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Oxidative stress in brain aging, neurodegenerative and vascular diseases: An overview

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Abstract

According to the free radical theory, aging can be considered as a progressive, inevitable process partially related to the accumulation of oxidative damage into biomolecules – nucleic acids, lipids, proteins or carbohydrates – due to an imbalance between prooxidants and antioxidants in favor of the former. More recently also the pathogenesis of several diseases has been linked to a condition of oxidative stress. In this review we focus our attention on the evidence of oxidative stress in aging brain, some of the most important neurodegenerative diseases – Alzheimer's disease (AD), mild cognitive impairment (MCI), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD) – and in two common and highly disabling vascular pathologies—stroke and cardiac failure. Particular attention will be given to the current knowledge about the biomarkers of oxidative stress that can be possibly used to monitor their severity and outcome.
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1. Introduction

According to the free radical theory of aging, formulated for the first time by Harman in 1956 [1], aging can be considered as a progressive, inevitable process partially related to the accumulation of oxidative damage into biomolecules. Consistent with this theory is the idea that oxidative damage occurring in nucleic acids, lipids, proteins or carbohydrates is due to a disturbance of the prooxidant–antioxidant balance of the organism in favor of the former [2].

A free radical is a very reactive atom with an unpaired electron, which can be in a reduced or oxidized state. The majority of free radicals that damage biological systems are oxygen radicals and other reactive oxygen species (ROS), the main byproducts formed in the cells of aerobic organisms. The amount of free radical production is determined by the balance of many different factors, while the source of ROS formation is mainly constituted by mitochondria during electron transport in the oxidative phosphorylation chain. Mitochondria are thought to play a crucial role in the aging process not only due to their role as main intracellular generators of ROS, but also because they are targets of ROS attack. Although defenses against damage produced by ROS are extensive, including enzymatic and small molecule antioxidants as well as repair enzymes, an increased production of ROS or a poor antioxidant defense network can lead to a progressive damage in the cell with a decline in physiological function. This impairment may in turn pave the way to and increase the risk for morbidity and mortality that is, indeed, the main characteristic of the aging process.

In addition to the interest in the potential causative role of ROS in the pathophysiology of several age-related processes and diseases, there is much discussion about the clinical implications of oxidative stress in terms of diagnostic use and therapeutic possibility. Despite the growing support for the free radical theory of aging, direct evidence for this theory

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is yet lacking, and a direct antioxidant intervention in delaying aging and prolonging lifespan in the absence of disease has not been found yet. In this review we will focus on the evidence, particularly in humans, of the role of oxidative stress in aging brain, in neurodegenerative conditions such Alzheimer's disease (AD), mild cognitive impairment (MCI), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD), and in two common and highly disabling vascular pathologies, stroke and cardiac failure. Particular attention will be given to the current knowledge about the biomarkers of oxidative damage that can be possibly used to monitor the severity and outcome of the disease.

2. Oxidative stress and the aging brain

From a theoretical point of view, the nervous system is particularly vulnerable to the deleterious effects of ROS. First of all, the brain contains high concentrations of polyunsaturated fatty acids (PUFAs) that are highly susceptible to lipid peroxidation; it utilizes, compared to other tissues, the highest amount of oxygen to produce energy; finally, the brain is relatively deficient in antioxidant systems with lower activity of glutathione peroxidase (GPx) and catalase (Cat) compared to other organs [3].

During normal aging, the brain suffers both morphological and functional modifications affecting dendritic trees and synapses, neurotransmission, circulation and metabolism that are reflected in the alteration of motor and sensory systems, sleep, memory and learning [4]. Among the neuronal changes taking place as a function of aging, decrements in calcium homeostasis and in the sensitivity of catecholaminergic, dopaminergic, cholinergic and opioid systems can be counted. The molecular mechanisms involved in these changes have yet to be determined. Growing evidence, however, appears to implicate increased susceptibility to the long-term effects of oxidative stress, mitochondrial dysfunction and inflammatory insults as major contributing factors [5,6]. Indeed, age-related deficits in brain function related to oxidative stress might be largely due to this increased vulnerability of the brain to the deleterious effects of oxidative damage, additionally enhanced by a decline in the normal antioxidant defense mechanisms in the brain with age [7,8].

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is a hydroxyl radical-damaged guanine nucleotide, excised from DNA by endonuclease repair enzymes, and is the most used biomarker of oxidative DNA alteration [9]. Its measurement by means of HPLC with electrochemical detection (EC) has been used by many researchers for evaluating oxidative damage to chromosomal and mitochondrial DNA (mtDNA), although other methods such as the comet assay [10] and the LC/MS/MS with electrospray ionization [11] have been considered. As far as the measurement of 8-OHdG is concerned, the use of the so-called chaotropic method for DNA extraction [12] seems to lower the artifactual production of the adducts observed with phenol extraction [13]. This method of DNA extraction has been adopted by European Standard Committee on Oxidative DNA Damage (ESCODD) [14], a group created in 1997 in order to standardized the methods for evaluating DNA oxidative damage. The human healthy average of 8-OHdG as assessed by HPLC-EC is reported in the order of 23.0 ng/ml in urine [15].

High levels of 8-OHdG were detected both in nuclear DNA (nDNA) and in mtDNA of the post-mortem brain of aged subjects [16]. MtDNA appears to be more prone to damage than nDNA [16], as recently summarized by Barja [17]. This damage was strictly related to aging, with a marked increase after the age 75. The damage in mtDNA was postulated to be a component in the impaired mitochondrial function leading to the reduced metabolic activity observed with age [16,18].

Lipid peroxidation is a central feature of oxidative stress and can be assessed by a number of methods including the quantification of either primary (conjugated dienes and hydroperoxides) or secondary (thiobarbituric acid-reactive substances – TBARS – gaseous alkanes and prostaglandin F_2-like compounds, also called F_2-isoprostanes) peroxidation end products. Among these biomarkers, F_2-isoprostanes are currently considered a far more accurate index of oxidative stress in vivo in humans than other available methods [19]. However, while it is not clear whether isoprostane formation is increased in human brain in normal aging, malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE), two aldehyde markers of lipid peroxidation, have been shown to increase with age in the cytoplasm of neurons and astrocytes [20] and in oculomotor neurons [21].

Most of the studies conducted to assess the role of protein oxidation in brain aging conclude that there is an increase of oxidized proteins. Among these studies, an important report showed a general logarithmic increase of protein oxidation, measured by assay of protein carbonyl groups, in human cerebral cortex along with age [22]. Protein carbonyl content may be high enough to explain the decline of cellular and tissue functions with age, i.e. 5% or less in the young versus 10–20% in the aged. Oxidative inactivation of enzymes is another index of age-dependent oxidative damage to proteins. The proteasome is a large intracellular protease, present in all cells of the central nervous system (CNS), that is responsible for the majority of intracellular protein degradation. Recent studies indicated that alterations in proteasome activity may occur during, and possibly contribute to, the aging process [23–25]. Such age-related alterations in the proteolytic pathway may contribute to the elevations in protein oxidation, protein aggregation and neurodegeneration evident in the aging CNS [26]. On the other hand, oxidized and cross-linked proteins can inhibit proteasome function [27,28].

3. Oxidative stress and neurodegenerative diseases

It is still unclear whether oxidative stress is the primary initiating event associated with neurodegeneration or a

secondary effect related to other pathological pathways but a growing body of evidence implicates it as being involved in the propagation of cellular injury leading to different damages observed in neurodegenerative diseases [29–31].

3.1. Alzheimer's disease

In AD, a "two-hit" hypothesis has been postulated for which, although either oxidative stress or abnormalities in mitotic signaling can independently serve as initiators, both processes are necessary to propagate disease pathogenesis [32]. AD constitutes the most prominent cause of dementia in the elderly and is clinically characterized by memory dysfunction, loss of lexical access, spatial and temporal disorientation and impairment of judgment. Histopathologically, AD is characterized by synaptic loss, nerve cell loss (mostly in the cerebral cortex, in the hippocampus and in the amygdala), extracellular deposition of β-amyloid (Aβ) protein (forming senile plaques) and intracellular precipitation of hyperphosphorylated tau protein (forming neurofibrillary tangles). The exact biochemical mechanism of the pathogenesis of AD is still unknown, but much attention is given to the role of the massive loss of the neurotransmitter acetylcholine (necessary for cognition and memory) and to the possible implication of oxidative stress in its development. Excitotoxicity and oxidative stress-induced triggering of degenerative signaling, including activation of stress kinases such as JNK, also appears to play an important role [33]. Age is a strong risk factor for AD, and several studies show logarithmic age-dependent increases in oxidized proteins, lipids and DNA in AD patients [34]. A large number of studies implicate metabolic defects in this neurodegenerative disease, and that abnormal mitochondria contribute to increased oxidative stress in AD [35] is evidenced by the strong positive correlation between mitochondrial abnormalities and the extent of oxidative damage in the cytoplasm [36].

Oxidative damage may play a role in amyloid deposition in AD, and the complex reciprocal relationships between A-beta deposition, excitotoxicity, calcium dysregulation and ROS production in AD have been recently elegantly summarized [37–39]. Oxidizing conditions cause protein cross-linking and aggregation of Aβ peptides [40] and also contribute to aggregation of tau [41] and other cytoskeletal proteins [42]. Behl and coll. [43] have demonstrated that Aβ aggregates upon interaction with the nerve cell membrane induce a sequence of events that lead to the intracellular accumulation of ROS. Aβ may cause the oxidation of the nonsaturated carbohydrate side chains of membrane lipids, disintegration of the neuronal membrane and, ultimately, cell lysis [43]. In addition to the direct induction of oxidative stress, Aβ can also indirectly generate an oxidative microenvironment, for example, via the induction of a local immune response. Indeed, cellular and soluble mediators of inflammation are found in post-mortem AD tissue [44]. It is worthy of note, however, a quite recent hypothesis that consider β-amyloid as a protective consequence to an underlying disease mechanism, viewing the known lesions of AD as a compensatory response places them in an environment that is both adaptive and protective [45,46]. β-Amyloid, in fact, has many physiological roles, some of which include redox-active metal sequestration [47], and SOD-like activity [48]. Furthermore, β-amyloid has been shown to be inversely correlated with oxidative stress markers [49], suggesting that it may have antioxidant effects [50].

Several markers of oxidative damage to DNA, lipids and proteins have been widely studied in AD [51]. A significant increase of 8-OHdG in nDNA and mtDNA isolated from three cortical areas and from cerebellum of AD patients was found in comparison to controls, particularly in the parietal cortex. These levels were much higher in mtDNA than in nDNA, showing an elevated susceptibility of mitochondria to oxidative stress [52]. Furthermore, elevated levels of 8-OHdG were also detected in lymphocyte DNA from AD donors [53]. Interestingly, the concentration of this oxidized base in lymphocyte DNA was found to be related to plasma carotenoid levels, namely lutein, lycopene, α- and β-carotene [54].

In AD brains, lipid peroxidation has been quantitatively assessed by measuring TBARS, HNE, MDA, lipid hydroperoxides, and isoprostanes. TBARS were found to be increased in AD frontal and temporal cortex compared with controls [55–58]. Other studies failed to find significant differences in TBARS between AD and controls at basal condition, whereas, after incubation with pro-oxidant, AD brains had higher TBARS concentration than controls [59,60]. HNE is toxic and a causative agent in protein aberrations in AD; several reports showed an increase in free HNE in multiple AD brain regions including cerebellum compared to controls [61]. The described HNE increases appear to be particularly significant in the amygdala and hippocampus [62]. Several studies reported no differences in MDA levels between AD and controls [63–65]. These data have been confirmed in two studies where there was also no difference in lipid hydroperoxide levels [66,67]. On the other hand, in cerebrospinal fluid, total F_2-isoprostanes were higher in demented patients [68]. Lipid peroxidation cause alterations in membranes [69–72]: in a study on mitochondria extracted from different cerebral areas we found a significant reduction of membrane fluidity compared to age-matched normal subjects; it was also related to the increase in 8-OHdG content in mtDNA [73].

Regarding protein oxidation in AD, Smith and coll. found that brain carbonyl levels were increased with age, but no difference was observed between aged and AD brains [22]. Likewise, AD brain had ortho-tyrosine levels, a protein carbonyl end product, another marker of protein oxidative damage, similar to controls [64]. On the contrary, increased 3-nitrotyrosine was found in both neurons containing neurofibrillary tangles and in those in which they were absent [74,75]. Increased amounts of carbonyl groups were found in AD brains by means of a chemiluminescence assay [76]. Protein carbonyls were significantly increased in both hippocampus and the inferior parietal lobule, but unchanged in the cerebellum, consistent with the regional pattern of histopathology

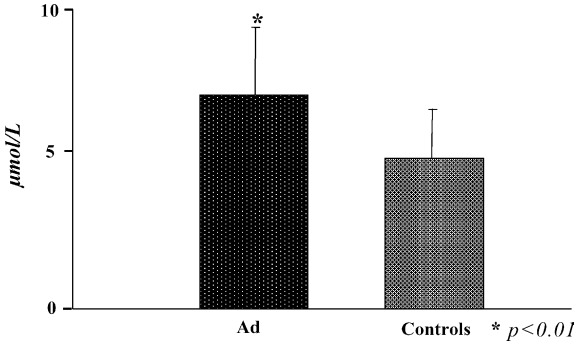

Fig. 1. Plasma IgG-dityrosine content in AD and cognitively normal elderly subjects.

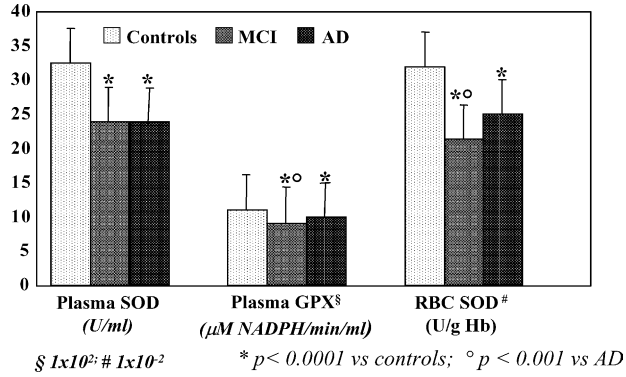

Fig. 2. Plasma superoxide dismutase, plasma glutathione peroxidase and red blood cell superoxide dismutase activity in healthy elderly subjects, Mild cognitive impairment (MCI) subjects and Alzheimer's disease (AD) patients.

in AD [77]. Two studies showed that there were increases in protein carbonyls both in neurofibrillary tangles as well as in the cytoplasm of tangle free neurons [78,79]. AD patients showed a significantly higher content of dityrosine in plasma immunoglobulin-G as compared to controls [80] (Fig. 1). Last but not least, an emerging issue in this field possessing high potential for the study of AD is proteomics. Proteomics is a developing tool used to identify specifically oxidized proteins in AD brain, because posttranslational modification of brain oxidized proteins may provide an effective means of screening a subset of proteins within the brain proteome able to reflect AD-related oxidative stress [81,82].

3.2. Mild cognitive impairment

An issue which is strictly related to AD development – particularly to the question whether oxidative stress plays a causative role in AD or is consequence of it – is MCI. MCI is a condition in which memory or other cognitive abilities are slightly abnormal but coexist with normal function in the activities of daily living, normal general cognitive function, and absence of dementia [83]. This condition is at significant increased risk of future conversion to dementia, especially to AD [84], and there is a large body of evidence suggesting that it may represent a pre-clinical stage of AD. While the evidence for a role of oxidative stress in the pathogenesis of AD is more extensive and solid, less so can be said for MCI [85].

An increased DNA oxidative damage in peripheral leukocytes of MCI subjects, as evaluated by comet assay, has been recently reported [86].

Increased levels of the isoprostane 8,12-iso-$PF_{2\alpha}$-VI – a specific marker of in vivo lipid peroxidation [87] – were found to be significantly elevated in CSF, plasma and urine of MCI subjects compared with controls [88], suggesting that lipid peroxidation may be an early event in the pathogenesis of the disease. Nevertheless, additional studies on the presence of elevated levels of biomarkers of oxidative stress in MCI are warranted, also in light of the discrepancies otherwise observed between brain/CSF versus plasma/urine F_2-isoprostanes and F_4-neuroprostanes levels [89,90].

Peripheral levels and activities of a broad spectrum of non enzymatic and enzymatic antioxidants were evaluated in a group of elderly subjects with MCI in comparison to AD patients and controls [91]. MCI and AD subjects showed lower means of non enzymatic antioxidants Vitamin A, Vitamin C, Vitamin E, uric acid and of the carotenoids lutein, zeaxanthin and α-carotene. In addition, patients with MCI and AD showed similarly lower activities of plasma and erythrocyte superoxide dismutase (SOD) as well as of plasma GPx as compared to controls (Fig. 2), suggesting that subjects developing MCI and subsequently AD may have an antioxidant enzymatic activity inadequate to counteract the hyperproduction of free radicals during a recently established condition of oxidative stress. Finally, the activity of aconitase, an enzyme of the Krebs cycle particularly sensitive to free radical damage [92] was also shown to be decreased in lymphocytes of MCI subjects [Mecocci et al., unpublished data]. An accumulation of oxidatively damaged aconitase in mitochondria might constitute a continuous source of free radical damage [93] able to modulate the progression of MCI to AD.

3.3. Parkinson's disease

PD, as a result of neurodegeneration occurring in the substantia nigra and in the striatum and of dopamine depletion, is clinically characterized by bradykinesia, postural instability, gait difficulty and tremor. Depigmentation, neuronal loss, and gliosis of the substantia nigra are typical brain abnormalities found in PD. The mechanisms of cell death in PD have not yet been fully elucidated, but increased oxidative stress, abnormal mitochondrial function and excitotoxicity are perhaps among the most important initiators or mediators of neuronal damage.

The etiology of PD remains unclear, but the evidence of an involvement of free radicals in PD comes from the observation that oxidation of dopamine yields potentially toxic semiquinones, and that the accelerated metabolism of dopamine by monoamine-oxidase-B may induce an excessive formation of hydrogen peroxide, superoxide anions, and hydroxyl radicals [94]. Further evidence for the role of oxidative stress in PD patients comes from studies regarding the

selective toxicity against the substantia nigra of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which induces Parkinson-like symptoms in primates. MPTP acts through its metabolite MPP^+ to inhibit Complex I of the mitochondrial respiratory chain. Post-mortem studies on PD brains have demonstrated that a disease-specific and drug-independent defect of mitochondrial Complex I accumulates in the substantia nigra of PD patients [95–97]. It has also been suggested that MPP^+ acts by increasing the vulnerability of cells to oxidative stress [98]. The role of a Complex I dysfunction in PD is supported by the finding that Complex I is inhibited in vivo and in vitro also by neuroleptic drugs which have extrapyramidal side effects, but not by clozapine, an atypic antipsychotic agent which has a minimal, if at all, extrapyramidal toxicity [99]. Mitochondrial complex I defects have been also found in muscle [99] and platelets [100] of PD patients. As for other neurodegenerative diseases, a fundamental molecular pathway to PD development is the abnormal folding, function and metabolism of proteins such as alpha-synnuclein and parkin, which are simultaneously source and target of oxidative and nitrative stresses [101].

PD was found to be associated with increased oxidative damage to DNA, with a marked enhance in 8-OHdG in caudatum and substantia nigra [102,103]. High 8-OHdG levels were found in the serum and cerebrospinal fluid of PD patients, and the mean values of serum 8-OHdG were significantly higher in female than in male PD patients [104]. Migliore and coll. found that, compared to controls, patients with untreated PD showed an increase in chromosomal, primary DNA damage and oxidative DNA damage in peripheral blood lymphocytes [105].

There are also evidences of increased lipid peroxidation in the PD brain [21]. MDA levels were increased in parkinsonian nigra compared with other PD brain regions and control tissue [106]. Also cholesterol lipid hydroperoxide, a marker of lipid peroxidation, has a 10-fold increase in the PD substantia nigra compares to control subjects [107]. Lipid peroxidation measured by means of lipoprotein oxidation in CSF and plasma was found to be increased in PD as compared to controls and was additionally used to monitor the effects of levodopa treatment, which disclosed a potential prooxidant role of levodopa and a possible protective role of dopamine in PD treatment [108].

Increases in protein carbonyls were found in many PD brain regions, including the substantia nigra, basal ganglia, globus pallidus, substantia innominata, frontal cortex and cerebellum [109]. Increased 3-nitrotyrosine immunostaining was shown in Lewy bodies and in amorphous deposits in intact and degenerating neurons in PD substantia nigra [110]. Other studies showed increased cerebrospinal fluid nitrate concentrations and nitrosyl adducts in brains of PD patients [111]. Other evidence for oxidative damage to proteins in PD is the increased expression of neural heme-oxygenase-1 and increased immunostaining of glycosylated proteins [112,113]. Other signs of oxidative damage in PD are elevated brain levels of iron and progressive siderosis of substantia nigra [114,115] and reduced levels of ferritin [116]. The iron-neuromelanin interaction may play an important role in the genesis of PD, as this complex is a source of cytotoxic free radicals and synthetic dopamino-melanin potentiates lipid peroxidation initiated by iron ions.

3.4. Amyotrophic lateral sclerosis

ALS is a progressive, fatal neurodegenerative disease characterized by gradual degeneration of motor neurons in the cortex, brainstem and spinal cord. The cause of neuronal death in sporadic ALS (SALS) is not known. However, in approximately 10% of all ALS cases, the disease is inherited. Familiar ALS (FALS) shows autosomal dominant inheritance and very high penetration. About 20% of FALS cases are associated with point mutations and lowered activity of CuZnSOD (SOD1) [117]. SOD1 catalyzed the formation of hydrogen peroxide through the dismutation of superoxide radical anions; by this reaction, SOD1 is thought to play an important role in regulating oxidative damage to cells [118]. Other reports have shown an increase in mitochondrial MnSOD (SOD2) protein and activity, in contrast with one study indicating potential gene defects of this enzyme in sporadic ALS [119]. Several studies suggest that the inclusion bodies, characteristic of ALS, differ in SALS versus SOD1-linked FALS. Analyzing SALS spinal cords, it was found immunoreactivity to HNE-histidine and crotonaldehyde-lysine, indicative of lipid peroxidation; carboxymethyl-lysine (CML), indicative of lipid peroxidation or protein glycoxidation; and pentosidine, indicative of protein glycoxidation [120]. In an other study it was also found that the pattern of immunoreactivity in FALS spinal cord was quite different, with intense immunoreactivity for CML bit also for pyrraline, marker of non oxidative protein glication, and in the Lewy body-like inclusion only and imidazolone – another marker of non oxidative protein glication – immunoreactivity in the cytoplasm of the residual motor neurons [121].

There is evidences of oxidative damage to DNA in ALS: increased levels of 8-OHdG were found in plasma, urine, and CSF of ALS patients. Plasma and urine 8-OHdG levels increased significantly with time in the ALS patients and the rate of increase in urine 8-OHdG levels with time was significantly correlated with disease severity [122].

As far as protein oxidation is concerned, a large increase in protein carbonyls was found both in ALS frontal and motor cortex [123]. In addition, there is substantial evidence for increased protein nitration in ALS, as increased immunocytochemical staining for 3-nitrotyrosine was observed in spinal cord motor neurons of both sporadic and FALS patients [124]. Biochemical measurements of 3-nitrotyrosine and 3-nitro-4-hydroxyphenylacetic acid showed significant increases in the lumbar and thoracic spinal cords of ALS patients. Marked increases of both free 3-nitrotyrosine and nitrated MnSOD in the cerebrospinal fluid of SALS patients have also been reported [125,126].

3.5. Huntington's disease

HD is a fatal neurodegenerative disorder with an autosomal dominant mode of inheritance. It leads to progressive dementia, psychiatric symptoms and an incapacitating choreiform movement disorder, culminating in premature death. HD is caused by an increased CAG repeat number in a gene coding for a protein with unknown function, called huntingtin. It has been proposed that glyceraldehyde-3-phosphate-dehydrogenase, which binds specifically to proteins implicated in the pathogenesis of neurodegeneration and in apoptosis, including huntingtin, may affect neuronal energy production [127]. Direct evidence for a defect in oxidative phosphorylation in HD was obtained applying proton nuclear magnetic resonance spectroscopy in HD patients. A three-fold increase in lactate concentrations in the occipital cortex was observed, and increases were also found in the basal ganglia [128].

Regarding oxidative DNA damage, one study found no elevations in the levels of 8-OHdG or of a wide range of other markers DNA oxidation, and no alterations in the levels of lipid peroxidation (as measured by lipid peroxides and thiobarbituric acid–MDA adducts) in the caudate nucleus, putamen, or frontal cortex of patients with HD compared with normal controls. Similarly, there was not an increased level of protein carbonyls in these tissues [129]. Several studies, however, have shown DNA strand breaks in HD post-mortem tissue as detected by in situ end labeling of DNA. Administration of 3-nitropropionic acid, a neurotoxic agent inhibiting succinate-dehydrogenase, caused brain lesions accompanied by increased levels of 8-OHdG and increased staining for 8-OHdG in the basal ganglia [130]. Increased 8-OHdG levels have been found in mtDNA from HD parietal cortex [131].

The contribution of oxidative stress to the pathogenesis of HD has also been studied by measuring the levels of F_2-isoprostanes in the CSF of 20 patients in the early phase of the disease [132]. F_2-isoprostane concentration was moderately but significantly higher in HD patients than in the control group (35% of increase). Several studies have also demonstrated that the disease is associated with increased levels of MDA, 3-nitrotyrosine and heme-oxygenase in areas of degeneration [133].

Increased 3-nitrotyrosine immunostaining has also been reported in a transgenic mouse model of HD [134]. It has been proposed that glyceraldehyde-3-phosphate-dehydrogenase, which binds specifically to proteins implicated in the pathogenesis of neurodegeneration and apoptosis, including huntingtin, may affect neuronal energy production [127].

4. Oxidative stress and vascular diseases

Traditional vascular risk factors only partly explain the excess risk of developing cerebrovascular and cardiovascular diseases and many studies support today the role of oxidative stress in their pathogenesis. In this section we focused our attention on two leading causes of disability and mortality, particularly in the elderly: stroke and congestive heart failure (CHF).

4.1. Stroke

Stroke is the main cause of disability and mortality in Western countries. Ischemic stroke accounts for about 75% of all cases while hemorrhagic stroke is responsible for almost 15% of all strokes. It has also been estimated that up to 30% of all ischemic strokes will eventually undergo hemorrhagic transformation. Brain ischemia, and especially the condition of ischemia and reperfusion occurring after stroke, has been shown to be associated with free radical-mediated reactions potentially leading to neuronal death [135].

Extensive evidence from experimental studies supports a role for free radical generation and oxidative injury in the pathogenesis of stroke. Several sources of free radicals have been proposed, including inflammatory cells, xanthine-oxidase, ciclooxygenase, and mitochondria [136]. The large increases in glutamate and aspartate that accompany ischemia may contribute to free radical generation by excitotoxic mechanisms [137,138].

Plasma levels of 8-OHdG were found to be increased in an animal model of ischemic stroke, with a significant association with brain content of 8-OHdG [139]. Regarding oxidative damage to DNA in humans, plasma levels of 8-OHdG were found to be increased after ischemic stroke, with a significant association with brain content of 8-OHdG [140].

Evidence for lipid peroxidation in cerebral ischemia comes from a large number of studies showing increased TBARS and fluorescent lipid peroxidation products in brain and peripheral tissues after ischemia [141–145] A correlation has been observed between MDA levels and infarct size, clinical stroke severity and patient's outcome [146,147]. It must be said, however, that TBARS and MDA are imprecise measures of lipid peroxidation [148]. More recently, several gas chromatography/mass spectroscopy (GC–MS) methods have been developed that overcome the limitations of previous assays but unfortunately they have not been applied yet to the measurement of lipid peroxidation in stroke patients [149–151]. A marked increase in plasma cholesteryl-ester-hydroperoxides (CEOOH) levels was observed in patients with cortical stroke compared to patients with lacunar stroke and normal controls, in whom levels of CEOOH were undetectable. CEOOH levels were also correlated with stroke severity, and there was a significant linear correlation between CEOOH levels and stroke volume [152]. However, this study has not been followed by other studies that could confirm these findings. Oxidized LDL (oxLDL) have been also used as biomarkers of lipid peroxidation in humans, since their source might be oxidized phospholipids released from brain tissue into circulation. Although plasma level of oxLDL is thought to reflect the oxidative status of the whole body, it remains unclear whether it can serve as a peripheral marker that is directly linked to the severity of oxidative damage in

the presence of ischemic brain lesions. Only three studies measured F_2-isoprostanes in acute stroke patients, achieving conflicting results. While it was. found that F_2-isoprostane levels are modestly increased in stroke patients compared to the normal range [153], recently it was demonstrated that PGF_2 increase in stroke patients when measured in serum few days after the event [154]. We found that plasma 8,12-iso-$PF_{2\alpha}$-VI levels are decreased in ischemic stroke patients treated with Vitamin C plus aspirin for up to three months as compared to patients treated with aspirin alone [155].

With respect to protein oxidation, there is a lack of studies assessing the presence and levels of biomarkers of oxidative damage against proteins and aminoacids in ischemic or hemorrhagic stroke in humans. One study did not find any difference in protein carbonyls between a small sample of stroke patients and controls [156].

4.2. Congestive heart failure

CHF constitutes the only major cardiovascular disease that has increasing incidence and prevalence [157]. It is a chronic, progressive disease representing the advanced stage of cardiac disease. Mortality rates are high, with less half of patients with overt signs of CHF surviving at five years [158]. The annual incidence of CHF is one to five per 1000 person, and the relative incidence doubles for each decade of life after the age of 45 [158]. It is worth noting that most of the components of neurohormonal and inflammatory activation observed in CHF induce oxidative stress through several mechanisms: angiotensin II [159], aldosterone [160], TNF-α [161], and proinflammatory cytokines [162] have all been implicated in the cascade of events leading to increased oxidative stress. Heart failure has been found to be associated with oxidative stress in animal studies, with a concomitant lowering in antioxidant enzyme activity [163–165], and with antioxidant depletion in plasma [165], and heart [163,164] in animal studies. Antioxidant might be depleted in CHF due to increased ROS scavenging [166,167], or be already deficient for life-style, disease, or age-related reasons and facilitate the worsening of oxidative stress [167,168]. Free radical production has been related in CHF to catecholamine autoxidation and recurrent episodes of ischemia and reperfusion [169]. Increased ROS production has been shown to be involved in myocardial apoptosis [170] and remodeling [171].

With respect to human studies, there is still a lack of data on presence and levels of biomarkers of oxidative stress in CHF patients [172]. Recently, we showed that plasma levels of some lipophilic antioxidants are significantly lower in CHF patients as compared to controls [173]. In this study, we also observed higher plasma levels of MDA in CHF patients with severe as compared to patients with moderate disease. As mentioned above, however, the application of MDA measurement in complex biological systems in vivo is criticized due to lack of specificity, and currently the best available biomarker of lipid peroxidation seems to be the isoprostanes, whose levels have been found increased in a variety of diseases where oxidative stress has been implicated. When levels of plasma 8,12-iso-$PF_{2\alpha}$-VI were measured in CHF patients and in controls, markedly increased levels significantly were found in patients. In contrast, plasma levels and activities of several water-soluble and lipophilic antioxidants were significantly lower in patients with CHF as compared to controls. Interestingly, plasma 8,12-iso-$PF_{2\alpha}$-VI levels were higher by more than 40% in patients with severe CHF than in patients with moderate disease, while mean plasma levels of Vitamin C, uric acid, Vitamin E, Vitamin A, lutein, zeaxanthin and lycopene were lower in patients with more severe disease as compared to patients with less severe cardiac failure [174]. Another important result of both studies is that there were no differences of biomarker and antioxidant levels between patients with CHF of ischemic and non ischemic origin [173,174], supporting the importance of myocardial tissue damage per se, which, once established, is progressive independent from the initial cause [175].

5. Conclusive remarks

There is a large amount of evidence indicating that oxidative stress plays a crucial role in aging as well as in neurodegenerative, cerebrovascular and cardiovascular diseases. All types of research models available to explore oxidant/antioxidant interactions and their relevance in these conditions, in fact, continue to confirm the deleterious effects of free radical and ROS hyperproduction in in vitro systems, cells, animals and humans. Assays of biomarkers of oxidative damage keep being searched, developed and ameliorated which allow to uncover conditions of oxidative stress in several age-related diseases. Currently, the best available oxidative stress biomarkers appear to be 8-OHdG and the comet assay for DNA damage; F_2-isoprostanes for lipid peroxidation; protein carbonyls and dityrosine for protein oxidation. Several rigorously conducted studies have shown a similar increase of these indexes in AD, PD, ALS, HD, stroke and CHF in humans. This is for instance the case of 8-OHdG, whose DNA content in different tissues and fluids (brain tissue, lymphocytes, plasma, CSF and urine) show an increase varying from 1.4-fold in ALS [122] to 2-fold in PD [104] to 3.0-fold in AD [52] to 3.6-fold in stroke [140]. Measurement of peripheral antioxidant levels has been also proven of great importance in the evaluation of age- and oxidative stress-related diseases in humans [167], including those discussed here but also other such as type-2 diabetes [176] and osteoporosis [177]. Despite this evidence, however, some key questions remain unanswered, such as those regarding the temporal occurrence of oxidative stress in the development of a specific disease, or the reasons for the lack of convincing, beneficial effects of antioxidant therapy in lowering incidence of cardiovascular [178] or neurodegenerative diseases [179].

One central issue that should be addressed when conducting a study on the oxidant/antioxidant balance of the organism in aging and in various disease states is the inclusion of

the measurement of an appropriate index of oxidative damage. This is extremely important, especially in light of the lack of possibility to evaluate qualitatively and quantitatively oxidative stress in human target tissues (brain, heart). For all diseases discussed in this review, the possibility that a biochemical stress other than the oxidative one – e.g. nitrative or chlorine stresses – occurs is never to be excluded, and this is also a factor that should be taken into account. Another biochemical issue able to exert a great influence on oxidant processes is the one related to non-antioxidant activities of antioxidant compounds, or else the biological functions of antioxidant metabolites. Since, as discussed in detail elsewhere [178], a condition of oxidative stress in the organism is influenced by a multitude of parameters including nutrition, comorbidity, drug therapy and lifestyle, each of these should be monitored. In particular, the evaluation of gene-nutrition interactions by means of nutrigenetic and nutrigenomic tools might fruitfully accompany the study of antioxidant effects in oxidative stress-related states.

From a pragmatic point of view – and while waiting that a larger number of well-performed studies leads to more clear-cut results on oxidative stress-lowering possibilities and significance – the importance of preventive strategies against age-related diseases should not be forgotten. The 2002 World Health Report [180] – describing the results of the WHO global burden of disease (GBD) 2000 Study [181] – suggests that increasing individual fruit and vegetable consumption could contribute to reducing the worldwide burden of disease for ischemic heart disease and ischemic stroke by 30 and 19%, respectively [181]. This report, along with the evidence that primary intervention is cost-efficient and of proven effectiveness [182], strongly encourage to effect lifestyle change and reduce prevalence of risk factors even in later life. For these reasons, the National Cancer Institute and the National Research Council recommend 5–9 servings of antioxidant-rich fruits and vegetables per day be part of daily nutrition, and the “at least five portions a day” (400–600 g fruits and vegetables/day) message has been used by the Europe Against Cancer program and adopted by several European large-scale public health campaigns. Nevertheless, 80% of American children and adolescents [183], almost 70% of American adults [184] as well as the populations of eight out of ten European countries [185] do not meet the recommended intake.

In conclusion, while the role of oxidative stress in aging, neurodegenerative and vascular diseases is getting more and more accepted, the value of antioxidant strategies, particularly with supplements, is still unclear although a well-balanced diet seems undoubtedly important. No single best biomarker of oxidative stress is available at the moment. A combination of at least two biomarkers of oxidative stress (appropriately chosen, i.e., indexes of lipid peroxidation in cardiovascular diseases) with the antioxidant profile (i.e. a broad spectrum of circulating antioxidant micronutrients such as Vitamins C and E and carotenoids) may give the most accurate information on the oxidant/antioxidant balance of the organism as well as on the nutritional needs of the patients and on the possible antioxidant strategies. More reliable and sensitive biomarkers of oxidative stress, furthermore, will be helpful in assessing the efficacy of antioxidant treatment or of antioxidant-rich nutritional intervention.

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