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Molecular mechanisms in cognitive frailty: potential therapeutic targets for oxygen-ozone treatment

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ABSTRACT

In the last decade, cognitive frailty has gained great attention from the scientific community. It is characterized by high inflammation and oxidant state, endocrine and metabolic alterations, mitochondria dysfunctions and slowdown in regenerative processes and immune system, with a complex and multifactorial aetiology. Although several treatments are available, challenges regarding the efficacy and the costs persist. Here, we proposed an alternative non-pharmacological, non-side-effect, low cost therapy based on anti-inflammation, antioxidant, regenerative and anti-pathogens properties of ozone, through the activation of several molecular mechanisms (Nrf2-ARE, NF-κB, NFAT, AP-1, HIFα). We highlighted how these specific processes could be implicated in cognitive frailty to identify putative therapeutic targets for its treatment.

The oxigen-ozone (O_2-O_3) therapy has never been tested for cognitive frailty. This work provides thus wide scientific background to build a consistent rationale for testing for the first time this therapy, that could modulate the immune, inflammatory, oxidant, metabolic, endocrine, microbiota and regenerative processes impaired in cognitive frailty.

Although insights are needed, the O_2-O_3 therapy could represent a faster, easier, inexpensive monodomain intervention working in absence of side effects for cognitive frailty.

1. Introduction

Frailty is an age-related clinical condition, highly prevalent in the older population, characterized by a multisystem dysregulation, leading to decreased physiological reserve and increased vulnerability for adverse health outcomes. Interestingly, this status predisposes multifarious less or more severe conditions, including dementia, acute illnesses, falls, hospitalization, disability, dependency, and mortality (Bandeen-Roche et al., 2006; Fried et al., 2004; Kojima et al., 2019; Moraes et al., 2018). Thus, the management of frailty is extremely challenging and further complicated by numerous comorbidities, which generate additional impairments and social costs (Vetrano et al., 2016). Considering that this condition is potentially preventable, detection of frailty as soon as possible is crucial before that this risk-state converts in a true pathological condition (Roland et al., 2014).

The complex pathophysiological picture of frailty causes the lack of a solid definition, worsening further by the clinical heterogeneity of aging. The clinical assessment is based on two main ways: the frailty phenotype (Fried et al., 2001) and the frailty index (Mitnitski et al., 2001; Rockwood and Mitnitski, 2007). Fried et al. (2001) defined the frailty as an age-associated "phenotypic phenomenon" expressing at least three specific clinical symptoms (weakness, weight loss, slow walking speed, fatigue, low level of physical activity). On the other hand, the group of Rockwood and Mitnitski (2007) elaborated a more complex perspective of this vulnerability status, considering biological and psychosocial aspects of frailty enclosed in a frailty index. In this case, the frailty index represents a measure of overall decline in health associated with age, due to accumulation of healthy related deficits.

One of the emerging clinical expression related to the multidimensional nature of frailty is represented by the cognition

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impairment. In the last decade, cognitive frailty has gained great attention from the scientific community, being more studied for its physiologic, epidemiologic, clinical and genetic aspects (Panza et al., 2018; Sugimoto et al., 2018; Young et al., 2016).

In 2001, cognitive frailty term was incidentally used by Paganini-Hill and colleagues in a study on the Clock Drawing Test (CDT) performance and its association with potential protective and risk factors for Alzheimer's Disease (AD) in an older cohort (Paganini-Hill et al., 2001). In 2004, the same term was used as a general descriptor for cognitive impairment occurring as people reach advanced age, or to refer to cognitive disturbances or pre-dementia occurring in association with other medical conditions (Chouliara et al., 2004). Only in 2006, cognitive frailty was used more faithfully as a clinical label for a particular state of cognitive vulnerability in mild cognitive impairment (MCI) and other similar clinical entities exposed to vascular risk factors with a subsequent increased progression to dementia (Panza et al., 2006). In 2013, a common consensus about the definition of cognitive frailty was reached thanks to the International Academy of Nutrition and Aging (IANA) and the International Association of Gerontology and Geriatrics (IAGG) (Kelaiditi et al., 2013). To date, cognitive frailty is considered a geriatric condition characterized by the coexistence of a physical frailty condition with a specific degree of cognitive impairment, anyhow in the absence of a clinical diagnosis of dementia (Kelaiditi et al., 2013). Different studies (for review Panza et al., 2018) support that cognitive frailty is a clinical entity distinct from the classical construct of the frailty.

Cognitive frailty is characterized by a complex multifactorial aetiology where several mediators/pathways are involved (Panza et al., 2018; Ticinesi et al., 2018) that could represent potential therapeutic targets for interventions. To date, multidomain treatments from physical to psychosocial activities are available influencing on: 1. nutrition (e.g. Mediterranean diet, use of antioxidants); 2. metabolic and vascular state (e.g. dyslipidemia, diabetes, blood pressure, maintenance of a proper body weight); 3. lifestyle (e.g. cessation of smoking, drinking, reduction of polypharmacy) (Morley, 2018; Ngandu et al., 2015; Panza et al., 2019; Tan et al., 2018). However, these therapeutic approaches could not completely resolve this condition mainly in relation to effectiveness and costs relative to benefits (Apostolo et al., 2018). Thus, new approaches are quickly emerging as highly promising novel avenues for treating frailty.

Oxygen-ozone (O_2-O_3) therapy is a non-invasive, non-pharmacological and no-side effect procedure based on regenerative capacity of O_3 applied in medicine for the treatment of more than 50 pathological processes. Noteworthy, different clinical trials evidenced the effectiveness of O_2-O_3 therapy in the treatment of cardiovascular, peripheral vascular, neurological, degenerative, orthopaedic, gastrointestinal and genitourinary pathologies (Bocci et al., 2011; Bocci, 2012; Braidy et al., 2018; Elvis and Ekta, 2011; Re et al., 2008; Smith et al., 2017); as well as multiple sclerosis (Ameli et al., 2019; Delgado-Roche et al., 2017; Smith et al., 2017); fibromyalgia (Moreno-Fernandez et al., 2019; Tirelli et al., 2019); skin diseases/wound healing (Fitzpatrick et al., 2018; Wang, 2018); diabetes/ulcers (Guclu et al., 2016; Izadi et al., 2019; Martinez-Sanchez et al., 2005; Ramirez-Acuna et al., 2019; Rosul and Patskan, 2016); infectious diseases (Mandhare et al., 2012; Smith et al., 2017; Song et al., 2018); dentistry (Azarpazhooh et al., 2009; Isler et al., 2018; Khatri et al., 2015; Srikanth et al., 2013); lung diseases (Hernandez Rosales et al., 2005); osteomyelitis (Bilge et al., 2018). *In vitro* and animal studies as well as isolated clinical reports suggest the potential role of O_2-O_3 as an adjuvant therapy for cancer treatment (Clavo et al., 2018). O_2-O_3 therapy is accepted by doctors as a non-traditional treatment for its safety, convenience and low cost, since the twentieth century (Hao et al., 2019).

In this review, we first describe the relevant, well known and documented molecular mechanisms targeted by O_3 administration. Secondarily we highlight how these specific processes could be implicated in cognitive frailty to identify putative therapeutic targets for its treatment and thus to prospectively provide a consistent rationale for applying the O_2-O_3 therapy in this complex geriatric syndrome

2. Oxygen-ozone (O_2-O_3) therapy

O_3 is a triatomic gaseous molecule which has been using as a powerful oxidant in medicine for more than 150 years (Elvis and 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 leaded to ascertain that our body produces oxidants to hurl at invading pathogens, not only as hydrogen peroxide (H_2O_2), superoxide, hypochlorite and O_2 but also O_3 as further part of its oxidative armamentarium in fighting infection (Babior et al., 2003; Lerner and Eschenmoser, 2003; Wentworth et al., 2002). These studies were performed on neutrophils isolated from human peripheral blood and coated with antibodies and demonstrated that the antibodies can catalyse the generation of O_3 by a water oxidation pathway, leading to efficient killing of bacteria.

Van Mauren was the first identifying the distinctive odor of O_3 in 1785. 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 et al., 2011). In 1860, Jacques-Louis Soret, a Swiss chemist demonstrated that the O_3 was made up of three atoms of oxygen (Altman, 2007). The first application of O_3 as an antiseptic for operating rooms and to disinfect surgical instruments dates to 1856, and in 1860 the first O_3 water treatment plant was built in Monaco to disinfect water (Altman, 2007). The first portable O_3 generator was patented by Nikola Tesla in 1896 in the United States.

The use of O_3 in the clinical practice was introduced in the past century (Wolff, 1915). During the World War I, from 1914 to 1918, doctors used O_3 to successfully treat post traumatic gangrene in German soldiers, bone fractures, inflammations and abscesses (Bocci et al., 2011). O_3 was also used to prevent infections in local medical procedures and to control wound infections because of its prophylactic proprieties (Merin et al., 2007). The invention of a reliable O_3 generator by a physicist, Joachim Hansler, was the major 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 (Schoop, 1982).

If the year 2019 brings the signature of the medicine Nobel for "discovery of how cells sense oxygen", O_2-O_3 therapy acquires to date a further prestigious significance. 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). If the low availability of O_2 leads the cell to rise *VEGF, PDGF, HIF* genes expression, some studies demonstrated that O_3 increases itself the levels of these factors (Curro et al., 2018; Re et al., 2010; Zhang et al., 2014), as if replacing the work of the cell with the role of cellular adapter to hypoxia.

O_3's action is omni various from immunoregulation and anti-inflammatory properties to antioxidant activity, antimicrobial effect, role in analgesic and vasodilation, blood flow and oxygenation promotion, modulator of regenerative processes and epigenetic modifications (Smith et al., 2017; Travagli et al., 2010; Zeng and Lu, 2018) (Fig. 1).

2.1. Immunoregulation and anti-inflammatory and anti-apoptosis properties

Low doses of O_3 have been shown to increase secretions of macrophages and leukocytes (Orakdogen et al., 2016), to enhance the

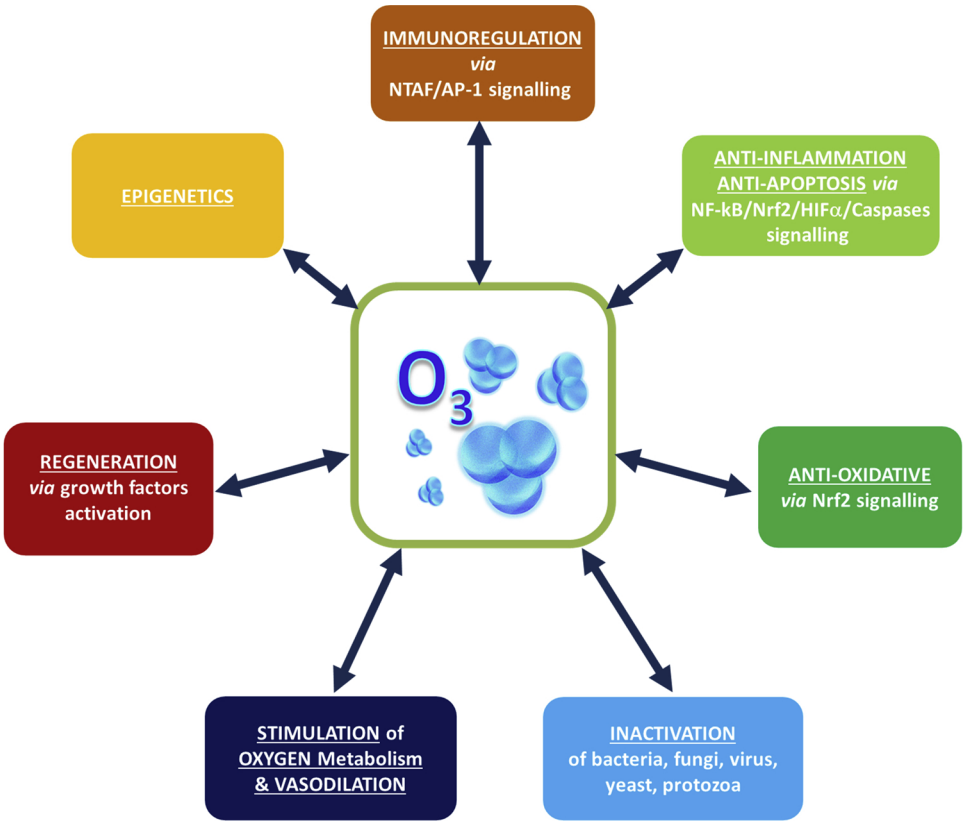

Fig. 1. The omini various properties of ozone and consequently the potentiality of Oxygen-Ozone Therapy: from immunoregulation and anti-inflammatory/anti-apoptotic activities (*via* NF-κB, NTAF, AP-1, HIFα, caspases pathways) to antioxidant function (*via* Nfr2 signalling), antimicrobial effect, role in analgesic and vasodilation, blood flow and oxygenation promotion, modulator of regenerative processes and epigenetic modifications.
Note: NF-κB (Nuclear Factor Kappa B Subunit 1), NTAF (nuclear factor activated T-cells), AP-1 (Activated Protein-1), HIFα (Hypoxia Inducible Factor 1α), Nfr2 (nuclear factor erythroid 2–related factor 2).

phagocytic capacity of granulocytes and to facilitate the formation of monocytes and activation of T-cells. T-cells defend the body against foreign pathogens: a tyrosine-phosphorylation response takes place immediately in the ZAP-70 molecule when the T-cell antigen receptor (TCR) recognizes any invaders, and then activates phospholipase Cγ1 (PLCγ1) (Smith-Garvin et al., 2009). Membrane lipid phosphatidylinositol-4,5-bisphosphate (PIP2) can be hydrolysed by the activation of PLCγ1, therefore, producing two critical second messengers: inositol triphosphate (IP3) and diacylglycerol (DAG). Then, IP3 binds to its receptor (IP3r) located in the endoplasmic reticulum (ER) membrane, leading to calcium (Ca^{2+}) from ER into the cytosol. The elevated levels of Ca^{2+} in cytosol will activate calcineurin, which dephosphorylates nuclear factor activated T-cells (NFAT) and transports it into the nucleus. NFAT then induces the transcription of cytokines, such as Interleukin-(IL)-2, IL-6, IL-8, Tumor Necrosis Factor-α (TNF-α), and Interferon-γ (IFN-γ), participating in the immune response of the body (Amasaki, 2010). These cytokines have pro-inflammatory actions that express chemiotactic ability to promote aggregation and infiltrations and enhance the phagocytosis of neutrophils, lymphocytes, macrophages and other inflammatory cells to kill local pathogens (Banerjee et al., 2017; Wu et al., 2017b). IL-6 is the most prominent interleukin, expressed in a variety of tissues and involved in numerous pathophysiological conditions not only immune/inflammation (Sindhu et al., 2015), but also in mitochondrial myopathy (Rue et al., 2014), insulin resistance, cell proliferation (Chen et al., 2018), apoptosis and cellular senescence (Zhuang et al., 2017).

Through another molecular mechanism, PLCγ1 activates Ras/Mitogen-activated Protein Kinases (MAPK) signalling to promote the translocation of the Activated Protein-1 (AP-1) in the nucleus and activate the immune response (Karin, 1995). AP-1 is a sequence-specific transcriptional activator composed of members of the Jun and Fos families, whose activity is induced by many stimuli, including not only T-cell activators but also growth factors, cytokines, neurotransmitters.

When O_3 is administered intravenously, it dissolves in biological fluids determining a mild oxidative stress (Bocci, 2006). Here, O_3 reacts with polyunsaturated fatty acids (PUFAs), low-weight molecules such as uric and ascorbic acid and molecules containing thiol groups (cysteine, reduced glutathione or albumin) (Guven et al., 2008). All these compounds can undergo oxidation by O_3, resulting in the formation of H_2O_2 and Lipid Oxidation Products (Inal et al., 2011). H_2O_2, a Reactive Oxygen Species (ROS), can act as an O_3 messenger for initiating therapeutic and biological effects (Groeger et al., 2009; Guven et al., 2008). It induces the activation of NFAT or AP-1 and in this way O_3 stimulates the innate immune system, helping the cells to survive injury (Reth, 2002) (Fig. 1 immunoregulation property *via* NTAF/AP-1 signalling; Fig. 1S for details in molecular pathways).

Members of the nuclear factor-κB (NF-κB) and Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) constitute an important network of transcription factors and regulatory proteins that control the expression of a broad array of genes, including those associated with immune and inflammatory responses (Antunes and Han, 2009; Kim et al., 2010). Pharmacological and genetic studies suggest that there is a functional cross-talk between these two important pathways (Ahmed et al., 2017).

Cytokines are generated when NF-κB is activated, but at the same time the pro-inflammatory cytokines TNF-α and IL-1β are activators of NF-κB, giving rise to vicious circle which perpetuate the chronic inflammatory process (Himmelfarb et al., 2002; Kim et al., 2010). In the absence of stimuli, NF-κB is found in cytoplasm bound to the inhibitory IκB proteins. In response to stimuli, IκB proteins are rapidly phosphorylated by IκB kinase and ultimately degraded by the 26S proteasome. The resulting release of NF-κB and subsequent translocation to the nucleus promote its action on target genes, involved with inflammation, immunity, and apoptosis (Baud and Jacque, 2008; Salminen et al., 2008). It has been demonstrated that O_3 can exert its anti-inflammatory ability, blocking the action of NF-κB, reducing the TNF-α concentrations, and causing a reduction of the inflammation and apoptotic cell death (Karouzakis et al., 2006; Vaillant et al., 2013) (Fig. 1 anti-inflammatory and anti-apoptotic properties *via* NF-κB

signalling; Fig. 2S for details in molecular pathways).

O_3 can perform its anti-inflammatory ability also activating the Nrf2, a member of the CNC-basic leucine zipper (CNC-bZIP) family of transcription factors. Several studies demonstrated that Nrf2 contributes to the inflammatory processes by orchestrating the recruitment of inflammatory cells and regulating gene expression through the antioxidant response element (ARE) (Ahmed et al., 2017). The kelch-like ECH-associated protein (keap1)/Nrf2 ARE signalling pathway mainly regulates anti-inflammatory gene expression and inhibits the progression of inflammation (Ahmed et al., 2017). Under basal condition, Nrf2 binds to its repressor Keap1, an adapter between Nrf2 and Cullin 3 protein, which leads to ubiquitination followed by proteasome degradation. Under moderate oxidative stress induced by O_3, after heterodimerization with the Muscoloaponeurotic Fibrosarcoma (MAFs), the Nrf2 translocates to the nucleus, where it dimerizes and binds to ARE genes such as heme oxygenase 1 (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 the NF-κB pathway 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 activating the anti-inflammatory cytokines, thus leads to balancing of the inflammatory process (Ahmed et al., 2017). Keap1 can also prevents NF-κB activity *via* IKK (IκB kinase) inhibition (Fig. 1 anti-inflammatory property *via* Nfr2 signalling; Fig. 2S for details in molecular pathways).

In summary, through its immunoregulation role against injury, different studies reported increased levels of IL-2, TNF-α, IL-8, IFN-γ and β, IL-1, T-box transcription factor 2, Granulocyte-Monocyte Colony Stimulating Factor (Bocci et al., 1994, 1998; Paulesu et al., 1991; Re et al., 2010; Yong et al., 2017), but also trophic factors such as transforming grow factor (TGF) β1 (Re et al., 2010; Xiao et al., 2017; Zhang et al., 2014) and α (Feng et al., 2016; Xie et al., 2016) after O_3 treatment.

Through its anti-inflammatory activity trigged by chronic inflammation, O_3 decreases the levels of the pro-inflammatory IL-6, TNF-α, IL-1β and Intercellular Adhesion Molecule 1 (ICAM1) (Chang et al., 2005; Xiao et al., 2017; Xie et al., 2016).

Thanks to its anti-apoptotic role and pro-cell survival and proliferation, O_3 administration decreases the expression of *caspases* 1-3-9, *HIFα*, *TNF-α*, *Bcl-2-associated X protein*(*Bax*) and *p53* genes (Guclu et al., 2016; Yong et al., 2017). 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). (Fig. 3S Molecular mechanisms for anti-apoptotic property *via* pro-apoptotic molecules inactivation). Moreover, O_3 stimulates the Kreb's cycle in the mitochondria by enhancing the oxidative carboxylation of pyruvate and stimulating the production of adenosine triphosphate (ATP) (Guven et al., 2008). It also causes a significant reduction of nicotinamide adenine dinucleotide (NADH), an increase of the coenzyme A levels to fuel the Kreb's cycle and oxidizes cytochrome C (Brigelius-Flohe and Flohe, 2011; Elvis and Ekta, 2011). The activation of these mechanisms with the blockage of apoptotic mechanisms promotes cell survival and proliferation. In general, as effect on well-being, administrated O_3 induces also a release of adrenocorticotropic hormone (ACTH), cortisol and corticotropin-releasing hormone (CRH), dehydroepiandrosterone (DHEA) and endorphins (Dardes et al., 2017).

2.2. Antioxidant property

The efficacy of O_3 therapy is not only through the actions of cytokines, but O_3 can also re-establish the cellular redox homeostasis (Vaillant et al., 2013). Oxidative stress, known as the imbalance between production of ROS and their elimination by protective mechanisms such as antioxidants, may be contributing to the neuronal damage and the abnormal neurotransmission. For this, it is implicated in the pathogenesis and progression of various diseases, where brain and mitochondria are the most involved due to their high sensitivity to oxidative damage caused by free radicals.

Nrf2 regulates the constitutive and inducible expression of antioxidant enzymes such as Superoxide dismutases, Glutathione peroxidase, Glutathione-S-Transferase (GST), Catalase, Heme oxygenase 1 (HO-1), NADPH quinone oxidoreductase 1 (NQO1), phase II enzymes of drug metabolism and Heat Shock Proteins (HSPs), via cis-acting DNA ARE elements. Many of these enzymes act as free radical scavengers clinically relevant to a wide variety of diseases (Inal et al., 2011). Under normal conditions, Nrf2 is sequestered in the cytoplasm by its repressor protein, the Keap 1. In response to moderate oxidative stress induced by O_3, the Nrf2 is targeted to the nucleus where it binds to the ARE elements of genes encoding the antioxidant and phase II detoxifying enzymes, GST and NQO1 and those that increase glutathione (GSH) biosynthesis (Bocci and Valacchi, 2015; Galie et al., 2018; Pedruzzi et al., 2012). The presence of Nrf2 can reduce NF-κB activity, *via* Keap1 protein, leading to increase of HO-1 expression, decrease of pro-inflammatory cytokines production, and increase of antioxidant defences. To further support this finding, our research group demonstrated that mild ozonisation, tested on *in vitro* systems, induced modulation of genes including HO-1 (Scassellati et al., 2017) and at mitochondria level, its effect increased the length of the mitochondrial cristae and the content of mitochondrial heat shock protein 70 (HSP 70) (Costanzo et al., 2018). Moreover, O_3 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 (Liu et al., 2015).

O_3 can protect against overproduction of nitric oxide (NO), when NO is a toxic oxidant. O_3 blocks the activity of NF-κB pathway, that in turn cannot stimulate the production of the inducible nitric oxide synthase (iNOS) to synthetize NO (Arias-Salvatierra et al., 2011; Karouzakis et al., 2006; Laskin et al., 1998; Manoto et al., 2018) (Fig. 1 antioxidant property *via* Nfr2 signalling; Fig. 2S for details in molecular pathways).

2.3. Pathogens inactivation role

Starting from the demonstrated evidence that our immune system produces O_3 by antibodies to dispatch their bactericidal activity (Babior et al., 2003; Lerner and Eschenmoser, 2003; Wentworth et al., 2002), it is clear the importance of its anti-pathogen role against bacteria (both Gram-positive and Gram-negative), fungi, virus, yeast and protozoa. In bacteria, O_3 disrupts the integrity of the bacterial cell wall through oxidation of phospholipids and lipoproteins (Polydorou et al., 2012; Thanomsub et al., 2002). As this occurs, the stability of the bacterial cell envelope is attenuated. In fungi, O_3 inhibits their growth interacting in the same way as bacteria (Brodowska et al., 2017; Gupta and Brintnell, 2013). In viruses, O_3 damages the viral capsid and breaks the reproductive cycle by disrupting the contact between the virus and the cell through the process of peroxidation. The cells vulnerable to the invasion of viruses are coated with weak enzymes, susceptible to oxidation and can be eliminated from the body when interacting with O_3 (Bocci et al., 2010) (Fig. 1 anti-pathogens property).

As reported above, O_3 treatment was extremely efficacious for different infectious diseases from human immunodeficiency virus type 1 to methicillin-resistant *Staphylococcus aureus* skin infection, *Mycobacterium Ulcerans* (Buruli Ulcer), acute bacterial infection-tick bite cellulitis (Lyme disease), candidiasis, gastrointestinal (hepatitis, cirrhosis, colitis) and genitourinary (cystitis, vaginitis) diseases (Altinel et al., 2011; Amin, 2018; Mandhare et al., 2012; Rowen, 2018; Smith et al., 2017; Song et al., 2018; Wells et al., 1991).

2.4. Role in stimulation of oxygen metabolism and in vascular modulation

It has been demonstrated that O_3 increases the red blood cells glycolysis rate (Bocci et al., 2011). In particular, O_3 stimulates 2,3-diphosphoglycerate (DPG) concentration, a direct inhibitor of the affinity of the haemoglobin (Hb) for O_2. DPG produces changes in the Hb dissociation curve and displaces the HbO_2/Hb equilibrium to the right: HbO_2 + 2,3-DPG → Hb - 2,3-DPG + O_2, so to increase the delivery of O_2 to the tissues. Also, in red blood cells O_3 stimulates the Kreb's cycle, reduces NADH, oxidizes cytochrome C, with consequent production of ATP and improvement in the blood circulation (Fig. 1 stimulation and modulation property on oxygen metabolism and vasodilation; Fig. 4S details for molecular pathways). Multiple studies provide evidence that the activation of antioxidant enzymes after O_3 administration can increase erythroblast differentiation. This leads to a progressive increase in erythrocytes and preconditions them to have resilience towards oxidative stress (for review see Elvis and Ekta, 2011). Additionally, O_3 can improve the flexibility of erythrocytes membranes and the rheological proprieties of blood, so to diminish blood viscosity and platelet aggregation (Giunta et al., 2001; Verrazzo et al., 1995).

O_3 induces the production of the vasodilator prostacyclin (Elvis and Ekta, 2011) and stimulates the production of NO by vascular endothelial cells exercising its function of strong endothelium-independent vasodilator (Foresti et al., 2006; Valacchi and Bocci, 2000). These events favour the vasodilation at the microcirculation level and decreasing of peripheral vascular resistance. More in detail, since HO-1-derived bilirubin has been demonstrated to interact with NO (Barone et al., 2009; Mancuso et al., 2008), O_3-induced HO-1 upregulation could modify NO production and alter vasodilation to maintain normal blood and to attenuate the release of inflammatory cytokines.

As already reported, O_3 positive effects have been observed in clinical trials for chronic limb, brain and heart ischemia (Bocci et al., 2011; Dardes et al., 2017; Smith et al., 2017) where reduced red blood cells deformability is a common risk factor.

2.5. Role in the regenerative processes

During regeneration process, the mesenchymal stem cells release various paracrine factors or growth cytokines (VEGF; Epidermal Growth Factor, EGF; Fibroblast Growth Factors 1-2, FGF; Insulin Growth Factor, IGF-1; PDGF; TGF-α, -β; Brain Derived Neurotrophic Factor, BDNF; IL-8) that play key roles in mitogenesis, apoptosis, angiogenesis and scarring (tissue regeneration). Several studies demonstrated the positive effect of O_3 increasing their levels during the injury (Feng et al., 2016; Re et al., 2010; Smith et al., 2017; Ustebay et al., 2017; Xiao et al., 2017; Xie et al., 2016; Yong et al., 2017; Zhang et al., 2014). It has been reported that O_3 treatment promotes also the fibroblasts migration, increases the levels of collagen, α-smooth muscle actin (α-SMA), TGF-β and inhibits the inflammation in injured fibroblasts (Kim et al., 2009; Re et al., 2010; Xiao et al., 2017).

VEGF is a growth factor that promotes new capillary generation, enriches the granulation tissue in the process of wound repair, improves the microcirculation in local wound and provides the nutrients needed for repairing (angiogenesis) (Lord et al., 2017). EGF is a growth cytokine that can specifically promote epidermal cell proliferation and can accelerate the epithelial process of wound, promote the connective tissue formation and contraction within wound (Li et al., 2016). FGFs are involved in wound repair, promoting effects on the proliferation of fibroblasts, endothelial cells and keratinocytes and accelerating tissue repair and angiogenesis in local tissues (Cha et al., 2017). BDNF is expressed in many tissues and it is known to regulate several aspects of neuronal development and function such as survival and differentiation of different neuronal populations, synaptic transmission and plasticity and neuronal repair following injury (Huang and Reichardt, 2001). It is an important modulator of inflammation (Gezen-Ak et al., 2013; Liang et al., 2015) with crucial antioxidant proprieties (Wu et al., 2017a), contributing to increase mitochondria performance and to mitigate neuronal metabolic defects following injury (Xu et al., 2018).

The mammalian or mechanistic target of rapamycin (mTOR) and associated phosphatidyl-inositiol 3-kinase (PI3K)/protein kinase B (AKT) pathway (PI3K/AKT/mTOR) regulate cell growth, differentiation, migration, and survival, as well as angiogenesis and metabolism. This signalling is also a master regulator of protein synthesis and autophagy. For its importance, recent studies support the development of PI3K/AKT/mTOR targeted therapies for groups of severe skin cancers (Chamcheu et al., 2019). Moreover, EGF and IGF fulfill their functions through the activation of this pathway. Interestingly, the stimulation of PI3K/AKT/mTOR signalling is modulated by O_3 treatment to promote wound healing both by increasing the migration of fibroblasts (Xiao et al., 2017) and rising the level of autophagy in a chondrocytes model of osteoarthritis (Zhao et al., 2018).

The mechanisms underlying the wound healing after O_3 treatment consist in two principal pathways (Di Mauro et al., 2019). O_3 can have a topic effect on the skin, producing an antioxidant response, blocking the pro-inflammatory pathway and promoting wound healing processes. On the other hand, O_3 can have also systemic effects on platelet activity increasing the production of VEGF, PDGF and TGF-β, blocking inflammation mechanisms and activating the wound healing. As reported above, its efficacy was observed for different skin diseases/wound healing both in clinical trials and in animal models (Alpan et al., 2016; Fitzpatrick et al., 2018; Guclu et al., 2016; Izadi et al., 2019; Kushmakov et al., 2018; Martinez-Sanchez et al., 2005; Ramirez-Acuna et al., 2019; Rosul and Patskan, 2016; Smith et al., 2017; Wang, 2018; Xie et al., 2016; Zhang et al., 2014).

In all these processes, O_3 promotes also the release of NO which increases blood circulation for tissue remodelling.

(Fig. 1 involvement in the regenerative processes).

2.6. Epigenetic modifications

Evidence supported that epigenetic modifications (i.e. DNA methylation, noncoding RNAs, histone modifications) play a critical role in the molecular mechanism of oxidative stress. Although it is known that chronic exposure of O_3 may be associated with adverse effects (Braidy et al., 2018), mild oxidative stress caused by therapeutic concentrations of O_3 on NF-kB-regulated genes can influence transcriptional/epigenetic regulators, such as sirtuins, that have been demonstrated to promote lifespan extension (Kawahara et al., 2009, 2011). This corroborates the ontological link between inflammation and aging and allows speculation that O_3-induced activation of Nf-KB might be a potential tool to prevent and prolong lifespan trough epigenetic modifications (Fig. 1. Epigenetic modulations).

3. Rationale of using Oxygen-Ozone therapy in cognitive frailty

Several evidences support the involvement of several systems in the etiopathogenetic mechanisms of cognitive frailty (Abdelhafiz and Sinclair, 2019; Barrera et al., 2018; Panza et al., 2018; Ticinesi et al., 2018): inflammation, oxidative stress processes, metabolic (diabetes, hypoglycaemia) and endocrine alterations, mitochondrial dysfunctions, stem cells/growth cytokines exhaustion, microbiota modifications are those of major relevance (Fig. 2). Studies have also found a strong connection with cardiovascular risk factors, cerebrovascular accident, neurodegenerative diseases (Panza et al., 2015).

3.1. Inflammation and oxidative stress systems and endocrine and metabolic dysfunctions

Different evidence supports that inflammaging (chronic low-grade inflammation) is an underlying mechanism of cognitive frailty (Fulop et al., 2010; Li et al., 2011). Alterations in the concentration of immune activation markers, pro-inflammatory molecules and in different

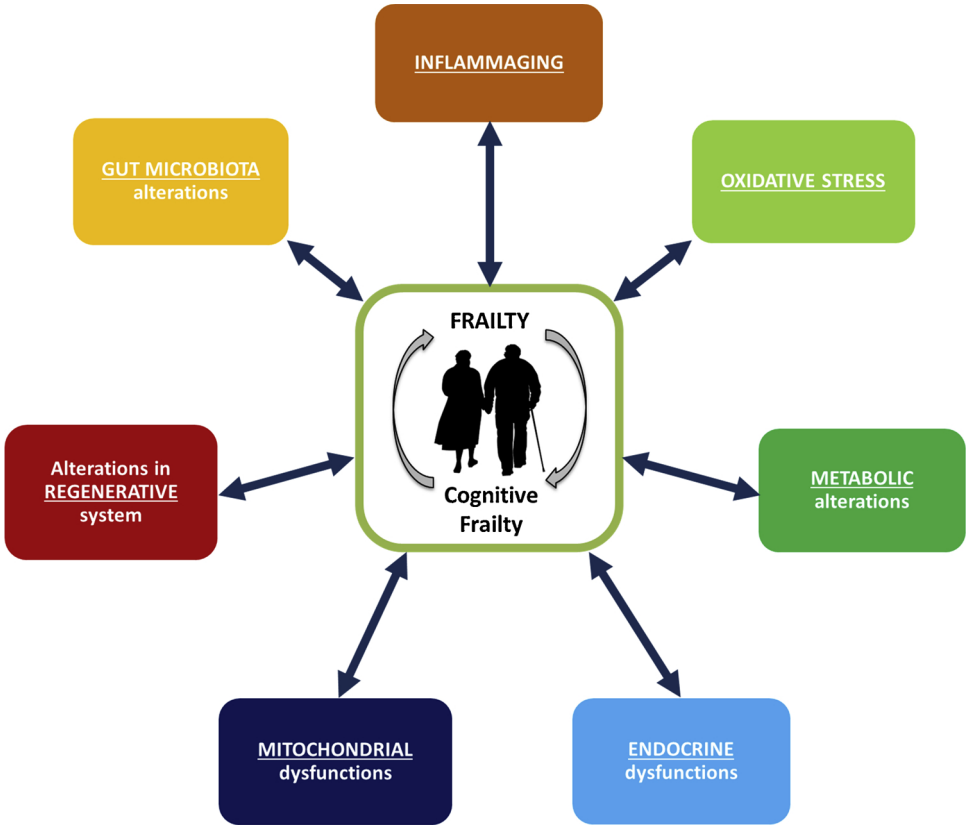

Fig. 2. The involvement of several systems in the etiopathogenetic mechanisms of cognitive frailty: inflammation, oxidative stress processes, metabolic (diabetes, hypoglycaemia) and endocrine alterations, mitochondrial dysfunctions, stem cells/growth cytokines exhaustion, microbiota modifications.

lymphocytes subpopulations have been observed in cognitive frail subjects (Baylis et al., 2013; Collerton et al., 2012; Hubbard and Woodhouse, 2010; Marcos-Perez et al., 2017) and chronically high levels of pro-inflammatory biomarkers (i.e TNF-α, IL-6, C-reactive protein, CRP) predict risk of morbidity and mortality in the elderly population (Gonzalez et al., 2015).

As reported in the systematic review of Sargent et al. (2018), some neuroinflammatory markers are associated with cognitive impairment and physical frailty. These included: elevated levels of IL-6, CRP, TNF-α, IL-1β, IL-1α, IL-1 Receptor A (IL-1RA), CD4, CD8, IL-6 Receptor, IL-18, TNF-α receptor I, ICAM-1, cortisol/DHEA ratio, uric acid, erythrocyte sedimentation rate, homocysteine, fibrinogen, circulating osteogenic progenitor cells, and beta 2-microglobulin. ICAM-1 and DHEA are known to affect changes in pro-inflammatory cytokines levels (Corcoran et al., 2010; Hofbauer et al., 1999; McCabe et al., 1993; Straub et al., 1998) and play a role in the endocrine system.

Interestingly, six genes were found to be associated with cognitive impairment, physical frailty, and sarcopenia in candidate genes association studies: *IL-6* with rs1800796, *TNF-α* with rs1800629, *IL-18* with rs360722, *IL-1β* with rs16944, and *COMT* with different SNPs, rs4680 for cognitive decline and rs4646316 for frailty. IL-6, TNF-α, IL-18, IL-1β showed corresponding alterations in serum markers associated with cognitive frailty (Panza et al., 2018; Sargent et al., 2018), suggesting also the well-known functional impact of these cytokines' polymorphisms on the production of circulating proteins. Cardoso et al. (2018) conducted a systematic search to identify biomarker candidates for a frailty biomarker panel. They suggested a core panel where IL-6 filled a central role.

On the oxidative stress side, evidence support that increased levels of oxidative stress markers and/or antioxidant deficiencies may pose risk factors for cognitive decline (Berr et al., 2000). Two biomarkers were associated to cognitive frailty: malondialdehyde (MDA) and protein carbonyls (Ingles et al., 2014). In addition, Ingles et al. (2017) found that plasma BDNF levels decreased significantly in cognitive frail people and a correlation between single nucleotide polymorphism (Met66Val) present in this gene and the decreased plasma BDNF levels in frail people was detected, indicating that the presence of this polymorphism influences its levels. BDNF was also added as high priority marker in the core panel proposed by Cardoso et al. (2018) along with IL-6.

(Fig. 2 Inflammation and oxidative stress systems and endocrine and metabolic dysfunctions).

Starting from the plethora of scientific evidence showing that the differential activation of NFAT, AP-1, NF-kB, Nrf2-ARE and HIFα pathways are the main molecular mechanisms underlying the therapeutic effects of O_2-O_3 therapy and that their stimulation leads to up-regulation of endogenous antioxidant systems, activation of immune functions as well as suppression of inflammatory processes, we can prospectively assume that O_3 administration in frail subjects could protect against the ROS increment, increasing the immune defences against unknown injury and cell survival and proliferation (anti-apoptosis) and at the same time decreasing the inflammaging processes. As the functional impairment in cell-mediated and humoral immunity in frailty (Pinti et al., 2016; Qin et al., 2016) leads to an increased vulnerability to infectious diseases (Mitnitski et al., 2015), O_3 administration could contribute also through its anti-microbial functions (Fig. 3). Oxidative stress is one of the major drivers of protein misfolding that, when they accumulate and aggregate as insoluble inclusions can determine multiple aging-related neurodegenerative and metabolic disorders (Knowles et al., 2014). If Nfr2 promotes the clearance of oxidized or otherwise damaged proteins through the autophagy mechanism (Tang et al., 2019), it is conceivable that O_3 by Nfr2 can modulate the degradation protein systems, also through the activation of PI3K/AKT/mTOR signalling (pro-autophagy role) (Zhao et al., 2018).

This rationale is strengthened by the consolidate evidence that O_3

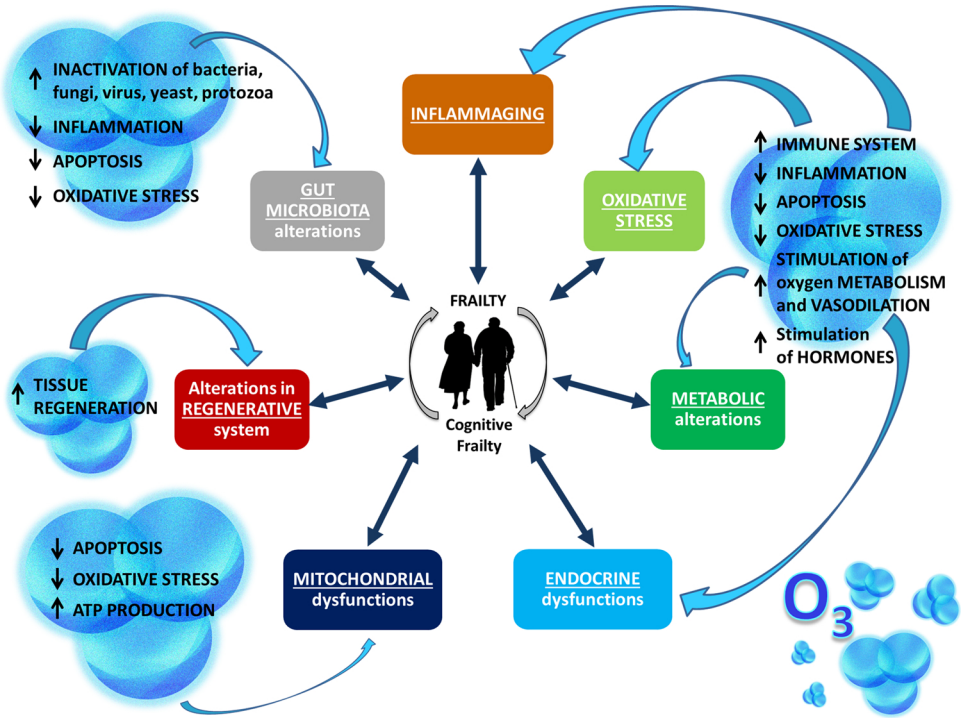

Fig. 3. Rationale of using Oxygen-Ozone therapy in cognitive frailty. Prospectively ozone (O_3) administration in frail subjects could protect against the Reactive Oxygen Species increment, increase the immune defences against unknown injury, increase cell survival and proliferation (anti-apoptosis/pro-authophagy) and decrease the inflammaging processes. O_3 treatment could correct the metabolic and endocrine functions impaired in cognitive frailty through its abilities to improve blood circulation and oxygen delivery to ischemic tissue, to increase 2,3-diphosphoglycerate level, reduce glutathione and basal metabolism through improved oxygen delivery, to induce heat shock protein-70 expression and to increase the release of growth factors and hormones (corticotropin-releasing hormone, adreno cortico tropic hormone, dehydroepiandrosterone, cortisol). In addition, the O_3 abilities of up-regulating endogenous antioxidant systems, activating the Kreb's cycle and reducing the nicotinamide adenine dinucleotide, and inducing anti-apoptotic mechanisms, permits to suggest its further potential role in the restoring of the mitochondria functions in cognitive frailty.
Through the O_3's action on growth cytokines (VEGF, PDGF, EGF, FGF, IGF, TGF-β, BDNF, PI3K/AKT/mTOR signalling), O_3 could improve the regeneration systems, angiogenesis, cellular proliferation, differentiation, tissue regeneration. Finally, if the manipulation of gut microbiota can improve cognitive functions, O_3 intervention could interfere with its properties of anti-inflammation, anti-oxidant and strong bactericidal, fungicidal, antiviral, and anti-protozoal activities.
Note: VEGF (Vascular Endothelial Growth Factor A), PDGF (Platelet Derived Growth Factor), EGF (Epidermal Growth Factor), FGF (Fibroblast Growth Factor), IGF (Insulin Like Growth Factor), TGF-β (Transforming Growth Factor Beta), BDNF (Brain Derived Neurotrophic Factor), PI3K/AKT/mTOR (The phosphatidylinositol-3-kinase (PI3K)/AKT and the mammalian target of rapamycin (mTOR) signalling pathways).

administration, thought its functions of antioxidant and anti-inflammation, has positive effects on different chronic diseases where it is important to correct inflammation and stress oxidative. Coppola et al. (2010) found that geriatric patients after O_3 treatment exhibited significant improvements in mood tones according to Montgomery-Asberg Depression Rating and Hamilton Depression Rating scales and this was also accompanied by an increase of BDNF levels, as if O_3 and BDNF acted as antidepressant medications. Moreover, it has been observed that a significant decline in sirtuin 1 levels was observed in individuals with cognitive frailty compared to age matched healthy individuals (Kumar et al., 2014a; 2014b), suggesting that O_3 could be involved in preventing aging and prolonging lifespan.

Taking in consideration other properties of O_3 such as the improvement of blood circulation and oxygen delivery to ischemic tissue, an increase in 2,3-DPG level, an increase in reduced glutathione, an enhancement in basal metabolism through improved oxygen delivery, the induction of HSP-70 and the increased release of growth factors (VEGF, PDGF, EGF, FGF, IGF, BDNF, CRH, ACTH, DHEA, cortisol), we prospectively can hypothesize that O_3 treatment could correct the metabolic and endocrine functions impaired in cognitive frailty and in the same way also those linked to the cardiovascular diseases, risk factors for frailty. It is well known the positive effect of O_3 on diabetes in clinical and animal models studies (Alpan et al., 2016; Guclu et al., 2016; Izadi et al., 2019; Martinez-Sanchez et al., 2005; Ramirez-Acuna et al., 2019; Rosul and Patskan, 2016; Xie et al., 2016), cardiovascular and peripheral vascular diseases (Dardes et al., 2017; Smith et al., 2017) (Fig. 3).

3.2. Mitochondrial dysfunctions

With aging and in cognitive frailty there is an increase in mitochondrial disfunctions with consequent increased in ROS for lowered oxidative capacity and antioxidant defence, and thus increased oxidative damage to protein and lipids, decreased ATP production and accumulation of DNA damage (Barrera et al., 2018; Boccardi et al., 2017). Interestingly, multiple lines of evidence have shown that Nfr2 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) (Fig. 2 Mitochondria dysfunctions). Thus, it is conceivable the role of O_3 on mitochondrial disfunctions in cognitive frailty, through the up-regulation of endogenous antioxidant systems *via* Nrf2 signaling. Also, its property to activate the Kreb's cycle and reduce the NADH promotes the production of ATP. The induction of anti-apoptotic mechanisms where O_3 treatment decreases the *Bax* gene expression level in mitochondria (Mac Nair et al., 2016) as well as restores mitochondrial damaging in animal models (Liu et al., 2015; Meng et al., 2017), permits to suggest its further potential role in the renewing mitochondria functions in cognitive frailty (Fig. 3).

3.3. Stem cell/growth cytokines exhaustion

An endogenous stem cell production and function decreases with age and this decline likely contributes to reduced ability to regenerate and repair organs and tissues (Jones and Rando, 2011; Yu and Kang, 2013; Zhuo et al., 2010). There is evidence that as mesenchymal stem cells (MSCs) undergo senescence, their properties of multilineage differentiation, immunomodulation and wound healing gradually disappear (Raggi and Berardi, 2012). Altered and dysfunctional stem cell niches have been implicated in frailty syndrome (Golpanian et al., 2016; Lopez-Otin et al., 2013). As such, it has been proposed that a regenerative medicine therapeutic approach has the potential to improve or reverse the signs and symptoms of frailty (Kanapuru and Ershler, 2009; Raggi and Berardi, 2012) (Fig. 2. Alterations in

regenerative system). Through the O_3's action on growth cytokines (VEGF, PDGF, EGF, FGF, IGF, TGF-β, BDNF, PI3K/AKT/mTOR signalling), we hypothesised that prospectively O_2-O_3 could be considered as a regenerative medicine approach for cognitive frailty, improving the regeneration systems, angiogenesis, cellular proliferation, differentiation, tissue regeneration (Fig. 3).

It is widely known the O_3 involvement and positive effects on skin disorders, wound healing (Di Mauro et al., 2019) characterized by three overlapping but distinct stages: inflammation (O_3 kills microorganism and activates immune system), tissue proliferation (O_3 increases the expression of TGF-β and VEGF) and remodelling (O_3 promotes the release of NO to increase blood circulation). Moreover, the evidence that Nfr2 is involved in the adipogenic differentiation of MSCs along with HO-1 (Vanella et al., 2012) and that low O_3 concentrations exerted an adipogenic effect on human adipose-derived adult stem cells (Costanzo et al., 2018) in the absence of damage in differentiated adipocytes (Cisterna et al., 2019) suggests the role of the mild ozonisation as an adjuvant tool for tissue regeneration and engineering.

3.4. Microbiota-gut-brain axis and microbiota alterations

Several lines of evidence suggest that the gut microbiota (ensemble of bacteria, fungi, viruses, protozoa and archaea symbiotically living in the distal human gastrointestinal tract) is an important part of the microbiota-gut-brain axis. This includes multiple bidirectional systems through which the gut microbiota and the brain communicate, encompassing hormonal Hypothalamic Pituitary Axis, (HPA), neuronal (vagus nerve), and immune systems. In a homeostatic state, a healthy gastro-intestinal tract has a normal and stable commensal intestinal microbiota and provides the host with nutrition and energy by producing vitamins. Aging associated alterations lead to variations in the composition of the intestinal microbiota probably contribute to immunosenescence and the development of a proinflammatory phenotype (Gomez-Gomez and Zapico, 2019). Inflammatory aging, in turn, can significantly alter brain function due to an increase in the expression of inflammatory cytokines and increase oxidative stress, the breakdown of the blood-brain barrier, the infiltration of peripheral immune cells, and glial cell activation. It is likely that these processes contribute to the cognitive frailty (Gomez-Gomez and Zapico, 2019). Some studies reviewed in Ticinesi et al., 2018) found overrepresentation of *Enterobacteriaceae* and *Fusobacteriaceae*, and identified some taxa, namely *Alcaligenaceae, Porphyromonadaceae, Lactobacillales* positively correlated with cognitive impairment. *Lactobacillus, Lactococcus, Streptococcus* and *Enterococcus* may produce histamine, which acts as a neurotransmitter and an important modulator of neuroinflammation, through reduction of TNF-α expression in the brain. *Bacillus, Lactobacillus and Bifidobacterium*, can synthesize neurotransmitters involved in memory and learning function regulation, such as gamma-aminobutyric acid, serotonin, norepinephrine and acetylcholine. *Clostridium* may also produce indole-3-propionic acid, a relevant antioxidant for neurons (Fig. 2 Gut microbiota alterations).

Thus, if the manipulation of gut microbiota can improve cognitive functions, thanks to its anti-inflammation, anti-oxidant, strong bactericidal, fungicidal, antiviral, and anti-protozoal activities (Duricic et al., 2015; Sato et al., 1990; Sugita et al., 1992), along with its positive effects on gastrointestinal (hepatitis, cirrhosis, ulcerative colitis) diseases (Elvis and Ekta, 2011; Smith et al., 2017; Sukhotnik et al., 2015) O_3 intervention could represent a potential preventive and therapeutic intervention for cognitive frailty (Fig. 3). Of note, it has been demonstrated a significant positive effect of O_3 in patients with intestinal dysbiosis (Loprete and Vaiano, 2017).

4. Concluding remarks

Looking for alternative and new interventions for cognitive frailty, we suggest that O_2-O_3 therapy could be a useful, safe, no-invasive, no-pharmacological, economical, effective treatment for this condition. The mechanisms of the positive effects of O_3 are mainly attributed to activation of the immune and anti-inflammatory systems, up-regulation of cellular antioxidant enzyme activity, enhancement in the release of growth factors from platelets, improvement in blood circulation and O_2 delivery to damaged tissues, and enhanced general metabolism (Bocci, 2004, 2011; Bocci, 2006), along with being a potent bactericide, fungicide and virucidal with potential effect on gut microbiota. Consequently, these combinatorial effects could impact on cognitive domains directly or indirectly through the mediation of gut microbiota. Nrf2-ARE, NF-κB, NFAT, AP-1, HIFα are principal signalling pathways on which O_3 exercises its effects, that could be sharable with those involved in cognitive frailty, where high inflammation and oxidant state, endocrine and metabolic alterations, mitochondria dysfunctions and slowdown in regenerative processes and immune system characterize this geriatric condition.

Recently, Cuadrado et al. (2019, 2018) reported extensive evidence about the central role playing by Nrf2 as master regulator of multiple cytoprotective responses and the key molecular node within a particular cluster of a wide spectrum of diseases: from cardiovascular (atherosclerosis coronary artery disease) to metabolic (diabetes, hyperglycemia), respiratory (emphysema, chronic obstructive pulmonary disease), neurodegenerative (Alzheimer's and Parkinson's Diseases) and digestive (steatosis, non-alcoholic steatohepatitis, cirrhosis) diseases. All these pathologies show sharable common mechanisms, including oxidative, inflammatory, and metabolic alterations, molecular systems implicated also in cognitive frailty. As it has been suggested, keeping in mind that Nrf2 could represent a new strategy for therapeutic approaches (Cuadrado et al., 2018, 2019), O_2-O_3 therapy acquires a significant value for its known and widely demonstrated interaction with Nfr2.

Medical O_3 is a mixture of O_3 in O_2 under therapeutic (10-40 μg O_3/ml of O_2) or antibiotic (60-100 μg/ml) concentrations, generated by certificated apparatus and applied according to local or systemic routes of administration (Dardes et al., 2017). O_3 medical preparations are classified into three types: ozonized water, ozonized oil and ozonized gas. At present, a randomized double-blind clinical trial has commenced with the aim to test the efficacy of this therapy in a cognitive frailty cohort, a project 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. We believe that this therapy could replace the multidomain approach currently available for cognitive frailty due to the omni various functions of the O_3: a monodomain intervention with O_2-O_3 therapy could be faster, easier, inexpensive, predictable and conservative with much more efficacy results. Importantly, several studies reported that this therapy works in absence of side effects (Delgado-Roche et al., 2017; Hao et al., 2019; Martinez-Sanchez and Re, 2012; Re et al., 2008, 2014), and this could overcome those linked to the use of anti-inflammatory and anti-oxidant medications for which high doses could cause toxicity (Gomez-Gomez and Zapico, 2019), whereas the efficacy of antioxidants in reducing the concentrations of aldehydes and/or protein-aldehyde adducts in blood or in tissues has not been evaluated yet (Gomez-Gomez and Zapico, 2019).

With the awareness that further studies are needed, this review reports substantial scientific evidence for building a rationale of using the O_2-O_3 therapy for the early stage of cognitive decline, exploiting well documented omni various functions of O_3. This therapy could represent a convenient, inexpensive monodomain intervention, working in absence of side effects that will permit to modulate the immune, inflammatory, oxidant, metabolic, endocrine, microbiota and regenerative processes impaired in cognitive frailty.

Author contributions

All authors contributed to the preparation of this review.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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.mad.2020.111210.

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