

Oxidative Stress in Parkinson's Disease

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Abstract: Parkinson's disease (PD) is a progressive, age-related movement disorder, whose neuropathology is characterized by degeneration of the afferent pigmented neurons of the substantia nigra. Also associated with PD neuropathology are disrupted iron homeostasis, oxidative stress, and intracellular deposition of alpha-synuclein protein in Lewy bodies. Here we review oxidative stress mechanisms in Parkinson's disease, with emphasis on the relationship between oxidative stress and alpha-synuclein gene expression.

Keywords: Alpha-synuclein, iron regulatory protein, iron responsive element, oxidative stress, Parkinson's disease.

INTRODUCTION

Reactive oxygen species (ROS) are natural byproducts of mitochondrial respiration and other cellular processes. Some ROS, such as superoxide anion, nitric oxide and hydrogen peroxide, are physiologic species, essential for redox signaling and cellular function. They are safely metabolized by cellular antioxidant mechanisms under normal conditions. However, if constitutive production of ROS begins to exceed the cellular antioxidant capacity, non-physiologic and toxic ROS, such as peroxynitrite and hydroxyl radical, may be liberated in a process referred to as oxidative stress [1]. In the most general sense, oxidative stress is characterized by cellular dysfunction or cell death due to deleterious and indiscriminate reactivity of non-physiologic ROS with proteins, nucleic acids carbohydrates, and lipids. Patterns of oxidative stress are cell-type and disease specific. They are commonly coupled to mitochondrial energy metabolism in disease mechanism, since most initial sources of ROS are formed due to dysfunctional electron flow in the mitochondrial inner membrane and then damage the mitochondria further [2]. Mitochondrial damage may eventually lead to cellular and organ system pathology through multiple mechanisms, including decreased ATP production, oxidative damage, abnormal calcium sequestration, and apoptosis [3]. The brain is particularly susceptible to oxidative stress due to its high-energy demand and the specialized redox activities

of neurons. Although the brain only constitutes 2 to 3% of total body mass, it utilizes 20% of basal oxygen supplied to the body.

IRON AND OXIDATIVE STRESS IN PARKINSON'S DISEASE

Transition metals including Fe mediate oxidative damage to cellular components through the one-electron transfer called the Fenton reaction ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}\cdot + \text{OH}^-$), which leads to production of the unstable hydroxyl radical ($\text{OH}\cdot$) that will oxidize nucleic acid, protein, carbohydrate, and lipid, whichever is proximate [4, 5].

Disrupted iron metabolism in Parkinson's disease (PD) was first reported in 1924 by Lehermitte and McAlpine [6]. Since then a body of literature has emerged implicating iron in pathophysiology of PD and other synucleinopathies [7, 8]. It is well established that iron levels are increased in the PD substantia nigra, the anatomic region most vulnerable to degenerate in the disease [9-11]. Additionally, in neurodegeneration with brain iron accumulation type 1, which is also associated with alpha-synuclein pathology, substantial iron deposits are associated with neuronal loss, gliosis, and Lewy body pathology. In multiple systems atrophy as well, alpha-synuclein frequently deposits with iron [12].

Using a modification of the Prussian blue reaction, Castellani, *et al.* visualized iron in association with Lewy bodies in substantia nigra pars compacta neurons in PD (Fig. 1) [13]. This histochemical method involves binding of iron(II) cyanide (ferrocyanide) to iron(III) in tissue to give a blue mixed-valence iron complex that can be directly visualized or can be observed directly or enhanced, based on the ability of the complex to oxidize 3,3-diaminobenzidine [14].

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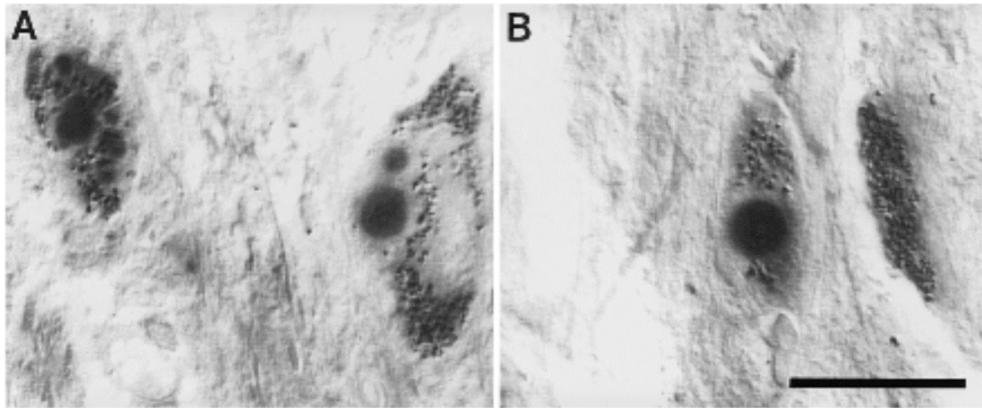


Fig. (1). Histochemically reactive iron in Lewy bodies. Viable pigmented neurons of the substantia nigra pars compacta in Parkinson's disease show strong iron (II or III) labeling of the Lewy bodies. The most pronounced staining involves the Lewy body core. Iron labeling of neuromelanin granules is also present, as expected. Bar = 25 μ m. Reproduced with permission from Elsevier Press (*Acta Neuropathol* 2000; 100: 111-4).

Iron sequestered in Lewy bodies and other pathobiologic pools of iron in PD brain has the potential to promote Fenton chemistry and oxidative damage to macromolecules, and, indeed, evidence of oxidative damage to macromolecules is abundant in postmortem PD tissue, with proteins, nucleic acids, lipids and sugars all showing evidence of oxidative modification [15].

The lipid peroxidation products 4-hydroxynonenal and N^ε-(carboxymethyl)lysine have been localized immunohistochemically to Lewy bodies [16]. Two advanced glycation end-products, pentosidine and pyrraline, also have been detected immunocytochemically in PD and Lewy body disease brain, particularly in association with filaments of cortical Lewy bodies [10]. Oxidative modifications to nucleic acids are also prominent in PD and Lewy body disease. 8-hydroxy-2'-deoxyguanosine and 8-hydroxyguanosine, are products of oxidized DNA and RNA, respectively. In the hippocampus and temporal neocortex in diffuse Lewy body disease, neuronal 8-hydroxyguanosine immunoreactivity has been shown to be prominent. RNase and DNase treatment prior to immunostaining confirms that RNA is the primary nucleic acid substrate for oxidative modification [17]. In PD brain, similar 8-hydroxyguanosine immunoreactivity in cytoplasmic RNA is seen in the substantia nigra, and to a lesser extent in neurons of the nucleus raphe dorsalis and oculomotor nucleus, and occasionally in glia [18]. 8-hydroxy-2'-deoxyguanosine and 8-hydroxyguanosine have also been found to be elevated in the serum and cerebrospinal fluid of patients with PD [19].

The primary toxic ROS is hydroxyl radical, which is formed from two substrates, hydrogen peroxide and a redox active metal (e.g., Cu⁺, Fe²⁺). There are a number of possible sources of toxic ROS in PD brain. Aberrant availability of redox active iron in neurons may be the primary source, whether it's cytosolic or compartmentalized, as for example in neuromelanin [20]. Because dopaminergic cells are uniquely vulnerable to degenerate, dopamine may provide a source for hydroxyl radical generation. Dopamine is known to coordinate iron and can regenerate Fe²⁺, allowing it to continue to donate electrons to hydrogen peroxide [21]. Alternatively, hydroxyl radical formation may be increased by

increased availability of superoxide anion and hydrogen peroxide from impaired oxidative phosphorylation in mitochondria, and there is good evidence that oxidative phosphorylation becomes increasingly inefficient in aging and in PD [22].

MITOCHONDRIAL ABNORMALITIES IN PARKINSON'S DISEASE

Abnormal function of complex I of the mitochondrial respiratory transport chain has been reported from PD brain, particularly in the substantia nigra [22, 23]. Deficient complex I function would likely increase production of superoxide anion by impairing electron flow from NADH to ubiquinone, promoting oxidative stress through subsequent superoxide dismutase and Fenton chemistry. Similar deficits in respiratory transport chain complex I have also been reported in peripheral cells, such as myocytes and platelets [24, 25]. Somatic mitochondrial DNA mutations have been reported in PD brain and also in the aging brain [7, 26-28]. These findings are significant because the mitochondrial DNA encodes components of the respiratory transport chain complexes, and such mutations may impair efficient electron flow from NADH to molecular oxygen.

Oxidative phosphorylation in the substantia nigra has been shown to become increasingly impaired as a function of age. This impairment is evidenced by increasing numbers of mitochondrial DNA deletions within substantia nigra neurons in individuals over the age of 65 [29, 30], with increasing numbers of deletions corresponding with decreased histochemical detection of cytochrome oxidase [29]. With quantitative single cell techniques, Bender, *et al.* showed high levels of mtDNA deletions in dopaminergic midbrain neurons during aging [27, 31]. These deletions were more prevalent in the substantia nigra neurons in PD as compared with neurologically intact controls [27].

In experimental systems, inhibitors of the mitochondrial respiratory transport chain can reproduce many features of PD. Rotenone, a complex I inhibitor induces dopaminergic cell loss in cell culture [32] and can cause PD type neuropathology and movement abnormalities [33-35]. Similarly, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP),

which inhibits complex II of the electron transport chain, causes dopaminergic neurodegeneration, similar to those found in PD, when administered to experimental animals [36, 37]. 6-hydroxydopamine can also cause dopaminergic specific oxidative stress and cell death [38].

ALPHA-SYNUCLEIN IN PARKINSON'S DISEASE

Alpha-Synuclein is the ~15 kd protein genetically linked to familial forms of PD. The protein is also implicated in pathogenesis of spectrum neurodegenerative diseases referred to as alpha-synucleinopathies [39-41], which may include, in addition to PD, diffuse Lewy body disease, dementia with Lewy bodies, Lewy body variant of Alzheimer's disease, multiple system atrophy, Parkinsonism dementia of Guam, and neurodegeneration with brain iron accumulation type I [42]. Thus, the immense suffering caused by alpha-synucleinopathy extends even beyond that attributed to PD alone [43-45]. In each of the synucleinopathies, alpha-synuclein is postulated to undergo conformational change and oligomerization, resulting in a toxic gain of function and subsequent neurodegeneration, coinciding with deposition of alpha-synuclein aggregates, most commonly in Lewy bodies, but also in dystrophic neurites, axonal spheroids, and glial cytoplasmic inclusions [46].

A causative rather than an epiphenomenal role for alpha-synuclein in disease is established in at least some forms of PD by the presence of alpha-synuclein mutations that segregate in rare families with autosomal dominant PD [47, 48]. Most patients with PD do not carry alpha-synuclein mutations, however, and the neurodegenerative cascade may result from increased concentrations of wild-type alpha-synuclein. Indeed, overproduction of wild-type alpha-synuclein is known to cause disease, because in some families with autosomal dominant PD, the disease segregates with alpha-synuclein gene multiplication [49]. Also, over expression of wild-type alpha-synuclein is sufficient to cause a degenerative Lewy body pathology in mice [50, 51]. Thus, controlled expression of alpha-synuclein is likely essential for neuronal health in the substantia nigra and other brain regions, and factors that up-regulate steady state levels of soluble alpha-synuclein even by 50% or less are potentially relevant to pathogenesis of alpha-synucleinopathies.

While the precise function of the alpha-synuclein protein remains to be elucidated, there is evidence supporting its involved in pre-synaptic membrane trafficking and neurotransmission [52-54]. Additional work has linked the normal function of alpha-synuclein to iron homeostasis at many levels. The alpha-synuclein protein itself has been shown to bind iron with high affinity [55], and its conformation is altered by iron concentrations [56]. alpha-synuclein-coordinated iron may be a source of free oxygen radicals [55]. alpha-synuclein gene expression is also linked to iron and redox metabolism at the transcriptional level through GATA hematopoietic factors. At the translational level iron and redox metabolism may regulate alpha-synuclein through an iron responsive element in the 5' untranslated region of the alpha-synuclein mRNA.

ALPHA-SYNUCLEIN GENE EXPRESSION

Although the normal biologic function of alpha-synuclein remains to be elucidated, it is clear that regulation of alpha-

synuclein expression is essential for healthy neuronal function. Even a 1.5 fold elevation in alpha-synuclein expression is sufficient to cause Lewy body disease [49]. Evidence is emerging that iron-dependent mechanisms are responsible for coordinated regulation of alpha-synuclein gene expression at the transcriptional, post-transcriptional, and translational levels.

Iron responsive elements (IREs) are mRNA stem loops critical to iron homeostasis [57, 58]. They post-transcriptionally regulate protein production, either by regulating translation or mRNA stability, by reversible binding to well characterized iron regulatory proteins (IRP1/IRP2), whose binding to the IREs is sensitive to cellular iron status [59]. IREs in the 5' UTR and 3' UTR have opposite effects on protein production. Under conditions of high cellular iron, IREs in the 5' UTR, such as in ferritin, decrease protein levels by suppressing translation [60]. The translation suppression is achieved by steric hindrance, impeding access of the small 40S ribosome subunit to the 5' end of the ferritin mRNA. IREs in the 3' UTR, such as in transferrin receptor, increase protein levels by increasing mRNA stability [61]. Examples of proteins regulated by IREs include transferrin receptor, mitochondrial aconitase, the erythroid isoform of 5-aminolevulinic synthase, ferroportin, the H- and L-subunits of ferritin, and A β PP [62].

Iron influx relieves repression of ferritin translation by removing IRPs from the IREs. Removal of IRPs from the ferritin IREs restores recruitment of the 40S ribosome at the 5' cap sites. Ferritin translation can take place after eIF4E promotes eIF4G binding to eIF3 and hence 40S ribosome recruitment. The 40S ribosome then scans ferritin mRNAs before eIF2-a dependent 60S ribosome subunit "joining step" when protein synthesis begins [62].

At the translational level, an IRE has been reported in the alpha-synuclein mRNA 5' UTR at the exon 1-2 splice junction [63] (Fig. 2). To assess the secondary structure of the 5' UTR of alpha-synuclein, computer generated folding was used with the Zucker RNA folding program [64]. The 46 base alpha-synuclein 5' untranslated region was predicted to form a single RNA stem loop, redrawn by hand in Fig. (2, panel A). (Gibbs free energy=-53 kcal/mol). The stem loop showed striking similarity to known IREs in H- ferritin (the best characterized IRE; (Fig. 2, panel A), L- ferritin, ferroportin, erythroid 5-aminolevulinic (eALAS), and mitochondrial aconitase, including 100% homology at the five nucleotide apex. Alignment of the canonical alpha-synuclein 5'CAGUG3' stem loop motif against the 5'CAGUG3' motif of the known IREs sequences of ferritin H- and L- chains, ferroportin, and erythroid 5-aminolevulinic (eALAS) reveals core homology residues among alpha-synuclein and the known type I IREs, with the homology most prominent in proximity to the canonical 5'GAGUG3' motif, particularly on the 5' aspect (Fig. 2, Panel B). Significantly, formation of the alpha-synuclein IRE requires precise ligation of exons 1 and 2. In fact the canonical CAGUG motif of the alpha-synuclein mRNA itself is precisely interrupted by intron 1 of the pre-mRNA [63].

Using microarray technology, Scherzer, *et al.* have found that expression of alpha-synuclein mRNA tracks with the heme metabolism genes ALAS2, FECH, and BLVRB [65], the same class of genes that show homology to alpha-

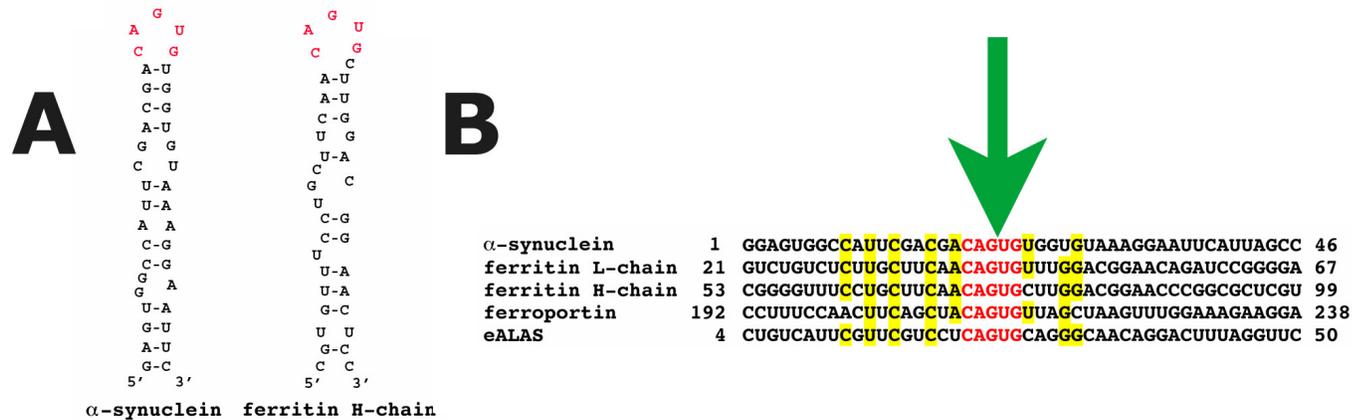


Fig. (2). The alpha-synuclein mRNA 5' UTR contains an iron responsive element at the exon 1-2 splice junction. (A) The 46 base alpha-synuclein 5' untranslated region is computer predicted to form a single RNA stem loop [64], as illustrated here (Gibbs free energy = -53 kcal/mol), with 100% homology at the apex to known IREs, such as the illustrated ferritin H-chain RNA. The loop region sequence in all known IREs encodes a 5'CAGUG3' motif. (B) Alignment of the alpha-synuclein 5'CAGUG3' motif against the 5'CAGUG3' motifs of the IRE sequences of ferritin H- and L- chains, ferroportin, and erythroid 5-aminolevulinatase (eALAS). Core homology residues among alpha-synuclein and the known IREs are most prominent in proximity to the predicted stem loop, particularly in the 5' aspect. The 5' and 3' aspects of the alpha-synuclein IRE stem loop derive, respectively, from exons 1 and 2 of the pre-mRNA. The alpha-synuclein RNA exon 1-2 splice junction indicated by the green arrow is within the canonical 5'CAGUG3' motif. With precise excision of intron 1 required for IRE formation, the implication is that regulation of alpha-synuclein protein production may also occur at the level of RNA splicing. Adapted with permission from Macmillan Publishers Ltd: (Molecular Psychiatry 2007; 12(3): 222-3).

synuclein the 5' UTR. Follow-up experiments identified specific binding of GATA hematopoietic transcription factors to the intron-1 region of alpha-synuclein DNA [65]. The latter finding is particularly intriguing, because attention to intron 1 was already suggested by work on the alpha-synuclein IRE. The linkage of alpha-synuclein intron 1 to iron-dependent gene expression raises the possibility that an additional level of regulation occurs at the post-transcriptional level through mRNA processing to form or preclude formation of the alpha-synuclein IRE.

CONCLUSION

Oxidative stress mechanisms are likely to play an important role in the neurodegenerative pathogenesis of PD and Lewy body disease. This evidence includes abnormalities of iron metabolism and markers of oxidative stress in PD brain. Mitochondrial defects, suggested by the presence of somatic mitochondrial DNA mutations and impaired respiratory transport chain function in PD brain further links redox metabolism to PD. alpha-synuclein gene expression has recently been linked to iron and redox metabolism at the transcriptional and post-transcriptional level, suggesting that alpha-synuclein over-production and Lewy body formation with neurodegeneration may in many cases occur secondary to a primary deficit in oxidative metabolism. Understanding the role of oxidative stress in PD and Lewy body pathology may lead to development of mechanism-based therapeutics and improved pharmacotherapy for presently incurable neurodegenerative diseases.

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