Mol Cell Biochem (2010) 345:91–104
DOI 10.1007/s11010-010-0563-x

Metals, oxidative stress and neurodegenerative disorders

Klaudia Jomova · Dagmar Vondrakova · Michael Lawson · Marian Valko

Received: 15 June 2010 / Accepted: 29 July 2010 / Published online: 22 August 2010

Abstract The neurodegenerative diseases, Alzheimer's disease (AD) and Parkinson's disease (PD), are age-related disorders characterized by the deposition of abnormal forms of specific proteins in the brain. AD is characterized by the presence of extracellular amyloid plaques and intraneuronal neurofibrillary tangles in the brain. Biochemical analysis of amyloid plaques revealed that the main constituent is fibrillar aggregates of a 39–42 residue peptide referred to as the amyloid-β protein (Aβ). PD is associated with the degeneration of dopaminergic neurons in the substantia nigra pars compacta. One of the pathological hallmarks of PD is the presence of intracellular inclusions called Lewy bodies that consist of aggregates of the presynaptic soluble protein called α-synuclein. There are various factors influencing the pathological depositions, and in general, the cause of neuronal death in neurological disorders appears to be multifactorial. However, it is clear, that the underlying factor in the neurological disorders is increased oxidative stress substantiated by the findings that the protein side-chains are modified either directly by reactive oxygen species (ROS) or reactive nitrogen species (RNS), or indirectly, by the products of lipid peroxidation. The increased level of oxidative stress in AD brain is reflected by the increased brain content of iron (Fe) and copper (Cu) both capable of stimulating free radical formation (e.g. hydroxyl radicals via Fenton reaction), increased protein and DNA oxidation in the AD brain, enhanced lipid peroxidation, decreased level of cytochrome c oxidase and advanced glycation end products (AGEs), carbonyls, malondialdehyde (MDA), peroxynitrite, and heme oxygenase-1 (HO-1). AGEs, mainly through their interaction with receptors for advanced glycation end products (RAGEs), further activate signaling pathways, inducing formation of proinflammatory cytokines such as interleukin-6 (IL-6). The conjugated aromatic ring of tyrosine residues is a target for free-radical attack, and accumulation of dityrosine and 3-nitrotyrosine has also been reported in AD brain. The oxidative stress linked with PD is supported by both postmortem studies and by studies showing the increased level of oxidative stress in the substantia nigra pars compacta, demonstrating thus the capacity of oxidative stress to induce nigral cell degeneration. Markers of lipid peroxidation include 4-hydroxy-trans-2-nonenal (HNE), 4-oxo-trans-2-nonenal (4-ONE), acrolein, and 4-oxo-trans-2-hexenal, all of which are well recognized neurotoxic agents. In addition, other important factors, involving inflammation, toxic action of nitric oxide (NO·), defects in protein clearance, and mitochondrial dysfunction all contribute to the etiology of PD. It has been suggested that several individual antioxidants or their combinations can be neuroprotective and decrease the risk of AD or slow its progression. The aim of this review is to discuss the role of redox metals Fe and Cu and non-redox metal zinc (Zn) in oxidative stress-related etiology of AD and PD. Attention is focused on the metal-induced formation of free radicals and the protective role of antioxidants [glutathione (GSH), vitamin C (ascorbic acid)],

K. Jomova (✉)
Department of Chemistry, Faculty of Natural Sciences, Constantine The Philosopher University, SK-949 74 Nitra, Slovakia
e-mail: kjomova@ukf.sk

D. Vondrakova
Department of Cardiology, Na Homolce Hospital, CZ-150 00 Prague, Czech Republic

M. Lawson · M. Valko (✉)
Faculty of Chemical and Food Technology, Slovak Technical University, SK-812 37 Bratislava, Slovakia
e-mail: marian.valko@stuba.sk

vitamin E (α-Tocopherol), lipoic acid, flavonoids [catechins, epigallocatechin gallate (EGCG)], and curcumin. An alternate hypothesis topic in AD is also discussed.

Keywords Iron · Copper · Zinc · Alzheimer's disease · Parkinson's disease · Oxidative stress · Free radicals · Antioxidants

Introduction

For two decades, the primary target in studies of Alzheimer disease has been centered on Amyloid beta (Aβ). Aβ is a peptide of 39–43 amino acids that appear to be the main constituent of amyloid plaques in the brains of Alzheimer's disease (AD) patients [1–5]. In fact, during the past 20 years, the amyloid cascade hypothesis has become the "Null Hypothesis" [6]. However, there is a group of scientists advocating the "Alternate Hypothesis," stating that amyloid-β (Aβ), while involved in the pathogenesis of disease, is not an initiating event but, rather, is a secondary event of the disease [7]. Regardless of this dilemma, many studies have arrived at the conclusion, that the Aβ peptide is capable of generating free radicals through the generation of hydrogen peroxide, as well as of stimulating inflammatory cells. In fact, the vast majority of articles reporting on the pathology of AD and to a lesser extent also to Parkinson's disease (PD), related the origin of these neurodegenerative disorders with direct evidence supporting increased oxidative stress of the brain [8–10].

Besides accumulation of Aβ and increased inflammatory markers, neurofibrillary tangles represent one of the pathologic hallmarks of the AD brain composed primarily of an aberrant form of the tau protein, a highly soluble microtubule-associated protein [4]. In addition, many lines of evidence suggest that mitogenic signaling and oxidative stress play a significant role in the pathogenesis of the disease. The main features of enhanced oxidative stress in the AD brain involve increased brain content of Cu and Fe capable of stimulating free radical generation, increased protein and DNA oxidation in the AD brain, enhanced lipid peroxidation in the AD brain, decreased levels of cytochrome c oxidase in the brain in AD, and advanced glycation end products (AGEs), carbonyls, malondialdehyde, peroxynitrite, and heme oxygenase-1 (OH-1) [11].

PD is associated with the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc). One of the pathological hallmarks of PD is the presence of intracellular inclusions called Lewy bodies that consist of aggregates of the presynaptic soluble protein called α-synuclein. Relatively high levels of basal oxidative stress in the SNc have been explored in the normal brain, but this has been found to be increased in PD patients. The features of enhanced oxidative stress linked with PD are supported by both, postmortem studies and by studies demonstrating the capacity of oxidative stress to induce nigral cell degeneration [12]. In addition, other important factors, involving inflammation, toxic action of nitric oxide, defects in protein clearance, and mitochondrial dysfunction all contribute to the etiology of PD [13].

The aim of this article is to give an overview of the role of oxidative stress in neurological disorders, AD and PD, with the emphasis on metallo-neurobiology and the role of antioxidant protective mechanisms. The role of redox (Cu, Fe) and non-redox metals (Zn) in the etiology of neurodegeneration will be discussed.

Biochemistry of metal-induced oxidative stress

Free radicals are molecules or molecular fragments containing one or more unpaired electrons. This unpaired electron(s) usually gives a considerable degree of reactivity to the free radical. The most important class of radicals generated in living systems represents those derived from oxygen [14]. These involve the superoxide anion radical ($O_2^{\bullet -}$), arising either through metabolic processes or following oxygen "activation" by physical irradiation. The superoxide radical is considered as the "primary" ROS, capable of further interaction with other molecules to generate "secondary" ROS. This could be achieved either directly or prevalently through enzyme- or metal-catalyzed processes. The superoxide radical ion does not react directly with polypeptides, sugars, or nucleic acids, and its ability to peroxidise lipids is controversial. Superoxide is depleted by a dismutation reaction [15]:

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \quad (1)$$

SOD enzymes accelerate this reaction in biological systems by about four orders of magnitude over free aqueous solution. The SOD enzymes work in conjunction with H_2O_2-removing enzymes, such as catalases and glutathione peroxidases.

The generation of free radicals is tightly linked with the participation of redox-active trace metals. The redox state of the cell is largely linked to iron (and copper) redox couples and is maintained within strict physiological limits. It has been documented that iron regulation ensures that there is no free intracellular iron; however, in vivo, under stress conditions, an excess of superoxide acts as an oxidant of [4Fe–4S] cluster-containing enzymes releasing "free iron" from iron-containing molecules [16]. In fact, the release of iron by superoxide has been demonstrated for [4Fe–4S] cluster-containing enzymes of the dehydratase-lyase family. The released Fe^{2+} can participate in the Fenton reaction, generating highly reactive hydroxyl radical

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^{-} \quad (2)$$

The superoxide radical participates in the Haber–Weiss reaction [17]

$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + {}^{\bullet}OH + OH^{-} \quad (3)$$

which combines a Fenton reaction (2) and the reduction of Fe^{3+} by superoxide, yielding Fe^{2+} and oxygen

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \quad (4)$$

Cells of the immune system produce both the superoxide anion and nitric oxide during the oxidative burst triggered during inflammatory processes. Under these conditions, nitric oxide and the superoxide anion may react together to produce significant amounts of a much more oxidatively active molecule, peroxynitrite anion ($ONOO^{-}$), which is an oxidizing free radical that can cause DNA fragmentation and lipid oxidation [18, 19]:

$$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^{-} \quad (5)$$

Reaction (5) has one of the highest rate constants known for reactions of $NO^{\bullet}$, $7.0 \times 10^9\ M^{-1}\ s^{-1}$. Thus, $NO^{\bullet}$ toxicity is linked to its ability to combine with superoxide anions.

Nitric oxide readily binds certain transition metal ions; in fact many physiological effects of $NO^{\bullet}$ are exerted as a result of its initial binding to Fe(II)-Heme groups in the enzyme *guanylate cyclase* [20]

$$Fe(II) + NO^{\bullet} \rightarrow Fe(II) - NO \quad (6)$$

Nitric oxide reacts fast with many radicals, e.g., with the tyrosyl radical. By contrast, nitric oxide is generally unreactive with most non-radicals.

Metallo-biology of Alzheimer's disease

The role of copper

As described above, the "null hypothesis" in studies of AD has been centered on Aβ [21]. Individuals with genetic alterations in one of the genes that code three transmembrane proteins, amyloid precursor protein (APP), presenilin-1 (PS1), and presenilin-2 (PS2), deposit large amounts of the amyloid fragment Aβ(1–42). Enhanced production of Aβ, as a preventive antioxidant for brain lipoproteins under the action of increased oxidative stress and neurotoxicity in ageing, is postulated to represent a major event in the development of AD [22].

The central tenet of Aβ toxicity is linked with the presence of redox metals, mainly copper and non-redox zinc [3, 11, 23]. There are three major copper transport pathways for copper imported into neuronal cells by high-affinity copper transporter Ctr1. Copper delivery to cuproenzymes from Ctr1 is mediated via known metallochaperone pathways, including (i) Atox1 to ATP7A for copper incorporation into cuproenzymes, (ii) Cox17 to Cox11 for copper incorporation into cytochrome c oxidase, and (iii) copper chaperone for superoxide dismutase (CCS) to Cu, Zn-SOD [23]. In addition, sequestration of intracellular copper involves glutathione, which can mediate transfer of copper to metallothionein.

The role of metals in Aβ has been substantiated by the recent findings based on the application of the three ion beam techniques, namely, scanning transmission ion microscopy, Rutherford back scattering spectrometry, and particle induced X-ray emission in conjunction with a high energy (MeV) proton microprobe. These techniques have shown that there is an increase in the metal concentrations within the amyloid plaques compared with the surrounding tissue as follows: iron (85 ppm compared with 42 ppm), copper (16 ppm compared to 6 ppm), and zinc (87 ppm compared to 34 ppm) [24].

The Aβ peptide toxicity depends on its conformational state and peptide length [25]. It is known, that Aβ aggregates into two different conformational states: (i) the non-β-sheet, an amorphous, non-fibrillar, state and (ii) the β-sheet, a highly ordered, fibrillar, state. While the non-β-sheet is benign, the highly ordered, and fibrillar Aβ is cytotoxic [26]. Aggregation of Aβ is fundamental to Aβ-mediated neurotoxicity. It is now widely acknowledged that species of soluble Aβ oligomers are the most toxic form of the peptide. The aggregated state and structure of Aβ peptide are influenced by the concentration of peptide, pH, and concentration of copper, zinc, and iron ions. The neurotoxicity of Aβ depends also on peptide length, with Aβ(1–42) being more toxic than Aβ(1–40). Aβ(1–40) represents the most common species, but Aβ(1–42) is the most abundant species in amyloid plaques and is the most likely candidate to generate hydrogen peroxide and other reactive species. An increase in the Aβ(1–42) to Aβ(1–40) ratio is associated with AD. The affinity for Cu^{2+}, neurotoxicity, and the propensity to aggregate for various Aβ species is greater for Aβ(1–42) than Aβ(1–40).

Copper is known to bind to Aβ with a high affinity via histidine (His13, His14, and His6) and tyrosine (Tyr10) residues [27]. Copper in abnormally high concentrations, and markers indicating oxidative stress have also been found in amyloid plaques. In addition to Cu(II), Aβ also binds Zn(II) and Fe(III) in vitro and the amounts of these metals are also markedly elevated in the neocortex and especially enriched in amyloid plaque deposits in individuals with AD. Zn(II) precipitates Aβ in vitro and Cu(II) interaction with Aβ promotes its neurotoxicity which correlates with the metal reduction [Cu(II) → Cu(I)] and the generation of hydrogen peroxide. Cu(II) promotes the

neurotoxicity of Aβ with the greatest effect for Aβ (1–42) > Aβ (1–40), corresponding to the capacity to reduce Cu(II) to Cu(I), respectively and form hydrogen peroxide. [28]. The copper complex of Aβ(1–42) has a highly positive reduction potential, characteristic of strongly reducing cupro-proteins.

The crucial factor in Aβ toxicity in the pathogenesis of AD has been linked with the idea, that Aβ peptide is capable of forming free radicals through the generation of hydrogen peroxide [23]. The clear evidence that Aβ toxicity is linked with the formation of ROS and consequently with increased oxidative stress has been documented by the attenuated toxicity after administration of antioxidants, such as vitamin E (α-tocopherol) and various free radical scavengers [29].

The significance of oxidative damage in AD is seen by the up-regulation of antioxidant enzymes. Heme oxygenase-1 (HO-1) is among the most sensitive and selective indicators of the cellular oxidative stress response in AD, and it has been demonstrated that both HO-1 protein and its mRNA are elevated in brains of AD patients [30]. RNA has been recently recognized as a major target of oxidation in AD (and also in PD) [31]. Together with the mitochondria dysfunction in AD, the cytoplasmic predominance of neuronal 8-hydroxy-Guanine supports mitochondria as the major source of ROS responsible for RNA oxidation.

In vivo experiments evidenced that pro-oxidant properties of Aβ are substantiated by the amyloid deposits associated with oxidative damage. Thus, while it is clear, that Aβ directly or indirectly promotes oxidative stress and that subsequent toxicity can be reduced by the antioxidants, the exact mechanism linking the amyloid depositions and increased oxidative stress is not yet clear. The protein deposition triggers a chronic inflammatory response in AD patients accompanied by activated microglia releasing free radicals as part of the respiratory burst.

The prevalence view that Aβ is itself toxic, has been recently reassessed, and the results have shown that Aβ is not toxic in the absence of redox metal ions [32]. Thus, the oxidative damage of Aβ is directly linked with the presence of redox metals, copper, and iron. Aβ has unusual high affinity for both transition metal ions copper and iron and has the capacity to reduce both these metals and subsequently produce hydrogen peroxide and oxidized amyloid.

A variety of oxidized Aβ species are enriched in amyloid plaques. Methionine35 (Met35) is particularly abundant in AD brain, which is consistent with the high propensity of the methionine sulfur atom to oxidation. Oxidative modifications of Aβ at other residues (histidine, lysine, and tyrosine) have also been identified. In particular, the conjugated aromatic ring of Tyr10 is a target for free-radical attack, and accumulation of dityrosine and 3-nitrotyrosine has been reported in AD brain.

As mentioned above, it has been shown that the N-terminal residues of His13, His14, His6, and Tyr10 are involved in the complexation of Cu in Aβ [33]. It has recently been proposed that N-terminally complexed Cu(II) is reduced by electrons originating from the C-terminal Met35 residues according to the reaction

$$\mathrm{MetS} + \mathrm{A}\beta - \mathrm{Cu(II)} \leftrightarrow \mathrm{MetS}^{\bullet+} + \mathrm{A}\beta - \mathrm{Cu(I)} \qquad (7)$$

forming the sulfide radical of Met35 ($\mathrm{MetS}^{\bullet+}$) and reducing Cu(II). Stoichiometrically, the process of reduction requires the one-electron oxidation of Methionine to its radical cation, $\mathrm{MetS}^{\bullet+}$ and it appears that specific chemical properties of $\mathrm{MetS}^{\bullet+}$ play an important role in the process underlying Aβ neurotoxicity and free radical formation.

While thermodynamic calculations based on the reduction potentials of the Cu(II)/Cu(I) and Met/$\mathrm{MetS}^{\bullet+}$ couples show that the reaction (7) is rather unfavorable, electron transfer between MetS and Aβ-Cu(II) may be accelerated by the subsequent exergenic reaction of deprotonation of $\mathrm{MetS}^{\bullet+}$, leaving behind the 4-methylbenzyl radical, thus making the reaction (7) viable in vivo [23].

The Methionine sulfide radical cations may stabilize their structure via sulfur–sulfur (S:S) three electron bond formation [33]. Theoretical calculations indicate the possibility of (S.:O) bond formation in Aβ. The notation .: indicates a three electron bond, in which two electrons are located in a bonding σ-orbital and one electron is located in a σ^* antibonding orbital. The sulfide radical $\mathrm{MetS}^{\bullet+}$ may also undergo very fast reactions with e.g., superoxide radical anions, originating from the reaction (8). This reaction leads to the formation of Met-sulphoxide (MetSO) which has been isolated from AD senile plagues [23, 33–35]

$$\mathrm{MetS}^{\bullet+} + \mathrm{O}_2^{\bullet-} \xrightarrow{\mathrm{Met}} 2\mathrm{MetSO} \qquad (8)$$

Methionin35 is strongly related to the pathogenesis of AD, since it represents the residue in Aβ most susceptible to oxidation in vivo. It has been proposed that Met35 oxidation to MetSO reduces toxic and pro-apoptotic effects of the Aβ protein fragment on isolated mitochondria.

Very recent spectroscopic results on rectification of the role of Met35 as a reducing agent in metallo-biology of AD using Aβ(1–20) and Aβ(1–16) fragments have been presented [36]. Since fragments Aβ(1–20) and Aβ(1–16) do not contain Methionine and the reduction process from copper(II) to copper(I) could not take place, the unexpectedly observed metal-centered oxidative catalysis of Copper-Aβ(1–20)/Aβ(1–16) cast doubts on the longstanding proposed redox role of Met35 in Aβ. Thus, if Met does not act as a reducing agent for the copper(II), then the observed oxidative catalysis of Cu-Aβ(1–20) must proceed via a non-redox mechanism.

Aβ-mediated reduction of $Cu^{2+} \rightarrow Cu^{+}$ using external sources of biological reductants such as cholesterol, fatty acids, dopamine, ascorbate, and others preserves the net oxidation of the peptide. Catalytic oxidation by the Cu^{2+}–Aβ complex includes mainly cholesterol and fatty acid chains forming oxysterols and lipid peroxidation products, such as 4-HNE that accumulate in AD and APP transgenic mice brains. In vitro studies demonstrated that oxidatively modified lipids can contribute to AD pathogenesis by promoting Aβ oligomerization [27].

Lipid peroxidation process, mediated by ROS, generates a variety of reactive carbonyl compounds. These carbonyl compounds can further react with cellular proteins, lipids, and DNA resulting in protein crosslinking and DNA damage. A variety of advanced glycation and advanced lipid peroxidation products (AGEs and ALEs) are produced during these complex reactions [4, 27]. AGEs and ALEs, mainly through their interaction with receptors for advanced glycation end products (RAGEs), further activate signaling pathways, inducing formation of proinflammatory cytokines such as interleukin-6 (IL-6) [23].

ROS-mediated lipid peroxidation of polyunsaturated fatty acids gives rise to formation of several reactive α,β-unsaturated aldehydes, among which 4-hydroxy-*trans*-2-nonenal (HNE), 4-oxo*trans*-2-nonenal (4-ONE), acrolein, and 4-oxo-*trans*-2-hexenal, all of which are well recognized neurotoxic agents.

Under in vitro conditions, the presence of copper and iron ions in the vicinity of Aβ catalyzes the formation of hydrogen peroxide further catalytically converted to the reactive hydroxyl radical [28]. In vitro studies have shown that chelation of copper and iron by a suitable ligand inhibited the formation of ROS. An effective chelator clioquinol (CQ, 5-chloro-7-iodoquinolin-8-ol), hydroxyquinoline antibiotic was found to be an effective high-affinity chelator in blocking the formation of hydrogen peroxide by Aβ [37–39]. Furthermore, it prevented precipitation of synthetic Aβ by zinc and copper ions and the release of Aβ (at least) from postmortem AD brains. Treatment of Chinese hamster ovary cells overexpressing amyloid precursor protein with CQ and Cu^{2+} or Zn^{2+} resulted in 85–90% reduction of secreted Aβ-(1–40) and Aβ-(1–42) compared with untreated controls [40]. Analogous effects were observed in amyloid precursor protein-overexpressing neuroblastoma cells. In addition, the effect of added CQ and Cu^{2+} on secreted Aβ led to rapid degradation through up-regulation of matrix metalloprotease-2 and matrix metalloprotease-3 [41, 42].

The role of Zinc

The role of zinc in the etiology of AD is very intriguing. A growing number of reports indicate that zinc in micromolar concentration inhibits Aβ-induced toxicity [28, 43]. The exact mechanisms of the protective effect of zinc against Aβ toxicity are unclear; however, one of the reasons might be cytoprotection through blockage of the membrane calcium channel pore formed by Aβ(1–40).

An additional role of zinc in AD is linked with copper. In the context of AD, the zinc has a clear relationship with copper [28]. The argument advocating the protective role of zinc is its competition with copper (or iron) to bind to Aβ. Binding of zinc to Aβ changes its conformation to the extent that copper ions cannot reach its metal-binding sites. Preventing copper from interacting with Aβ may preclude the Cu-Aβ induced formation of hydrogen peroxide and free radicals.

On the other hand, a trigger caused by endogenous (genetic) and exogenous (e.g., environmental) factors results in oxidative and nitrosative stress which in turn leads to abnormal metabolism of Aβ accompanied by uncontrolled flooding of the vesicular zinc pool [44]. Thus, while low levels of zinc protect against Aβ toxicity, the excess of zinc released by oxidants could trigger neuronal death that is independent or even synergistic with the toxic effect of Aβ. This conclusion is in agreement with other studies documenting that at higher concentrations of zinc its binding to Aβ force the Aβ to precipitate over a wide range of pH (6–8) [45]. Zinc binding has been found to preserve the α-helical conformation of Aβ(1–40) and highly ordered conformational state of Aβ(1–40) upon binding of zinc and has been interpreted as producing toxic, fibrillar, Aβ aggregates. Consequently, immunological/inflammatory responses to nonsoluble Aβ plagues are disruption of zinc homeostasis followed by uncontrolled cerebral zinc release, typical for oxidative stress. Thus, it can be hypothesized that under normal physiological conditions a sensitive balance exists between zinc, copper and Aβ metabolism. However, oxidative and nitrosative stress may perturb this balance which leads to uncontrolled zinc elevation and amyloid deposition. Uncontrolled accumulation of zinc or Aβ may lead to zinc-induced and Aβ-mediated oxidative stress and cytotoxicity.

The hypercholesterolemia is also a potential trigger of AD, and is thought to increase brain levels of Aβ and iron [46]. The experimental study using laboratory animals has shown that iron preferentially accumulates around Aβ plaques in the adjacent cortex, but not in the hippocampus [47]. Further, it has been demonstrated that the cholesterol diet-induced apoptosis is mediated by the activation of the endoplasmic reticulum stress pathway, involving the downregulation of the endoplasmic reticulum chaperones, calreticulin, grp78 and grp94, and the activation of the growth arrest DNA damage protein, gadd153. These results suggest that BBB damage and disturbances in iron metabolism may render the cortex more vulnerable than the hippocampus to the cholesterol-induced cellular stress.

Alzheimer's disease and antioxidants

Vitamin C

Vitamin C (ascorbic acid) is regarded as the major aqueous phase antioxidant. Ascorbic acid has two ionisable hydroxyl groups and, therefore, is a di-acid ($AscH_2$). At physiological pH, 99.9% of Vitamin C is present as $AscH^-$, and only very small proportions as $AscH_2$ (0.05%) and Asc^{2-} (0.004%). The antioxidant chemistry of Vitamin C is thus the chemistry of $AscH^-$ (Fig. 1) [48–50].

Recent evidence suggests that vitamin E (α-tocopherol) and ascorbic acid function together in a cyclic-type of process [17]. During the antioxidant reaction, α-tocopherol is converted to an α-tocopherol radical by the donation of a labile hydrogen to a lipid or lipid peroxyl radical [51]. The α-tocopherol radical can thus be reduced to the original α-tocopherol form by ascorbic acid.

Recently, it has been reported that neurotoxic forms of Amyloid-β, Aβ(1–42), Aβ(1–40), and also Aβ(25–35) stimulated copper-mediated oxidation of ascorbate, whereas nontoxic Aβ(40–1) did not [52–54]. Based on this study, it was concluded that toxic Aβ peptides stimulate copper-mediated oxidation of ascorbate ($AscH^-$) and generation of hydroxyl radicals; therefore, cupric-amyloid peptide-stimulated free radical generation may be involved in the pathogenesis of AD. This can be described by the following set of equations

$$\begin{aligned}
&A\beta - Cu(II) + AscH^- \leftrightarrow A\beta - Cu(I) + Asc^{\bullet -} + H^+ \\
&A\beta - Cu(II) + Asc^{\bullet -} \leftrightarrow A\beta - Cu(I) + Asc \\
&A\beta - Cu(I) + H_2O_2 \rightarrow A\beta - Cu(II) \\
&\quad + {}^{\bullet}OH + OH^- \text{ (Fenton)} \\
&A\beta - Cu(I) + \ O_2 \ \leftrightarrow \ A\beta - Cu(II) + \ O_2^{\bullet -}
\end{aligned} \tag{9}$$

In the presence of oxygen or H_2O_2, Cu(I) may catalyze free radical oxidation of the peptide *via* the Fenton reaction.

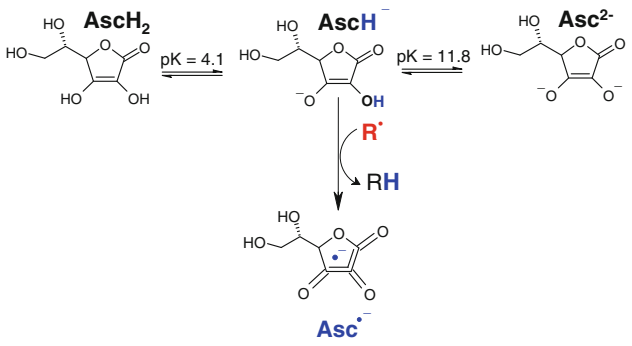

Fig. 1 Forms of Vitamin C at various pH

Ascorbate levels in plasma in AD patients have been found to be decreased as compared to control patients, in levels corresponding to dementia [55–58]. Interestingly, CSF levels of ascorbate were found to be decreased in AD patients as compared to control subjects which may hinder the reduction of α-tocopherol radical back to α-tocopherol [59]. The synergistic vitamins C and E were chosen in a study in which 400 IU vitamin E and 1000 mg vitamin C were given daily to AD patients [60]. The combination of both vitamins E and C led to increase of vitamins E and C in plasma and CSF, making thus CSF and plasma lipoproteins less vulnerable to in vitro oxidation. However, the plasma and CSF of patients given only vitamin E were not protected against in vitro oxidation. This study highlights the importance of the synergism between vitamin E and C in AD patients.

Some in vitro studies explored the pro-oxidant properties of ascorbate (for reviews see [23]). The pro-oxidant effect of ascorbate was attributed to the release of metal ions from damaged cells. However, detailed in vivo studies using appropriate methodologies and biomarkers have shown, that even in the presence of iron, vitamin C predominantly reduced in vivo oxidative damage, despite its well known pro-oxidant properties in vitro in buffer systems containing iron [49].

Vitamin E

Vitamin E is quantitatively the major lipophilic antioxidant in the brain, however, the low concentrations of this antioxidant are observed in cerebrospinal fluid of AD patients, suggesting that supplementation with vitamin E might delay the development of AD [61]. In a placebo-controlled trial, vitamin E (2000 IU/day, 2 years) slowed (−53%) functional deterioration in patients with moderate AD [62]. Recently, combined intake of vitamins E and C supplements was found to be linked with reduced prevalence (−78%) and incidence (−64%) of AD in elderly populations [62]. Vitamin E appears to act in concert with other antioxidants, such as ubiquinol-10, vitamin C, or monomeric Aβ to protect against oxidative damage. Whereas monomeric Aβ can function as a preventive antioxidant by chelating redox-active metals, such as iron and copper, both ubiquinol-10 and vitamin C are able to recycle α-tocopheroxyl radicals back to α-tocopherol, regenerating thus the vitamin E. Such combinations of preventive and chain-breaking antioxidants could prevent brain lipoproteins against oxidative stress.

Some conflicting reports for the clinical efficiacy of lipid soluble vitamins E, C, and beta-carotene may be due to the variations in uptake and local concentrations for effected tissues [63]. In addition, the differences between genetic, environmental, and behavioral differences of individuals

play a role. The pro-oxidant, antioxidant, or non-antioxidant properties attributed to vitamin E under in vitro conditions have not proven to take place in vivo and it is not clear if and at what time in life, or what amounts and combinations of vitamin E may be the most beneficial in preventing neurodegenerative disorders [63].

It appears that vitamin E treatments should be started much earlier, continue for a longer period, and be consumed together with vitamin C for its effect to become measurable in AD patients. Noteworthy, α-Tocopherol is beginning to reveal important, non-antioxidant, cell signaling functions [61]. It is possible that novel reactions and novel genes, found to be under α-tocopherol control, may help and clarify the relationships between molecular and clinical events in AD.

It can be concluded, that intake of vitamins E and C under certain conditions may lower the risk of developing and progression of AD. This conclusion is consistent with the view that oxidative stress underlies the molecular pathogenesis of this dementing disorder.

Thiol antioxidants—glutathione

GSH in the nucleus maintains the redox state of critical protein sulphydryls that are necessary for DNA repair and expression [64].

Modification of protein sulphydryls (Protein-SHs) involves two-electron oxidation yielding sulphenic acids (Protein-SOH) and one-electron oxidation yielding thiyl radicals (Protein-S$^{\bullet}$). The oxidized products react with GSH to form *S*-glutathiolated protein (Protein-SSG), which is reduced further by the glutathione cycle through glutathione reductase and small proteins such as glutaredoxin and thioredoxin, to restore protein sulphydryls (Protein-SHs) [65]. However, if the process of oxidation of protein sulphydryls is not trapped by GSH, then further oxidation leads to the formation of irreversibly oxidized forms such as sulphinic (Protein-SO_2H) and sulfonic (Protein-SO_3H) acids (Fig. 2) [66].

The most significant changes in the level of GSH in AD patients were observed in the synaptosomal fraction. Both mitochondrial and synaptosomal fractions had significant declines in antioxidants (glutathione, glutathione peroxidase, and glutathione-S-transferase) [67]. Levels of oxidative markers significantly correlated with Mini-Mental Status Examination scores. Oxidative stress was more localized to the synapses, with levels increasing in a disease-dependent fashion. These correlations implicate an involvement of oxidative stress in Alzheimer disease-related synaptic loss.

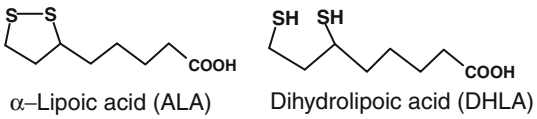

Fig. 3 Structure of α-lipoic acid and dihydrolipoic acid

Thiol antioxidants—Lipoic acid

α-Lipoic acid (ALA), a disulfide derivative of octanoic acid, is a natural compound also referred to as thioctic acid and has the full chemical name 1,2-dithiolane-3-pentanoic acid ($C_8H_{14}O_2S_2$) (Fig. 3). α-Lipoic acid is both water and fat soluble and, therefore, is widely distributed in both cellular membranes and the cytosol [68, 69].

α-Lipoic acid is readily absorbed from the diet and is converted rapidly in many tissues to its reduced dithiol form, dihydrolipoic acid (DHLA) [69]. Lipoic acid has been shown to have a variety of properties which may interfere with the pathogenesis of AD. Aside from its enzymatic cofactor role, in vitro and in vivo studies suggest that LA is a powerful micronutrient with diverse antioxidant properties [70]. LA has been documented to increase acetylcholine production by activation of choline acetyltransferase, increase glucose uptake, scavenge free radicals and by increasing the concentration of reduced glutathione, chelate redox-active metals, suppressing thus the formation of hydrogen peroxide and reactive hydroxyl radicals via Fenton chemistry [71].

The first indication of a beneficial effect of LA in AD patients and related dementias came from a rather

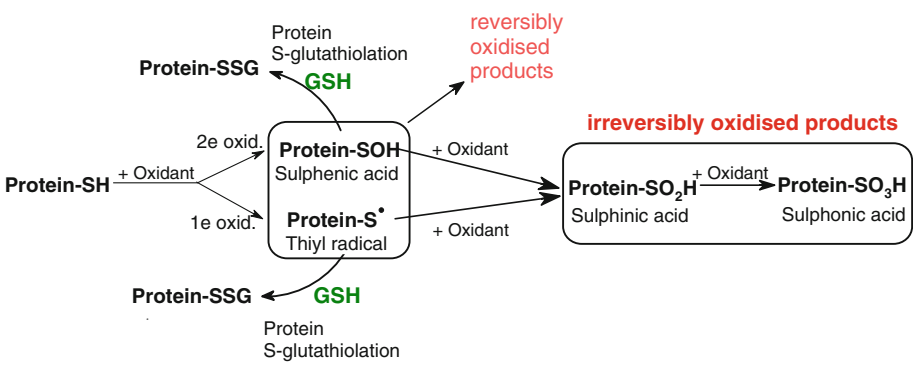

Fig. 2 Protective role of GSH in oxidation of protein sulphydryl groups

surprising case study. 600 mg LA was given daily to nine patients with probable AD (age: 67 ± 9 years) receiving a standard treatment with AChE inhibitors over an observation period of 337 ± 80 days [72]. The cognitive performance of the patients before and after addition of LA to their standard medication was compared. Before initiation of LA treatment, a steady decrease in cognitive performance in the AD assessment scale was observed. Treatment with LA led to a stabilization of cognitive functions, demonstrated by scores in two neuropsychological tests for nearly a year [72]. Further study has been conducted to determine the effect of fish oil and the antioxidant LA on factors in the blood that are associated with the progression of AD. The study was completed in February 2007. However, the results have not been posted yet. For more results see http://www.clinicaltrials.gov/show/NCT00090402.

A long-term therapy of neurological disorders with high doses of LA chelating metals may lead to a decline of metal-containing enzymes, such as insulin degrading enzyme or SOD. In millimolar concentrations, LA and also DHLA are unable to remove the metals from protein active sites [73]. Metal chelator clioquinol has been demonstrated to decrease vitamin B12 pools in brain and blood; therefore, high-dose long-term clinical trials with LA should be carefully monitored in this regard [74].

Various pathways for the management of oxidative stress by GSH and other antioxidants are shown in Fig. 4.

Flavonoids

Catechins

The main flavonoid phytochemical compounds present in green tea are catechins, in particular epigallocatechin gallate (EGCG), in the amount of 30–130 mg per cup of tea [75, 76]. Other polyphenolic compounds such as quercetin, kaempfenol, and myricetin and their glycosides are found in lower concentration. In hippocampal neurons, tea polyphenols show a protective effect against ischemic insult, while neurotoxicity induced by Aβ(1–42), whose deposition in the brain accompanies neuronal loss in AD, was attenuated in the presence of EGCG [77]. The protective antioxidant effect of these natural compounds was also confirmed by other studies in synaptosomes [78].

Epigallocatechin gallate (Fig. 5) is currently being studied for its role as a chemoprotective agent [79–82]. Catechins have been reported to possess chelating ability of divalent metal ions, antioxidant, and anti-inflammatory activities, to penetrate the brain barrier and to protect neuronal death in a wide array of cellular and animal models of neurological disorders [79]. Catechins directly scavenge ROS and RNS and excert indirect antioxidant effects via activation of transcription factors and antioxidant enzymes, modulating thus the cellular redox state.

Structurally important chelating groups in EGCG are the 3′,4′-dihydroxyl group in the B ring as well as the gallate group which may neutralize ferric iron to form redox-inactive iron, protecting thus the neuronal cells against damage [81, 82]. The ability of catechins to neutralize a surplus of free iron may play a direct role in AD which is directly linked with the nature of APP as an iron regulator protein [83, 84]. The reduction of the free-iron labile pools by EGCG chelation may lead to suppression of APP mRNA translation [83]. This is achieved by targeting the IRE-II sequence in the APP-5 untranslated regions (UTR), as was recently shown for desferrioxamine and the amyloid-binding/metal chelating drug XH1 [85]. XH1 has no significant neurotoxicity at micromolar concentrations but acute animal toxicity at high concentrations. XH1 specifically reduced APP protein expression in human SH-SY5Y neuroblastoma cells and attenuated cerebral amyloid pathology in PS1/APP transgenic mice [86].

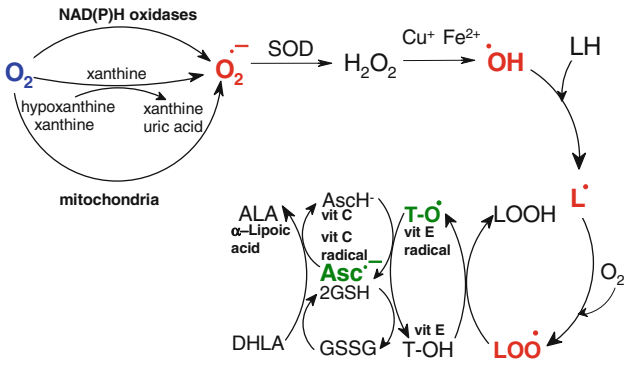

Fig. 4 The various pathways of thiol antioxidants (glutathione, lipoic acid) and vitamin E and vitamin C in the management of oxidative stress

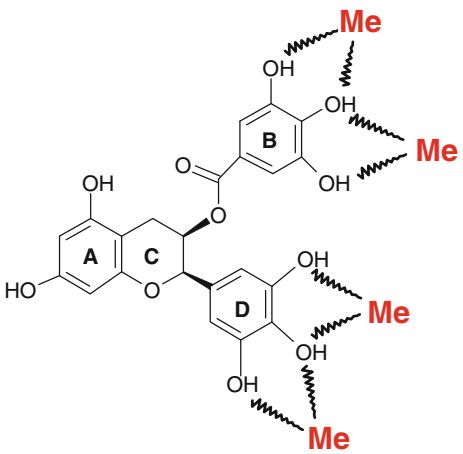

Fig. 5 Potential metal (Me) binding sites in Epigallocatechin gallate

Very recently, it has been shown that EGCG has the ability to convert large, mature Aβ fibrils and alpha-synuclein (see below) into smaller, amorphous protein aggregates that are nontoxic to mammalian cells [80]. Mechanistic studies revealed that the compound directly binds to beta-sheet-rich aggregates and mediates the conformational change without their disassembly into monomers or small diffusible oligomers. These findings suggest that EGCG is a potent remodeling agent of mature amyloid fibrils [80].

Iron chelation by catechins affects not only the post-transciptional regulation of iron homeostasis-related RNAs, but also the induction of genes regulated by the hypoxia inducible factor-1 (HIF-1), one of the key regulators of oxygen homeostasis, regulating the physiological responses to low oxygen levels and the pathophysiology of heart attack, cancer, stroke, and chronic lung disease [87]. Both HIF-1 and IRP2 share a common iron-dependent proteasomal degradation pathway which becomes inactivated by iron chelation. The decrease of the labile iron pool by chelation may lead to the inhibition of prolyl hydroxylases and consequently, in the concerted action activation of both HIF and IRP2 [88].

Curcumin

Curcumin is the principal curcuminoid of the popular Indian spice turmeric and is responsible for the yellow color of turmeric. The aromatic ring systems, which are polyphenols are connected by two α,β-unsaturated carbonyl groups [89].

Curcumin has a neuroprotective effect as documented by a reversal lipid peroxidation in ethanol-induced brain damage in rats. Curcumin also increased glutathione levels and the activity of γ-glutamyl-cysteinyl synthetase and other GSH-linked detoxifying enzymes [90]. Curcumin was shown to protect PC12 cells useful as a model system for neuronal differentiation and human endothelial cells from Aβ(1–42) oxidative insult [91].

Recent epidemiological studies have raised the possibility that the properties of this molecule are responsible for the significantly reduced (4.4-fold) prevalence of AD in India compared to USA [92]. Curcumin administrated to an Alzheimer transgenic APPSw mouse model (Tg2576) for 6 months resulted in a suppression of indices of inflammation and oxidative damage in the brains of these mice. Indeed, a significant decrease of oxidized protein and interleukin-1β, a proinflammatory cytokine usually elevated in the brains of these mice, was observed in association with a 43–50% reduction in insoluble Aβ, soluble Aβ, and plaque burden [93].

"Alternate hypothesis" in AD

The amyloid hypothesis is the best defined and most studied concept in AD [7]. The presence of amyloid plaques is considered as the main feature of AD. Aβ peptides have been identified as the major constituents of plaques. They are protolytically derived products of APP, and cloning the APP gene has allowed the disease to be examined at biochemical and molecular levels [94].

As outlined above, originally it was proposed that the core of the amyloid hypothesis is that the difference between increased production and decreased clearance of Aβ peptides causes the disease [95]. Accumulation of the hydrophobic Aβ40 and Aβ42 peptides results in aggregation and formation of insoluble plaques, which trigger a cascade of deleterious changes, leading to neuronal death and consequently AD [96]. Since the Aβ42 levels are higher in AD patients, it was proposed that increased levels of Aβ42 trigger the cascade of the deleterious events described above [6, 97, 98].

Aβ is known to interact with the signaling pathways that regulate the phosphorylation of the microtubule-associated protein tau [6, 98]. Hyperphosphorylation of tau disrupts its normal function in regulating axonal transport and leads to the accumulation of neurofibrillary tangles and toxic species of soluble tau. Accumulation of all these events results in synaptic dysfunction causing AD.

An alternative view suggests that the deleterious events leading to AD are triggered by Aβ as well as non-Aβ factors [6, 7]. Thus, in this view synaptic dysfunction causing AD is due to the same factors as in the original hypothesis of AD (aberrant activity, synaptic loss, and neuroinflammation). However, their initiation is partly due to the factors not related to Aβ. This is in agreement with the current data on the correlation between Aβ and the disease severity, exploring the fact, that the therapeutical strategies directed at Aβ in the advanced stage of disease are ineffective. It appears that the therapeutical strategies beyond the AD hypothesis, e.g., multiple tau-targeted therapies would be more effective in combating AD.

Parkinson's disease

Parkinson's disease (PD) was first described by James Parkinson in 1817. PD is a chronic progressive neurodegenerative movement disorder characterized by a profound and selective loss of nigrostriatal dopaminergic neurons [99]. Clinical manifestations of PD include motor impairments involving resting tremor, a slowing of physical movement (bradykinesia), postural instability, gait difficulty, and rigidity.

The most striking pathological feature of PD is a progressive loss of dopaminergic neurons in the SNc leading to a dopamine deficit in the striatum [100]. The most probable origin of the etiology of dopaminergic neuronal demise is a combination of genetic susceptibilities and environmental factors. The majority of PD cases are sporadic (90–95%), while familial cases account for 5–10% of PD. One of the pathological hallmarks of PD is the presence of intracellular inclusions called Lewy bodies that consist of aggregates of the presynaptic soluble protein called α-synuclein [101, 102]. The toxic effects of α-synuclein include impaired endoplasmic reticulum (ER) to Golgi vesicular trafficking and ER stress, Golgi fragmentation, sequestration of anti-apoptotic proteins into aggregates, and the formation of pores on cellular membranes [103].

Oxidative stress in PD is especially substantiated by the complex I mitochondrial dysfunction [104]. In fact, α-synuclein decreased mitochondrial complex I activity and increased ROS production in human fetal dopaminergic primary neuronal cultures overexpressing wildtype α-synuclein.

The onset of PD is accompanied by the dramatic depletion of levels of the glutathione in substantia nigra, resulting in a selective decrease in mitochondrial complex I activity (a major hallmark of PD) and a marked reduction in overall mitochondrial function [105]. GSH depletion-driven inhibition of complex I most probably occurs via thiol oxidation of critical residues within the complex itself, as demonstrated by treatment with the thiol-reducing agent dithiothreitol. In fact, the current state of knowledge suggests that mitochondrial complex I inhibition may be the central tenet of sporadic PD. The harm to mitochondrial complex I causes α-synuclein aggregation, which contributes to the death of dopamine neurons.

An evidence for mitochondrial alterations was found in various models overexpressing wildtype or mutant α-synuclein, reduced COX and complex IV activity, a decrease in the mitochondrial membrane potential and oxidation of mitochondria-associated metabolic proteins [106–108]. More than 50% decrease in mitochondrial complex I activity was found both in patients with parkin mutations (see below) and sporadic PD patients, whereas complex IV activity was only reduced in sporadic PD patients. A good marker of protein oxidation, protein nitration of tyrosine residues within the α-synuclein protein has been demonstrated to be elevated in Lewy bodies in cases of PD. Treatment of glutathione-depleted, cultured dopaminergic cells with inhibitors of nitric oxide synthetase (NOS), the enzyme that makes $NO^{\bullet}$, prevents mitochondrial complex I inhibition.

The link between oxidative stress and PD is supported by postmortem analysis showing the oxidative stress-induced nigral cell degeneration [99]. We noted that it has been documented, that the markers of oxidative stress in the SNc in the normal brain are rather high. However, in PD patients, the markers are significantly increased. In addition, other factors, apart of oxidative stress, involving inflammation, toxic action of nitric oxide, excitotoxic mechanisms, as well as mitochondrial dysfunction, all play roles in the etiology of PD [109]. Signs of oxidative damage have been largely observed also in peripheral tissues of PD individuals.

The role of trace metals, in particular, increased iron levels have been reported to be elevated in the PD midbrain, leading to subsequent neurodegeneration [105]. The iron chelation has been shown to be effective in preventing or delaying PD progression. Pharmacologically chelated iron, for example the Fe-clioquinol complex, in a chelated form in which it cannot participate in oxidative events prevents degeneration of dopaminergic midbrain neurons [110]. The consequence of the above mentioned biochemical abnormalities results in aberrant oxidation of dopamine to 6-hydroxydopamine or dopamine-quinone, both neurotoxic either directly or in conjugation with cystein [4]. The entry and release of iron from the iron-storage protein, ferritin, occurs via the "free-iron (ferrous) labile pool," active in Fenton chemistry. Besides superoxide, ferritin iron can be released by 6-hydroxydopamine a neurotoxin implicated in PD.

Following α-synuclein, described as the first gene associated with familial PD, four other genes have conclusively been linked to autosomal recessive (parkin, PINK-1, DJ-1) or dominant (LRRK2) Parkinsonism [111, 112].

Mutations in the LRRK2 gene are the most common cause of genetic PD. The LRRK2 gene encodes a large multidomain protein of 2527 amino acids, including a kinase domain, a Roc domain, a COR domain, a WD40-repeat domain, and leucinerich repeats [113, 114]. Whether LRRK2 has an impact on mitochondrial integrity, needs to be determined.

PINK1 is an N-terminal mitochondrial targeting sequence and a serine/threonine kinase domain containing 581 amino acids [104]. Increased expression of PINK1 is related to protection from apoptosis. Mutations in PINK1 (PTEN-induced putative kinase) gene are the second most common cause of autosomal recessive, early onset Parkinsonism following the parkin mutations [115]. Enhanced PINK1 expression is linked with protection against apoptotic cell death under stress conditions and conversely, loss of PINK1 function increases the propensity of cells to oxidative stress-induced cell death and a dysbalance of calcium homeostasis. The experiments suggest that the impairment of mitochondrial calcium efflux promotes ROS formation that inhibits glucose uptake, resulting in reduced

substrate delivery and respiration [116]. The dysbalance in calcium homeostasis was placed at the top of these events.

The parkin gene represents a cytosolic 465 amino acid protein with a ubiquitin-like domain at the N terminus and a RBR domain close to the C terminus [117]. The pathogenic mutations induce a loss of parkin function, leading to the hypothesis that the accumulation of parkin substrates causes neurotoxicity and results in the death of dopaminergic neurons. Parkin gene expression is up-regulated in various stress examples and has a wide range of neuroprotective capacities, including protection against mitochondrial dysfunction, endoplasmatic reticulum stress, exitotoxicity, proteasome inhibition, and overexpression of α-synuclein, tau, and others.

Future studies of the biochemical interactions between PINK1 and parkin and identification of other components in this pathway are likely to provide insight into PD pathogenesis, and might identify new therapeutic targets [112].

DJ-1 has structural similarities with the stress-inducible *Escherichia coli* chaperone Hsp31 and mutations in the DJ-1 gene (encoding a 189-amino acid protein) are associated with rare cases of early onset autosomal recessive PD [108]. DJ-1 is present in cytosolic, mitochondrial, and nuclear compartments; mitochondrial localization of DJ-1 was enhanced by oxidative stress.

The recent results indicate that the exposure to various environmental toxins acting through oxidative stress seems to be associated with PD [118]. It has been explored, that the loss of DJ-1 leads to striking sensitivity to the herbicide paraquat and the insecticide rotenone, which suggests that DJ-1 may have a role in protection from oxidative stress from environmental toxins. It has been clearly demonstrated that while overexpression of DJ-1 protects neurons from oxidative stress-induced damage, DJ-1 deficiency renders cells more susceptible to oxidative injury. It has been explored that DJ-1 is converted into a more acidic pI variant in response to oxidative stress, due to the formation of cysteine–sulfinic acid at cysteine 106. Observed mitochondrial alterations in parkin- or PINK1-deficient cells could not be compensated by DJ-1, suggesting thus that DJ-1 does not interfere with the PINK1/parkin pathway.

Conclusions

The fact that oxidative stress plays an important role in AD pathogenesis is apparent, given all the evidence the research has recently provided. Markers of oxidative damage, including HO-1, 8-hydroxy-Guanine, oxidative modification of proteins, lipids, and nucleic acids, mainly through lipid peroxidations are increased in the AD brain as compared with controls. Markers of lipid peroxidation detected in AD brains include 4-hydroxy-trans-2-nonenal (HNE), 4-oxo-trans-2-nonenal (4-ONE), acrolein, and 4-oxo-trans-2-hexenal, all of which are well recognized neurotoxic agents.

Markers of lipid peroxidation, including HNE and malondialdehyde, have been identified in the substantia nigra of PD patients. Together with the mitochondria dysfunction in AD and PD, the cytoplasmic predominance of neuronal 8-hydroxy-Guanine supports mitochondria as the major source of ROS responsible for RNA oxidation.

In addition, NFTs and senile plaques are altered in ways characteristic of oxidative damage including AGE-modification, protein cross-linking and carbonyl- and acyl-modification. Although the source of the shift in oxidative homeostasis is still unclear, current evidence points to changes in the balance of redox transition metals, especially iron and copper. Both Fe and Cu are present at significantly elevated levels in AD neuropil, and detection of redox activity in the AD brain can be attenuated by chelators of these key metals. To counter the effects of oxidative stress in these pathologies, therapeutic strategies involving AGE inhibitors and anti-inflammatory antioxidants appear to be most promising.

The future research direction will have to critically examine the importance of redox imbalance in the pathogenesis of AD, and reveal chelating agents that take a primary role in clinical intervention of neurodegenerative diseases.

Acknowledgments We thank the Slovak Grant Agency (Projects VEGA/1/0575/08 and 1/0213/08) for financial support. This study was also supported by the Slovak Research and Development Agency under the contract No. VVCE-0004-07.

References

1. Selkoe DJ (2001) Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 81:741–766
2. Barnham KJ, Masters CL, Bush AI (2004) Neurodegenerative diseases and oxidative stress. Nat Rev Drug Discov 3:205–214
3. Bush AI (2003) The metallobiology of Alzheimer's disease. Trends Neurosci 26:207–214
4. Sayre LM, Smith MA, Perry G (2001) Chemistry and biochemistry of oxidative stress in neurodegenerative disease. Curr Med Chem 8:721–738
5. Varadarajan S, Yatin S, Aksenova M, Butterfield DA (2000) Review: Alzheimer's amyloid beta-peptide-associated free radical oxidative stress and neurotoxicity. J Struct Biol 130: 184–208
6. Lee HG, Zhu XW, Castellani RJ, Nunomura A, Perry G, Smith MA (2007) Amyloid-beta in Alzheimer disease: the null versus the alternate hypotheses. J Pharmacol Exp Ther 321:823–829
7. Pimplikar SW (2009) Reassessing the amyloid cascade hypothesis of Alzheimer's disease. Int J Biochem Cell Biol 41:1261–1268
8. Devi L, Anandatheerthavarada HK (2010) Mitochondrial trafficking of APP and alpha synuclein: relevance to mitochondrial

dysfunction in Alzheimer′s and Parkinson′s diseases. Biochim Biophys Acta 1802:11–19
9. Tillement JP, Lecanu L, Papadopoulos V (2010) Amyloidosis and neurodegenerative diseases: current treatments and new pharmacological options. Pharmacology 85:1–17
10. Block ML, Calderon-Garciduenas L (2009) Air pollution: mechanisms of neuroinflammation and CNS disease. Trends Neurosci 32:506–516
11. Bush AI, Curtain CC (2008) Twenty years of metallo-neurobiology: where to now? Eur Biophys J Biophys Lett 37:241–245
12. Jenner P, Olanow CW (1998) Understanding cell death in Parkinson′s disease. Ann Neurol 44:S72–S84
13. Dawson TM, Dawson VL (2003) Molecular pathways of neurodegeneration in Parkinson's disease. Science 302:819–822
14. Cadenas E, Davies KJA (2000) Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 29:222–230
15. Halliwell B, Gutteridge JMC (2007) Free radicals in biology and medicine, 4th edn. Oxford University Press, Oxford
16. Liochev SI, Fridovich I (1994) The role of O2 in the production of HO: in vitro and in vivo. Free Radic Biol Med 16:29–33
17. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J (2004) Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 266:37–56
18. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84
19. Ghafourifar P, Cadenas E (2005) Mitochondrial nitric oxide synthase. Trends Pharmacol Sci 26:190–195
20. Denninger JW, Marletta MA (1999) Guanylate cyclase and the (NO)-N-/cGMP signaling pathway. Biochim Biophys Acta 1411:334–350
21. Braak H, Braak E (1991) Neuropathological staging of Alzheimer-related changes. Acta Neuropathol 82:239–259
22. Hardy J, Selkoe DJ (2002) Medicine—the amyloid hypothesis of Alzheimer′s disease: progress and problems on the road to therapeutics. Science 297:353–356
23. Valko M, Morris H, Cronin MTD (2005) Metals, toxicity and oxidative stress. Curr Med Chem 11:1161–1208
24. Rajendran R, Ren MQ, Ynsa MD et al (2009) A novel approach to the identification and quantitative elemental analysis of amyloid deposits-insights into the pathology of Alzheimer's disease. Biochem Biophys Res Commun 382:91–95
25. Atwood CS, Moir RD, Huang XD et al (1998) Dramatic aggregation of Alzheimer abeta by Cu(II) is induced by conditions representing physiological acidosis. J Biol Chem 273:12817–12826
26. Chafekar SM, Hoozemans JJM, Zwart R et al (2007) Abeta (1–42) induces mild endoplasmic reticulum stress in an aggregation state-dependent manner. Antioxid Redox Signal 9:2245–2254
27. Hung YH, Bush AI, Cherny RA (2010) Copper in the brain and Alzheimer's disease. J Biol Inorg Chem 15:61–76
28. Cuanjungco MP, Goldstein LE, Nunomura A et al (2000) Evidence that the beta-amyloid plaques of Alzheimer's disease represent the redox-silencing and entombment of A beta by zinc. J Biol Chem 275:19439–19442
29. Halliwell B (2001) Role of free radicals in the neurodegenerative diseases—therapeutic implications for antioxidant treatment. Drugs Aging 18:685–716
30. Premkumar DR, Smith MA, Richey PL, Petersen RB, Castellani R, Kutty RK, Wiggert B, Perry G, Kalaria RN (1995) Induction of heme oxygenase-1 messenger-RNA and protein in neocortex and cerebral vessels in Alzheimer's-disease. J Neurochem 65:1399–1402
31. Nunomura A, Perry G, Zhang J, Montine TJ, Takeda A, Chiba S, Smith MA (1999) RNA oxidation in Alzheimer and Parkinson diseases. J Anti-Aging Med 2:227–230
32. Huang XD, Cuajungco MP, Atwood CS et al (1999) Cu(II) potentiation of Alzheimer abeta neurotoxicity—correlation with cell-free hydrogen peroxide production and metal reduction. J Biol Chem 274:37111–37116
33. Cerpa WF, Barria MI, Chacon MA, Suazo M, Gonzalez M, Opazo C, Bush AI, Inestrosa NC (2004) The N-terminal copper-binding domain of the amyloid precursor protein protects against Cu2+ neurotoxicity in vivo. FASEB J 18:1701
34. Pogocki D (2003) Alzheimer's beta-amyloid peptide as a source of neurotoxic free radicals: the role of structural effects. Acta Neurobiol Exp 63:131–145
35. Schoneich C, Pogocki D, Hug GL, Bobrowski K (2003) Free radical reactions of methionine in peptides: mechanisms relevant to beta-amyloid oxidation and Alzheimer's disease. J Am Chem Soc 125:13700–13713
36. da Silva GF, Lykourinou V, Angerhofer A, Ming LJ (2009) Methionine does not reduce Cu(II)-beta-amyloid!—rectification of the roles of methionine-35 and reducing agents in metal-centered oxidation chemistry of Cu(II)-beta-amyloid. Biochim Biophys Acta 1792:49–55
37. Hider RC, Ma Y, Molina-Holgado F et al (2008) Iron chelation as a potential therapy for neurodegenerative disease. Biochem Soc Trans 36:1304–1308
38. Bush AI (2008) Drug development based on the metals hypothesis of Alzheimer's disease. J Alzheimers Dis 15:223–240
39. Smith DG, Cappai R, Barnham KJ (2007) The redox chemistry of the Alzheimer's disease amyloid beta peptide. Biochim Biophys Acta 1768:1976–1990
40. White AR, Du T, Laughton KM, Volitakis I et al (2006) Degradation of the Alzheimer disease amyloid beta-peptide by metal-dependent up-regulation of metalloprotease activity. J Biol Chem 281:17670–17680
41. Crouch PJ, Tew DJ, Du T et al (2009) Restored degradation of the Alzheimer's amyloid-beta peptide by targeting amyloid formation. J Neurochem 108:1198–1207
42. Ritchie CW, Bush AI, Mackinnon A (2003) Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease—a pilot phase 2 clinical trial. Arch Neurol 60:1685–1691
43. Garai K, Sahoo B, Kaushalya SK et al (2007) Zinc lowers amyloid-beta toxicity by selectively precipitating aggregation intermediates. Biochemistry 46:10655–10663
44. Cuajungco MP, Lees GJ (1998) Nitric oxide generators produce accumulation of chelatable zinc in hippocampal neuronal perikarya. Brain Res 799:118–129
45. Cuajungco MP, Faget KY (2003) Zinc takes the center stage: its paradoxical role in Alzheimer's disease. Brain Res Rev 41: 44–56
46. Ong WY, Halliwell B (2004) Iron, atherosclerosis, and neurodegeneration–a key role for cholesterol in promoting iron-dependent oxidative damage? Ann N Y Acad Sci 1012:51–64
47. Ghribi O, Golovko MY, Larsen B et al (2006) Deposition of iron and beta-amyloid plaques is associated with cortical cellular damage in rabbits fed with long-term cholesterol-enriched diets. J Neurochem 99:438–449
48. Kojo S (2004) Vitamin C: basic metabolism and its function as an index of oxidative stress. Curr Med Chem 11:1041–1064
49. Carr A, Frei B (1999) Does vitamin C act as a pro-oxidant under physiological conditions? FASEB J 13:1007–1024
50. Kasparova S, Brezova V, Valko M et al (2005) Study of the oxidative stress in a rat model of chronic brain hypoperfusion. Neurochem Int 46:601–611
51. Cuzzorcrea S, Thiemermann C, Salvemini D (2004) Potential therapeutic effect of antioxidant therapy in shock and inflammation. Curr Med Chem 11:1147–1162

52. Dikalov SI, Vitek MP, Mason RP (2004) Cupric-amyloid beta peptide complex stimulates oxidation of ascorbate and generation of hydroxyl radical. Free Radic Biol Med 36:340–347
53. Shearer J, Szalai VA (2008) The amyloid-beta peptide of Alzheimer's disease binds Cu-I in a linear bis-his coordination environment: insight into a possible neuroprotective mechanism for the amyloid-beta peptide. J Am Chem Soc 130:17826–17835
54. Ryglewicz D, Rodo M, Kunicki PK, Bednarska-Makaruk M et al (2002) Plasma antioxidant activity and vascular dementia. J Neurol Sci 203–204:195–197
55. Butterfield DA, Castegna A, Pocernich ChB et al (2002) Nutritional approaches to combat oxidative stress in Alzheimer's disease. J Nutr Biochem 13:444–461
56. McGrath LT, McGleenon BM, Brennan S et al (2001) Increased oxidative stress in Alzheimer's disease as assessed with 4-hydroxynonenal but not malondialdehyde. QJM 94:485–490
57. Riviere S, Birlouez-Aragon I, Nourhashemi F (1998) Low plasma vitamin C in Alzheimer patients despite an adequate diet. Int J Geriatr Psychiatry 13:749–754
58. Foy CJ, Passmore AP, Vahidassr MD et al (1999) Plasma chain-breaking antioxidants in Alzheimer's disease, vascular dementia and Parkinson's disease. QJM 92:39–45
59. Schippling S, Kontush A, Arlt S et al (2000) Increased lipoprotein oxidation in Alzheimer's disease. Free Radic Biol Med 28:351–360
60. Kontush A, Mann U, Arlt S et al (2001) Influence of vitamin E and C supplementation on lipoprotein oxidation in patients with Alzheimer's disease. Free Radic Biol Med 31:345–354
61. Petersen RC, Thomas RG, Grundman M et al (2005) Vitamin E and donepezil for the treatment of mild cognitive impairment. N Engl J Med 352:2379–2388
62. Kontush A, Schekatolina S (2006) Vitamin E in neurodegenerative disorders: Alzheimer's disease. Ann N Y Acad Sci 1031:249–262
63. Azzi A, Gysin R, Kempna P, Ricciarelli R, Villacorta L, Visarius T, Zingg J-M (2003) The role of α-tocopherol in preventing disease: from epidemiology to molecular events. Mol Asp Med 24:325–336
64. Masella R, Di Benedetto R, Vari C et al (2005) Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. J Nutr Biochem 16:577–586
65. Ji YB, Akerboom TPM, Sies H, Thomas JA (1999) *S*-nitrosylation and *S*-glutathiolation of protein sulfhydryls by *S*-nitroso glutathione. Arch Biochem Biophys 362:67–78
66. Karoui H, Hogg N, Frejaville C et al (1996) Characterization of sulfur-centered radical intermediates formed during the oxidation of thiols and sulfite by peroxynitrite ESR-spin trapping and oxygen uptake studies. J Biol Chem 271:6000–6009
67. Ansari MA, Scheff SW (2010) Oxidative stress in the progression of Alzheimer disease in the frontal cortex. J Neuropathol Exp Neurol 69:155–167
68. Packer L, Witt EH, Tritschler HJ (1995) Alfa-lipoic acid as a biological antioxidant. Free Radic Biol Med 19:227–250
69. Smith AR, Shenvi SV, Widlansky M, Suh JH, Hagen TM (2004) Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. Curr Med Chem 11:1135–1146
70. Packer L, Tritschler HJ, Wessel K (1997) Neuroprotection by the metabolic antioxidant alpha-lipoic acid. Free Radic Biol Med 22:359–378
71. Holmquist L, Stuchbury G, Berbaum K et al (2007) Lipoic acid as a novel treatment for Alzheimer′s disease and related dementias. Pharmacol Ther 113:154–164
72. Maczurek A, Hagera K, Kenkliesa K et al (2008) Lipoic acid as an anti-inflammatory and neuroprotective treatment for Alzheimer′s disease. Adv Drug Deliv Rev 60:1463–1470
73. Suh JH, Zhu BZ, De Szoeke E, Frei B, Hagen TM (2004) Dihydrolipoic acid lowers the redox activity of transition metal ions but does not remove them from the active site of enzymes. Redox Rep 9:57–61
74. Cavalli A, Bolognesi ML, Minarini A et al (2008) Multi-target-directed ligands to combat at neurodegenerative diseases. J Med Chem 51:347–372
75. Rice Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20:933–956
76. Thielecke F, Boschmann M (2009) The potential role of green tea catechins in the prevention of the metabolic syndrome–a review. Phytochemistry 70:11–24
77. Choi YT, Jung CH, Lee SR et al (2001) The green tea polyphenol (−)-epigallocatechin gallate attenuates beta-amyloid-induced neurotoxicity in cultured hippocampal neurons. Life Sci 7:603–614
78. Guo QN, Zhao BL, Li MF, Shen SR, Xin WJ (1996) Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. Biochim Biophys Acta 1304:210–222
79. Mandel S, Amit T, Reznichenko L (2006) Green tea catechins as brain-permeable, natural iron chelators-antioxidants for the treatment of neurodegenerative disorders. Mol Nutr Food Res 50:229–234
80. Bieschke J, Russ J, Friedrich RP, Ehrnhoefer DE, Wobst H, Neugebauer K (2010) EGCG remodels mature alpha-synuclein and amyloid-beta fibrils and reduces cellular toxicity. Proc Natl Acad Sci USA 107:7710–7715
81. Kumamoto M, Sonda T, Nagayama K et al (2001) Effects of pH and metal ions on antioxidative activities of catechins. Biosci Biotechnol Biochem 65:126–132
82. Grinberg LN, Newmark H, Kitrossky N et al (1997) Protective effects of tea polyphenols against oxidative damage to red blood cells. Biochem Pharmacol 54:973–978
83. Rogers JT, Randall JD, Cahill CM et al (2002) An iron-responsive element type II in the 5′-untranslated region of the Alzheimer's amyloid precursor protein transcript. J Biol Chem 277:45518–45528
84. Mandel SA, Amit T, Zheng H et al (2006) The essentiality of iron chelation in neuroprotection: a potential role of green tea catechins. In: Luo Y, Packer L (eds) Oxidative stress and age-related neurodegeneration. Taylor & Francis Group, Boca Raton, pp 277–299
85. Dedeoglu A, Cormier K, Payton S et al (2004) Preliminary studies of a novel bifunctional metal chelator targeting Alzheimer's amyloidogenesis. Exp Gerontol 39:1641–1649
86. Hsiao K, Chapman P, Nilsen S et al (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science 274:99–102
87. Hanson ES, Rawlins ML, Leibold EA (2003) Oxygen and iron regulation of iron regulatory protein 2. J Biol Chem 278:40337–40342
88. Wang J, Chen G, Muckenthaler M et al (2004) Iron-mediated degradation of IRP2, an unexpected pathway involving a 2-oxoglutarate-dependent oxygenase activity. Mol Cell Biol 24:954–965
89. Preetha A, Thomas SG, Kunnumakkaraa AB et al (2008) Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochem Pharmacol 76:1590–1611
90. Singhal SS, Awasthi S, Pandya U et al (1999) The effect of curcumin on glutathione-linked enzymes in K562 human leukemia cells. Toxicol Lett 109:87–95
91. Kim DS, Park SY, Kim JK (2001) Curcuminoids from Curcuma longa L. (Zinggiveraceae) that protect PC12 rate

pheochromocytoma and normal human umbilical vein endothelial cells from betaA (1–42) insult. Neurosci Lett 303:57–61
92. Ganguli M, Chandra V, Kamboh MI (2000) Apolipoprotein E polymorphism and Alzheimer disease: the Indo-US Cross-National Dementia Study. Arch Neurol 57:824–830
93. Lim GP, Chu T, Yang F, Beech W, Frautschy SA, Cole GM (2001) The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J Neurosci 21:8370–8377
94. Glenner GG, Wong CW (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 120:885–890
95. Hardy JA, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. Science 256:184–185
96. Terry RD, Masliah E, Salmon DP et al (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 30:572–580
97. Selkoe DJ, Wolfe MS (2007) Presenilin: running with scissors in the membrane. Cell 131:215–221
98. Gong CX, Iqbal K (2008) Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. Curr Med Chem 15:2321–2328
99. Jenner P (2003) Oxidative stress in Parkinson′s disease. Ann Neurol 53:S26–S36
100. Ebadi M, Srinivasan SK, Baxi MD (1996) Oxidative stress and antioxidant therapy in Parkinson's disease. Prog Neurobiol 48:1–19
101. Eriksen J, Dawson T, Dickson D, Petrucelli L (2003) Caught in the act: α-synuclein is the culprit in Parkinson's disease. Neuron 40:453–456
102. Gasser T (2001) Genetics of Parkinson's disease. J Neurol 248:833–840
103. Cooper AA, Gitler AD, Cashikar A et al (2006) Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science 313:324–328
104. Winklhofer KF, Haass C (2010) Mitochondrial dysfunction in Parkinson's disease. Biochim Biophys Acta 1802:29–44
105. Chinta SJ, Andersen JK (2008) Redox imbalance in Parkinson′s disease. Biochim Biophys Acta 1780:1362–1367
106. Kraytsberg Y, Kudryavtseva E, McKee AC et al (2006) Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. Nat Genet 38:518–520
107. Bender A, Krishnan KJ, Morris CM et al (2006) High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat Genet 38:515–517
108. Andersen JK (2004) Oxidative stress in neurodegeneration: cause or consequence? Nat Med 10:S18–S25
109. Jenner P, Olanow CW (2006) The pathogenesis of cell death in Parkinson's disease. Neurology 66:S24–S36
110. Kaur D, Yantiri F, Rajagopalan S et al (2003) Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity in vivo: a novel therapy for Parkinson's disease. Neuron 37:899–909
111. Bogaerts V, Theuns J, van Broeckhoven C (2008) Genetic findings in Parkinson's disease and translation into treatment: a leading role for mitochondria? Genes Brain Behav 7:129–151
112. Henchcliffe C, Beal MF (2008) Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. Nat Clin Pract 4:600–609
113. Paisan-Ruiz C, Jain S, Evans EW et al (2004) Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 44:595–600
114. Zimprich A, Biskup S, Leitner P et al (2004) Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 44:601–607
115. Valente EM, Abou-Sleiman PM, Caputo V et al (2004) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 304:1158–1160
116. Gandhi S, Wood-Kaczmar A, Yao Z (2009) PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. Mol Cell 33:627–638
117. Moore DJ (2006) Parkin: a multifaceted ubiquitin ligase. Biochem Soc Trans 34:749–753
118. Meulener M, Whitworth AJ, Armstrong-Gold CE et al (2005) Drosophila DJ-1 mutants are selectively sensitive to environmental toxins associated with Parkinson's disease. Curr Biol 15:1572–1577