpinar, M.T., Toman, H., Sahin, H., Uzun, M., 2016. Major Ozonated Autohemotherapy Preconditioning Ameliorates Kidney Ischemia-Reperfusion Injury. *Inflammation* 39, 209–217.
- Scassellati, C., Ciani, M., Galoforo, A.C., Zanardini, R., Bonvicini, C., Geroldi, C., 2020. Molecular mechanisms in cognitive frailty: potential therapeutic targets for oxygen-ozone treatment. *Mech. Ageing Dev.* 186, 111210.
- Scassellati, C., Costanzo, M., Cisterna, B., Nodari, A., Galie, M., Cattaneo, A., Covi, V., Tabaracci, G., Bonvicini, C., Malatesta, M., 2017. Effects of mild ozonisation on gene expression and nuclear domains organization in vitro. *Toxicol. In Vitro* 44, 100–110.
- Schaffert, L.N., Carter, W.G., 2020. Do Post-Translational Modifications Influence Protein Aggregation in Neurodegenerative Diseases: A Systematic Review. *Brain Sci.* 10 <https://doi.org/10.3390/brainsci10040232>.
- Schipper, H.M., 2010. Biological markers and Alzheimer disease: a canadian perspective. *Int. J. Alzheimers Dis.* 2010 <https://doi.org/10.4061/2010/978182>.
- Schipper, H.M., 2007. Biomarker potential of heme oxygenase-1 in Alzheimer's disease and mild cognitive impairment. *Biomark. Med.* 1, 375–385.
- Schipper, H.M., Chertkow, H., Mehindate, K., Frankel, D., Melmed, C., Bergman, H., 2000. Evaluation of heme oxygenase-1 as a systemic biological marker of sporadic AD. *Neurology* 54, 1297–1304.
- Schipper, H.M., Song, W., Tavitian, A., Cressatti, M., 2019. The sinister face of heme oxygenase-1 in brain aging and disease. *Prog. Neurobiol.* 172, 40–70.
- Schmidlin, C.J., Dodson, M.B., Madhavan, L., Zhang, D.D., 2019. Redox regulation by Nrf2 in aging and disease. *Free Radic. Biol. Med.* 134, 702–707.
- Schwartz-Tapia, A., Martínez-Sánchez, G., Sabah, F., Alvarado-Guérrez, F., Bazzano-Mastrelli, N., Bikina, O., Borroto-Rodríguez, V., Cakir, R., Clavo, B., González-Sánchez, E., Grechkanov, G., Najm Dawood, A.H., Izzo, A., Konrad, H., Masini, M., Peretiagyn, S., Pereyra, V.R., Ruiz Reyes, D., Shallenberger, F., Vongay, V., Xirezhati, A., Quintero-Marino, R., 2015. Madrid Declaration on Ozone Therapy, 2nd Madrid ed. ISCO3.
- Scuto, M., Di Mauro, P., Ontario, M.L., Amato, C., Modafferi, S., Ciavardelli, D., Trovato Salinaro, A., Maiolino, L., Calabrese, V., 2019. Nutritional Mushroom Treatment in Meniere's Disease with Coriolus versicolor: A Rationale for Therapeutic Intervention in Neuroinflammation and Antineurodegeneration. *Int. J. Mol. Sci.* 21 <https://doi.org/10.3390/ijms21010284>.
- Selkoe, D.J., 2001. Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein. *J. Alzheimers Dis.* 3, 75–80.
- Shan, Y., Schoenfeld, R.A., Hayashi, G., Napoli, E., Akiyama, T., Iodi Carstens, M., Carstens, E.E., Pook, M.A., Cortopassi, G.A., 2013. Frataxin deficiency leads to defects in expression of antioxidants and Nrf2 expression in dorsal root ganglia of the Friedreich's ataxia YG8R mouse model. *Antioxid. Redox Signal.* 19, 1481–1493.
- Shehata, N.I., Abd-Elgawad, H.M., Mawsouf, M.N., Shaheen, A.A., 2012. The potential role of ozone in ameliorating the age-related biochemical changes in male rat cerebral cortex. *Biogerontology* 13, 565–581.
- Silva, T.O., Jung, I.E., Moresco, R.N., Barbisan, F., Ribeiro, E.E., Ribeiro, E.A., Motta, K., Britto, E., Tasch, E., Bochi, G., Duarte, M.M., Oliveira, A.R., Marcon, M., Bello, C., dos Santos Montagner, G.F., da Cruz, I.B., 2015. Association between advanced oxidation protein products and 5-year mortality risk among amazon riparian elderly population. *Free Radic. Res.* 49, 204–209.
- Silva-Palacios, A., Ostolga-Chavarria, M., Zazueta, C., Konigsberg, M., 2018. Nrf2: Molecular and epigenetic regulation during aging. *Ageing Res. Rev.* 47, 31–40.
- Singh, A., Kukreti, R., Saso, L., Kukreti, S., 2019. Oxidative Stress: A Key Modulator in Neurodegenerative Diseases. *Molecules* 24. <https://doi.org/10.3390/molecules24081583>.
- Siniscalco, D., Trotta, M.C., Brigida, A.L., Maisto, R., Luongo, M., Ferraraccio, F., D'Amico, M., Di Filippo, C., 2018. Intraperitoneal Administration of Oxygen/Ozone to Rats Reduces the Pancreatic Damage Induced by Streptozotocin. *Biology (Basel)* 7. <https://doi.org/10.3390/biology7010010>.
- Sivandzade, F., Prasad, S., Bhalerao, A., Cucullo, L., 2019. NRF2 and NF- κ B interplay in cerebrovascular and neurodegenerative disorders: Molecular mechanisms and possible therapeutic approaches. *Redox Biol.* 21, 101059.
- Smith, N.L., Wilson, A.L., Gandhi, J., Vatsia, S., Khan, S.A., 2017. Ozone therapy: an overview of pharmacodynamics, current research, and clinical utility. *Med. Gas Res.* 7, 212–219.
- Son, T.G., Zou, Y., Yu, B.P., Lee, J., Chung, H.Y., 2005. Aging effect on myeloperoxidase in rat kidney and its modulation by calorie restriction. *Free Radic. Res.* 39, 283–289.
- Spillantini, M.G., Crowther, R.A., Jakes, R., Hasegawa, M., Goedert, M., 1998. alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6469–6473.
- Srikanth, A., Sathish, M., Sri Harsha, A.V., 2013. Application of ozone in the treatment of periodontal disease. *J. Pharm. Bioallied Sci.* 5, S89–94.
- Stadlbauer, T.H., Eisele, A., Heidt, M.C., Tillmanns, H.H., Schulz, S., 2008. Preconditioning with ozone abrogates acute rejection and prolongs cardiac allograft survival in rats. *Transplant. Proc.* 40, 974–977.
- Sun, W., Pei, L., 2012. Ozone preconditioning and exposure to ketamine attenuates hepatic inflammation in septic rats. *Arch. Med. Sci.* 8, 918–923.
- Tang, Z., Hu, B., Zang, F., Wang, J., Zhang, X., Chen, H., 2019. Nrf2 drives oxidative stress-induced autophagy in nucleus pulposus cells via a Keap1/Nrf2/p62 feedback loop to protect intervertebral disc from degeneration. *Cell. Death Dis.* 10, 510-019-1701-3.
- Tarafdar, A., Pula, G., 2018. The Role of NADPH Oxidases and Oxidative Stress in Neurodegenerative Disorders. *Int. J. Mol. Sci.* 19 <https://doi.org/10.3390/ijms19123824>.
- Tasdoven, I., Emre, A.U., Gultekin, F.A., Oner, M.O., Bakkal, B.H., Turku, U.O., Gun, B. D., Tasdoven, G.E., 2019. Effects of ozone preconditioning on recovery of rat colon anastomosis after preoperative radiotherapy. *Adv. Clin. Exp. Med.* 28, 1683–1689.
- Teskey, G., Abraham, R., Cao, R., Gyurjian, K., Islamoglu, H., Lucero, M., Martinez, A., Paredes, E., Salaiz, O., Robinson, B., Venketaraman, V., 2018. Glutathione as a Marker for Human Disease. *Adv. Clin. Exp. Med.* 87, 141–159.
- Tieu, K., Ischiropoulos, H., Przedborski, S., 2003. Nitric oxide and reactive oxygen species in Parkinson's disease. *IUBMB Life* 55, 329–335.
- Tirelli, U., Cirrito, C., Pavanello, M., Piasentin, C., Lleshi, A., Taibi, R., 2019. Ozone therapy in 65 patients with fibromyalgia: an effective therapy. *Eur. Rev. Med. Pharmacol. Sci.* 23, 1786–1788.
- Toda, N., Ayajiki, K., Okamura, T., 2009. Cerebral blood flow regulation by nitric oxide in neurological disorders. *Can. J. Physiol. Pharmacol.* 87, 581–594.
- Trovato Salinaro, A., Pennisi, M., Di Paola, R., Scuto, M., Crupi, R., Cambria, M.T., Ontario, M.L., Tomasello, M., Uva, M., Maiolino, L., Calabrese, E.J., Cuzzocrea, S., Calabrese, V., 2018. Neuroinflammation and neurohormesis in the pathogenesis of Alzheimer's disease and Alzheimer-linked pathologies: modulation by nutritional mushrooms. *Immun. Ageing* 15, 8-017-0108-1. eCollection 2018.
- Trovato, A., Siracusa, R., Di Paola, R., Scuto, M., Fronte, V., Koverech, G., Luca, M., Serra, A., Toscano, M.A., Petralia, A., Cuzzocrea, S., Calabrese, V., 2016a. Redox modulation of cellular stress response and lipoxin A4 expression by Coriolus versicolor in rat brain: Relevance to Alzheimer's disease pathogenesis. *Neurotoxicology* 53, 350–358.

- Trovato, A., Siracusa, R., Di Paola, R., Scuto, M., Ontario, M.L., Bua, O., Di Mauro, P., Toscano, M.A., Petralia, C.C.T., Maiolino, L., Serra, A., Cuzzocrea, S., Calabrese, V., 2016b. Redox modulation of cellular stress response and lipoxin A4 expression by *Hericium Erinaceus* in rat brain: relevance to Alzheimer's disease pathogenesis. *Immun.Ageing* 13, 23-016-0078-8. eCollection 2016.
- Tuney, I., Sanchez-Lopez, F., Aguera, E., Fernandez-Bolanos, R., Sanchez, F.M., Tasset-Cuevas, I., 2011. Important role of oxidative stress biomarkers in Huntington's disease. *J. Med. Chem.* 54, 5602-5606.
- Tusat, M., Mentese, A., Demir, S., Alver, A., Imamoglu, M., 2017. Medical ozone therapy reduces oxidative stress and testicular damage in an experimental model of testicular torsion in rats. *Int. Braz. J. Urol.* 43, 1160-1166.
- Uysal, B., Yasar, M., Ersoz, N., Coskun, O., Kilic, A., Cayc, T., Kurt, B., Oter, S., Korkmaz, A., Guven, A., 2010. Efficacy of hyperbaric oxygen therapy and medical ozone therapy in experimental acute necrotizing pancreatitis. *Pancreas* 39, 9-15.
- Vaillant, J.D., Fraga, A., Diaz, M.T., Mallok, A., Viebahn-Hansler, R., Fahmy, Z., Barbera, A., Delgado, L., Menendez, S., Fernandez, O.S., 2013. Ozone oxidative postconditioning ameliorates joint damage and decreases pro-inflammatory cytokine levels and oxidative stress in PG/PS-induced arthritis in rats. *Eur. J. Pharmacol.* 714, 318-324.
- Veal, E., Jackson, T., Latimer, H., 2018. Role/s of 'Antioxidant' Enzymes in Ageing. *Subcell. Biochem.* 90, 425-450.
- Vikram, A., Anish, R., Kumar, A., Tripathi, D.N., Kaundal, R.K., 2017. Oxidative Stress and Autophagy in Metabolism and Longevity. *Oxid Med.Cell.Longev* 2017, 3451528.
- Vina, E.R., Fang, A.J., Wallace, D.J., Weisman, M.H., 2005. Chronic inflammatory demyelinating polyneuropathy in patients with systemic lupus erythematosus: prognosis and outcome. *Semin. Arthritis Rheum.* 35, 175-184.
- Vonsattel, J.P., DiFiglia, M., 1998. Huntington disease. *J. Neuropathol. Exp. Neurol.* 57, 369-384.
- Wang, J., Zhang, Y., Zhu, Q., Liu, Y., Cheng, H., Zhang, Y., Li, T., 2016. Emodin protects mice against radiation-induced mortality and intestinal injury via inhibition of apoptosis and modulation of p53. *Environ. Toxicol. Pharmacol.* 46, 311-318.
- Wang, L., Chen, H., Liu, X.H., Chen, Z.Y., Weng, X.D., Qiu, T., Liu, L., 2014a. The protective effect of ozone oxidative preconditioning against hypoxia/reoxygenation injury in rat kidney cells. *Ren.Fail.* 36, 1449-1454.
- Wang, L., Chen, H., Liu, X.H., Chen, Z.Y., Weng, X.D., Qiu, T., Liu, L., Zhu, H.C., 2014b. Ozone oxidative preconditioning inhibits renal fibrosis induced by ischemia and reperfusion injury in rats. *Exp.Ther.Med.* 8, 1764-1768.
- Wang, L., Chen, Z., Liu, Y., Du, Y., Liu, X., 2018a. Ozone oxidative postconditioning inhibits oxidative stress and apoptosis in renal ischemia and reperfusion injury through inhibition of MAPK signaling pathway. *Drug Des.Devel.Ther.* 12, 1293-1301.
- Wang, L., Chen, Z., Weng, X., Wang, M., Du, Y., Liu, X., 2019a. Combined Ischemic Postconditioning and Ozone Postconditioning Provides Synergistic Protection Against Renal Ischemia and Reperfusion Injury Through Inhibiting Pyroptosis. *Urology* 123, 296.e1-296.e8.
- Wang, X., 2018. Emerging roles of ozone in skin diseases. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 43, 114-123.
- Wang, X., Wang, W., Li, L., Perry, G., Lee, H.G., Zhu, X., 2014c. Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. *Biochim.Biophys.Acta* 1842, 1240-1247.
- Wang, Y., Li, H., Li, Y., Zhao, Y., Xiong, F., Liu, Y., Xue, H., Yang, Z., Ni, S., Sahil, A., Che, H., Wang, L., 2019b. *Coriolus versicolor* alleviates diabetic cardiomyopathy by inhibiting cardiac fibrosis and NLRP3 inflammasome activation. *Phytother.Res.* 33, 2737-2748.
- Wang, Z., Bai, Z., Qin, X., Cheng, Y., 2019c. Aberrations in Oxidative Stress Markers in Amyotrophic Lateral Sclerosis: A Systematic Review and Meta-Analysis. *Oxid Med. Cell.Longev* 2019, 1712323.
- Wang, Z., Han, Q., Guo, Y.L., Liu, X.H., Qiu, T., 2018b. Effect of ozone oxidative preconditioning on inflammation and oxidative stress injury in rat model of renal transplantation. *Acta Cir.Bras.* 33, 238-249.
- Wang, Z., Zhang, A., Meng, W., Wang, T., Li, D., Liu, Z., Liu, H., 2018c. Ozone protects the rat lung from ischemia-reperfusion injury by attenuating NLRP3-mediated inflammation, enhancing Nrf2 antioxidant activity and inhibiting apoptosis. *Eur.J. Pharmacol.* 835, 82-93.
- Wentworth Jr, P., McDunn, J.E., Wentworth, A.D., Takeuchi, C., Nieva, J., Jones, T., Bautista, C., Ruedi, J.M., Gutierrez, A., Janda, K.D., Babior, B.M., Eschenmoser, A., Lerner, R.A., 2002. Evidence for antibody-catalyzed ozone formation in bacterial killing and inflammation. *Science* 298, 2195-2199.
- WHO, 2011. **Global Health and Aging [Online]. Available from:** <https://www.who.int/ageing/publications/global-health/en/>.
- Wyss-Coray, T., 2016. Ageing, neurodegeneration and brain rejuvenation. *Nature* 539, 180-186.
- Xing, B., Chen, H., Wang, L., Weng, X., Chen, Z., Li, X., 2015. Ozone oxidative preconditioning protects the rat kidney from reperfusion injury via modulation of the TLR4-NF-kappaB pathway. *Acta Cir.Bras.* 30, 60-66.
- Yanar, K., Atayik, M.C., Simsek, B., Cakatay, U., 2020. Novel biomarkers for the evaluation of aging-induced proteinopathies. *Biogerontology*.
- Yeo, E.J., 2019. Hypoxia and aging. *Exp.Mol.Med.* 51, 1-15.
- Yong, L., Lyu, X., Huang, C., Xu, Y., 2017. "Effect of local ozone treatment on inflammatory cytokine, growth cytokine and apoptosis molecule expression in anal fistula wound".
- Yu, G., Bai, Z., Chen, Z., Chen, H., Wang, G., Wang, G., Liu, Z., 2017. The NLRP3 inflammasome is a potential target of ozone therapy aiming to ease chronic renal inflammation in chronic kidney disease. *Int.Immunopharmacol.* 43, 203-209.
- Zamora, Z.B., Borrego, A., Lopez, O.Y., Delgado, R., Gonzalez, R., Menendez, S., Hernandez, F., Schulz, S., 2005. Effects of ozone oxidative preconditioning on TNF-alpha release and antioxidant-prooxidant intracellular balance in mice during endotoxic shock. *Mediators Inflamm.* 2005, 16-22.
- Zamora, Z.B., Borrego, A., Lopez, O.Y., Delgado, R., Menendez, S., Schulz, S., Hernandez, F., 2004. Inhibition of tumor necrosis factor-alpha release during endotoxic shock by ozone oxidative preconditioning in mice. *Arzneimittelforschung* 54, 906-909.
- Zhang, H., Davies, K.J.A., Forman, H.J., 2015. Oxidative stress response and Nrf2 signaling in aging. *Free Radic.Biol.Med.* 88, 314-336.
- Zhang, J., Guan, M., Xie, C., Luo, X., Zhang, Q., Xue, Y., 2014. Increased growth factors play a role in wound healing promoted by noninvasive oxygen-ozone therapy in diabetic patients with foot ulcers. *Oxid Med.Cell.Longev* 2014, 273475.
- Zhao, X., Li, Y., Lin, X., Wang, J., Zhao, X., Xie, J., Sun, T., Fu, Z., 2018. Ozone induces autophagy in rat chondrocytes stimulated with IL-1beta through the AMPK/mTOR signaling pathway. *J.Pain Res.* 11, 3003-3017.
- Zhao, Y., Zhao, B., 2013. Oxidative stress and the pathogenesis of Alzheimer's disease. *Oxid Med.Cell.Longev* 2013, 316523.
- Zheng, Z., Dong, M., Hu, K., 2020. A preliminary evaluation on the efficacy of ozone therapy in the treatment of COVID-19. *J.Med.Virol.*
- Zhou, M., Hou, J., Li, Y., Mou, S., Wang, Z., Horch, R.E., Sun, J., Yuan, Q., 2019. The pro-angiogenic role of hypoxia inducible factor stabilizer FG-4592 and its application in an in vivo tissue engineering chamber model. *Sci. Rep.* 9, 6035-019-41924-5.