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Oxidative Stress in Parkinson's disease

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Abbreviations:
Cat: Catalase
CNS: Central nervous systems
CuZnSOD: Copper zinc superoxide dismutas
DA: Dopamine
ETC: Electron transport chain
GPx: Glutathione peroxidase
GSH: Glutathione;
GSSG: Glutathione disulfide
4HNE: 4-Hydroxynonenal
MnSOD: Manganese superoxide dismutas
MAO-B: Monoamine oxidase B
MPP^+: 1-methyl-4-phenylpyridinium
MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
Nac: Nucleus accumbens
NADH-DH: NADH-dehydrogenase activity
NMDA: *N*-methyl-D-aspartate
NO: Nitric oxide
3-NT: 3-nitrotyrosine
OXPHOS: Oxidative phosphorylation systems
ONOO-: peroxynitrite
PD: Parkinson's disease
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
SN: Substantia nigra
SOD: Superoxide dismutas
ST:striatum

VTA: ventral tegmental area

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Abstract:

Parkinson's disease (PD), the second most common neurodegenerative disease, occurs when dopaminergic neurones in the substantia nigra (SN) lose in the brain. The discovery of the drug 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the early 1980s, a neurotoxin that causes a parkinsonism-like syndrome, fostered the concern that environmental toxins could be a prominent cause of PD. MPTP has also found associated with oxidative stress and a mitochondrial dysfunction in PD. MPTP metabolizes to MPP+ by MAO-B in the brain is found to generate free radicals, blocks complex I activity in the respiratory chain, lead to a rapid loss of ATP, and neurons cell death. MPTP induces free radicals accumulation and decreases antioxidant enzyme levels in brain cells. Increased reactive oxygen species (ROS) production has been implicated in oxidative stress and mitochondrial dysfunction in cell death following brain injury. So, oxidative stress and free radical may play an important role in PD pathogenesis. Hence, future research needs to be done to test whether antioxidant enzymes and ROS production can be involved in PD.

Introduction

Among the most common aged- related neurodegenerative diseases, PD is the second common disease affecting approximately 500,000 people in the United States, with as many as fifty thousand new cases each year. As the elderly population increases, this disease is likely to increase. PD usually begins in a person's late fifties or early sixties and causes a progressive decline in movement control, affecting the ability to control initiation, speed, and

smoothness of motion. Parkinson's disease has a prevalence of approximately 0.5 to 1 percent among persons 65 to 69 years of age, rising to 1 to 3 percent among persons 80 years of age and older [1]. It is chronic and progressive and its underlying disease mechanisms remain poorly understood. While both genetic and environmental factors have been investigated, no clear candidate has emerged. Theories include: mitochondial dysfunction, free radical-induced oxidative damage, toxins in the environment, inherited tendencies and accelerated aging. Research in the recent years has accumulated substantial evidence supporting the hypothesis that oxidative stress triggers a cascade of events leading to the death of neuronal cells during PD [2].

Background on PD

PD is a movement disorder affecting all ethnic groups and both genders. The major risk factors for PD are aging and environmental factors, although known genetic forms and a male predominance of disease support a genetic component [1].

1) Clinical features

PD is manifested clinically by tremors, braykineasia, rigidity, slow movements, and postural instability. Symptoms are responsive, at least in the early stages, to replacement of the neurotransmitter dopamine with levodopa therapy. However, several years after disease onset, this therapy loses efficacy and side effects predominate. Death occurs on average a decade after initial symptom onset [3], usually as a result of complications of immobility.

2) Neuronal pathology

PD occurs when nerve cells in a certain part of the brain die or stop working properly. The substantia nigra is one of the principle movement control centers in the brain. By

releasing the neurotransmitter known as dopamine(DA), it helps to refine movement patterns throughout the body. Dopamine normally transmits signals to another part of the brain that allows controlled muscle movement. Without enough dopamine, the cells in this part of the brain fire out of control [4].

Pathologically, PD is characterized by the death of dopaminergic neurones in the substantia nigra. Its pathological features also include the presence of intracytoplasmic inclusions known as Lewy bodies [5]. There are two major dopaminergic pathways in the central nervous system (CNS). One is the nigrostriatal pathway, which arises from the SN and terminates in the striatum (ST); another is the mesolimbic pathway, which arises from the ventral tegmental area (VTA) and terminates in the nucleus accumb (NAc) and other limbic areas [6].

Many reports on Parkinson's disease (PD) have shown that the most severe damage to DA neurons occurs in the nigrostriatal pathway, while the mesolimbic pathway is relatively spared [7].

3) Genetic feature:

The role of genetics in PD is always widely discussed and controversial. However, familial aggregation studies suggest that late-onset PD has a significant genetic etiology [8]. Three gene mutations: α synuclein, parkin and ubiquitin C-terminal hydrolase L1 are involved in heritable forms of PD. Several additional loci are shown to be associated with familial forms of PD [9]. Autosomal dominant PD results in mutations in the α-synuclein gene and autosomal recessive PD is due to mutations in the parkin gene [10].

4) Environment factors:

Free radical- induced oxidative damage and toxin such as MPTP can induce the risk of PD. There are other environmental factors such as pesticides that are involved in idiopathic PD. However, in a case control study, tea and cola drinks were observed that may reduce the risk of PD [11].

Mechanisms of MPTP:

An association between neurodegeneration and mitochondrial dysfunction or oxidative damage, or both, stems from studies of 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP)-induced parkinsonism.

MPTP is known as a dopaminergic neurotoxin that causes a parkinsonism-like syndrome in humans, primates, and mice [12]. Because MPTP is a highly lipophilic compound that rapidly crosses the blood–brain-barrier, it is retained in the brain in the form of its metabolite, 1-methyl-4-phenylpyridinium (MPP^+). After the oxidation of MPTP to MPP^+ by monoamine oxidase B (MAO-B) [13], MPP+ is specifically taken up by dopaminergic neurons and is actively accumulated in the mitochondria. Inside the mitochondria MPP^+ reduces the mitochondrial respiration rate and the NADH-dehydrogenase activity (NADH-DH) of the respiratory chain complex I [14]. The high concentration of MPP^+ inside the mitochondria blocks complex I activity in the respiratory chain. MPP^+ seems to inhibit complex I, both in state 4 (without ADP) and in state 3 (with ADP) of respiration, acting at the same site of rotenone, between NADH-DH and coenzyme Q, without affecting complex II activity [15].

This process is reversible and leads to a rapid loss of ATP. On the other hand, oxidation of MPTP to MPP^+ by MAO-B in the brain was found to generate free radicals and then can initiate apoptotic cell death through a decrease in mitochondrial membrane potential (Figure1). Incubation of MPP^+ with mitochondrial enzymes also induces free radical production; while the increased free radicals can further inhibit the function of complex I. In idiopathic Parkinson's disease (PD), there is a 30–40% decrease in complex-I activity in the substantia nigra. These results suggest that neuronal death occurs not only due to the loss of energy, but also due to the damage resulting from free radicals [12]. In particular, neuronal cells in the brain are highly sensitive to free radicals, because of high concentrations of polyunsaturated fatty acids that can be easily oxidized by ROS; the high rate of oxygen consumption(approximately 20%), the presence of Ca^{2+}-permeable channels, and low levels of catalase [4, 16].

Beside inducing the production of free radical, many evidence point that MPTP has a significant contribution of apoptosis to cell death by apoptotic DNA strand breaks, activation of the JNK pathway and caspases [17]. Insights into the mechanisms underlying the neurotoxicity of MPTP have significantly contributed to the understanding of processes involved in neuronal cell death in PD. This provides support for a role of environmental toxins in the aetiology of PD.

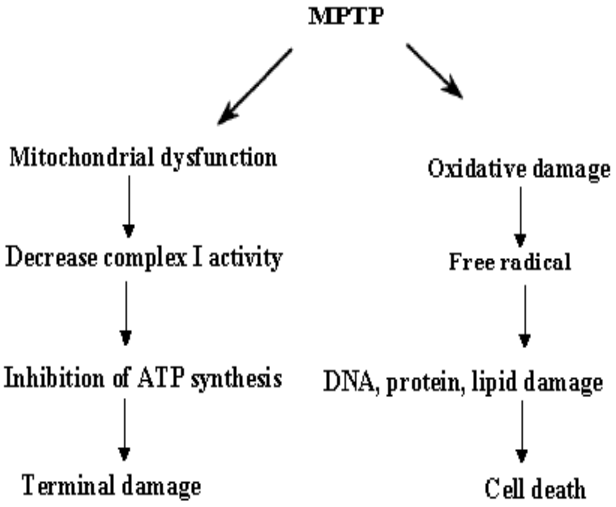

Figure 1: The mechanism of MPTP involving in cell death [12].

Oxidative stress

The accumulation of molecular alterations resulting from oxidative stress has been hypothesized to underlie the physiological and physical changes associated with neurodegenerative diseases including PD [18].

1) ROS

ROS are generated as a result of normal metabolism, but a deleterious condition, termed oxidative stress, can occur when their production is accelerated or when the mechanisms involved in maintaining the normal reductive cellular milieu are impaired. ROS include radical species such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), and reactive nitrogen species such as nitric oxide ($NO\bullet$) and peroxynitrite ($ONOO^-$).

The major route of metabolism of O_2 occurs in mitochondria, O_2 accepts electrons one at a time, the sequential univalent reduction of $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and water (reaction 1-4). Iron accumulating has been observed in specific brain regions including substantia nigra. The increased concentration of iron in the basal ganglia may account for its higher vulnerability to

chronic oxidative stress because H_2O_2 generates OH- radicals at a fast kinetic rate through the Fenton reaction [18].

$$O_2 + e \rightarrow O_2^{\bullet -} \quad 1)$$

$$O_2^{\bullet -} + e + 2H^+ \rightarrow H_2O_2 \quad 2)$$

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

$$OH^{\bullet} + OH^- + e \rightarrow H_2O + O_2 \quad 4)$$

2) NOS in brain

Reactive nitrogen species (RNS) can be generated by biochemical reactions of nitric oxide (NO) or by enzymatic catalysis of NO metabolism The generation of NO from arginine by nitric oxide synthase is a process which is involved in neurotransmission, regulation of vascular relaxation, and in inflammatory processes. The generation of NO• is catalyzed by three isoforms of nitric oxide synthase: neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS), and inducible nitric oxide synthase (iNOS). In PD and other neurogenerative diseases, the neuron injury results in extracellular glutamate concentration increase followed by opening of *N*-methyl-D-aspartate (NMDA)-gated channels. In this process, the excessive intracellular calcium are accumulated that leads to an abnormal activation of Ca^{2+}-dependent enzymes, including NOS. Then, the excess NO that has a short half-life in many biological systems can highly favorablly react with $O_2^{\bullet -}$ and produces peroxynitrite ($ONOO^-$)(reaction 5). The formation of hydroxyl radicals by MPP^+ reaction pathway is showed in figure 2. Since the toxicity associated with NO generation can in many cases be prevented by the scavenging of $O_2^{\bullet -}$, formation of $ONOO^-$ is considered to be an important factor in causing cellular damage [19].

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

Peroxynitrite can cause damage to macromolecules including DNA oxidation and single-strand breakage, lipid peroxidation, formation of protein carbonyls, and oxidation of cysteine, methionine, tryptophan, phenylalanine, and tyrosine amino acids [20]. More specifically, peroxynitrite can nitrate or oxidative tyrosine residues to form 3-nitrotyrosine (3-NT) and o-o'-dityrosine, respectively. 3-NT and o-o'-dityrosine also can result from the catalytic activity of peroxidases, such as myeloperoxidase using nitrite as a substrate [21]. Peroxynitrite plays a key role in neuronal damage associated with excitotoxicity. Some evidence shows that inhibitors of neuronal NOS block MPTP induced dopaminergic toxicity in mice, and that MPTP neurotoxicity is attenuated in mice deficient in neuronal NOS. Neuronal NOS inhibitors blocked MPTP neurotoxicity accompanied by an inhibition of 3-NT staining. MPTP neurotoxicity and 3-NT generation are also attenuated in mice deficient in iNOS [20].

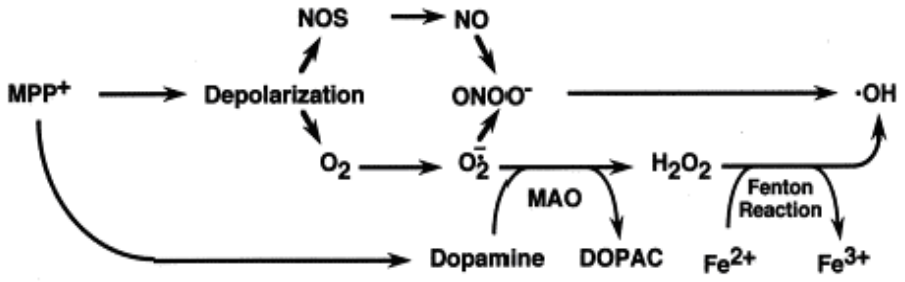

Figure 2: The reaction pathway in rat brain illustrates the formation of hydroxyl radical by depolarization-induced NO [20].

3) Molecular targets of oxidative damage

Proteins, lipids, and nucleic acids can be modified by ROS or RNS [22]. Nucleic acids are particularly reactive with strong oxidants such as hydroxyl radicals, which can attack pyrimidines, sugars, and purines. Exposure of nucleic acids to reactive species can result in

strand breakage, nucleic acid-protein cross-linking, and nucleic acid base modification [23]. Amino acids are also sensitive to oxidative damage, particularly aromatic amino acids, histidine, methionine, and cysteine [24]. Fatty acids, especially when polyunsaturated, are susceptible to damage by reactive species through the abstraction of hydrogen atoms from methylene groups. The resulting carbon radical group can react with molecular oxygen to generate a peroxyl radical that can itself abstract a hydrogen atom from another fatty acid molecule in a self-perpetuating process termed lipid peroxidation [24].

4) Oxidative stress in PD

The role of oxidative stress in neuronal degeneration in PD is substantiated by pathological findings and animal models that have provided experimental paradigms to delineate the possible mechanisms. Perhaps the main factor in the vulnerability of dopaminergic neurons is their intrinsic predisposition to generate reactive species. The normal enzymatic metabolism of dopamine results in the generation of hydrogen peroxide H_2O_2 by MAO-B. The nonenzymatic autoxidation of dopamine at neutral pH results in the formation of reactive quinones and semiquinones, this process is enhanced in the presence of iron, leading to the further formation of hydrogen peroxide, superoxide anions, and hydroxyl radicals [25]. It also has been suggested that the oxidation of dopamine results in the formation of 6-hydroxy-dopamine, which readily undergoes rapid autoxidation with molecular oxygen to generate reactive free radical species [26]. Although the importance of the formation of 6-hydroxy-dopamine under normal physiological conditions is still unknown, it has been widely used as a model of dopaminergic neuronal injuries. These findings have provided further credence to the proposal that dopamine metabolism results in oxidative stress [27].

Markers of oxidative stress, such as products of lipid peroxidation and oxidation of mitochondrial DNA and cytoplasmic RNA, are increased in dopaminergic neurons of PD brains [28].

Mitochondrial dysfunction

It has been hypothesized that mitochondrial dysfunction and consequent production of ROS may cause neuronal death evolving in the process of PD [18].

1) The mitochondrial electron transport chain:

Each mitochondrion consists of two phospholipid bilayers, the outer membrane and the inter membrane. The mitochondrial electron transport chain (ETC) is located in the inner mitochondrial membrane. Between the two bilayers is intermembrane space. The primary function of ETC is ATP synthesis. It comprises a series of electron carriers grouped into four enzyme complexes: (i) complex I (NADH ubiquinone reductase), (ii) complex II (succinate ubiquinone reductase), (iii) complex III (ubiquinol cytochrome *c* reductase), and (iv) complex IV (cytochrome *c* oxidase). In brief, flow of electrons along the ETC from NADH or FADH2 to molecular oxygen is coupled to the pumping of protons across the inner mitochondrial membrane, resulting in the formation of a proton gradient. Dissipation of this proton gradient drives ATP synthesis [30].

The brain is dependent on mitochondrial energy supply to maintain normal brain function. Therefore, damage to one or more of the respiratory chain complexes may lead to

impairment of cellular ATP synthesis. However, each of the complexes exerts varying degrees of control over respiration, and substantial loss of activity of an individual respiratory chain complex may be required before ATP synthesis is compromised [31].

2) Mitochondrial DNA and oxidative phosphorylation system

MtDNA is a circular double stranded molecule comprising a heavy (H) and a light (L) chain but without any histone coat. MtDNA encodes a full complement of 22 transfer RNAs (tRNAs) and 12S and 16S ribosomal RNAs in addition to 13 proteins, all of which are part of the respiratory chain and oxidative phosphorylation system (OXPHOS). Five different complexes involve in OXPHOS system: complex I, II, III, IV and ATP systhase. MtDNA is dependent upon the cell nucleus for encoding its replication, transcription, translation, repair and regulatory factors [31].

3) Interaction between oxidative phosphorylation and oxidative damage:

There are significant interactions between oxidative damage and mitochondrial energy metabolism, especially the OXPHOS. OXPHOS generates most of free radicals in the cells. Inhibition of ETC induces free radicals generation. In addition toproducing free radicals, OXPHOS itself is vulnerable to damaged by free radicals. There are two mechanisms involved in this process: one is it can be injured through damage to mtDNA. This susceptibility is probably because of it lack of protective histones, limited repair ability, and proximity to ECT [32]; the other mechanism is that ECT can also be affected directly by free radicals. Among those complexes, complex I is particularly sensitive to $OH^{\bullet}$ and $O_2^{\bullet -}$. The vulnerability of ECT may due to the damage to protein and phospholipids [33].

A cycling process may occur between oxidative damage and oxidative phosporylation,due to the fact that free radicals attack this system that also generate them (

Figure 3). When oxidative phosporylation generates free radials, these radicals damage mtDNA, proteins, lipids and induce the greater level of free radicals that may result in addition oxidative damage. This process may also reduce ATP levels, excessive release and reuptake of mitochondrial calcium [34]. In the cell, the mitochondrion is both a target and source of free radicals.

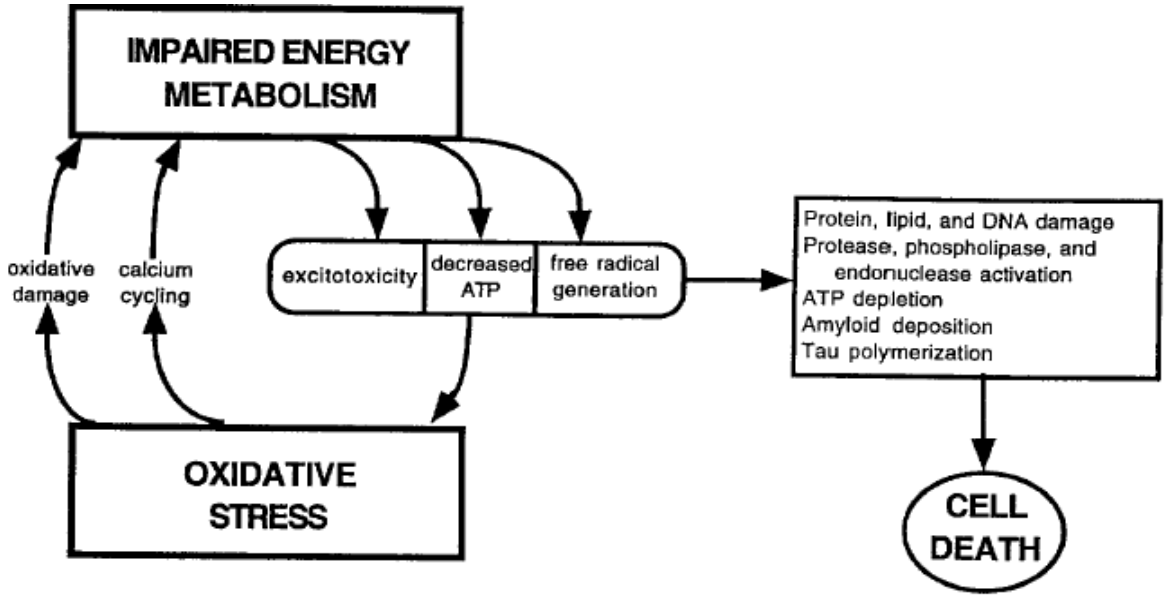

Figure 3: Possible cycling mechanism between impaired energy metabolism and oxidative stress. Oxidative stress may produce oxidative damage to macromolecules and " calcium cycling," both of which may impair energy metabolism. Impaired energy metabolism may the result in increased free radicals generation. These processes may facilitate cycling by further increasing oxidative stress. In addition, these processes may play an important role in cell death through oxidative damage to macromolecules, excitotoxic mechanisms, ATP depletion, amyloid aggregation, and tau polymerization [35].

4) Mitochondrial dysfunction in PD

A consequence of mitochondrial dysfunction is increased by generation of free radicals and oxidative damage, which are strongly implicated in the pathogenesis of neurodegenerative diseases including PD.

Substantial evidence has shown a mitochondrial defect in PD. It has demonstrated that MPP^+ inhibits complex I in SN and is not altered in other brain regions [36]. Increased oxidative stress in PD also induces increases lipid peroxidation in SN in patients [37]

It has been hypothesized that oxidative stress in substantia nigra is caused by increased iron level. By Fenton reaction, iron can react with H_2O_2 to produce $OH^{\bullet}$. Total iron levels are elevated in PD substantia nigra. Iron is also found increased in MPTP-treated-primate [38]. Mitochondrial dysfunction in PD also might be a consequence of either nuclear DNA or mtDNA [39].

Antioxidant enzymes Systems in PD

Free radicals are constitutively produced under normal physiological conditions; therefore, organisms develop various defense mechanisms to protect themselves against free radical injury. Such defense mechanisms include the antioxidant enzymes, free radical scavengers, and metal chelating agents. The antioxidant enzymes include catalase (Cat), glutathione peroxidase (GPx), and superoxide dismutase (SOD). SOD catalyzes the dismutation of superoxide ($O_2^{\cdot -}$) to hydrogen peroxide (H_2O_2), while catalase and GPx convert H_2O_2 to H_2O. The scavengers include ascorbate, a-tocopherol and glutathione (GSH). GSH not only acts as a scavenger, but also regenerates other scavengers and serves as a substrate in the GPx reaction [12].

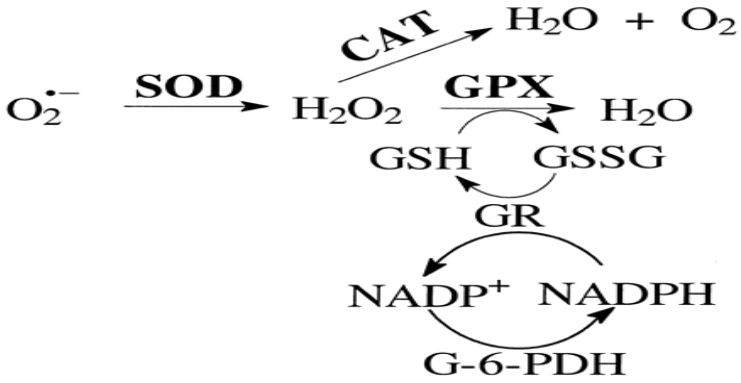

Figure 4: Scheme of antioxidant system [29].

Compared to other organs in the body, the brain has lower lever activities of detoxifying enzymes like SOD, catalase and GR. It also contains excess unsaturated fatty acids which are targets for lipid peroxidation. Mitochondria, nitric oxide synthase, arachidonic acid metabolism, xanthine oxidase, MAO and P450 enzymes are all sources of ROS in the brain. GPx is the major enzyme for the detoxification of H_2O_2 in the brain since the brain has very low catalase activity[40].

1) SOD

Several different superoxide dismutases (SOD) have evolved to inactivate both intracellular and extracellular superoxide. There are two types of intracellular SODs: the manganese SOD (MnSOD) and the copper/zinc SOD (CuZnSOD). MnSOD is localized within the mitochondrial matrix, while CuZnSOD is confined predominantly in the cytoplasm or the nuclear [41]. Klivenyi *et al* [42] found that overexpressing the human MnSOD gene in mice showed significant neuroprotection against MPTP-induced depletion of dopamine levels, as well as peroxynitrite-mediated oxidative damage. In previous study, transgenic mice with increased Cu/ZnSOD activity are also found resistant to MPTP-induced neurotoxicity. Further, inhibition of SOD activity was reported to enhances MPTP toxicity *in vivo* [40].

2) GPX

The role of the key hydrogen peroxide converting enzyme, GPX, is controversial. Early studies report decreased GPX activity in the substantia nigra, caudate and putamen in PD patient [43], however, they are not supported by subsequent investigations [44]. Importantly, in vitro assessment of GPx levels in post-mortem tissues may not reflect events occurring in vivo.

3) GSH

GSH plays an important role in the adult brain by removing H_2O_2 formed during normal cellular metabolism. In general, SN has lower levels of GSH compared to other regions in the brain. It has been observed that during PD, there is a further reduction in GSH levels within the SNpc. In fact, GSH depletion is the first indicator of oxidative stress during PD progression suggesting a concomitant increase in ROS. As figure 5 shows, the magnitude of GSH depletion occurs prior to other hallmarks of the disease including decreased activity of mitochondrial complex I, decreased enzyme activities in mitochondrial as well as losses in ATP production. GSH may protect neurons against the build-up of protein aggregation which form Lewy bodies within the cell, the deleterious effects of the lipid peroxidation by-product 4-hydroxynonenal (4-HNE) [45], and protein oxidation [46].

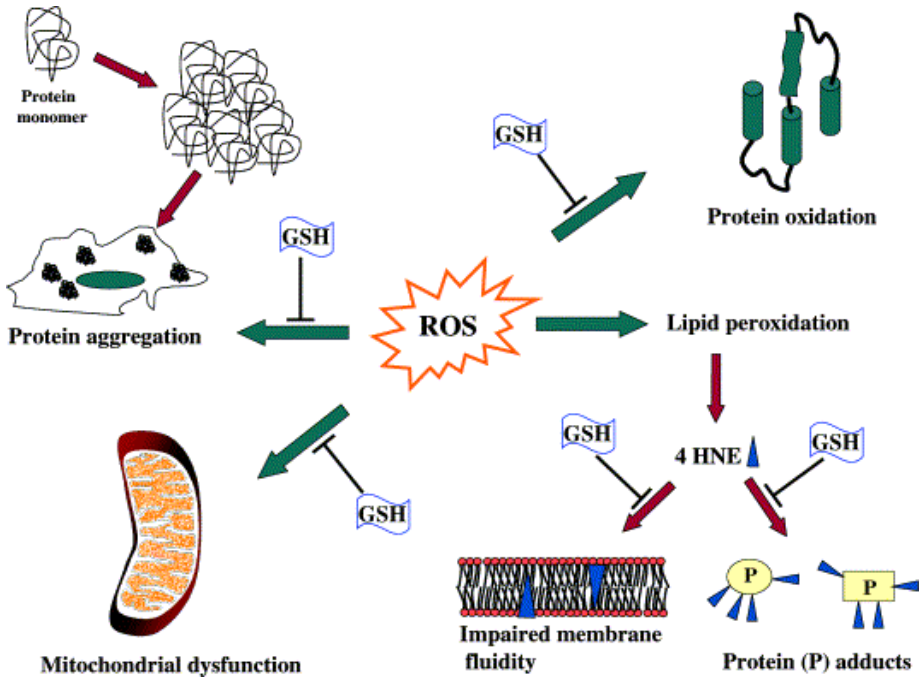

Figure 5: The different roles of GSH: a schematic representation of the antioxidant properties of GSH as relevant to SN dopaminergic neuronal cells in PD [40].

Future research direction

Based on the above discuss, many factors seem to be involved in the pathogenesis of PD, such as MPTP regulation, oxidative stress, ROS/RNS, and mitochondrial dysfunction. Free radicals that are generated and accumulated in those pathways may play an important and primary role in PD. However, the exact mechanism of PD remains unclear. Moreover, the role of antioxidant enzymes in MPTP model is not defined. Hence, the future direction can be designed and focused on the free radical generation and antioxidant system. Using the information gathered, three main areas will be reached:

Special aims I: Antioxidant enzymes level in PD pathogenesis:

It has demonstrated that SOD, Gpx and GSH levels are low in PD. An imbalance in antioxidant system leading to oxidative stress in the brain cells is identified in PD. MPTP, an neurotoxin, which selectively destroy dopaminergic neuron, can induce PD -type symptoms. MPTP not only cause mitochondrial dysfunction, but also produce excessive free radicals. If the mitochondria is the primary source of $O_2^{\bullet -}$ generated by MPTP, the antioxidant enzymes should show an attenuation of toxin. Here, we can test this hypothesis that these MPTP might induce cell death in nigral dopaminergic cell line, SN4741 [47] and overexpressing antioxidant enzymes such as MnSOD, CuZnSOD and GPx can decrease the cell death.

1) *In vitro* experiments, MnSOD, CuSOD and GPx adenoviruses are added separately in each group after MPTP incubation with cells. Growth curve, plating efficiency, soft agar, and activity assay and protein levels should be examined.
2) *In vivo* experiment, transgenic mice with high-level DA are used. Control group is injected with MPTP alone in brain area, and other groups are injected with MPTP and

adenovirus. The mice are killed and the brain is removed. Mitochondria can be separated form the brain. The respiratory chain enzyme activities and antioxidant enzyme assays should be examined.

Special aims II: ROS/RNS involved in PD pathogenesis:

In this area, the function of $ONOO^-$ and $O_2^{\bullet-}$ will be studied. As mentioned before, $ONOO^-$ can cause DNA oxidation, single-strand breakage and lipid peroxidation. In postmortem studies of PD, lipid peroxidation was elevated in SN, while GPx and GSH level were reduced [2-5]. $ONOO^-$can be generated from the near-diffusion limited reactivity of NO with $O_2^{\bullet-}$. NO is produced in various cell types by NOS, while $O_2^{\bullet-}$ is generated during normal metabolic processes in mitochondria and removed by SOD. So using antisense NOS, RNAi, or a NOS inhibitor, such as L-NMA [48], the production of NO will be hindered. To reduce the production of $O_2^{\bullet-}$, *AdMnSOD* or *AdCuZnSOD* will be added. Therefore, the $ONOO^-$ production will decrease. Previous study also shows that inhibition of neuronal NOS can protect against MPTP-induced neurotoxicity in mice. So, if *AdMnSOD* or *AdCuZnSOD* combination with NOS inhibitor can successful decrease the level of $ONOO^-$ in cells, this method would be clinically tested in patients.

1) *In vitro,* the SN4741 cell line will be grown in culture. The $ONOO^-$ formation in different groups that added MPTP, NOS inhibitor L-NMA or antisense NOS gene adenovirus, *AdMnSOD* or *AdCuZnSOD* alone and added those combinational

treatment will be compared. This process can be measured and quantified using electron paramagnetic resonance to trap the radical.

2) *In vivo* experiment, NOS knockout mice can be used as replacement of antisense NOS gene adenovirus. Using clinical markers of PD, the percentage of mice with and without PD symptoms will be determined in each group with different treatment as *in vitro* experiment.

Special aims III: GSH level in PD pathogenesis:

In PD, a profound GSH depletion is associated with decrease dopaminergic cell death in the SN. Although there may exist a link between the fall in GSH concentration, mitochondrial damage and cellular death, it is still not known whether GSH depletion in the SN represents a primary cause of neurodegeneration in PD. The relationships between those fators remain to be resolved and may provide important insights into the pathogenesis of Parkinson's disease. To know the effect of GSH deficiency on the mitochondrial damage, ROS production, and celluar death, buthionine sulfoximine (BSO), a specific inhibitor of γ-glutamylcysteine synthetase that is the rate-limiting enzyme in GSH synthesis, can be used. Also, 1,3-*bis*-Chloroethyl-1-nitrosourea (BCNU), an inhibitor of glutathione reductase (GR) that acts as an important antioxidant [50], reduces glutathione disulfide (GSSG) back to primary GSH can be used to reduce the incelluar GSH level.

1) *In vitro,* the SN4741 cell line will be grown in culture. The GSH level in different groups that added MPTP, BSO or BCNU alone and added those combinational treatments will be compared. Quantification of GSH activity can measured by the method Spitz and Oberley descried [51]. The soft agar, plating efficiency, and cell

growth curve also be determined. The radicals can be measured and quantified using electron paramagnetic resonance.

3) *In vivo* experiment, using clinical markers of PD, the percentage of mice with and without PD symptoms will be determined in each group with different treatment as *in vitro* experiment. The mice are killed and the brain is removed. Mitochondria can be separated form the brain. The respiratory chain enzyme activities and GSH activity should be examined.

Summary

Parkinson's disease is a common and progressive neurodegenerative disease. The mechanism of Parkinson's disease is still unknown. Two factors, oxidative stress and mitochondrial dysfunction have been proposed, which is concerned by the study of MPTP, a neurotoxin that causes a parkinsonism-like syndrome and produce excessive free radicals in metabolism. MPTP also inhibits complex I activity and further leads a loss of ATP synthesis resulting cell death. Although ROS have been shown to accelerate Parkinson's disease progression, the role of antioxidant enzymes is not defined. Elucidating the pathways in formation of oxidative stress and mitochondrial dysfunction and the relationship between ROS and other PD attributive factor may help us to better understand the mechanism of Parkinson's disease.

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