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Review

Ozone Atmospheric Pollution and Alzheimer's Disease: From Epidemiological Facts to Molecular Mechanisms

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Abstract. Atmospheric pollution is a well-known environmental hazard, especially in developing countries where millions of people are exposed to airborne pollutant levels above safety standards. Accordingly, several epidemiological and animal studies confirmed its role in respiratory and cardiovascular pathologies and identified a strong link between ambient air pollution exposure and adverse health outcomes such as hospitalization and mortality. More recently, the potential deleterious effect of air pollution inhalation on the central nervous system was also investigated and mounting evidence supports a link between air pollution exposure and neurodegenerative pathologies, especially Alzheimer's disease (AD). The focus of this review is to highlight the possible link between ozone air pollution exposure and AD incidence. This review's approach will go from observational and epidemiological facts to the proposal of molecular mechanisms. First, epidemiological and postmortem human study data concerning residents of ozone-severely polluted megacities will be presented and discussed. Then, the more particular role of ozone air pollution in AD pathology will be described and evidenced by toxicological studies in rat or mouse with ozone pollution exposure only. The experimental paradigms used to reproduce in rodent the human exposure to ozone air pollution will be described. Finally, current insights into the molecular mechanisms through which ozone inhalation can affect the brain and play a role in AD development or progression will be recapitulated.

Keywords: Alzheimer's disease, atmospheric pollution, neuroinflammation, oxidative stress, ozone

INTRODUCTION

Atmospheric pollution is a well-known environmental hazard, especially in developing countries where millions of people are frequently, if not consistently, exposed to airborne pollutant levels above safety standards [1]. Accordingly, several epidemiological and animal studies confirmed its role in respiratory and cardiovascular pathologies [2–9] and identified a strong link between ambient air pollution exposure and adverse health outcomes such as hospitalization and mortality [10, 11]. More recently, the potential deleterious effect of air pollution inhalation on the central nervous system (CNS) was also investigated and mounting evidence supports a link between air pollution exposure and neurodegenerative pathologies, especially Alzheimer's disease (AD) and Parkinson's disease.

Atmospheric pollution implies the presence of a diverse and complex mixture of particulate matter, gases, organic compounds, or metals that can cause

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damage or discomfort to human beings or other living organisms. Among these, ozone and particulate matter appear to be the most widespread and harmful airborne pollutants [10]. Ozone can be naturally formed in the upper stratosphere by photodissociation of highly energetic ultraviolet (UV) lights from the sun, on oxygen molecules. Nonetheless ozone can also be abnormally produced on ground levels (troposphere) as a result of photochemical transformation of primary pollutants like nitrogen oxides (NO_x) and volatile organic compounds, produced by human activities. Ozone (O_3) is one of the most powerful oxidizing molecule to which living beings can be exposed. Accordingly, ozone inhalation may cause oxidative damages and inflammation, which could expand from the respiratory system to the periphery and to the brain [12], for which the nose and olfactory pathway is another portal of entry [13]. Repeated or lifelong ozone pollution exposures could then contribute to the development of brain oxidative stress and neuroinflammation, which are two important pathogenic processes associated with AD.

The focus of this review is to highlight the possible link between ozone air pollution exposure and AD incidence. This review's approach will go from observational facts to the proposal of molecular mechanisms. First, epidemiological and postmortem human study data concerning residents of ozone-severely polluted megacities will be presented and discussed. Then, the more particular role of ozone air pollution in AD pathology will be described and evidenced by toxicological studies in rat or mouse with ozone pollution exposure only. Finally, current insights into the molecular mechanisms through which ozone inhalation can affect the brain and play a role in AD development or progression will be recapitulated.

OZONE AIR POLLUTION AND AD: EVIDENCE FROM POSTMORTEM STUDIES IN HIGHLY EXPOSED URBANITIES

First evidence from autopsy and necropsy studies in human and canine subjects

The first study exploring a potential role of air pollution exposure in the development of AD-related brain lesions or pathological processes, was a necropsy study on canine residents of South West Mexico City [14], an area that is severely and regularly polluted by ozone and particulate matter. In this study, the authors observed an increased expression of inflammatory and oxidative markers in endothelial cells of cortical sections of young dogs of Mexico City [e.g., early and persistent activation of NFκB and strong expression of inducible nitric oxide synthase (iNOS)]. They also found additional damages including blood-brain barrier alteration, degenerating neurons surrounded by apoptotic glial cells, and other AD-related lesions such as amyloid plaques and neurofibrillary tangles (NFTs) in older dogs. Finally, tissue damages were observed in target brain regions of the olfactory pathway, in a gradient fashion from olfactory mucosa to olfactory bulbs and then frontal cortex, implicating the nose as portal of entry. Interestingly, those damages observed along the olfactory pathway were in a striking similarity with the early olfactory pathology observed in AD.

The first study on human individuals was also a postmortem study [15], which confirmed the neuroinflammatory phenotype associated with chronic air pollution exposure, observed in Mexico City dogs. Highly exposed human residents indeed displayed an increased cyclooxygenase-2 (COX-2) expression in frontal cortex, hippocampus, and olfactory bulbs and greater accumulation of neuronal and astrocytic amyloid-β (1-42) peptide ($A\beta_{42}$). Interestingly, autopsy studies on children and young adults from Mexico City also showed elevated indices of neuroinflammation and oxidative stress in frontal cortex, and 40% of these children already exhibited tau hyperphosphorylation with pre-tangle material and 51% had Aβ diffuse plaques compared with 0% in controls [16]. This preliminary evidence from postmortem studies was sufficient to raise concern about the potential detrimental effect of chronic air pollution exposure on human brain and cognitive functions, both in adults and in developing (children) brains.

Insights from epidemiological studies

Evidence from children and adult human studies with populations chronically exposed to high ozone air pollution levels came also mainly from epidemiological studies in Mexico [16–29]. Mexico City Metropolitan Area indeed represents an extreme of urban growth and environmental pollution [30]. The critical air pollutants are mainly particulate matter and ozone (O_3) [30]. As the climatic conditions in Mexico City are stable throughout the seasons, air pollutant concentrations are relatively consistent from year to year, and ozone consistently exceed the current US EPA (United States Environmental

Protection Agency) National Ambient Air Quality Standards (NAAQS) most of the year. Lifelong residents of Mexico City involved in those epidemiological studies have then been chronically exposed to significant concentrations of O_3 for the last decades [28, 31]. Children from Mexico city displayed early markers of neurodegeneration (e.g., decreases in right hippocampus N-acetylaspartate/Creatinine (NAA/Cr) ratio which is considered reflective of neuronal density, functional integrity, loss of synapses, and higher pTau burden), neuroinflammation (e.g., elevated MIF, IL-6, IL-1ra, IL-2, and PrP^C in cerebrospinal fluid levels, increased IL-6, NFκB, TNF, INF, and TLRs expression in frontal cortex), olfactory dysfunction (with soap being the predominantly failed odor in urban children), and cognitive deficits (e.g., decrements on attention, short-term memory and below-average scores in verbal and full scale Intelligence Quotient) compared to control children from nearby non-polluted cities. Noteworthy, the cognitive deficits in Mexico City children predominantly impacted females, apolipoprotein ε4 (APOE4) carriers and children with high body mass index (BMI). Two other epidemiological studies were performed in adult populations exposed to significant ozone (and particulate matter) air pollution levels [32, 33]. A population-based cohort study in Taiwan also investigated the association between long-term exposure to O_3 and particulate matter and newly diagnosed AD in 95,690 individuals of at least 65 years old over a long follow-up period (2001–2010). They estimated a 211% risk of increase of AD per increase of 10.91 ppb in O_3 over the 10 years follow-up period [34]. More recently, two publications from the same group revealed that large population-based cohorts, living in Ontario, Canada, were associated with a higher incidence of dementia [35, 36], even though air pollution levels in Ontario are among the lowest in the world [36]. But here, only nitrogen dioxide (NO_2) and fine particulate matter were significantly associated with higher risk of dementia.

Finally, these results tend to support a role for chronic air pollution exposure (including the main component ozone) in the alteration of brain structure integrity and cognitive functions, both in adulthood and during brain development. However, it is extremely difficult to discriminate the particular role played by ozone, alone or in synergism with other air pollutants, in those human studies due to the multiple confounding factors (e.g., other air pollutants, genetic factors, lifestyle factors, diseases states, individual exposure to each pollutants). It was therefore obvious to evaluate the contributory role of ozone in the deleterious effects of chronic air pollution exposure on the brain, explaining numerous toxicological studies in mice or rats experimentally exposed to this gas.

OZONE AIR POLLUTION AND AD: EVIDENCE FROM ANIMAL STUDIES

In the stratosphere, ozone is naturally produced by photochemical dissociation of dioxygen molecules (O_2) of the air under the action of UV light, to form the protective ozone layer. Following this principle, an ozone atmospheric pollution exposure can be experimentally reproduced in rodents by exposing them into a hermetic and ventilated chamber, equipped with an UV lamp which, placed in the ventilation system air flow, generates ozone from oxygen photochemical dissociation. Control exposures are performed in similar chambers provided with the same filtered room air at the same flow rate, but without UV light.

Ozone and brain oxidative stress

As evoked previously, ozone is a very powerful oxidizing molecule [12]. When inhaled, ozone can react with biomolecules of the respiratory tract and produce reactive oxygen species (ROS) [12, 13, 37]. As long as lung antioxidant defenses can buffer those reactive species, the oxidative injury remains localized to the respiratory system. However, when the inhaled ozone dose overwhelms lung antioxidant capacity, ROS and other ozone mediators including pro-inflammatory cytokines can reach others tissues (including the brain) through the bloodstream, and eventually expand oxidative stress and inflammation to those tissues if their defense mechanisms are exceeded (Figs. 1 and 2) [13]. The ozone dose depends on ozone air concentration, exposure duration, and on the respiratory frequency of the subject. Being exposed transiently to a high ozone concentration or regularly to a medium-low ozone air concentration may both have detrimental consequences, even if different in nature, reversibility, or severity. Accordingly, results from both chronic and acute studies on ozone exposure effect on rat brain will be discussed.

According to the results of the acute rat studies, ozone-inhalation induced oxidative stress occurs in some brain regions from medium to high doses of ozone (0.4–1.5 ppm for 4 h, or 1.0 ppm for 1–9 h) [13,

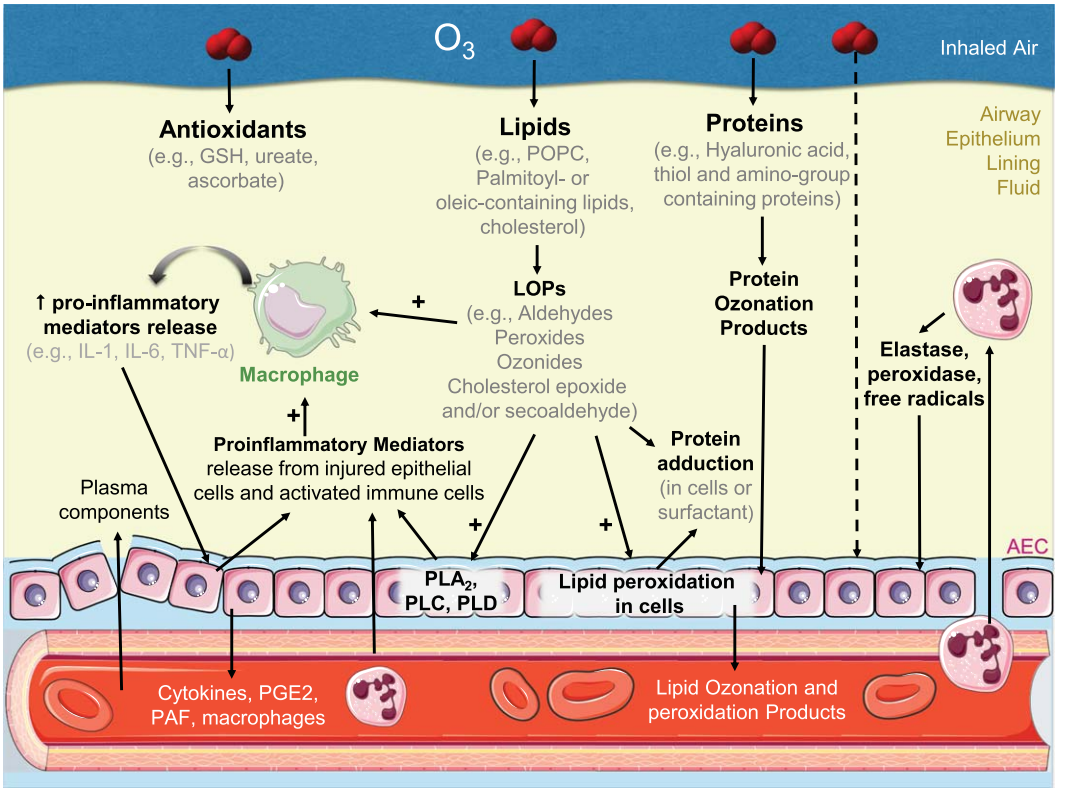

Fig. 1. Inhaled ozone reactivity with lung surfactant components: production of mediators of O_3 pulmonary and extra-pulmonary toxicity. Inhaled ozone dissolves in airway epithelium lining fluid (AELF) and rapidly reacts with its components (mainly antioxidants, lipids, and proteins). This reaction produces primary lipid (LOPs) and protein (POPs) ozonation products. These products may then lead to further production of O_3 toxicity mediators by activating immune cells, promoting lipid peroxidation, protein adduction, and the production of pro-inflammatory precursors within cells by activating phospholipases (PLA_2, PLC, and PLD). Ozone may also directly react with cholesterol and phospholipids of the airway epithelial cells membranes, which may injure those cells and further increase the production of LOPs. Injured epithelial cells release additional pro-inflammatory mediators to attract and activate immune cells, further amplifying lung inflammation. Circulating mediators of O_3 extra-pulmonary effects thereby mainly include pro-inflammatory signals and immune cells, and lipid ozonation and peroxidation by-products (e.g., POPC ozonation derivatives, HNE, HHE, oxidized cholesterol, etc.). AEC, airway epithelial cells; HHE, hydroxyhexenal; HNE, hydroxynonenal; LOP, lipid ozonation/oxidation product; PLA2/C/D, phospholipase A2, C, or D; POPC, 1-palmitoyl-2-oleyl-sn-glycero-3-phosphatidylcholine.

38–41], but no increase in lipid peroxidation level was noticed after a single low dose of ozone (0.25 ppm, 4 h) [41] because lung and brain antioxidant defenses were upregulated (increased brain and lung level of the antioxidant enzyme superoxide dismutase (SOD) [42]) which was sufficient to prevent ROS production amplification. At higher ozone doses (0.5 and 1.0 ppm, 4 h), SOD brain and lung levels decreased and dose-dependently returned to baseline levels [42], explaining the brain oxidative stress observed from medium to high ozone doses [38, 41]. Logically, ozone-induced oxidative stress level increases with ozone air concentration and exposure time duration.

Noteworthy, the different brain regions are not affected alike by ozone inhalation. Interestingly, higher lipid peroxidation levels were observed in hippocampus, the first and main region affected in AD, compared to other brain regions (striatum, cortex, cerebellum, pons, etc.) for a same ozone dose [38]. Hippocampus was also the first region affected by ozone inhalation-induced oxidative stress: lipid peroxidation markers were increased in hippocampus as early as after 1 h of exposure to 1.0 ppm O_3, while the other brain regions started to be affected after 3 h of exposure to the same O_3 air level, pons being one of the last brain region affected (from 6 h of exposure) [39]. Those differences could be related to different antioxidant capacities between brain regions, and/or to the close connection of hippocampus with olfactory bulbs, that are particularly exposed and subjected to ozone inhalation oxidative damages and could then transfer reactive species to hippocampus through olfactory nerves.

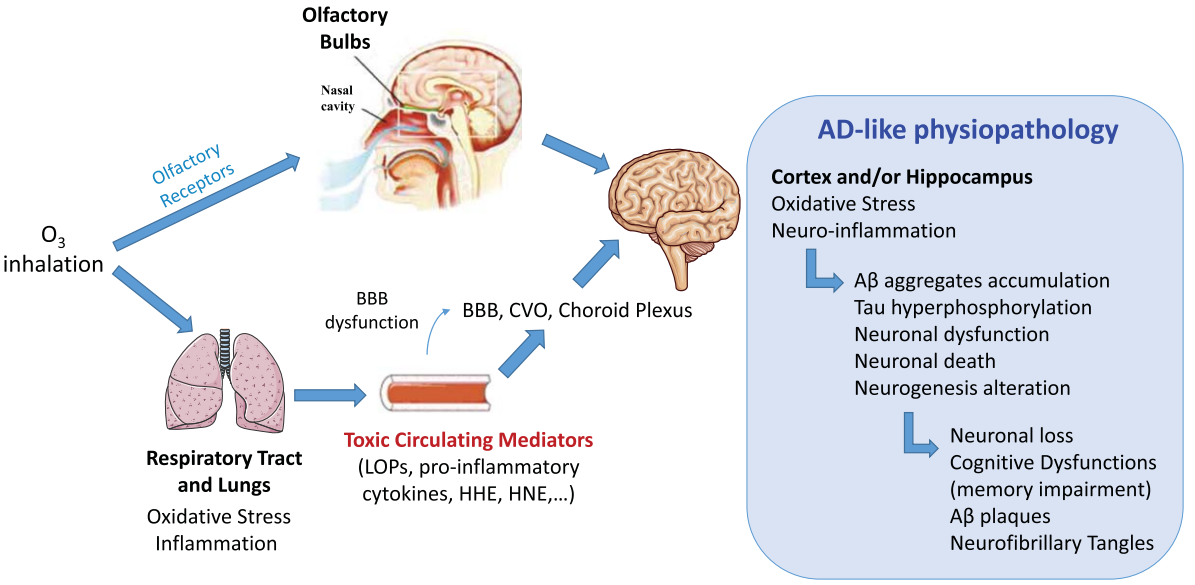

Fig. 2. Putative pathways and mediators of inhaled ozone-induced brain effects. Ozone inhalation causes an oxidative injury to the respiratory tract and lungs, causing lung inflammation and the production and release of toxic mediators into the bloodstream (mainly ozonation and peroxidation products of lipids from the lung surfactant and from airway epithelial cells membranes, as well as pro-inflammatory mediators). These toxic circulating mediators can alter the BBB function and may reach the brain through an intact or altered BBB, or through the CVO or choroid plexus. The olfactory pathway (nasal cavity and olfactory receptors and bulbs) may be another putative pathway for the mediation of ozone brain effects. This pathway could be involved in the propagation of O_3-induced oxidative stress from the olfactory bulbs to deeper brain regions (e.g., cortex, hippocampus). Both pathways and their associated ozone mediators may then lead to oxidative stress and inflammation development in some brain areas (e.g., cortex, hippocampus, and striatum) which may then contribute to the development of the other AD-like brain defects depending on the ozone inhaled dose and exposure frequency. BBB, blood-brain barrier; CVO, circumventricular organs; HHE, hydroxyhexenal; HNE, hydroxynonenal; LOPs, lipid oxidation products; O_3, ozone.

In case of chronic/repeated ozone exposures, ozone-induced oxidative stress occurs in rat hippocampus even at low ozone doses (0.25 ppm, 4 h/day, from 7 consecutive days of exposure [43, 44]) and increases with exposure occurrences [13, 43, 44]. As suggested by the increased nitrotyrosine tissue level after ozone exposure in some studies [45, 46], chronic ozone exposure was also associated with a nitrosylative stress. This nitrosylative stress could result at least in part from an increased mitochondrial nitric oxide (NO) production by mitochondrial iNOS, itself overactivated due to oxidative-stress induced mitochondrial dysfunction in hippocampus of ozone exposed rats [44]. Pathways and nature of ROS and reactive nitrogen species (RNS) mediating ozone deleterious effects in the brain are still poorly described. Putative implication of ROS generating enzymatic systems, such as myeloperoxidase (MPO), xanthine oxidase, or NAPDH oxidase, in the mediation of ozone-induced brain oxidative stress was not studied. Nonetheless, rats chronically exposed to ozone present a neuroinflammatory phenotype associated with microglial activation [13] and MPO is known to be abundantly secreted by activated microglia and astrocytes in inflammatory conditions, and produces highly damaging ROS. A putative implication of MPO in ozone-induced brain damages is thereby not unlikely and could even contribute to the neurological alterations associated with ozone exposure. Regarding antioxidant defenses, mitochondrial SODs were upregulated until 7 days of repeated exposures to a low ozone dose but then progressively reduced from 15 to 60 days of ozone exposure, reflecting a progressive overwhelming of antioxidant defenses by the sustained ROS production [44, 47]. In accordance with this progressive antioxidant defenses overflow, reduced glutathione peroxidase and succinate dehydrogenase activities were observed in hippocampus from 30 and 7 days of ozone exposure, respectively [44]. The authors of this study thereby suggested that a chronic and irreversible oxidative stress had settled from 30 days of daily exposure to a low ozone dose. In the same study, this oxidative stress was associated with a mitochondrial dysfunction that probably consecutively lead to nitrosylative stress and neuronal cell death [44].

On the whole, in both acute and chronic studies, a dose- and time-dependent increase in ROS production is observed, which is in a first time dampened by an upregulation of antioxidant defenses. However, when ozone concentration and/or exposure duration reach(es) a certain level, antioxidant defenses are overtaken, leading to uncontrolled ROS production and so to chronic oxidative stress and related cellular damages. Noteworthy, before chronic oxidative stress has settled, ROS production is transient and ozone-induced oxidative damages thereby remain reversible. Finally, the fact that hippocampus is a brain region particularly sensitive to ozone inhalation effects gives credence to the idea that ozone air pollution exposure could play a role in memory alteration and AD development or aggravation.

Ozone and neuroinflammation

Both peripheral and central inflammation are associated with many neurodegenerative diseases, including AD. Although inflammation may not typically represent an initiating factor in AD, there is emerging evidence in animal models that sustained inflammatory responses involving microglia and astrocytes contribute to disease progression [48]. Air pollution is a well-known pro-inflammatory stimulus to both peripheral tissues and CNS that had been largely but wrongly neglected as a risk factor for neurodegenerative diseases. As mentioned before, the few available human studies showed inflammatory responses in brains of subjects exposed to severe air pollution during childhood or adulthood. However, it is unclear whether those inflammatory processes are beneficial (resolving inflammatory response) or detrimental (dysregulated inflammatory response) to brain health, and/or from which point of pollution level and exposure duration it switches from a beneficial to a noxious process. Contribution of each air pollutant to the inflammatory processes observed in human studies also remains unclear. Complementary animal studies are then useful to get more answers to those questions.

Animal studies evaluating the particular role of ozone-pollution exposure on brain and systemic inflammatory status mainly confirmed the results of human studies and pointed out the contribution of ozone to air pollution induced-inflammatory processes. Single or repeated but short exposures of rats to extreme levels of ozone air pollution (1.0 ppm ozone) triggers a rapid systemic and central inflammatory response, as evidenced by the increases in pro-inflammatory cytokines (TNF-α and IL-6) levels in lungs and cerebral cortex [49]. In cerebral cortex, this increase in cytokines levels is associated with an upregulation of the pro-inflammatory transcription factor NFκB and of glial fibrillary acidic protein (GFAP) [49], a marker of astrocytes activation.

Interestingly, after acute exposure to high but less extreme ozone air levels (0.8 or 0.4 ppm ozone for 4 h), cytokines (IL-6, IL-1β, TNF-α, CCl2) genes were found downregulated or unchanged in rat lungs, pituitary, cerebral hemispheres, and/or kidneys [50, 51]. The authors of this study suggested that this inflammatory gene downregulation could be a response to glucocorticoids due to an activation of the HPA axis by ozone stimulus. In support of this hypothesis, other studies showed that inhalation of high doses of ozone activated stress-responsive regions in the brain [37, 52]. Of note, in nearly all the acute studies on rat, sacrifices were performed just after the end of ozone exposure. However, when sacrifices were performed 24 h after the end of ozone exposure (i.e., after 24 h recovery in fresh air), ozone exposed rats displayed control phenotypes [50], indicative of a transient/reversible response to acute oxidative injury.

If the effect of an acute high dose ozone inhalation on brain inflammatory phenotype is unclear, results of the few chronic studies performed in rat and exploring neuroinflammation tend to show the progressive development of a sustained and time-dependent brain inflammatory phenotype. An upregulation of COX-2 was indeed reported from 7 days until at least 30 days of exposure to low ozone doses (0.25 ppm, 4 h/day) in hippocampus of rats, and especially in CA1 and CA3 layers [43]. COX-2 is well-known to be upregulated in response to oxidative damage, but it is also a well-known inducer of ROS production, inflammation, and apoptosis that likely contributes to the amplification of ozone exposure damage to the brain. Accordingly, this COX-2 overexpression was associated with increased lipoperoxide levels and neuronal loss in rats hippocampus [43]. Another study from the same research group confirmed and completed those results, demonstrating a time-dependent increase in ozone-induced hippocampal damage (oxidative stress, neuroinflammation, neurogenesis disruption, neuronal loss, and cognitive dysfunction) [13]. In particular, they observed a time-dependent activation of astrocytes and microglia, in rat hippocampus from 15 and 30 days of ozone exposure, respectively. Those results are consistent with human studies in populations chronically exposed to high air

pollution levels [15, 27], at least regarding astroglia activation.

Finally, through induction or aggravation of pre-existing oxidative and neuroinflammatory processes, chronic exposure to ozone air pollution likely contribute to AD progression or severity, even from low ozone pollution levels. Indeed, although some inflammatory stimuli are beneficial because they induce resolving mechanisms, sustained and uncontrolled inflammation may result in production of neurotoxic factors and tissue damages that amplify underlying disease states [48]. In support of this hypothesis, the neuroinflammatory phenotype of rats chronically exposed to low ozone levels is associated with AD-like brain lesions development and cognitive dysfunction, as exposed thereafter.

Ozone and neurodegenerative processes

AD is related to neurodegenerative processes, including neurogenesis alteration and neuronal dysfunction and death, that progressively lead to cognitive dysfunction and in particular learning capacity reduction and memory loss, as hippocampus is the first and main brain region affected in this disease. Besides, numerous damage-response pathways are regulated by the redox state in the CNS, so that oxidative stress plays a major role in neuronal death caused by severe vulnerability of the brain to a lost oxidation-reduction balance [13, 53]. Maintenance of oxidation-reduction balance is also necessary to maintain neurogenesis and thus brain repair ability and plasticity. Accordingly, oxidative stress accompanied by neurogenesis alteration has been involved in the physiopathology of neurodegenerative diseases such as AD [13, 54]. Oxidative damage related to normal aging processes was also shown to cause hippocampal function alteration and cognitive deficits such as memory loss and impaired ability to perform daily tasks [13, 55, 56].

As ozone inhalation can cause an oxidation-reduction imbalance in the CNS and as such brain oxidative stress state has been associated with neurodegenerative processes, Rivas-Arancibia et al. investigated whether repetitive exposure of rats to a low ozone dose (0.25 ppm) were able to cause progressive damage in the hippocampus and alter adult neurogenesis in a neurodegenerative process [13]. Results of their study showed that neurogenesis, estimated through double-cortin (DCX) immunopositive cell number, was firstly stimulated by ozone brain injury at 30 days of ozone exposure (and was unchanged at 7 and 15 days); however, from 60 days of exposure, DCX-positive cells number decreased below control level and continued to decline time-dependently until 90 days of exposure, evidencing progressive neurogenesis disruption due to rising and uncontrolled oxidative insult. In addition to progressive neurogenesis alteration, a progressive increase in neuronal death by apoptosis was observed, which was logically associated with the progressive hippocampal neuronal loss. As memory loss is associated with neurogenesis alteration and neuronal dysfunction and loss [57], the study also revealed the decline of memory function with ozone exposure duration [13]. Those effects of chronic low dose ozone inhalation on hippocampus oxidative status, neuronal death, and memory function were further confirmed in several later studies [44–47], with identification of mitochondrial dysfunction as mediator of oxidative stress effects on neurons function and death [44]. Alteration of acetylcholine neurotransmission was also observed in one of these later studies [45], alongside with memory function deficits. Cholinergic neurons form the septal-hippocampal pathway are indeed important for learning and memory function and acetylcholine neurotransmission abnormalities are a major characteristic of AD, together with senile plaques, NFTs, and extensive neuronal loss [58]. Those studies thereby further points toward a possible role of chronic ozone pollution exposure in AD-like pathological processes and lesions development.

Acute exposure of rats to ozone also induces hippocampal neuronal damage and memory function alterations from an exposure to 0.7 ppm ozone for 4 h, with maximal effect at 1.0 or 1.5 ppm ozone air level [38, 40, 59–61].

Noteworthy, males and females seem to be differently affected by ozone pollution exposure. A study performed on a well-established transgenic mouse model of AD (Amyloid Precursor Protein/Presenilin 1 (APP/PS1) overexpressing mouse) reported that exposure of APP1/PS1 mice to cyclic ozone pollution episodes (8 cycles of 5 days exposure to 0.8 ppm ozone 7 h/day) accelerated memory deficits and learning capacity impairment in male but not female transgenic mice [62]. In agreement with this observation, male APP/PS1 mice displayed lower antioxidant capacity, increased induction of NADPH oxidases and lipid peroxidation, and greatly increased neuronal apoptosis in cortex and hippocampus, compared to APP/PS1 female mice [62]. Although further studies would be needed to clarify the mechanisms underlying the different sensitivity of male and

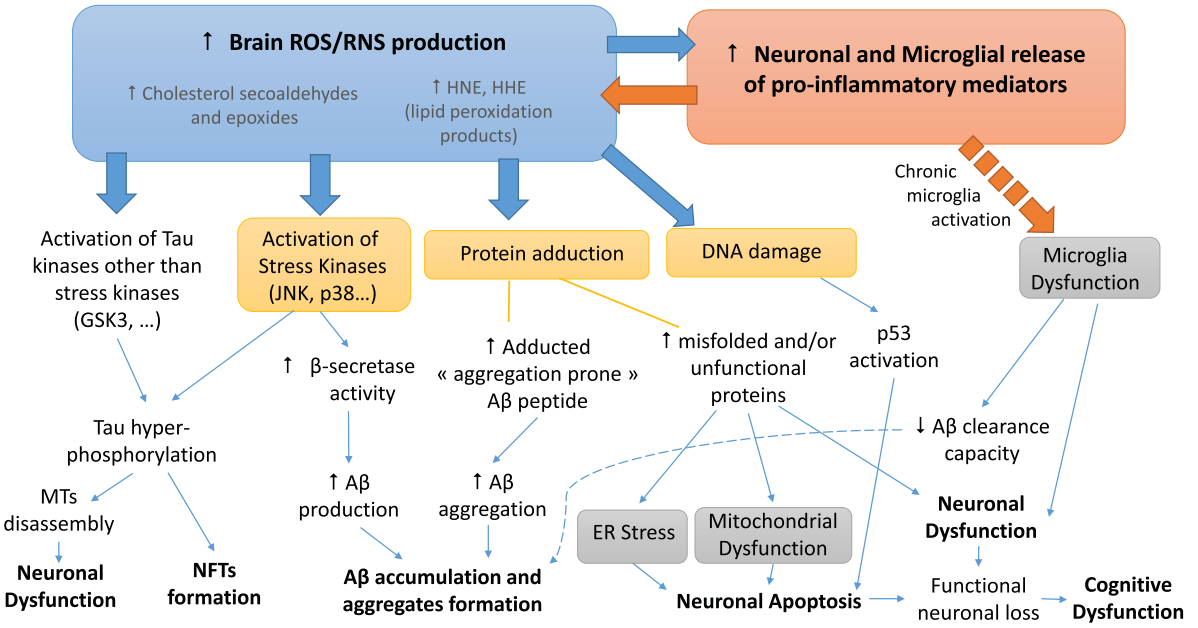

Fig. 3. Putative mechanisms of ozone-induced AD-like brain physiopathology. Inhaled ozone mediators (produced within the lungs or through the olfactory pathway) propagate the oxidative and inflammatory insult from the periphery to the brain (in particular in olfactory bulbs, cortex and hippocampus). Within the brain, the increase in ROS and RNS production by neuronal cells and/or activated immune cells (e.g., microglia) triggers the activation of cellular stress and other Tau kinases, favors protein misfolding and induces DNA damages. Tau kinases (e.g., GSK3, JNK, etc.) activation favors tau hyperphosphorylation and therefore tau aggregation and MTs disassembly, possibly contributing to the formation of NFTs like structures and to neuronal dysfunction due to impaired neurotransmissions. Activation of stress kinases may also favor neuronal apoptosis and increase the activity of the pro-amyloidogenic enzyme β-secretase, possibly contributing to Aβ peptide overproduction. Protein adduction, especially by HNE or cholesterol secoaldehydes may contribute to protein misfolding, aggregation, and/or dysfunction, which can result in facilitated Aβ aggregates accumulation, ER stress, and/or mitochondrial and/or neuronal dysfunction. Mitochondrial or nuclear oxidative DNA damage may also result in mitochondrial and/or neuronal dysfunction and apoptosis. Finally, despite being involved in detoxification and resolving processes at first, microglia may also contribute to the production of pro-inflammatory cytokines and of ROS/RNS, which may amplify the neuronal insult. If the ozone exposure persists, the over- and/or chronic activation of microglia may lead to microglial dysfunction and so to impaired Aβ clearance capacity and increased neuronal toxicity. Aβ, amyloid-β peptide; GSK3, glycogen synthase kinase 3; JNK, c-Jun N-terminal kinases; MTs, microtubules; NFT, neurofibrillary tangles; ROS/RNS, reactive oxygen/nitrogen species.

female APP/PS1 mice to ozone, it is likely that the higher sensitivity of males is related to their reduced brain antioxidant capacity, especially because males experience more dramatic aging-related decline in reduced glutathione in many organs than females [62, 63]. Although origin of this sex difference is still unclear, a difference in estrogens levels has been suggested [62, 64]. Another additional explanation to this sex difference may be that the APP/PS1 female mice already displayed a more advanced AD phenotype than males before ozone exposure. Finally, ozone exposure may also have different consequences depending on the genetic background of the subject because the same cyclic exposure of healthy non-transgenic littermates (C57BL/6J mice) to ozone did not trigger cognitive deficits.

In summary, both acute and chronic studies showed that ozone pollution inhalation *per se* can cause neuronal death and neurogenesis alteration in hippocampus, with associated memory deficits in healthy rat. Vulnerability to ozone-induced oxidative stress and injury degree thereby depended on the inhaled dose of ozone, ozone exposure duration and periodicity, on the brain region considered and on rodent age, sex, and genetic susceptibility to AD.

Ozone and pathological protein aggregation

Accumulation of Aβ peptide in the brain (through increased production, aggregation, and/or reduced clearance) is a major pathological feature of AD, although the mechanisms underlying Aβ accumulation in sporadic AD patients remains unclear. As ozone inhalation can cause oxidative stress and neuroinflammation, it could potentially reduce Aβ peptide clearance and/or increase Aβ production or aggregation, contributing to Aβ accumulation in

some sporadic AD patients. Data from epidemiological studies is scarce but tends to show a greater level of amyloid-positive material in olfactory bulbs and/or other brain regions from Mexico City residents (dogs, adults, and children) chronically exposed to high air levels of ozone and particulate matter, compared to low-polluted city residents. However, as pointed out before, the specific role of ozone cannot be discriminated from other air pollutants contribution in those epidemiological or autopsy studies on human and canine subjects.

Regarding experimental evidence, to the best of our knowledge, three animal studies evaluated the effect of ozone inhalation on Aβ brain accumulation in the hippocampus: one on APP/PS1 overexpressing mice [62] and the two others on Wistar rats [65, 66]. In the study on APP/PS1 overexpressing mouse [62], an increased level in SDS-insoluble $A\beta_{40}$, and in SDS-soluble and SDS-insoluble $A\beta_{42}$ was observed in the cortex and hippocampus of male transgenic mice compared to female mice. Cyclic exposure of those mice to ozone, however, had no additional effect on Aβ plaques or $A\beta_{40/42}$ brain amount, suggesting that ozone-induced oxidative stress did not impact Aβ production, aggregation, or clearance in a model of AD genetic susceptibility. This result also implies that the other effects of ozone exposure observed in this study (i.e., increased neuronal apoptosis and memory and learning capacity loss in male APP/PS1 exposed to ozone vs male APP/PS1 exposed to fresh air) were independent of a putative increase in amyloidogenesis and were mostly the direct consequence of oxidative injury. In the first study on Wistar rats [65], an increase of $A\beta_{42}$-positive signal was measured in hippocampal dentate gyrus cells from ozone-exposed rats (from 60 to 90 days of exposure to 0.25 ppm ozone, 4 h/day) colocalizing with a mitochondrial marker (OPA1), suggesting Aβ accumulation in the mitochondrial fraction. Supportive of these results, immunodetection of $A\beta_{42}$ in the mitochondrial fraction of rat hippocampi after subcellular fractionation also showed an increased level of $A\beta_{42}$. The same research group then confirmed in an independent study [66] the increased production of $A\beta_{42}$ in the rat hippocampus after chronic exposure of Wistar rats to low doses of ozone. In this study, they also showed by Raman spectroscopy that the secondary structure of $A\beta_{42}$ in the brain of ozone-exposed rat evolved progressively from an alpha-helix to a β-sheet secondary structure, prone to polymerization, misfolding, and aggregation into protofibrils, similarly to what may happen in AD patient's brain.

Finally, the effect and mechanisms of ozone air pollution exposure on brain amyloid deposition are still unclear and would deserve further researches, with different animal models of AD susceptibility and/or aging, and may be with more precise and specific techniques than immunohistochemistry or western blotting (which are semi-quantitative techniques) to quantify Aβ level in the rodent hippocampus. The effect of ozone on tau hyperphosphorylation and NFTs formation has never been reported and would also need further investigations.

Ozone and other air pollutants interactions

Although experimental exposure of rodents to ozone recapitulates most of the features of AD, ozone air pollution alone may not be sufficient to induce the development of the disease in humans. However, it is probable that ozone interaction with other factors (e.g., BMI, gender, age, genetic background, other predisposing disease states like diabetes or stroke, or other environmental factors) can trigger and/or accelerate the development of neurodegenerative processes and AD. In particular, ozone can probably exacerbate the toxicity of other air pollutants like particulate matter or nitrogen oxide, by potentiating oxidative stress and inflammation and so consecutive tissue damages, and by contributing to the alteration of the barriers that normally prevent or limit the entry of these pollutants into the brain. However, experimental evidence is scarce regarding the combined effects on the brain, and human studies cannot distinguish the putative synergistic effect of different air pollutants from their individual effects. Still noteworthy, co-exposure to inhaled particles and O_3 causes a synergistic effect on airway responsiveness and allergic inflammation in a murine (BALB/c) model of ovalbumin-induced asthma [67]. Moreover, ambient concentrations of O_3 can increase the biological potency of diesel exhaust particles [68–71]. Indeed ozone being a powerful oxidant, it can increase the toxicity and production of other atmospheric and indoor air pollutants like volatile organic compounds from diesel exhaust particles or laser printers, or like terpenoids, by oxidizing these and producing secondary organic aerosols [70]. Exposure to O_3 can also alter regional function and particle dosimetry in human lung (significantly enhanced the fraction of respired aerosol retained by the lung and altered the distribution pattern of deposited aerosol by increasing particle deposition to the middle lung region) [72]. Finally, combined exposure to O_3 and dust in

office air could cause significantly stronger effects than either O_3 or dust exposure alone [73].

Ozone and genes interaction

Individual differences that were observed upon exposure to the same polluted ambient air suggest that genetic susceptibility is likely to play a role in response to air pollution [74]. The Apolipoprotein E (ApoE) 4 allele, the most prevalent genetic risk for AD, indeed increases the risk of AD development in response to air pollution in humans, especially in young girls and in combination with a high BMI. APOE4 heterozygous females with >75% to <94% BMI percentiles are at the highest risk of severe cognitive deficits (1.5–2 SD from average IQ) [31, 18, 75]. Some, but still too few, research groups investigated the effect of certain gene products on the susceptibility to damage by air pollutants using genetically modified animals. To the best of our knowledge, the only study investigating ozone-gene interaction effect on the brain was the one from Akhter et al. described previously and showing an increased neuronal apoptosis and accelerated memory loss in APP/PS1 mouse exposed cyclically to ozone versus APP/PS1 mouse exposed only to fresh air [62]. Some studies used ApoE knockout ($ApoE^{-/-}$) mice [76–78] and showed that ApoE deficiency enhances particulate matter-induced neuroinflammation and neurotoxicity but the specific effect of ozone on this genetic background was not investigated. Another susceptibility gene for the effects of air pollution may be the glutathione-S-transferase gene (GSTP1) because of its important role as radical scavenger, but again, its interaction with ozone air pollution in the brain has not yet been studied. More globally, oxidative stress and inflammatory pathway genes including Glutathione S-transferase Mu 1 (GSTM1), GSTP1, NAD(P)H dehydrogenase quinone 1 (NQO1), TNF, and TLR4 are further logical candidates for the study of the association with the susceptibility to air pollutants since oxidative stress, and inflammatory processes are common denominators of air pollution-induced neuropathology [10].

CURRENT INSIGHT INTO MOLECULAR MECHANISMS OF OZONE-INDUCED NEURODEGENERATIVE PROCESSES

Ozone air pollution exposure is associated with several neurobiological and other extra-pulmonary biological effects in both human and animal subjects. However, ozone is a gaseous and highly reactive substance so that it cannot deeply penetrate into tissues or reach the bloodstream. Although how inhaled ozone affects the brain remains a major uncertainty, here are the current insights into possible molecular mechanisms of ozone-induced neurodegenerative processes (Figs. 1–3).

Ozone mediators-induced brain oxidative stress and neuroinflammation

Ozone mediators-induced brain oxidative stress

Because of its high content of lipids, its high requirement for oxygen (high metabolic activity), and its low antioxidant defenses, the brain is highly susceptible to oxidative stress development. O_3-oxidation by-products that are able to cross the blood-brain barrier and reach the brain may include hydrophobic low molecular weight substances such as malondialdehyde, hydoxy-2-nonenal (HNE), hydroxyl-2-hexenal (HHE), and lipid oxidation products (cholesterol oxidation products). Within the brain or other tissues, aldehydes such as HNE or HHE are highly reactive species prone to forming covalent adducts on many classes of biomolecules (e.g., proteins, phospholipids, nucleotides) [79, 80]. For example, HNE is able to form Michael adducts on proteins by reacting with amine groups of lysine or histidine residues through its carbonyl group. HNE or HHE adduction on proteins may cause their misfolding and thus their dysfunction and/or aggregation. Alteration of mitochondrial enzyme activity due to their covalent adduction by lipid peroxidation product can cause mitochondrial dysfunction [81], and therefore ROS generation and reduced ATP production, and this can ultimately lead to neuronal dysfunction and apoptosis. Besides, misfolded proteins can be degraded or handled by chaperon proteins in the endoplasmic reticulum (ER). However, if the production of misfolded proteins overwhelms the capacity of the ER to take them up, ER stress arises and the unfolded protein response is triggered. Although the molecular mechanisms are still unclear, ER stress and oxidative stress are coupled and the accumulation of unfolded protein in the ER lumen is sufficient to produce ROS [82]. Through protein adduction/carbonylation, hydroxyalkenals (HHE/HNE) may then contribute to ROS production amplification by contributing to mitochondrial dysfunction and ER stress development, and may be mediators of ozone-induced oxidative stress in the brain. Interestingly, Aβ peptide can

be adducted *in vitro* by hydroxyalkenals or products of cholesterol oxidation by ozone (ozonlysis cholesterol by-products such as some secoaldehydes) and adducted Aβ peptide is more prone to aggregation than its non-adducted form [83–85]. Those findings suggest that some ozone mediators (HNE, secoaldehydes) could contribute to the amyloid pathology *in vivo* by triggering and/or accelerating Aβ peptide aggregation. Adducted Aβ peptide aggregates could then further amplify ROS production and brain oxidative stress, through different mechanisms depending of the aggregation state (monomeric, oligomeric, fibrillary, plaque, etc.). For example, amyloid aggregates can induce microglial activation, neuronal mitochondrial dysfunction, ER stress,* or proteasome impairment through NMDA receptor interaction with amyloid oligomers [86, 87]. Microglia activation facilitated by some ozone mediator action may also contribute to ROS production and brain oxidative stress development. Indeed, one of the most invariant features of microglial activation is the production of ROS and RNS such as superoxide anion ($O_2^{\cdot -}$) and NO [88, 89]. Macrophages are effectively well known to express a membrane bound NADPH oxidase associated with the respiratory burst and an iNOS, that when activated contribute to the generation of oxidative and/or nitrosylative stress in tissues. How ozone mediators can contribute to microglial activation will be discussed in the following sections.

Ozone mediators induced neuroinflammation

Although some inflammatory stimuli induce beneficial effects and inflammation contribute to tissue repair processes, uncontrolled and sustained inflammation may result in the production of neurotoxic factors that amplify underlying disease state. Persistent stimuli may result from environmental factors (e.g., air pollution exposure, diet, systemic infection) or the formation of endogenous factors (e.g., protein aggregates) that are perceived by the immune system as 'danger' signals. Increasing evidence suggests that AD pathogenesis is not restricted to the neuronal compartment, but includes strong interactions with immunological mechanisms in the brain [48]. Misfolded and aggregated proteins can indeed bind to pattern recognition receptors on microglia and astroglia, and trigger an innate immune response characterized by the release of inflammatory mediators, which contribute to disease progression and severity. Ozone pollution exposure could contribute to AD pathogenesis by aggravating an ongoing neuro-inflammatory process or by facilitating neuroinflammation development through microglia and/or astroglia priming, as discussed thereafter.

Microglia priming by ozone circulating mediators

Increasing evidence points to a role of ozone-induced circulating mediators in microglial priming. Indeed, ozone inhalation has been shown to cause persistent microglial activation *in vivo* in rat; and *in vitro* exposure of primary rat microglia cells or HAPI rat microglial cell line to the serum of O_3-exposed rats, resulted in an increased TNF-α and H_2O_2 production in response to lipopolysaccharide (LPS) and $A\beta_{42}$, respectively [90]. Interestingly, the circulating mediators present in the serum of O_3-exposed rats and involved in microglial priming and activation were independent from traditional cytokine triggers that commonly transfer inflammation from the periphery to the brain (i.e., TNF-α, IL-6, IL-1β, and MCP-1). Instead, results from this study suggest that macrophage Ag complex-1 (MAC-1) receptor ligands are critical suspects for microglial priming induced *ex vivo/in vitro* by serum of O_3-exposed rats [90]. Of note, it has been reported in animal experiments and postmortem human studies that the increase in MAC-1 expression corresponds to microglial activation in numerous neurodegenerative diseases [91].

Finally, those findings suggest that inhalation of ozone instigate a circulating signal that is independent of conventional cytokines but activates and reprograms microglia to a primed state, making it more sensitive to additional pro-inflammatory stimuli and potentially emphasizing ongoing neurotoxicity (e.g., Aβ peptide-induced neurotoxicity). The exact nature of the circulating signal still needs to be defined.

Microglia and astroglia activation by central/local mediators

Microglia and astroglia cells can be activated by diverse signals, ever through the binding of pathogen-associated molecular patterns (e.g., LPS from gram negative bacteria) to a diverse range of pattern recognition receptors (e.g., Toll-like receptors, NOD-like receptors), through the binding of ATP released from dead or injured cells to purinergic receptors or through the activation of the so-called 'scavenger receptors' by oxidized lipids, proteins or apoptotic cells [48]. As ozone exposure is associated with

brain oxidative injury, increased neuronal cell death, and microglial activation, it can be presumed that microglia activation in this context is related to the activation of scavenger receptors and/or purinergic receptors by local ozone-induced oxidation by-products, ATP and/or apoptotic cells. Ligation of pattern recognition receptors then leads to the activation of signal transduction pathways that result in part in the activation of downstream kinases including MAPK and IκB Kinases and to further activation of pro-inflammatory signaling pathways. Ozone exposure may also promote the production of advanced glycation endproducts (AGEs) that can activate their receptors (RAGEs) on the surface of microglia, astrocytes, vascular endothelial cells and neurons, contributing to glial cells activation. Aβ peptide has also been suggested to activate microglia and astroglia through TLR4 and RAGEs. Finally, both ozone pollution exposure by-products and Aβ peptide aggregates in the brain can promote glial cells activation and contribute to the induction or exacerbation of an ongoing inflammatory process in the brain which may then contribute to neuronal dysfunction and death, and thus to AD pathogenesis.

Reactive astrogliosis

Reactive astrogliosis is a ubiquitous feature of CNS pathologies [92]. At later stages of CNS disorders, astrocytes become activated and contribute to neuroinflammation and neurodegeneration. Astroglia were reported to be activated in humans that were chronically exposed to high levels of air pollution, as evidenced by enhanced GFAP expression [15, 28]. Animal studies investigating ozone exposure showed that astroglial cells that are located close to brain capillaries have enhanced expression of IL-6 and TNFα [93] or are increased in number [13]. However, it is unclear whether the astroglia respond to components of air pollution, to the inflammation and oxidative stress produced from other cell types, or to cellular damage [10].

From O3-induced brain oxidative stress and neuroinflammation to AD-like pathology

AD pathology is characterized by the presence of extracellular Aβ peptide aggregates (amyloid plaques) and intracellular hyperphosphorylated tau protein aggregates (NFTs) in the brain, especially hippocampus and cortex. This disease is also associated with brain atrophy and impaired memory function due to neuronal dysfunction and death associated with impaired compensatory neurogenesis. O_3 pollution exposure is also associated with the presence of such AD-like features and processes in both human and animal subjects as described in part 1 and 2 of this review. Despite little being known regarding the putative underlying mechanisms, the actual knowledge and insight in this field will be presented and discussed in the following sections.

O_3 and brain Aβ peptide deposition

According to the amyloid hypothesis, AD implies the overproduction of the Aβ peptide, as consequence of the disruption of the homeostatic processes that regulate the proteolytic cleavage of the amyloid-β protein precursor (AβPP). Indeed, AβPP is normally snipped by α- and γ-secretases; which represents the "non-amyloidogenic pathway", leading to the production of the truncated nontoxic sAβPPα and p3 peptides. However, when AβPP is cleaved by β-secretase and γ-secretase, i.e., the "amyloidogenic pathway", $A\beta_{1\text{-}40}$ or the more toxic form $A\beta_{1\text{-}42}$ is produced, depending on the cleavage site used by the γ-secretase, and the extracellular Aβ level rises. Due to its high lipophilic and hydrophobic nature, $A\beta_{1\text{-}42}$ monomer rapidly misfolds and forms spheric structures that are prone to aggregate into higher order structures (dimers, oligomers, protofibrils, and fibrils) that can ultimately aggregate to form the core of the senile plaque lesions. Of note, $A\beta_{1\text{-}40}$ monomer can also aggregate into higher order structures but, contrary to $A\beta_{1\text{-}42}$, it does not misfold and aggregate spontaneously. Secondly, after its production and release in the extracellular compartment, Aβ peptide monomers or aggregates can be cleared from the brain by phagocytic processes mediated by microglial cells and proteolytic cleavage by cell surface proteases such as neprilysin, the insulin degrading enzyme, or endothelin-converting enzymes. If those clearance processes are altered by pathophysiological processes and/or overwhelmed due to an excessive production of Aβ peptide, Aβ peptide accumulates in the extracellular space which favors its aggregation. Consequently, processes and mechanisms that favor Aβ peptide overproduction and aggregation and/or that reduce Aβ peptide clearance capacity may favor amyloid brain deposition and lead to AD.

Oxidative stress induced by ozone pollution exposure may contribute to the amyloid pathology through all of these mechanisms (i.e., increased amyloidogenesis, increased Aβ aggregation propensity, and alteration of Aβ clearance). Indeed, mitochondrial and ER accumulation of $A\beta_{42}$ in rat hippocampal

cells was recently reported [65, 94]) from and after 60 days of sub-chronic exposure to low doses of O_3. The mitochondrial $A\beta_{42}$ accumulation was associated with an increase in γ-secretase expression and with a decrease in that of α-secretase, suggesting that low doses of O_3 can induce an over-activation of the amyloidogenic pathway and therefore contribute to the overproduction and accumulation of Aβ peptide in the mitochondria of hippocampal cells. In support of this hypothesis, previous studies have shown that oxidative stress or ROS overproduction *per se* can induce an increase in presenilin (γ-secretase) expression and activity levels [95–98].

The lipid peroxidation by-product 4-HNE has also been shown to upregulate the expression of β-secretase through the activation of the stress-activated kinases JNK and p38 MAPK [99]. Ozone pollution exposure could then also contribute to $A\beta_{42}$ accumulation through HNE brain overproduction and consecutive stress kinases activation and β-secretase expression upregulation. Secondly, and as mentioned previously, some oxidative stress metabolites (HNE, HHE, secoaldehydes) have been shown to form adducts on amyloid proteins, which consecutively accelerated their aggregation and fibrillation rate *in vitro* [83, 100, 101]. In particular, the carbonyl group of 4-HNE can react with thiol and amine groups of proteins to form Michael adducts and Schiff bases, both of which possibly leading to protein cross-linking, cyclization, and rearrangements of adducts. For example, the double bond of Michael adducts or Schiff bases can undergo epoxidation, and the 4-hydroxy group can be further oxidized to an aldehyde, either of which can lead to protein cross-linking by reaction with amino groups on adjacent molecules. Thus, 4-HNE *initially* increases the hydrophobicity of a protein and *subsequently* leads to protein cross-linking, both of which being able to increase Aβ aggregation, from the mechanistic point of view. Adduction of HNE on $A\beta_{1\text{-}40}$ peptide indeed modifies $A\beta_{1\text{-}40}$ conformational energy landscape to the extent that it becomes prone to spontaneous aggregation, like $A\beta_{1\text{-}42}$ [85]. Of note, adduction of HNE on other proteins like p53, low density lipoprotein-apoB100, or prion protein also triggered the misfolding of those proteins and turned them into amyloidogenic proteins [101]. As ozone exposure induces the production of HNE and HHE in the brain and especially in hippocampus, it can be presumed that ozone pollution exposure contributes to Aβ aggregation acceleration in sporadic AD cases through HNE adduction of proteins.

Finally, ozone pollution exposure is associated with microglia activation and may contribute to the development of microglia dysfunction due to chronic and excessive activation of immune cells [13, 102]. As dysregulated microglia is less efficient to clear the brain from abnormal substances such as Aβ peptide aggregates [103, 104], ozone pollution exposure could also contribute to Aβ peptide accumulation through microglia dysfunction. However, the effect of ozone pollution exposure on Aβ clearance mechanisms has never been reported to date and would also need further investigation.

O_3 and NFTs formation

NFTs are another characteristic lesion of AD. They are formed as a result of tau protein hyperphosphorylation and consecutive self-assembly in the intracellular compartment of neuronal cells. Tau is a microtubule-associated protein, abundant in neurons of the CNS and that binds tubulin to form and stabilizes microtubules. Phosphorylation of tau has been reported on approximately 30 sites in physiological tau proteins and can trigger tau detachment from tubulin, thereby inducing microtubule physiological disassembly. However, in AD conditions, an imbalance in the function of several protein kinases and phosphatases is thought to be responsible for tau hyperphosphorylation. Although the identity of the kinases involved in the physiological or pathological phosphorylation of tau *in vivo* has so far remained elusive, these may include the GSK3β, CDK5, JNK, MAPK, PKA, SAPK, Fyn, and/or c-Abl non receptor Tyrosine kinases [105].

The mechanisms through which ozone pollution exposure can contribute to NFT formation are still undiscovered. However, several lines of evidence indicate that oxidative stress *per se* can promote tau hyperphosphorylation and aggregation [106]. The mechanism by which oxidative stress affects tau phosphorylation remains controversial but it could include GSK3β and/or stress-activated protein kinases JNK or p38 activation (for example by HNE) [106]. Another possible link between oxidative stress and pathologic tau phosphorylation is the reduction of the peptidyl prolyl cis-trans isomerase 1 (PPIase1 or Pin1) activity, leading to increased tau phosphorylation. Pin1 has indeed been implicated in dephosphorylation of tau protein, and this enzyme has been shown to be oxidized and significantly downregulated in AD hippocampus [107].

GSK3β activity is downregulated by the insulin IRS/PI3K/Akt canonical signaling pathway so that a

reduction in brain insulin signaling (due to insulin resistance and/or insulin insufficiency) leads to GSK3β activity upregulation. Brain insulin signaling reduction was associated to both brain oxidative stress and AD [108–113]. In addition, ozone exposure was recently shown to induce skeletal muscle insulin resistance with impaired Akt activation due to oxidative-stress mediated activation of stress kinases [37]. Ozone pollution exposure could possibly contribute to NFTs formation through oxidative stress mediated brain insulin-resistance and GSK3β upregulation, although such phenomenon has never been investigated or reported to date. Finally, it is known that an inflammatory environment might activate some tau kinases to promote tau hyperphosphorylation and consecutive NFT formation [48, 114]. O_3 pollution exposure could thus contribute to NFT formation by inducing neuroinflammatory processes.

O_3 and neuronal dysfunction, death, and neurogenesis impairment

Neurodegeneration refers to the progressive loss of neuronal function and structure. It includes the pathological processes of neuronal dysfunction, neuronal death, and impaired neurogenesis. The exact sequence of events that leads to neurodegeneration in AD is still not fully understood. In particular, which pathological mechanism (e.g., amyloidogenesis, tauopathy, oxidative stress, neuro-inflammation, mitochondrial dysfunction, etc.) first triggers AD disease development remains unclear. All of these mechanisms are, however, at least involved in AD associated neurodegeneration progression and could be promoted by chronic ozone pollution exposure.

First, ozone pollution exposure could contribute to neurodegenerative processes through the induction of brain oxidative stress and consecutive neuronal dysfunction and apoptotic or necrotic cell death. At high levels of oxidative stress, perturbation of the mitochondrial permeability transition pore and the electron transfer chain indeed cause mitochondrial dysfunction and apoptotic or necrotic cell death, depending on the intensity of the oxidative insult. More precisely, oxidative stress leads to lipid peroxidation, protein oxidation and dysfunction, and to DNA damage due to nucleic acid oxidation. DNA damage activates p53 which triggers the expression of the Bcl-2 proteins family including pro- and anti-apoptotic proteins. Activation of the pro-apoptotic proteins (e.g., Bax, Bad, PUMA) can then impair mitochondrial membrane permeability to trigger the release of cytochrome C in the cytosol, and induce caspase activation cascade, leading to neuronal apoptosis. Besides, protein oxidation and mitochondrial DNA damage may lead to the impairment of glycolytic and/or mitochondrial protein function and synthesis, and therefore to the disruption of electron transport, mitochondrial membrane potential, and ATP production (Fig. 3). This may also result in mitochondrial dysfunction and in impaired brain energy metabolism, and may contribute to neuronal dysfunction and death. Accumulation of oxidized and/or adducted proteins can also result in ER stress mediated neuronal apoptosis (through PERK pathway). Finally, membrane lipid peroxidation can modify membrane fluidity and permeability, and such changes in the inner mitochondrial membrane can result in an opening of the mitochondrial permeability transition pore, loss of the mitochondrial transmembrane potential, and release of normally sequestered pro-apoptotic proteins (including cytochrome C) from the mitochondrial intermembrane space into the cytosol [115–117].

Ozone pollution exposure could also mediate mitochondria-induced neuronal death through the extrinsic apoptotic pathway, by promoting TNF-α production by activated microglia and other neuroinflammatory processes [115, 118]. Besides, exposure of rat to low doses of ozone (0.25 ppm, 4 h/day) promotes $A\beta_{42}$ mitochondrial accumulation in hippocampal cells and increases mitochondrial damages, reduces mitochondrial respiratory control, and triggers neuronal apoptotic death from 60 days of exposure [65]. $A\beta_{42}$ mitochondrial accumulation is thereby probably another mechanism of ozone-induced mitochondrial dysfunction and mitochondria-induced apoptotic neuronal death. In addition, nonfibrillar small aggregates (oligomers) of Aβ (rather than large aggregates/plaques) are major contributors to neurotoxicity. In particular, they can induce cell-death or neuronal dysfunction by interacting with cell-surface receptors, forming pores or channels in plasma membranes, or by aggregating intracellularly, leading respectively to cell signaling disturbances, ionic homeostasis disruption, and proteasome, mitochondria, and/or autophagy dysfunction [87]. Chronic exposure to even low doses of ozone could then contribute to neuronal dysfunction and death by promoting Aβ production, accumulation, and neurotoxicity.

Neurogenesis impairment observed in rat exposed to ozone is probably also a consequence of oxidative stress. Although the molecular mechanisms are still unclear, recent studies have reported alterations

of adult neurogenesis due to changes in oxidative state, and mitochondria-mediated apoptosis of neuronal progenitor cells could be one putative molecular mechanism [119]. Finally, neuronal dysfunction, death and impaired neurogenesis may all contribute to a progressive loss of functional neuronal mass in subjects chronically exposed to ozone. Such neuronal loss, which seemed to mainly affect the hippocampal brain region in animal studies, may be responsible for the impairment of cognitive functions such as learning and memory functions observed in both animal and human studies with ozone pollution exposure, and which are characteristic of AD pathology.

CONCLUSIONS

Both human and animal subjects exposed chronically or acutely to ozone air pollution displayed markers of brain oxidative stress, neuroinflammation, neuronal alteration and death, and impaired cognitive functions (altered short term and/or long term memory), in particular in the hippocampal brain region. In addition, inhabitants of a large city, chronically exposed to air pollution including ozone, presented with increased Aβ and tau protein deposits in their brains or olfactory tract, sometimes associated with olfactory dysfunction. Ozone pollution exposure, especially chronic low dose exposure, recapitulates most, if not all, of the features of AD pathology, at least in experimental studies. Indeed, it is important to note that it will be extremely difficult to establish a direct link of causality between neuropathological findings and ozone exposure in humans due to the complex environmental exposures, genetics susceptibilities, concomitant pathological processes, and other environmental/health variables impacting each individual. However, even if it may not be sufficient alone to induce the disease, ozone pollution exposure can be considered a probable risk factor or aggravating/accelerating factor for this neurodegenerative disease, at least in combination with other factors such as other air pollutants exposure, other diseases (inflammatory diseases, diabetes, etc.) and/or genetic predispositions. Additional clinical and experimental studies, however, are needed to confirm in other citizen populations, with or without genetic susceptibility to develop AD, and to better understand and identify the molecular mechanisms involved in ozone inhalation brain effects.

Despite the important lack of knowledge regarding the molecular mechanisms of ozone-induced AD-like lesions and pathological processes, it is highly probable that these all result from the production of pro-oxidant and pro-inflammatory mediators in the olfactory and respiratory tracts, as a consequence of ozone reaction with biological molecules from cell membranes and surfactant. These mediators (cytokines, lipid oxidation products, etc.) would then propagate oxidative stress and inflammation to the brain, through the olfactory pathway, altered blood-brain barrier regions, and choroid plexus or through the circumventricular organs. Oxidation of lipids, proteins, and nucleic acids may then contribute to mitochondrial dysfunction and ER stress and lead to neuronal dysfunction, death, and neurogenesis impairment. Neuroinflammation may also contribute to aggravated oxidative damage and neuronal death and dysfunction. Finally, ozone-induced brain oxidative stress and inflammation may also increase amyloidogenesis and tau hyperphosphorylation, leading to further neuronal dysfunction and death and to the appearance of AD characteristic brain lesions. Further studies are however needed to identify the exact nature of the mediators of ozone extrapulmonary effects, as well as the exact sequence of events and mechanisms occurring within the brain.

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