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Redox Imbalance in Parkinson's Disease

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Abstract

Parkinson's disease (PD) is an adult-onset neurodegenerative disorder characterized by preferential loss of dopaminergic neurons in an area of the midbrain called the substantia nigra (SN) along with occurrence of intraneuronal inclusions called Lewy bodies. The majority of cases of PD are sporadic in nature with late onset (95% of patients); however a few PD cases (5%) are seen in familial clusters with generally earlier onset. Although PD has been heavily researched, so far the exact cause of the rather selective cell death is unknown. Multiple lines of evidence suggest an important role for oxidative stress. Dopaminergic neurons (DA) are particularly prone to oxidative stress due to DA metabolism and auto-oxidation combined with increased iron, decreased total glutathione levels and mitochondrial complex I inhibition-induced ROS production in the SN which can lead to cell death by exceeding the oxidative capacity of DA-containing cells in the region. Enhancing antioxidant capabilities and chelating labile iron pools in this region therefore constitutes a rational approach to prevent or slow ongoing damage of DA neurons. In this review, we summarize the various sources of reactive oxygen species that may cause redox imbalance in PD as well as potential therapeutic targets for attenuation of oxidative stress associated with PD.

1. Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder in the US after Alzheimer's disease, affecting ~1% of the population over the age of 65. Clinical symptoms of the disease include rigidity, resting tremor, bradykinesia and postural instability. Pathological hallmarks include the preferential loss of dopaminergic neurons within the substantia nigra pars compacta (SNpc) and the presence of intracytoplasmic inclusions called Lewy bodies whose primary components include fibrillar α-synuclein and ubiquitin [1]. Clinical symptoms of PD appear only when dopamine levels are reduced to greater than 60% that of normal [2]. The majority of PD cases so far identified are sporadic in nature; however recent studies have described several mutations in specific genes that are highly correlated with PD suggesting the presence of rare hereditary forms of the disease [3]. Although PD has been heavily researched in the last several decades, the precise etiology of the disease is still unknown. However, research in recent years has provided substantial evidence supporting the generally held hypothesis in the field that oxidative stress plays a major role in disease pathogenesis [4]. Oxidative stress is caused by the excess formation of various reactive oxygen species (ROS) in cells and has been implicated in the pathogenesis of many neurodegenerative diseases besides Parkinson's disease (PD) including Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis [5]. All these disorders exhibit distinct pathological and

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symptomatic features but there is overwhelming evidence that oxidative stress contributes to subsequent neuropathogenesis [6–7].

Oxidative stress is classically defined as a redox imbalance with an excess formation of oxidants or a defect in antioxidants [8]. Although the body in general has developed several defense mechanisms to counteract oxidative stress, the brain appears to be more susceptible to this damage than any other organ. Although the brain comprises only 2% of the total body weight, it is especially prone to oxidative stress as it consumes about 20% of the resting total body oxygen. The ability of the brain to withstand oxidative stress is limited because of the presence of high amounts of polyunsaturated fatty acids, low levels of antioxidants such as glutathione and vitamin E and the elevated content of iron in specific areas such as the globus pallidus and the substantia nigra (SN). Moreover, being postmitotic, neurons in the brain once damaged may be permanently dysfunctional [9]. Post-mortem studies on brains from PD patients have consistently implicated the role of oxidative damage in the pathogenesis of PD It is not clear whether accumulation of ROS in PD is a primary event or a consequence of other cellular dysfunctions. Mitochondria are both the target and an important source of ROS. Mitochondrial dysfunction has also widely been hypothesized to play a major role in cell death associated with PD [10]. Studies on postmortem samples from PD patients have revealed a selective mitochondrial complex I deficiency both in the SN and in peripheral tissues [11–14]. A complex I defect could contribute to neuronal degeneration in PD not only via decreased ATP synthesis but also excess production of ROS [15].

2. Types of reactive oxygen and nitrogen species (ROS/RNS) and their possible role in subsequent PD neuropathology

Under normal physiologic conditions, superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$), collectively known as ROS, are generated as byproducts of metabolism of molecular oxygen by the mitochondria. Normally during oxidative phosphorylation, electrons are transferred to molecular oxygen and H_2O is produced by complex IV via a sequential four-electron transfer. However, a proportion of oxygen is reduced only partially by the mitochondria and this one-electron reduction results in the generation of superoxide. Superoxide anion radical is considered to be the “primary” ROS, which can further interact with other molecules to generate “secondary” ROS, either directly or commonly through enzyme- or metal-catalysed processes [16]. Superoxide is produced from both mitochondrial complexes I and III of the electron transport chain and, once in its anionic form, it is too strongly charged to readily cross the inner mitochondrial membrane [17]. Superoxide produced by the mitochondria can be reduced to hydrogen peroxide (H_2O_2) which is also produced by peroxisomes. These peroxisomes also contain catalase which decomposes hydrogen peroxide and presumably prevents accumulation of this toxic compound. During pathological conditions, peroxisomes can be damaged and their H_2O_2 consuming enzymes down regulated, leading to the release of H_2O_2 into the cytosol which can contribute to oxidative stress [16]. In the presence of reduced metals such as ferrous iron (Fe^{2+}), H_2O_2 can be converted into hydroxyl radicals by the Fenton reaction. The hydroxyl radical is highly reactive, making it the most harmful of all ROS. When produced *in vivo* ·OH immediately reacts with other molecules close to its site of formation [18]. Reactive nitrogen species such as NO and its metabolite peroxynitrite (PN) may also play a major role in PD. NO is known to inhibit several enzymes including complexes I and IV of the mitochondrial electron transport chain and aconitase. As a free-radical, NO can contribute to oxidative stress by reacting with proteins to form S-nitrosothiols thereby altering their function and with lipids, thereby inducing lipid peroxidation [19]. Nitric oxide also reacts with superoxide anion radical to produce significant amounts of the much more oxidatively active molecule PN, a potent oxidizing agent that can cause DNA fragmentation and lipid peroxidation [20]. PN can also cause protein damage by

modifying tyrosine (3-nitrotyrosine formation, 3NT), cysteine (*S*-nitrosylation or SNO formation), or tryptophan (via formation of *N*-formylkynurenine) residues.

Excessive formation of reactive oxygen and nitrogen species in PD may damage key cellular components such as lipids, proteins, and DNA. Evidence for oxidative damage in PD brains includes an increase in the amount of lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal; an increase in the extent of protein oxidation as evidenced by protein cross-linking and fragmentation as well as carbonyl group formation, and an increase in the concentration of 8-hydroxy-2'-deoxyguanosine, a product of DNA oxidation. Due to these observed alterations, the "free radical hypothesis" has become prominent in attempting to explain the etiology of PD [21]. Studies with parkinsonian mimetics further suggest a possible role for free oxygen and/or nitrogen species in selective loss of SN dopaminergic neurons in the disease. For example, 6-hydroxydopamine (6-OHDA) is known to destroy dopaminergic neurons through free radical–mediated mechanisms. Similarly, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced impairment of the mitochondrial respiratory chain via inhibition of complex I enhances superoxide formation that then can initiate neuronal death. This can result in increases in, amongst other things, levels of PN. 3-NT-modified proteins have been reported to accumulate in the brains of MPTP-injected mice [22,23] as well as in tissues isolated from the Lewy bodies of PD patients [24]. PN has been demonstrated to be capable of inhibiting cell respiration via complex I inhibition [25]. Recently, Murray *et al*. [26] have shown that incubation of purified bovine heart mitochondria with PN results in 3-NT-modifications of specific subunits within complex I. This is further supported by recent studies from our laboratory demonstrating that glutathione depletion-mediated complex I inhibition is via a peroxynitrite mediated event [27,28]. This suggests that PN-mediated oxidative damage might play an important role in PD pathology, perhaps via damaging nitrative affects on mitochondrial complex I.

3. The role of dopamine, glutathione (GSH) and iron in ROS/RNS generation and PD neuropathology

There is much evidence to suggest that ROS derived from the combined presence of dopamine, low GSH, and high iron are a major cause of the loss of dopaminergic cells in the brains of individuals with PD [29,30]. Dopamine is chemically unstable and undergoes auto-oxidation to form dopamine quinones (DAQs) and superoxide anion radicals. This reaction is catalyzed by the presence of metals, oxygen, or enzymes such as tyrosinase. The electrophilic quinones themselves can act as oxidants thus supporting ROS formation. Auto-oxidation of dopamine may be increased in the early stages of the disease when dopamine turnover is increased to compensate for dying dopaminergic neurons [31]. Other dopamine metabolites such as 3, 4-dihydroxyphenylacetic acid (DOPAC) can undergo further two-electron oxidation to generate ROS and DOPAC-quinones [32]. DOPAC-quinone levels may be elevated in PD due to decreases in GSH in the SN including within dopaminergic neurons in the diseased brain [33–35]. Although GSH is not the only antioxidant molecule reported to be altered in PD, the magnitude of GSH depletion appears to parallel the severity of the disease and is the earliest known indicator of nigral degeneration, reportedly preceding detectable losses in both mitochondrial complex I activity and striatal dopamine [36,37]. GSH depletion has been demonstrated to result in complex I inhibition likely via direct thiol oxidation of important residues within complex I itself; inhibition is reversible by treatment with thiol reducing agent dithiothreitol [38,39]. Depletion of GSH has also been reported to result in inhibition of glutathione reductase activity (the enzyme which converts oxidized glutathione or GSSG into reduced GSH) via direct oxidation of its two active site cysteine residues [40]. This would result in further increases in GSH loss and alteration of the cellular redox state as the GSH/GSSG ratio is further decreased. Glutaredoxin, another GSH dependant-enzyme which maintains thiol homeostasis by reducing glutathione-containing mixed disulfides and also is

required for maintenance of complex I function, is reported to be inhibited in toxin models of PD [41], indicating a critical role for GSH in PD.

DOPAC-quinones which fail to conjugate with GSH due to reduced levels of the latter may be converted rather into 5-*S*-cys-DOPAC, which further undergoes oxidation to produce molecular species that are also capable of inhibiting mitochondrial complex I activity [42, 43]. Along with decreased GSH levels, elevated levels of 5-*S*-cysteinyl-catecholamine conjugates such as 5-*S*-cys DA, 5-*S*- cysDOPAC have also been reported in the SN of individuals with PD [44]. These data indicate that not only can complex I inhibition result in increased dopamine oxidation, but dopamine oxidation itself might affect complex I function leading to mitochondrial dysfunction.

In glial cells, dopamine and other related substrates are metabolized enzymatically by monoamine oxidases (MAOs) into H_2O_2. The MAO-B isoform of the enzyme increases with age [45]. Levels of MAO-B appear to be highest in the substantia nigra (SN). Large numbers of MAO-B-positive astrocytes are present in this region and this may contribute to local oxidative stress [46]. H_2O_2 produced in glial cells by MAO-B is highly membrane-permeable and can cross into neighboring dopaminergic cells where it may react with free iron (Fe^{++}) to produce toxic hydroxyl radical which can damage cellular components. The glial cells themselves are protected from toxic levels of H_2O_2 by possessing high levels of glutathione and glutathione peroxidase which act to detoxify H_2O_2 to water. Once H_2O_2 has crossed into neighboring dopaminergic cells, it can oxidize dopamine.

In PD, the iron content of the SN is elevated compared to aged-matched controls with an increase of the Fe(III)/Fe(II)-ratio from 2:1 to almost 1:2 [47]. Increased levels of iron and Fe (II) enhance the conversion of H_2O_2 to ·OH via the Fenton reaction and favor a greater turnover in the Haber–Weiss cycle which leads to an amplification of oxidative stress [48]. Furthermore, oxidative stress may enhance the levels of free iron via, for example, enhanced release of iron from ferritin by $O_2^{\cdot-}$, from heme proteins like haemoglobin and cytochrome c by peroxides, and from iron–sulfer proteins by $ONOO^{-}$ [49]. Whether iron accumulation in the PD SN is an early or late event and whether increased iron and iron-induced free radical reactions are the cause of the neurodegeneration or the consequence of a pathological process is still under debate. Since iron accumulation in the brain occurs in a number of neurodegenerative disorders it has been suggested to be a non-specific or secondary consequence of the disease. However, several pieces of biochemical and genetic data also suggest that iron accumulation and subsequent oxidative stress may be a primary event in the degenerative process [50]. A number of iron chelators have been shown to attenuate MPTP toxicity suggesting that iron either mediates or accentuates subsequent neuropathological events associated with its administration [51,52]. Recent studies from our own laboratory, demonstrated that transgenic expression of ferritin or administration of the bioavailable metal chelator clioquinol (CQ) in dopaminergic midbrain neurons protected them from MPTP-mediated neurodegeneration and resulted in an attenuation of motor deficits [53]. Moreover, studies on iron infusion via unilateral injection or feeding of high iron diet to month old weanling mice demonstrated a significant increase in striatal iron associated with decreases in total glutathione (GSH + GSSG) and increases in hydroxyl radical levels in both the striatum and brainstem suggesting that increases in midbrain iron may be upstream of neurodegeneration associated with PD [54,55]. Recent reports from our laboratory also demonstrated that neonatal iron exposure results in Parkinson-like neurodegeneration with age suggesting that iron accumulation is an early event in dopaminergic cell loss [56]. Recently, it has been discovered that nitrosylated iron regulated protein (IRP2) is also present in Lewy bodies in the SN [57,58], indicating the possible involvement of oxidative/nitrosative iron dysregulation in the neurodegenerative process associated with PD.

4. ROS/RNS, dopamine, iron, and α-synuclein

Alpha-synuclein is a prominent component of Lewy body aggregates [59], a pathological hallmark of the disorder, and mutations in the α-synuclein gene have been linked to familial cases of PD [60,61]. Previous studies have implicated the role of increased oxidative or nitrosative stress in the formation of synuclein aggregates [62,63]. Conjugation of dopamine with α-synuclein impedes the protofibril-to-fiber transition and therefore potentially more cytotoxic protofibrils may accumulate in dopamine neurons making them more sensitive to PD-induced cell death. Addition of antioxidants reversed the formation of adducts suggesting that catechol oxidation can contribute to formation of protofibrils [64]. Iron-related oxidative stress has also been suggested in several recent studies to promote α-synuclein aggregation [65,66]. Iron catalyzed oxidative reactions convert the protein's α-helical structure into a β-sheet secondary structure leading to partially-folded intermediates that are more susceptible to aggregation [67]. This may result from either preferential binding of iron to an intermediate conformation of the protein or the positive charge of the metal masking negatively charged groups responsible for native unfolded α-synuclein conformation. Several reports of α-synuclein nitration in synucleopathies have been published [68,24], as well as following MPTP administration [69], which may also contribute to oligomer formation and toxicity. Nitration of α-synuclein can significantly enhance fibril formation *in vitro* similar to the biophysical properties of α-synuclein isolated from PD brains [70]. Indeed, soluble nitrated α-synuclein is able to activate microglia to produce copious amounts of ROS through modulation of specific ion channels [71]. The use of specific antibodies that recognize only the nitrated α-synuclein demonstrated that the majority of the Lewy bodies and protein inclusions contain nitrated and possibly oxidized α-synuclein, indicating that oxidative processes may participate in the formation of these inclusions [72]. Aberrant protein conformations of modified nitrated α-synuclein can also potentially overload the cellular proteasome and, by doing so, increase cellular stress associated with the accumulation of misfolded proteins in affected neurons [73]. Based on data from cellular model systems and *in vitro* biochemical studies, it is likely that oxidative and nitrosative processes stabilize the formation of α-synuclein aggregates in a manner that is resistant to proteolysis, thereby allowing the formation of highly insoluble protein aggregates [74].

α-Synuclein itself appears to increase ROS levels in dopaminergic cells. This presynaptic protein can interact with the dopamine transporter (DAT) and facilitate its clustering at the plasma membrane. Consequently, dopamine uptake becomes accelerated leading to increased susceptibility to dopamine-induced apoptosis [75]. The re-uptake of more dopamine intracellularly can be a source of increased ROS due to the metabolism of this catecholamine. Overexpression of mutant α-synuclein induces a significant increase in sensitivity of dopaminergic neurons to mitochondrial toxins such as MPP^+ and 6-hydroxydopamine, resulting in increased protein carbonylation and lipid peroxidation *in vitro* and *in vivo* [76, 77]. Conversely, studies with a-synuclein knockout mice demonstrate a marked resistance to MPTP as well as other mitochondrial toxins including malonate and 3-nitropropionic acid [78,79]. The mechanism of this resistance seems to be due to a reduction in oxidative stress following α-synuclein deficiency, implicating a role for α-synuclein as a modulator of oxidative damage perhaps via modulation of mitochondrial function [80].

5. Innate antioxidant alterations in PD

Organisms have developed several innate defense mechanisms to counteract the impact of increased oxidative stress including antioxidants to reverse their formation. Enzymatic antioxidant defenses include glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). Non-enzymatic antioxidants are represented by ascorbic acid (Vitamin C), glutathione (GSH), α-tocopherol (Vitamin E), and other antioxidants. During oxidative stress,

increased activity of several of these mechanisms would be expected to counteract the toxicity of free radicals. A specific increase in SOD levels has been observed in the SN of PD patients where as no change in activities of catalase, glutathione peroxidase and glutathione reductase were found when compared to age matched-controls [81].

6. Therapeutic strategies for maintaining redox status in PD

Maintenance of redox potential within cells is a primary component of homeostasis underlying neuronal survival. There is overwhelming evidence in PD that oxidative/nitrosative stress leads to an increase in pathological damage in the SN. There are many possible mechanisms of ROS/RNS formation in dopaminergic neurons. MAO-induced oxygen radical formation, for example, appears to be important in PD. MAO-B inhibition by deprenyl is already an established widely-used clinical therapy for the disease. Animal and cellular models of PD have proven that deprenyl protects neurons from cell death, but clinical trials failed to confirm this. However, recent reports from preliminary clinical trials have confirmed the protective role of another more specific MAOB inhibitor rasagiline, suggesting that MAO B inhibition may be an important target for protecting DA neurons against oxidative stress [82]. Another important potential target to minimize oxidative stress in the SN would be reduction of the content of iron in this region. The use of various iron chelator including desferoxamine and a newer brain permeable iron chelator V-28 have yielded promising results particularly in combination with other therapies including MAO-B inhibition [83]. Recently, the micronutrient coenzyme Q10, a fundamental component of the mitochondrial electron transport chain, was studied as a putative neuroprotective agent for PD [84]. Although initially promising, the results from this study require validation through longer and larger studies [85]. Several other agents that exhibit anti-oxidative properties which are currently being investigated for their antiparkinsonian effects include phytochemicals such as Ginkgo biloba, L-carnitine, cannabis, estrogen and nicotinamide. Agents such as polyphenols found in green tea are also in the testing stages [86] based on initial studies that suggested that green tea may be efficacious as a possible adjunct to conventional levodopa therapy for patients.

Another important way to counteract oxidative stress in the PD SN may be to replenish lost GSH levels by either by increasing synthesis of GSH or by slowing its degradation. GSH replacement can also be achieved by administration of thiol reagents such as GSH itself or GSH analogs [87]. As GSH does not easily cross the blood brain barrier due to its charged cysteine -SH group, GSH esters have been explored as an alternative. Recent studies from our laboratory and others have demonstrated that the GSH precursor glutamyl cysteine ethyl ester (GCEE) and glutathione ethyl ester (GEE) both significantly elevate intracellular glutathione levels in neuronal cells and provide significant protection of dopamine cells against oxidative/nitrosative stress or mitochondrial impairment both *in vitro* and *in vivo* [88,89]. Thiol antioxidants such as α-lipoic acid have also been shown to be effective in both *in vitro* and *in vivo* models of PD by reducing glutathione disulfides and thus increasing intracellular glutathione levels [90,91]. A pilot study in which a small group of untreated PD patients were given daily intravenous infusions of glutathione over the period of a month reportedly resulted in a significant improvement in disability [92].

Whatever the specific strategy taken, it is clear that antioxidant therapies have been and will continue to be an important avenue of investigation in PD therapeutics given the rich body of evidence indicating the role of oxidative stress in disease neuropathology.

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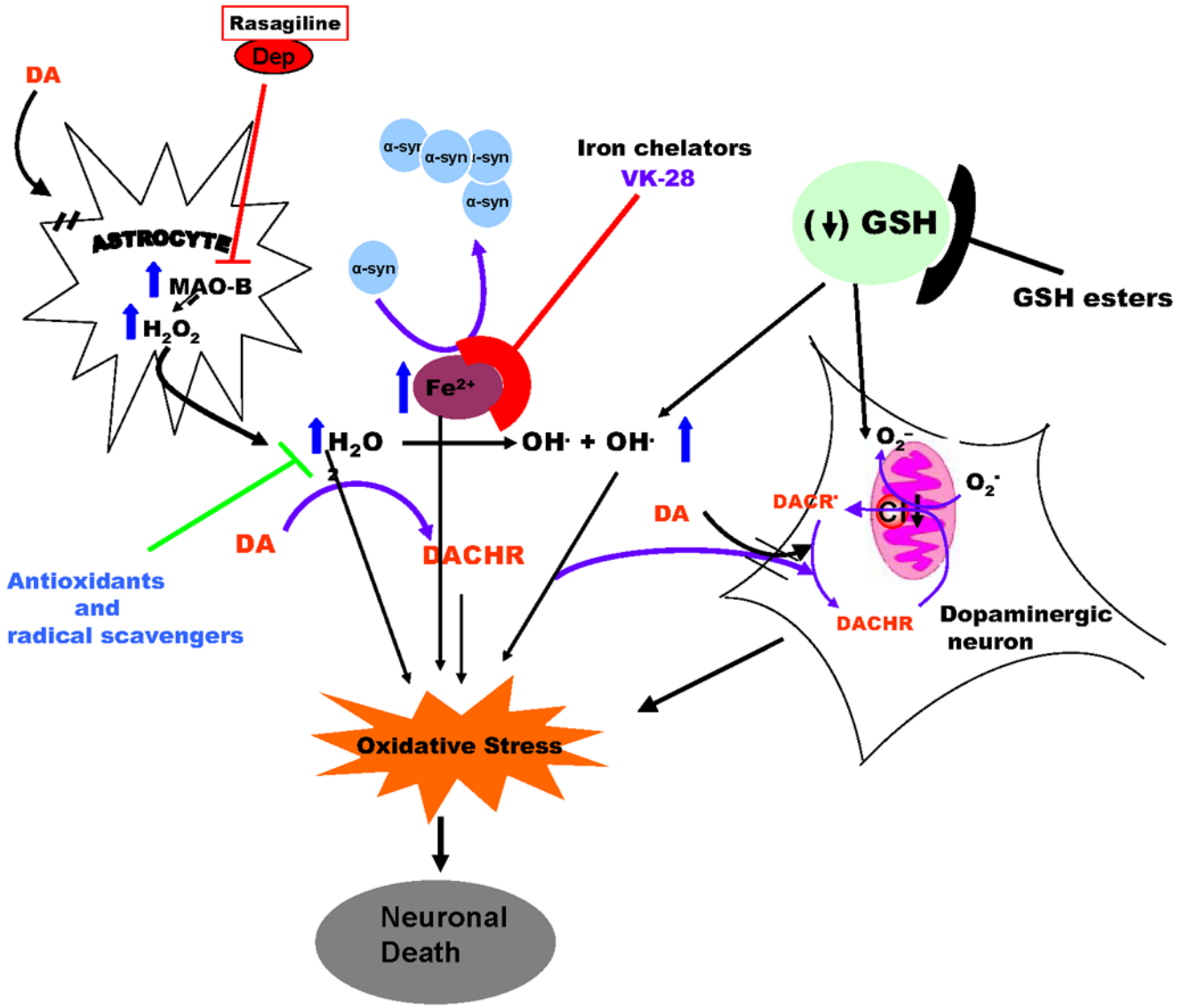

Fig. 1. Schematic illustrating the various sources of ROS that causes redox imbalance in PD and targets of therapeutic intervention to attenuate oxidative stress and dopaminergic cell loss

MAO-B, monoamine oxidase-B enzyme; Dep, deprenyl; α-syn, alpha-synuclein; H_2O_2, hydrogen peroxide; VK-28, 5-[4-(2hydroxyl) piperazine-1-ymethyl]-quinoline-8-ol; GSH, glutathione; CI, mitochondrial complex I enzyme; DA, dopamine; DACHR, dopaminochrome; $DACR^{-}$, dopaminochrome radical.