Review Article

Iron Redox Chemistry and Implications in the Parkinson's Disease Brain

Dinendra L. Abeyawardhane and Heather R. Lucas (ORCID)

Department of Chemistry, Virginia Commonwealth University, Richmond, VA 23284, USA

Correspondence should be addressed to Heather R. Lucas; hrlucas@vcu.edu

The etiology of Parkinson's disease (PD) is linked with cellular inclusions in the *substantia nigra pars compacta* region of the brain that are enriched in the misfolded presynaptic protein α-synuclein (αS) and death of the dopaminergic neurons. Brain iron homeostasis governs both neurotransmission and neurodegeneration; hence, the role of iron in PD progression and neuronal health is apparent. Elevated iron deposits become prevalent in the cerebral region upon aging and even more so in the PD brain. Structural as well as oxidative modifications can result from coordination of αS with redox active iron, which could have functional and/or pathological implications. In this review, we will discuss iron-mediated αS aggregation, alterations in iron metabolism, and the role of the iron-dopamine couple. Moreover, iron interactions with N-terminally acetylated αS, the physiologically relevant form of the human protein, will be addressed to shed light on the current understanding of protein dynamics and the physiological environment in the disease state. Oxidative pathways and biochemical alterations resulting from aberrant iron-induced chemistry are the principal focus of this review in order to highlight the plethora of research that has uncovered this emerging dichotomy of iron playing both functional and disruptive roles in PD pathology.

1. Introduction

Parkinson's disease (PD) is identified as the second most prevalent neurodegenerative disorder in the world, and it is an age-related progressive disease [1]. It has been estimated that approximately 1 million people in the United States alone are suffering from PD, where the majority of that population is over the age of 60. The key symptoms of PD include resting tremors, slow movements, muscle stiffness, and difficulties performing voluntary movements [2]. The main characteristic feature associated with PD is the loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc), and these neurons are the principal source of dopamine (DA), a neurotransmitter that regulates motor behavior [3]. PD etiology is also linked to the presence of cellular inclusions, known as Lewy bodies (LBs), which have been identified as postmortem hallmarks of PD [4, 5]. The major constituent of LBs is aggregated α-synuclein (αS), an intrinsically unfolded protein predominantly localized in the presynaptic regions of dopaminergic neurons. A tetrameric

form of αS has also been identified in human tissues that has been postulated as the inactive storage form of this structurally dynamic protein due to its aggregation resistance [6, 7]. Although the cellular mechanisms and normal functions associated with αS have not yet been completely resolved, the increased levels of αS in the brains of patients with PD suggest a relationship with the neurotransmitter DA [8].

It is well known that metal homeostasis plays an important role in regulating cellular functions [9]. Copper, iron, manganese, and zinc are essential transition metal ions for neurotransmission, enzymatic reactions, and mitochondrial functions regulated by the central nervous system (CNS) [10]. Thus, an imbalance of metal ions has an impact on disease states, among which are neurodegenerative disorders. *In vivo* magnetic resonance imaging (MRI) has highlighted the accumulation of iron in the SNpc region of the PD brain, which disrupts iron circulation pathways to create an imbalance of the metal [11, 12]. In a separate study, meta-analysis of literature data on blood serum iron levels also pinpointed an increase in this metal [13]. However, a direct correlation
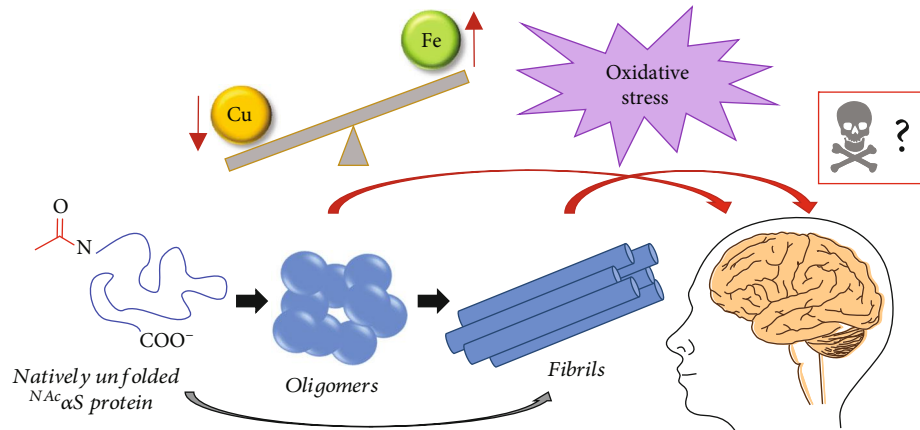
FIGURE 1: Schematic diagram of αS aggregation.
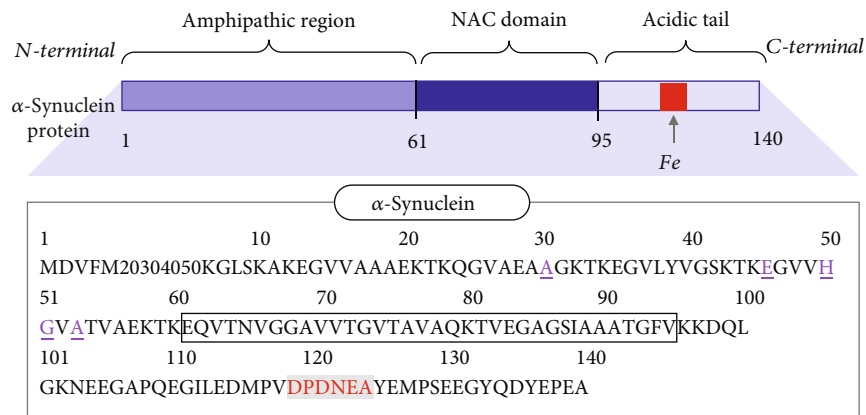
FIGURE 2: Cartoon representation of the N-terminal region, NAC region (highlighted within the box), and C-terminal region of αS with the primary amino acid sequence shown underneath. The iron binding site is highlighted at the C-terminal region in red text; genetic PD mutations are colored and underlined in purple.

between iron deposition and iron transportation within the brain has not been established in PD patients [14]. Beyond PD, *in vivo* MRI mapping of iron content among neurodegenerative tremulous diseases has also indicated a deposition of iron in the SNpc as compared to healthy controls [15, 16]. It is worth noting that iron dyshomeostasis associated with neurodegeneration is influenced by age, race, and gender. Therefore, iron-sensitive MRI mapping could be a powerful tool to diagnose and differentiate neurodegenerative diseases at an early stage. Evidence for iron accumulation within the SNpc region of the PD brain along with abundant misfolded αS inclusions implies a direct relationship between iron and αS in the pathogenesis of PD [17]. As shown in Figure 1, αS found in LBs exists in the form of highly ordered aggregated species that can be described as oligomers and/or fibrils. Under an oxidatively stressed environment, the aggregation processes of αS are majorly affected by interaction with redox active iron [18].

In this review, structural and biochemical consequences associated with iron-bound αS and alterations in the intracellular iron composition within dopaminergic neurons will be discussed. Key roles of iron will be delineated with respect to both the healthy brain and the PD brain. Cerebral iron levels are often associated with the modulation of dopamine-related biochemical pathways in the brain; therefore, the impact of the iron-dopamine couple and the disruption of biological cascades associated with these two components will be addressed in this review. Moreover, the impact of iron redox chemistry on the αS structure will also be highlighted.

2. α-Synuclein Structure and Iron Coordination

αS, encoded by the SNCA gene, consists of 140 amino acids and contributes approximately 1% of the protein content in cytosol [4, 5]. There are three distinct regions of αS protein (Figure 2). The N-terminal region, which contains 11 imperfect amino acid repeats with a consensus sequence KTKEGV, adopts an amphipathic α-helix conformation upon membrane interaction [8, 19]. The central non-amyloid-β component (NAC region) is involved in aggregation pathways and contains the hydrophobic core of αS. The C-terminal region is an acidic segment rich in negatively charged amino acid residues, namely, aspartic acid (D) and glutamic acid (E), and is highly dynamic. Although the pathogenesis of sporadic PD is mainly driven by the vulnerability of dopaminergic neurons, several genetic point mutations in αS have been identified as associated with PD, including A30P, E46K, H50Q,

G51D, A53E, and A53T (mutation sites are highlighted in Figure 2) [20]. In fact, enhanced aggregation properties have been reported for E46K, H50Q, and A53T as compared to wild-type, suggesting that these single point mutations initiate dramatic structural changes that seed fibrillation. The familial mutant A53T has also been reported to possess increased neurotoxicity upon elevated iron levels in comparison to wild-type αS due to selective loss of DA neurons as well as motor impairment [21].

Postmortem analyses of brain tissues isolated from patients with PD or dementia with Lewy bodies (DLB) have indicated that αS is acetylated at the N-terminus [22, 23]. Thus, it has been confirmed that N-terminally acetylated αS is the physiologically relevant form of the protein [24]. N-terminal acetylation is the transfer of an acetyl group from acetyl coenzyme A to the α-amino group of the first amino acid residue of a protein, as in methionine (M) for αS. Although it is a common posttranslational modification catalyzed by different N-terminal acetyltransferases (NATs) in that approximately 84% of human-derived proteins carry this modification [25–27], the majority of αS research has been carried out with the nonacetylated αS variant. The consequences of this modification are becoming increasingly more apparent with respect to membrane interactions, metal coordination, and the protein folding tendencies [18, 28–31]. The innate structural dynamics and behavioral patterns of αS are strongly influenced by N-terminal capping; for example, this modification can further stabilize the tetrameric structural orientation of αS [7].

The inherent metal binding properties of αS have been vastly studied [9, 10, 32, 33]. Among the prevalent redox active transition metals in the brain, copper has shown the highest binding affinity to αS [34]. Copper levels are diminished in the PD brain, while iron levels are elevated [35]. Thus, iron-αS interactions would be more prevalent under disease conditions. The primary binding site of iron at the C-terminus, ^{119}DPDNEA^{124} (Figure 2), is a motif rich in negatively charged amino acids that will facilitate hard acid/hard base interactions via potential iron-oxygen coupling. The presence of a proline residue can also facilitate protein folding dynamics to form a more stable global configuration upon iron binding. It can also be postulated that binding of iron at the C-terminus of αS (pI of 4.7) causes a neutralization of the negative charges [36]. Such charge pairing can result in electrostatic shielding, which may impact protein folding dynamics by further altering the structure upon protein oxidation, crosslinking, aggregation, etc.

Notably, the metal coordination sites for iron and copper are located at opposite termini, where iron preferentially interacts within the αS C-terminal region, and copper has two separate sites within the N-terminus that are dependent on the copper redox state. Davies et al. have shown that there are only subtle changes in the binding affinity upon iron(III) interaction with copper(II)-saturated αS, indicating that the binding of the two metals is independent from each other [37]. Copper was further proposed as a cofactor for αS due to the measured ferrireductase activity in the presence of an electron acceptor such as NADH [37].

The ability of αS to transform iron(III) to iron(II) highlights a ferrireductase activity of αS that could uncover a functional role. Conversely, the overexpression of αS could lead to excessive generation of iron(II) that may eventually result in an oxidative stress environment due to reactive oxygen species (ROS) production mediated by the Fenton reaction. Recently, we also reported evidence to support the ferrireductase activity of αS upon binding to iron(III) under anaerobic conditions as well as an increase in the antiparallel β-sheet composition as is characteristic of αS aggregates formed under oxidizing conditions in the presence of Fe^{II} [18]. On a similar note, Ortega and coworkers have reported that intracellular overexpression of αS in neurons promotes the accumulation of iron in the perinuclear region [38], accentuating that iron binding to αS correlates with αS aggregation and iron deposition as is reported for PD. Detection of iron in these regions could be acknowledged as an indirect biomarker for PD.

3. Iron-Mediated α-Synuclein Aggregation

The typical αS aggregation pathway involves propagation of natively unfolded monomers to higher-ordered oligomeric species which eventually form fibrillar structures [39, 40]. Specifically, the cardinal conformational alteration occurs when the disordered, "random coil" structure of the native protein is transformed into the well-known "β-sheet" structure concentrated in both oligomeric and fibrillar macromolecular aggregates. The orientation patterns of β-sheet (parallel or antiparallel) are distinguishable within certain contexts [41]. Although the in vivo toxic form of aggregates is not yet fully identified, antiparallel β-sheets are believed to be a characteristic of the toxic form [41]. The least compact oligomeric intermediates are reported to be toxic due to disruptive cellular functions including cellular leakages generated by membrane pores [42]. Hence, a correlation between oligomers and antiparallel β-sheets is expected. Based on nuclear magnetic resonance (NMR) spectroscopy and high-resolution cryoelectron microscopy techniques, the fold of fibrils is proposed to be parallel β-sheets [43]; however, the antiparallel versus parallel conformations of αS oligomers and/or fibrils have not yet been fully discerned. Traditionally, the mechanistic folding pathway is thought to involve an oligomer state that precedes fibrillation [44]; however, off-pathway aggregation routes have also been reported [18]. Fibrillation pathways via oligomer intermediates have also been thought to progress from antiparallel to parallel β-sheets [45].

The redox activity of metals like copper and iron is linked with oxidative and/or nitrosative stress and contributes as a major factor to the aggregation of αS [18, 30, 31, 46, 47]. For example, rat neuronal cultures rich in αS aggregates have indicated excessive free radical formation, implying oligomer-induced oxidative stress which is also metal-dependent [48]. It has also been suggested that iron(III)-induced SDS-resistant oligomers of αS form pores in the lipid planar bilayer in the presence of ethanol or DMSO, which eventually lead to toxicity as a result of permeability [49, 50]. Previously, it was reported that fibrils
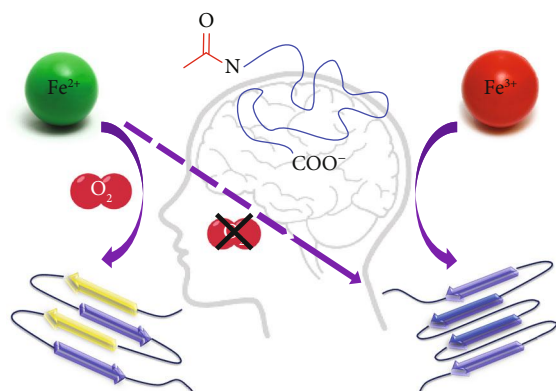
FIGURE 3: Graphical representation of N-terminally acetylated αS structural consequences upon aggregation mediated by iron-oxygen chemistry.

generated over the course of aggregation with iron(III) adopt a distinctly different morphology when compared to analogous fibrils with Cu(II) [36]. Transition electron microscopy (TEM) analysis of the stoichiometric Fe^{III}-αS aggregates indicated the presence of a short, thick, network-like fibrillar structure without any amorphous material. Growth patterns and morphology of fibrils are a potential biomarker which can aid in understanding structural factors affecting cell viability in neurodegeneration.

Oligomer formation can be coupled with potential membrane interactions related to pore-forming proteins which regulate metal homeostasis [49]. Oligomers generated in the presence of both iron(III) and iron(II) have also been suggested as nonharmful and to instead possess a potential functional role [51–53]. The Xie group has reported that the aggregation of αS induced by iron is dose- and time-dependent [54]. The cell viability assay conducted with SK-N-SH human neuroblastoma cells indicated that the higher the iron concentration (>1 mmol/L) and longer the period of aggregation (>24 h), the more toxicity to cells [54, 55]. Diminished mitochondrial transmembrane potential and elevated ROS production indicated the pivotal role of iron in inducing cytotoxicity. Cell toxicity due to iron-induced αS aggregation was treated by silencing the intracellular expression of αS using siRNA [56]. As it is evident that iron-mediated oxidative stress leads to cytotoxicity, it will be beneficial to look into iron chelators (Section 6 in this review), which could attenuate disease progression.

In order to shed some light on the iron-αS interaction, our group focused on iron-mediated aggregation of αS, marking the first publication to address the relationship of the iron-αS couple on the native N-terminally acetylated form of the protein [18]. A distinct change in the protein conformation was noted demonstrating the iron-oxygen-driven generation of an oligomer-locked iron-αS structure rich in right twisted antiparallel β-sheets (Figure 3). The PD relevance of this oligomeric motif was determined by its positive response to the anti-oligomer A11 polyclonal antibody, which selectively identifies soluble oligomeric epitopes present in common amyloidogenic proteins such as αS (PD), amyloid-β (Alzheimer's disease), IAPP (type II diabetes), and

prion protein (Creutzfeldt-Jakob disease or mad cow disease) [57]. Hence, our results highlight the major role of iron redox chemistry in the process of αS oligomerization.

4. Iron Metabolism and Alterations in the PD Brain

Iron plays a functional role in brain biochemistry by acting as a cofactor for tyrosine hydroxylase (TyrH), an enzyme that initiates the conversion of tyrosine to DA in the cytosol [58, 59]. Iron additionally serves as an essential element in various fundamental processes within the CNS, including mitochondrial respiration, DNA synthesis, myelin production, neurotransmission, and metabolism [60]. The innate redox nature of iron is coupled with electron transfer processes. Hence, the redox state of iron, whether ferrous (Fe^{2+}) or ferric (Fe^{3+}) ions, governs the feasibility of various iron-dependent biological functions. Dysfunction or imbalance of the equilibrium between iron(II) and iron(III) ions can disrupt processes due to the generation of ROS as is commonly associated with Fenton chemistry. Therefore, iron homeostasis plays a pivotal role in regulating cellular functions as is briefly illustrated in Figure 4.

Brain iron uptake is mainly driven by the glycoprotein transferrin (Tf), the primary iron transport protein in the CNS [61, 62]. Studies have shown that non-transferrin-bound iron levels are high in the cerebrospinal fluid due to controlled Tf transportation through the blood-brain barrier [63]. Tf possesses high affinity iron(III)-binding sites, and transferrin-bound ferric ions are engulfed into cells with the aid of transferrin receptor-1 (TfR-1) via endocytosis [62]. Newly imported ferric ions are subsequently reduced to ferrous ions and released into the cytosol by divalent metal transporter-1 (DMT-1). Intracellular iron levels are further controlled by iron regulatory proteins (IRP) that act in concert with DMT-1 and TfR-1. In iron-deficient cells, IRPs selectively bind to an iron responsive element (IRE) that facilitates iron uptake by stabilizing the mRNA coding for TfR-1 and DMT-1 [64]. IRE adopts a loop-like structure consisting of 26-30 nucleotides, often present in $3'$ or $5'$ untranslated regions ($3'$-UTR or $5'$-UTR) of eukaryotic mRNA for iron-dependent translational control. Another iron transporter, transferrin receptor-2 (TfR-2), which does not have an IRE, is also found in the dopaminergic neurons of the SNpc and concentrated more within the mitochondria of these cells [65]. Mitochondrial dysfunction has been accompanied by elevated Tf and TfR-2 levels in PD, suggesting oxidative stress promoted by iron redox chemistry [17, 62, 65].

Translation of ferritin, the main iron storage protein in the body, is also regulated by the availability of intracellular iron, as an IRE is found in the $5'$-UTR of Tf mRNA [66]. Interestingly, a region in the $5'$-UTR of human αS mRNA is reported to possess a high resemblance to the IRE present in Tf mRNA [67]. A potential IRE motif in αS mRNA suggests the possibility of iron-dependent posttranscriptional regulation of αS protein generation. Polysomal RNA analysis conducted after treatment with iron chelators has confirmed
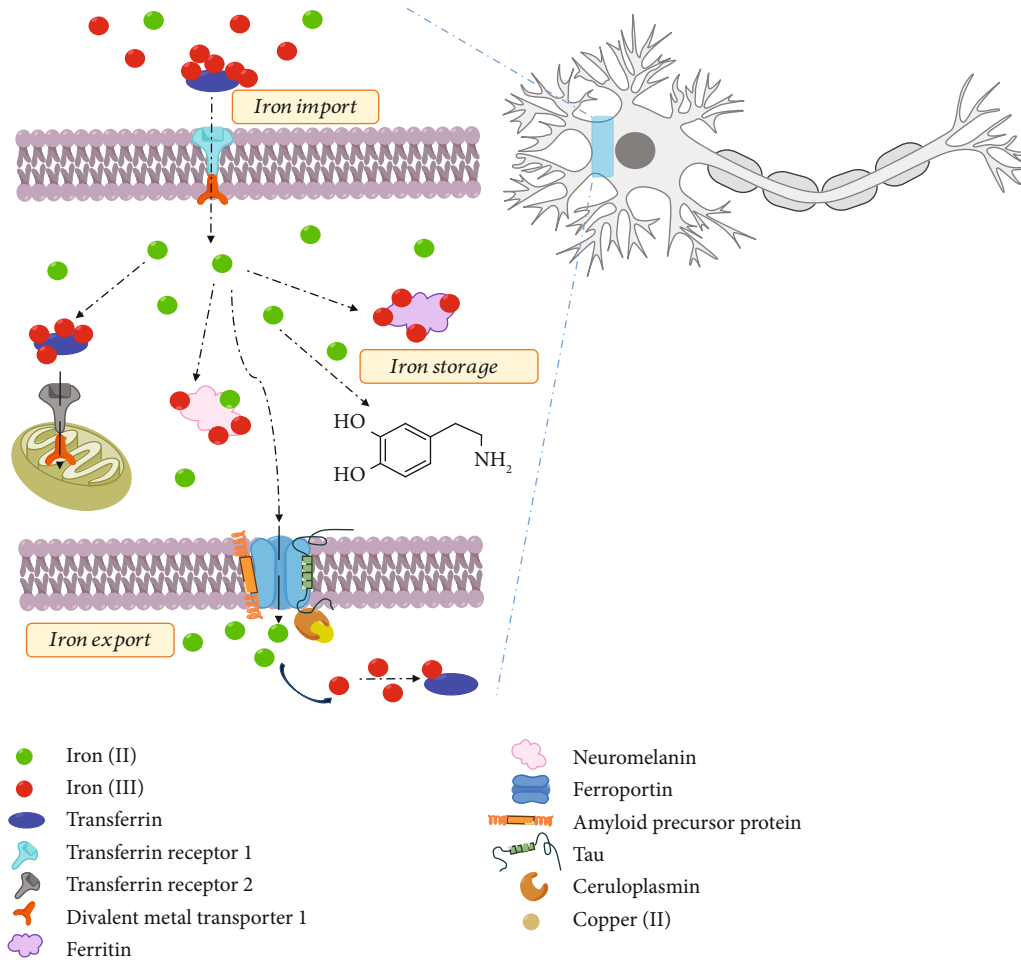
FIGURE 4: Iron metabolism in the neuron.

that expression of αS is influenced at a translational level by iron availability [66]. Another essential iron storage macromolecule in neurons, neuromelanin, has also been detected to accumulate in the SNpc of PD patients [68]. Both ferritin and neuromelanin possess dense iron cores [69, 70]. In the dopaminergic neurons, the ferric ions are easily reduced to ferrous ions by cytotoxic by-products of DA oxidation [17, 58, 59, 62, 71]. The ability of some cellular components, such as melanin, to reduce iron(III) to iron(II) can stimulate the hydroxyl radical formation mediated by Fenton chemistry [61, 69].

Physiological iron levels are expected to rise with aging; however, a drastic elevation is noted in PD patients [72]. Pathological iron dyshomeostasis affects the progression of PD, resulting from cumulative events that affect the capacity for neuronal survival. Suppressed expression of ferroportin and increased expression of DMT-1 mainly contribute to the elevated levels of iron in the body. Ferroportin, an iron efflux pore, is predominantly responsible for neuronal iron export, yet the efficiency of ferroportin is not governed by cellular iron levels alone [62]. There are several additional factors contributing to inefficient iron export from the neurons. For example, hepcidin is an iron regulatory hormone responsive to iron overload and inflammation. Binding of hepcidin to surface ferroportin impedes iron export via cellular internalization and degradation of ferroportin [73]. The ferroxidase activity of ceruloplasmin, a multicopper oxidase enzyme, can facilitate iron efflux coupled to ferroportin, enabling iron(III) to become readily available for binding with extracellular transferrin [74]. Notably, the low levels of copper in the SNpc as has been reported in PD patients correlate with low activity of ceruloplasmin, thus contributing to the intracellular accumulation of iron [17, 35, 75]. In addition, tau protein interacts with amyloid precursor protein to promote ferroportin-mediated iron export, and reduced levels of both of these neuronal proteins have been reported in PD brains [76]. Hence, the consequences of iron accumulation collectively disrupt cellular pathways that are dependent on our metabolism. Disruption of intracellular homeostasis could potentially be utilized to develop an indirect set of biomarkers for the diagnosis of PD based on various cellular components and proteins that are less commonly linked to PD. However, quantitative statistical analyses based upon external variables, such as age and ethnicity, have to be taken into account to establish a standard biomarker identification system.
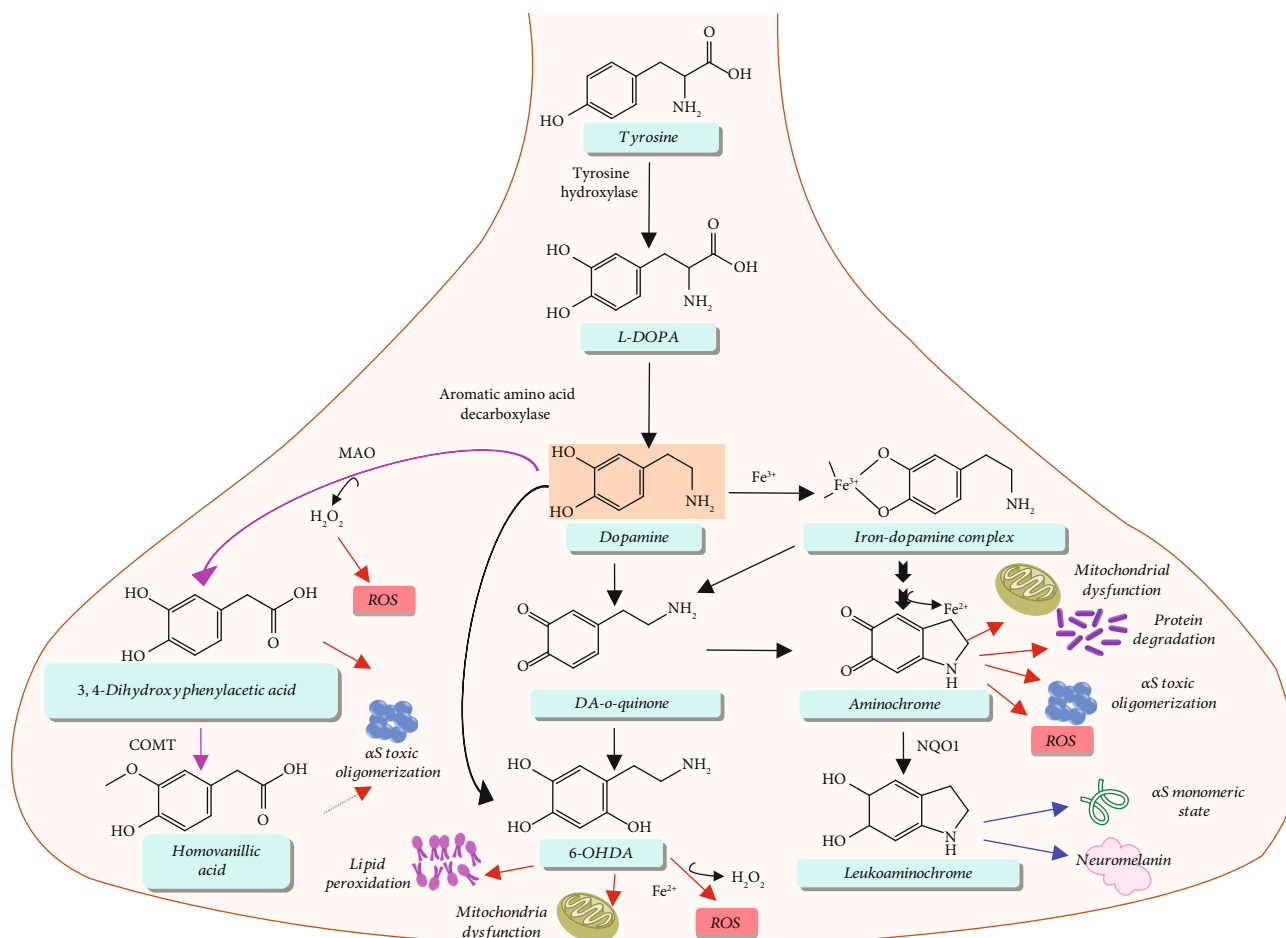
FIGURE 5: Schematic representation of dopamine metabolism in the synapse and toxic cascades associated with iron.

5. Toxic Consequences of the Interplay between Iron and Dopamine

Biosynthesis of DA is initiated from the amino acid tyrosine following its import into dopaminergic neurons by amino acid transporters (Figure 5) [77]. The rate-limiting step of DA synthesis is the conversion of tyrosine to dihydroxyphenylalanine (L-DOPA), which is driven by TyrH [77]. The active site of TyrH requires an iron atom in its ferrous form as a cofactor; thus, a deficiency of ferrous ions debilitates DA synthesis [77, 78]. The subsequent conversion of L-DOPA to DA is driven by aromatic amino acid decarboxylase (AADC, dopa decarboxylase), and it is selective to L-amino acid substrates [77]. Due to the dependency of the DA metabolic pathway on iron, changes in the redox state balance of Fe(III)/Fe(II) in PD brains and/or alterations in the iron flux can directly impact the health of dopaminergic neurons.

The major metabolites of enzymatic DA degradation are 3,4-dihydroxyphenylacetic acid and homovanillic acid (Figure 5) [79, 80]. DA degradation is prompted by monoamine oxidase (MAO) enzyme which results in 3,4-dihydroxyphenylacetaldehyde and hydrogen peroxide. Both substances are highly reactive and potential candidates for neurotoxicity. Under physiological conditions, 3,4-dihydroxyphenylacetaldehyde is readily oxidized to 3,4-dihydroxy-

phenylacetic acid, eventually leading to formation of homovanillic acid via catechol-o-methyl transferase (COMT) [79]. Elevated levels of both of these metabolites are identified in cerebrospinal fluid in patients with motor disorders or early PD symptoms. Hence, clinical studies emphasize that 3,4-dihydroxyphenylacetic acid and homovanillic acid could be pivotal biomarkers in PD progression [81]. In particular, the highly reactive nature of 3,4-dihydroxyphenylacetaldehyde can initiate hydroxyl radical generation leading to in $vitro$ and in $vivo$ neurotoxicity [82]. In fact, immunoanalyses have demonstrated that aggregation of αS both in $vitro$ and in $vivo$ is enhanced by 3,4-dihydroxyphenylacetaldehyde in a dose-dependent manner [83]. Furthermore, the aggregation is provoked by this metabolite to potentially form toxic oligomers [83].

In the presence of ferric ions, DA undergoes oxidation to generate DA-o-quinone (Figure 5), which can enter into neurotoxic pathways and eventually promote degeneration of dopaminergic neurons [84]. DA oxidation can be governed by several factors, such as oxygen, inorganic reagents, and redox active metals (primarily manganese, copper, and iron) [85–89]. Iron-facilitated DA oxidation forms another neurotoxic byproduct called 6-hydroxydopamine (6-OHDA) [90, 91], which perturbs mitochondrial functions and consequently promotes acute cell death due to disruption in ATP

synthesis [92]. It also contributes to oxidative stress by producing H_2O_2, eventually triggering lipid peroxidation and cell apoptosis [92].

Conversely, DA oxidation is also an essential step in the synthesis of neuromelanin [84], which is rapidly generated in the presence of iron(III) via formation of an iron-DA complex (Figure 5). While promoting biological reactions, the redox chemistry of iron can simultaneously influence the ROS and reactive nitrosative species (RNS) formation leading to lipid peroxidation, DNA/protein degradation, and ultimately cell death. Neurotoxicity coupled with the iron-DA complex depends upon its cellular uptake. The stable precursor of neuromelanin synthesis, aminochrome, can lead to subsequent ROS generation mediated by Fenton reactions and αS aggregation [93–95]. In addition, aminochrome can progress mitochondrial dysfunction, protein degradation, and oxidative stress in the neurons [96–98]. Toxic oligomerization of αS associated with aminochrome can be prevented by DT-diaphorase (NQO1), a flavoenzyme involved in the quinone reduction pathways [93]. This enzyme attenuated aggregation by stabilizing the monomeric state of αS upon catalyzing the reduction of quinone (aminochrome) to leukoaminochrome. In a separate study, the fibrillization pathway of αS has also been altered to spherical oligomer formation following DA oxidation [94]. The function of αS is also believed to be associated with DA metabolism [99]. DA interaction with αS leads to formation of SDS-resistant soluble oligomeric species upon oxidation of methionine residues in the αS amino acid sequence (Met locations: 1, 5, 116, and 127), consequently thwarting αS fibrillation [100, 101]. Modulation of aggregation pathways associated with αS by DA causes an increase in αS oligomers in the extracellular environment. Even though the disruptive mechanism behind the oligomerization is still under debate, these soluble oligomers are believed to be neurotoxic. Hence, a more detailed analysis of *in vivo* oligomerization and its potential to form cytotoxic membrane pores will be essential to understand the pathophysiology of the disease state.

6. Inhibition of Iron-Mediated Aggregation

Iron accumulation in the brain is implicated in diseases beyond PD, including rare synucleinopathies such as neurodegeneration with brain iron accumulation type 1 (NBIA1), multiple system atrophy (MSA), essential tremor (ET), progressive supranuclear palsy (PSP), and tremor in dystonia [12, 16, 102]. As aforementioned, increasing evidence supports the fact that the aggregation propensity of αS is affected by the redox activity of iron, and exposure to iron and oxygen stimulates a likely toxic oligomeric form of αS [18]. Therefore, inhibition of aggregation and iron chelation as a prevention technique has been briefly addressed in this section.

Baicalein and N′-benzylidene-benzohydrazide (NBB) derivatives, such as 293G02 and 301C09, have been shown to inhibit iron-induced oligomer formation and/or fibrillation (Figure 6). Low concentrations of baicalein have proven to be an excellent candidate to block the aggregation of αS initiated by treatment with organic solvent
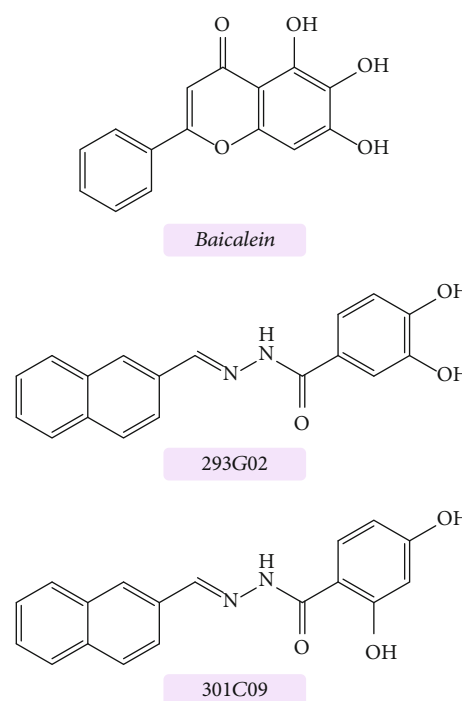

FIGURE 6: Structures of inhibiting agents.

[49]. The catechol moiety of NBB derivatives governs the inhibitory activity, highlighting a key structure-activity relationship [103, 104]. In particular, the compound 293G02 was highly active at controlling oligomerization inhibition, with cytotoxicity assays indicating a significant reduction in toxicity [104].

Aggregation promoted by iron can also be affected by the presence of redox inactive metal ions under physiological conditions. For example, Golts and coworkers have reported that iron-mediated αS aggregation can be inhibited by the presence of magnesium(II) by negatively modulating the iron(II) affinity [105]. It was suggested that an altered conformation enabled resistance to aggregation rather than competing with the same iron coordination site. Therefore, other metals that can compete for the same binding site in αS could act as potential therapeutic agents by mitigating the harmful effect of iron-promoted oligomerization.

Exposure of iron at early stages of life has been identified as a potential risk factor of PD [106]; however, the toxicity imparted upon early exposure to iron is irreversible even in the presence of a moderate chelator. Hence, the iron-αS interaction window is critical in PD progression as well as in the clinical aspects of disease prevention. Treatment with an iron chelator such as clioquinol has been shown to reduce nigral iron resulting in an increase in the cell viability [107]. In a separate *in vivo* study, treatment of iron-induced αS aggregates with deferiprone, a ferric ion chelator, has displayed improved motor functions in mouse models implying that the clinical application of iron chelation holds promise [108]. Structure-activity relationships among iron chelators will require careful attention as a means to open up new avenues in neurodegenerative drug discovery.

7. Concluding Remarks

Brain iron dyshomeostasis plays a crucial role in neurodegeneration associated with PD. Oxidative and conformational modifications of αS have a clear link to PD etiology, designating this structurally dynamic protein as a major target for therapeutic studies among the research community. The involvement of iron with αS biochemistry has been studied less extensively in comparison to research on copper-αS interactions; however, many studies have begun to address the potential structural and oxidative consequences that lead to αS deposits as a result of iron accumulation in the PD brain. Iron regulation in neurons has already shed light into clinical applications, and new research highlighted in this review may provide an avenue towards future therapeutic studies and/or inspire new biomarkers for PD.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

References

[1] C. Marras, J. C. Beck, J. H. Bower et al., "Prevalence of Parkinson's disease across North America," *NPJ Parkinson's Disease*, vol. 4, no. 1, p. 21, 2018.

[2] S. Sharma, C. S. Moon, A. Khogali et al., "Biomarkers in Parkinson's disease (recent update)," *Neurochemistry International*, vol. 63, no. 3, pp. 201–229, 2013.

[3] S. J. Chinta and J. K. Andersen, "Dopaminergic neurons," *The International Journal of Biochemistry & Cell Biology*, vol. 37, no. 5, pp. 942–946, 2005.

[4] W. S. Kim, K. Kågedal, and G. M. Halliday, "Alpha-synuclein biology in Lewy body diseases," *Alzheimers Research & Therapy*, vol. 6, no. 5-8, p. 73, 2014.

[5] A. D. Stephens, M. Zacharopoulou, and G. S. Kaminski Schierle, "The cellular environment affects monomeric α-synuclein structure," *Trends in Biochemical Sciences*, vol. 44, no. 5, pp. 453–466, 2019.

[6] T. Bartels, J. G. Choi, and D. J. Selkoe, "α-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation," *Nature*, vol. 477, no. 7362, pp. 107–110, 2011.

[7] R. D. Fernández and H. R. Lucas, "Isolation of recombinant tetrameric N-acetylated α-synuclein," *Protein Expression and Purification*, vol. 152, pp. 146–154, 2018.

[8] H. A. Lashuel, C. R. Overk, A. Oueslati, and E. Masliah, "The many faces of α-synuclein: from structure and toxicity to therapeutic target," *Nature Reviews Neuroscience*, vol. 14, no. 1, pp. 38–48, 2013.

[9] E. Carboni and P. Lingor, "Insights on the interaction of alpha-synuclein and metals in the pathophysiology of Parkinson's disease," *Metallomics*, vol. 7, no. 3, pp. 395–404, 2015.

[10] H. Kozlowski, M. Luczkowski, M. Remelli, and D. Valensin, "Copper, zinc and iron in neurodegenerative diseases (Alzheimer's, Parkinson's and prion diseases)," *Coordination Chemistry Reviews*, vol. 256, no. 19-20, pp. 2129–2141, 2012.

[11] P. Costa-Mallen, C. Gatenby, S. Friend et al., "Brain iron concentrations in regions of interest and relation with serum iron levels in Parkinson disease," *Journal of the Neurological Sciences*, vol. 378, pp. 38–44, 2017.

[12] J.-H. Lee and M.-S. Lee, "Brain iron accumulation in atypical parkinsonian syndromes: in vivo MRI evidences for distinctive patterns," *Frontiers in Neurology*, vol. 10, 2019.

[13] J. Jiao, H. Guo, Y. He, J. Wang, J. Yuan, and W. Hu, "Meta-analysis of the association between serum iron levels and Parkinson's disease: evidence from 11 publications," *Brain Research*, vol. 1646, pp. 490–493, 2016.

[14] Q. Chen, Y. Chen, Y. Zhang et al., "Iron deposition in Parkinson's disease by quantitative susceptibility mapping," *BMC Neuroscience*, vol. 20, no. 1, p. 23, 2019.

[15] F. Novellino, A. Cherubini, C. Chiriaco et al., "Brain iron deposition in essential tremor: a quantitative 3-tesla magnetic resonance imaging study," *Movement Disorders*, vol. 28, no. 2, pp. 196–200, 2013.

[16] N. Homayoon, L. Pirpamer, S. Franthal et al., "Nigral iron deposition in common tremor disorders," *Movement Disorders*, vol. 34, no. 1, pp. 129–132, 2019.

[17] D. J. Hare and K. L. Double, "Iron and dopamine: a toxic couple," *Brain*, vol. 139, no. 4, pp. 1026–1035, 2016.

[18] D. L. Abeyawardhane, R. D. Fernández, C. J. Murgas et al., "Iron redox chemistry promotes antiparallel oligomerization of α-synuclein," *Journal of the American Chemical Society*, vol. 140, no. 15, pp. 5028–5032, 2018.

[19] H. R. Lucas and J. C. Lee, "Copper(II) enhances membrane-bound α-synuclein helix formation," *Metallomics*, vol. 3, no. 3, pp. 280–283, 2011.

[20] P. Flagmeier, G. Meisl, M. Vendruscolo et al., "Mutations associated with familial Parkinson's disease alter the initiation and amplification steps of α-synuclein aggregation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 113, no. 37, pp. 10328–10333, 2016.

[21] Z. J. Zhu, K. C. Wu, W. H. Yung, Z. M. Qian, and Y. Ke, "Differential interaction between iron and mutant alpha-synuclein causes distinctive parkinsonian phenotypes in *Drosophila*," *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, vol. 1862, no. 4, pp. 518–525, 2016.

[22] A. Öhrfelt, H. Zetterberg, K. Andersson et al., "Identification of novel α-synuclein isoforms in human brain tissue by using an online NanoLC-ESI-FTICR-MS method," *Neurochemical Research*, vol. 36, no. 11, pp. 2029–2042, 2011.

[23] J. P. Anderson, D. E. Walker, J. M. Goldstein et al., "Phosphorylation of Ser-129 is the dominant pathological modification of α-synuclein in familial and sporadic Lewy body disease," *Journal of Biological Chemistry*, vol. 281, no. 40, pp. 29739–29752, 2006.

[24] F.-X. Theillet, A. Binolfi, B. Bekei et al., *Nature*, vol. 530, no. 7588, pp. 45–50, 2016.

[25] T. Arnesen, P. van Damme, B. Polevoda et al., "Proteomics analyses reveal the evolutionary conservation and divergence of N-terminal acetyltransferases from yeast and humans," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 20, pp. 8157–8162, 2009.

[26] T. Arnesen, "Towards a Functional Understanding of Protein N-Terminal Acetylation," *PLoS Biology*, vol. 9, no. 5, 2011.

[27] B. Fauvet, M. Fares, F. Samuel et al., "Characterization of semisynthetic and naturally N^{α}-acetylated α-synuclein *in vitro* and in intact cells implications for aggregation and cellular properties of α-synuclein," *Journal of Biological Chemistry*, vol. 287, no. 34, pp. 28243–28262, 2012.

[28] L. Kang, G. M. Moriarty, L. A. Woods, A. E. Ashcroft, S. E. Radford, and J. Baum, "N-Terminal acetylation of α-synuclein induces increased transient helical propensity and decreased aggregation rates in the intrinsically disordered monomer," *Protein Science*, vol. 21, no. 7, pp. 911–917, 2012.

[29] I. Dikiy and D. Eliezer, "N-terminal acetylation stabilizes N-terminal helicity in lipid- and micelle-bound α-synuclein and increases its affinity for physiological membranes," *Journal of Biological Chemistry*, vol. 289, no. 6, pp. 3652–3665, 2014.

[30] D. L. Abeyawardhane, R. D. Fernández, D. R. Heitger, M. K. Crozier, J. C. Wolver, and H. R. Lucas, "Copper induced radical dimerization of α-synuclein requires histidine," *Journal of the American Chemical Society*, vol. 140, no. 49, pp. 17086–17094, 2018.

[31] D. L. Abeyawardhane, D. R. Heitger, R. D. Fernández, A. K. Forney, and H. R. Lucas, "C-Terminal Cu^{II} coordination to α-synuclein enhances aggregation," *ACS Chemical Neuroscience*, vol. 10, no. 3, pp. 1402–1410, 2019.

[32] D. Valensin, S. Dell'Acqua, H. Kozlowski, and L. Casella, "Coordination and redox properties of copper interaction with α-synuclein," *Journal of Inorganic Biochemistry*, vol. 163, pp. 292–300, 2016.

[33] K. Joppe, A.-E. Roser, F. Maass, and P. Lingor, "The contribution of iron to protein aggregation disorders in the central nervous system," *Frontiers in Neuroscience*, vol. 13, p. 15, 2019.

[34] I. Gentile, H. A. Garro, S. Delgado Ocaña et al., "Interaction of Cu(I) with the Met-X$_3$-Met motif of alpha-synuclein: binding ligands, affinity and structural features," *Metallomics*, vol. 10, no. 10, pp. 1383–1389, 2018.

[35] K. M. Davies, S. Bohic, A. Carmona et al., "Copper pathology in vulnerable brain regions in Parkinson's disease," *Neurobiology of Aging*, vol. 35, no. 4, pp. 858–866, 2014.

[36] Bharathi, S. S. Indi, and K. S. J. Rao, "Copper- and iron-induced differential fibril formation in α-synuclein: TEM study," *Neuroscience Letters*, vol. 424, no. 2, pp. 78–82, 2007.

[37] P. Davies, D. Moualla, and D. R. Brown, "Alpha-synuclein is a cellular ferrireductase," *PLoS One*, vol. 6, no. 1, article e15814, 2011.

[38] R. Ortega, A. Carmona, S. Roudeau et al., "α-synuclein overexpression induces increased iron accumulation and redistribution in iron-exposed neurons," *Molecular Neurobiology*, vol. 53, no. 3, pp. 1925–1934, 2016.

[39] V. N. Uversky, J. Li, and A. L. Fink, "Evidence for a partially folded intermediate in α-synuclein fibril formation," *Journal of Biological Chemistry*, vol. 276, no. 14, pp. 10737–10744, 2001.

[40] S. W. Chen, S. Drakulic, E. Deas et al., "Structural characterization of toxic oligomers that are kinetically trapped during α-synuclein fibril formation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 112, no. 16, pp. E1994–E2003, 2015.

[41] M. S. Celej, R. Sarroukh, E. Goormaghtigh, G. D. Fidelio, J. M. Ruysschaert, and V. Raussens, "Toxic prefibrillar α-synuclein amyloid oligomers adopt a distinctive antiparallel β-sheet structure," *Biochemical Journal*, vol. 443, no. 3, pp. 719–726, 2012.

[42] S. K. Sharma and S. Priya, "Expanding role of molecular chaperones in regulating α-synuclein misfolding; implications in Parkinson's disease," *Cellular and Molecular Life Sciences*, vol. 74, no. 4, pp. 617–629, 2017.

[43] M. Vilar, H. T. Chou, T. Luhrs et al., "The fold of α-synuclein fibrils," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 25, pp. 8637–8642, 2008.

[44] A. L. Fink, "The aggregation and fibrillation of α-synuclein," *Accounts of Chemical Research*, vol. 39, no. 9, pp. 628–634, 2006.

[45] J. I. Gallea, R. Sarroukh, P. Yunes-Quartino, J. M. Ruysschaert, V. Raussens, and M. S. Celej, "Structural remodeling during amyloidogenesis of physiological N$^{\alpha}$-acetylated α-synuclein," *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, vol. 1864, no. 5, pp. 501–510, 2016.

[46] G. Ferrer-Sueta, N. Campolo, M. Trujillo et al., "Biochemistry of peroxynitrite and protein tyrosine nitration," *Chemical Reviews*, vol. 118, no. 3, pp. 1338–1408, 2018.

[47] R. Burai, N. Ait-Bouziad, A. Chiki, and H. A. Lashuel, "Elucidating the role of site-specific nitration of α-synuclein in the pathogenesis of Parkinson's disease via protein semisynthesis and mutagenesis," *Journal of the American Chemical Society*, vol. 137, no. 15, pp. 5041–5052, 2015.

[48] E. Deas, N. Cremades, P. R. Angelova et al., "Alpha-synuclein oligomers interact with metal ions to induce oxidative stress and neuronal death in Parkinson's disease," *Antioxidants & Redox Signaling*, vol. 24, no. 7, pp. 376–391, 2016.

[49] M. Kostka, T. Högen, K. M. Danzer et al., "Single particle characterization of iron-induced pore-forming α-synuclein oligomers," *Journal of Biological Chemistry*, vol. 283, no. 16, pp. 10992–11003, 2008.

[50] F. Schmidt, J. Levin, F. Kamp, H. Kretzschmar, A. Giese, and K. Botzel, "Single-channel electrophysiology reveals a distinct and uniform pore complex formed by α-synuclein oligomers in lipid membranes," *PLoS One*, vol. 7, no. 8, article e42545, 2012.

[51] K. M. Danzer, S. K. Krebs, M. Wolff, G. Birk, and B. Hengerer, "Seeding induced by α-synuclein oligomers provides evidence for spreading of α-synuclein pathology," *Journal of Neurochemistry*, vol. 111, no. 1, pp. 192–203, 2009.

[52] E. Illes-Toth, M. R. Ramos, R. Cappai, C. Dalton, and D. P. Smith, "Distinct higher-order α-synuclein oligomers induce intracellular aggregation," *Biochemical Journal*, vol. 468, no. 3, pp. 485–493, 2015.

[53] M. Ingelsson, "Alpha-synuclein oligomers—neurotoxic molecules in Parkinson's disease and other Lewy body disorders," *Frontiers in Neuroscience*, vol. 10, 2016.

[54] W. J. Li, H. Jiang, N. Song, and J. X. Xie, "Dose- and time-dependent α-synuclein aggregation induced by ferric iron in SK-N-SH cells," *Neuroscience Bulletin*, vol. 26, no. 3, pp. 205–210, 2010.

[55] W. Li, H. Jiang, N. Song, and J. Xie, "Oxidative stress partially contributes to iron-induced alpha-synuclein aggregation in SK-N-SH cells," *Neurotoxicity Research*, vol. 19, no. 3, pp. 435–442, 2011.

[56] Q. He, N. Song, H. Xu, R. Wang, J. Xie, and H. Jiang, "Alpha-synuclein aggregation is involved in the toxicity induced by ferric iron to SK-N-SH neuroblastoma cells," *Journal of Neural Transmission*, vol. 118, no. 3, pp. 397–406, 2011.

[57] R. Kayed, E. Head, J. Thompson et al., "Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis," *Science*, vol. 300, no. 5618, pp. 486–489, 2003.

[58] J. A. Duce, B. X. Wong, H. Durham, J. C. Devedjian, D. P. Smith, and D. Devos, "Post translational changes to α-synuclein control iron and dopamine trafficking; a concept for neuron vulnerability in Parkinson's disease," *Molecular Neurodegeneration*, vol. 12, no. 1, p. 45, 2017.

[59] J. Sian-Hülsmann, S. Mandel, M. B. H. Youdim, and P. Riederer, "The relevance of iron in the pathogenesis of Parkinson's disease," *Journal of Neurochemistry*, vol. 118, no. 6, pp. 939–957, 2011.

[60] B. X. Wong and J. A. Duce, "The iron regulatory capability of the major protein participants in prevalent neurodegenerative disorders," *Frontiers in Pharmacology*, vol. 5, 2014.

[61] D. Berg, M. Gerlach, M. B. H. Youdim et al., "Brain iron pathways and their relevance to Parkinson's disease," *Journal of Neurochemistry*, vol. 79, no. 2, pp. 225–236, 2001.

[62] D. W. Lee and J. K. Andersen, "Iron elevations in the aging Parkinsonian brain: a consequence of impaired iron homeostasis?," *Journal of Neurochemistry*, vol. 112, no. 2, pp. 332–339, 2010.

[63] T. Moos and E. H. Morgan, "Evidence for low molecular weight, non-transferrin-bound iron in rat brain and cerebrospinal fluid," *Journal of Neuroscience Research*, vol. 54, no. 4, pp. 486–494, 1998.

[64] P. Piccinelli and T. Samuelsson, "Evolution of the iron-responsive element," *RNA*, vol. 13, no. 7, pp. 952–966, 2007.

[65] P. G. Mastroberardino, E. K. Hoffman, M. P. Horowitz et al., "A novel transferrin/TfR2-mediated mitochondrial iron transport system is disrupted in Parkinson's disease," *Neurobiology of Disease*, vol. 34, no. 3, pp. 417–431, 2009.

[66] F. Febbraro, M. Giorgi, S. Caldarola, F. Loreni, and M. Romero-Ramos, "α-synuclein expression is modulated at the translational level by iron," *NeuroReport*, vol. 23, no. 9, pp. 576–580, 2012.

[67] A. L. Friedlich, R. E. Tanzi, and J. T. Rogers, "The $5'$-untranslated region of Parkinson's disease α-synuclein messengerRNA contains a predicted iron responsive element," *Molecular Psychiatry*, vol. 12, no. 3, pp. 222-223, 2007.

[68] L. Zecca, R. Fariello, P. Riederer, D. Sulzer, A. Gatti, and D. Tampellini, "The absolute concentration of nigral neuromelanin, assayed by a new sensitive method, increases throughout the life and is dramatically decreased in Parkinson's disease," *FEBS Letters*, vol. 510, no. 3, pp. 216–220, 2002.

[69] M. Fasano, B. Bergamasco, and L. Lopiano, "Modifications of the iron-neuromelanin system in Parkinson's disease," *Journal of Neurochemistry*, vol. 96, no. 4, pp. 909–916, 2006.

[70] L. Zecca, M. Gallorini, V. Schünemann et al., "Iron, neuromelanin and ferritin content in the substantia nigra of normal subjects at different ages: consequences for iron storage and neurodegenerative processes," *Journal of Neurochemistry*, vol. 76, no. 6, pp. 1766–1773, 2001.

[71] K. L. Double, M. Maywald, M. Schmittel, P. Riederer, and M. Gerlach, "In vitro studies of ferritin iron release and neurotoxicity," *Journal of Neurochemistry*, vol. 70, no. 6, pp. 2492–2499, 1998.

[72] A. A. Belaidi and A. I. Bush, "Iron neurochemistry in Alzheimer's disease and Parkinson's disease: targets for therapeutics," *Journal of Neurochemistry*, vol. 139, pp. 179–197, 2016.

[73] E. Nemeth, M. Tuttle, J. Powelson et al., "Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization," *Science*, vol. 306, no. 5704, pp. 2090–2093, 2004.

[74] G. Musci, F. Polticelli, and M. C. Bonaccorsi di Patti, "Ceruloplasmin-ferroportin system of iron traffic in vertebrates," *World Journal of Biological Chemistry*, vol. 5, no. 2, pp. 204–215, 2014.

[75] T. Skjørringe, L. B. Møller, and T. Moos, "Impairment of interrelated iron- and copper homeostatic mechanisms in brain contributes to the pathogenesis of neurodegenerative disorders," *Frontiers in Pharmacology*, vol. 3, 2012.

[76] P. Lei, S. Ayton, D. I. Finkelstein et al., "Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export," *Nature Medicine*, vol. 18, no. 2, pp. 291–295, 2012.

[77] J. D. Elsworth and R. H. Roth, "Dopamine synthesis, uptake, metabolism, and receptors: relevance to gene therapy of Parkinson's disease," *Experimental Neurology*, vol. 144, no. 1, pp. 4–9, 1997.

[78] Y. Y. Yien and B. H. Paw, "A role for iron deficiency in dopaminergic neurodegeneration," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 113, no. 13, pp. 3417-3418, 2016.

[79] J. Meiser, D. Weindl, and K. Hiller, "Complexity of dopamine metabolism," *Cell Communication and Signaling*, vol. 11, no. 1, p. 34, 2013.

[80] N. Song and J. Xie, "Iron, dopamine, and α-synuclein interactions in at-risk dopaminergic neurons in Parkinson's disease," *Neuroscience Bulletin*, vol. 34, no. 2, pp. 382–384, 2018.

[81] A. Stefani, M. Pierantozzi, E. Olivola et al., "Homovanillic acid in CSF of mild stage Parkinson's disease patients correlates with motor impairment," *Neurochemistry International*, vol. 105, pp. 58–63, 2017.

[82] S. W. Li, T. S. Lin, S. Minteer, and W. J. Burke, "3,4-Dihydroxyphenylacetaldehyde and hydrogen peroxide generate a hydroxyl radical: possible role in Parkinson's disease pathogenesis," *Molecular Brain Research*, vol. 93, no. 1, pp. 1–7, 2001.

[83] W. J. Burke, V. B. Kumar, N. Pandey et al., "Aggregation of α-synuclein by DOPAL, the monoamine oxidase metabolite of dopamine," *Acta Neuropathologica*, vol. 115, no. 2, pp. 193–203, 2008.

[84] F. A. Zucca, J. Segura-Aguilar, E. Ferrari et al., "Interactions of iron, dopamine and neuromelanin pathways in brain aging and Parkinson's disease," *Progress in Neurobiology*, vol. 155, pp. 96–119, 2017.

[85] W. Linert, E. Herlinger, R. F. Jameson, E. Kienzl, K. Jellinger, and M. B. H. Youdim, "Dopamine, 6-hydroxydopamine, iron, and dioxygen - their mutual interactions and possible implication in the development of Parkinson's disease," *Biochimica et Biophysica Acta-Molecular Basis of Disease*, vol. 1316, no. 3, pp. 160–168, 1996.

[86] M. Jimenez, F. Garcia-Carmona, F. Garcia-Canovas, J. L. Iborra, J. A. Lozano, and F. Martinez, "Chemical intermediates in dopamine oxidation by tyrosinase, and kinetic-studies of the process," *Archives of Biochemistry and Biophysics*, vol. 235, no. 2, pp. 438–448, 1984.

[87] Z. D. Zhou, Y. H. Lan, E. K. Tan, and T. M. Lim, "Iron species-mediated dopamine oxidation, proteasome inhibition, and dopaminergic cell demise implications for iron-

related dopaminergic neuron degeneration," *Free Radical Biology & Medicine*, vol. 49, no. 12, pp. 1856–1871, 2010.

[88] J. Segura-Aguilar and C. Lind, "On the mechanism of the Mn^{3+}-induced neurotoxicity of dopamine: prevention of quinone-derived oxygen-toxicity by DT diaphorase and superoxide-dismutase," *Chemico-Biological Interactions*, vol. 72, no. 3, pp. 309–324, 1989.

[89] I. Paris, A. Dagnino-Subiabre, K. Marcelain et al., "Copper neurotoxicity is dependent on dopamine-mediated copper uptake and one-electron reduction of aminochrome in a rat substantia nigra neuronal cell line," *Journal of Neurochemistry*, vol. 77, no. 2, pp. 519–529, 2001.

[90] L. Zhang, G. Yagnik, D. Jiang, S. Shi, P. Chang, and F. Zhou, "Separation of intermediates of iron-catalyzed dopamine oxidation reactions using reversed-phase ion-pairing chromatography coupled in tandem with UV-visible and ESI-MS detections," *Journal of Chromatography B*, vol. 911, pp. 55–58, 2012.

[91] J. Rodriguez-Pallares, J. A. Parga, A. Muñoz, P. Rey, M. J. Guerra, and J. L. Labandeira-Garcia, "Mechanism of 6-hydroxydopamine neurotoxicity: the role of NADPH oxidase and microglial activation in 6-hydroxydopamine-induced degeneration of dopaminergic neurons," *Journal of Neurochemistry*, vol. 103, no. 1, pp. 145–156, 2007.

[92] D. Blum, S. Torch, N. Lambeng et al., "Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease," *Progress in Neurobiology*, vol. 65, no. 2, pp. 135–172, 2001.

[93] P. Munoz, S. Cardenas, S. Huenchuguala et al., "DT-diaphorase prevents aminochrome-induced alpha-synuclein oligomer formation and neurotoxicity," *Toxicological Sciences*, vol. 145, no. 1, pp. 37–47, 2015.

[94] E. H. Norris, B. I. Giasson, R. Hodara et al., "Reversible inhibition of α-synuclein fibrillization by dopaminochrome-mediated conformational alterations," *Journal of Biological Chemistry*, vol. 280, no. 22, pp. 21212–21219, 2005.

[95] M. Bisaglia, S. Mammi, and L. Bubacco, "Kinetic and structural analysis of the early oxidation products of dopamine - analysis of the interactions with α-synuclein," *Journal of Biological Chemistry*, vol. 282, no. 21, pp. 15597–15605, 2007.

[96] R. Xiong, D. Siegel, and D. Ross, "Quinone-induced protein handling changes: implications for major protein handling systems in quinone-mediated toxicity," *Toxicology and Applied Pharmacology*, vol. 280, no. 2, pp. 285–295, 2014.

[97] C. Arriagada, I. Paris, M. J. Sanchez de las Matas et al., "On the neurotoxicity mechanism of leukoaminochrome o-semiquinone radical derived from dopamine oxidation: mitochondria damage, necrosis, and hydroxyl radical formation," *Neurobiology of Disease*, vol. 16, no. 2, pp. 468–477, 2004.

[98] P. Aguirre, P. Urrutia, V. Tapia et al., "The dopamine metabolite aminochrome inhibits mitochondrial complex I and modifies the expression of iron transporters DMT1 and FPN1," *Biometals*, vol. 25, no. 4, pp. 795–803, 2012.

[99] S. Arreguin, P. Nelson, S. Padway, M. Shirazi, and C. Pierpont, "Dopamine complexes of iron in the etiology and pathogenesis of Parkinson's disease," *Journal of Inorganic Biochemistry*, vol. 103, no. 1, pp. 87–93, 2009.

[100] S. L. Leong, C. L. L. Pham, D. Galatis et al., "Formation of dopamine-mediated α-synuclein-soluble oligomers requires methionine oxidation," *Free Radical Biology & Medicine*, vol. 46, no. 10, pp. 1328–1337, 2009.

[101] H.-J. Lee, S. M. Baek, D. H. Ho, J. E. Suk, E. D. Cho, and S. J. Lee, "Dopamine promotes formation and secretion of non-fibrillar alpha-synuclein oligomers," *Experimental and Molecular Medicine*, vol. 43, no. 4, pp. 216–222, 2011.

[102] J. E. Duda, V. M. Y. Lee, and J. Q. Trojanowski, "Neuropathology of synuclein aggregates: new insights into mechanisms of neurodegenerative diseases," *Journal of Neuroscience Research*, vol. 61, no. 2, pp. 121–127, 2000.

[103] U. Bertsch, K. F. Winklhofer, T. Hirschberger et al., "Systematic identification of antiprion drugs by high-throughput screening based on scanning for intensely fluorescent targets," *Journal of Virology*, vol. 79, no. 12, pp. 7785–7791, 2005.

[104] A. S. Hillmer, P. Putcha, J. Levin et al., "Converse modulation of toxic α-synuclein oligomers in living cells by N$^{\prime}$-benzylidene-benzohydrazide derivates and ferric iron," *Biochemical and Biophysical Research Communications*, vol. 391, no. 1, pp. 461–466, 2010.

[105] N. Golts, H. Snyder, M. Frasier, C. Theisler, P. Choi, and B. Wolozin, "Magnesium inhibits spontaneous and iron-induced aggregation of α-synuclein," *Journal of Biological Chemistry*, vol. 277, no. 18, pp. 16116–16123, 2002.

[106] N. Ostrerova-Golts, L. Petrucelli, J. Hardy, J. M. Lee, M. Farer, and B. Wolozin, "The A53T α-synuclein mutation increases iron-dependent aggregation and toxicity," *Journal of Neuroscience*, vol. 20, no. 16, pp. 6048–6054, 2000.

[107] J. L. Billings, D. J. Hare, M. Nurjono et al., "Effects of neonatal iron feeding and chronic clioquinol administration on the parkinsonian human A53T transgenic mouse," *ACS Chemical Neuroscience*, vol. 7, no. 3, pp. 360–366, 2016.

[108] E. Carboni, L. Tatenhorst, L. Tönges et al., "Deferiprone rescues behavioral deficits induced by mild iron exposure in a mouse model of alpha-synuclein aggregation," *Neuromolecular Medicine*, vol. 19, no. 2-3, pp. 309–321, 2017.