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Review Article

Dopamine (DA) Dependent Toxicity Relevant to DA Neuron Degeneration in Parkinson's Disease (PD)

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Abstract

Parkinson's Disease (PD) is a common incurable neuron degenerative disease characterized by progressive dopamine (DA) neuron degeneration in the pars compacta of the Substantia Nigra (SN) plus Lewy body formation in affected brain areas. DA is the neuron transmitter for DA neurons and progressive DA neuron degeneration will significantly decrease DA content in SN, contributing to PD onset. DA can be catabolized to form inactivate metabolites by multiple enzymes accompanied with generation of Reactive Oxygen Species (ROS). Furthermore DA is unstable and can undergo auto-oxidation or oxidation mediated enzymes or metal ions. The oxidation of DA can generate small molecular ROS and highly reactive DA Quinones (DAQ). Accumulative evidence demonstrates that highly reactive DAQ seems to play more important pathological roles than small molecular ROS in DA neuron degeneration in PD. The small molecular ROS can induce oxidative stress in DA neurons via reversible oxidative modification of macromolecule including proteins, lipids and nucleic acids. However, DAQ is highly reactive and can induce DA neuron vulnerability *via* multiple toxic mechanisms. The DAQ can irreversibly and covalently conjugate with cysteine residues of proteins, leading to protein misfold, inactivation and aggregation. Furthermore free DAQ and DAQ conjugated proteins can undergo redox cycling to generate deleterious ROS. We observe that endogenous DA derived DAQ can induce irreversible inhibition of Ubiquitin-Proteasome System (UPS). We also find that iron ions can significantly mediate DA oxidation and promote DAQ generation and subsequent toxicity to DA neurons. However conjugation of DAQ with proteins can be abrogated by sulfhydryl groups containing agents, including GSH and N-Acetyl-Cysteine (NAC). The toxicity of DAQ is also relevant to genetic factors induced DA neuron degeneration in Familial form PD (FPD). The α-synuclein (α-syn) induced toxicity can be DA dependent. The conjugation of DAQ with α-syn can enhance α-syn toxicity. On the other hand, the mutations of PINK1 can up-regulate Tyrosine Hydroxylase (TH) and DA levels, leading to DA dependent DA neuron vulnerability and degeneration under PINK1 mutations. In summary, DA, especially DA derived DAQ; induced toxicity to DA neurons can be a centre event in PD onset and development. In this short review, some major previous achievements on DA dependent toxicity relevant to environmental and genetic factors induced DA neuron degeneration in PD have been summarized and discussed.

Keywords: Dopamine oxidation; Dopamine quinone; GSH; Parkinson's disease; Pathogenesis; Reactive oxygen species

Abbreviations

PD: Parkinson's Disease; SN: Substantia Nigra Pars Compacta; LBs: Lewy bodies; DA: Dopamine; SPD: Sporadic PD; FPD: Familial Form PD; α-Syn: α-Synuclein; UCHL1: Ubiquitin C-Terminal Hydrolase L-1; ROS: Reactive Oxygen Species; DAQ: Dopamine Quinones; NE: Norepinephrine; EPI: Epinephrine; MAO: Monoamine Oxidase; COMT: Catechol-O-Methyl Transferase; ALDH: Aldehyde Dehydrogenase; HVA: Homovanillic Acid; AM: Aminochrome; UPS: Ubiquitin Proteasome System; NAC: N-Acetyl-Cysteine; L-Cys: L-cysteine; AA: Ascorbic Acid; SOD: Superoxidase Dismutase; CAT: Catalase; $K_4Fe(CN)_6$: Potassium Ferrocyanide; $K_3Fe(CN)_6$: Potassium Ferricyanide; DFO: Deferoxamine; BBB: Blood Brain Barrier; WT: Wild-Type; TH: Tyrosine Hydroxylase; α-MT: α-Methyltryptamine

Introduction

Parkinson's Disease (PD) is the second most common neurodegenerative disorder that affects 1% of the general population over the age of 60 [1]. In addition, it has been estimated that the number of PD affected individuals globally will double by 2030 [2]. PD is characterized by selective loss of dopaminergic neurones in the Substantia Nigra pars compacta (SN) as well as formation of protein inclusions called Lewy Bodies (LBs) in affected brain areas [3]. The progressive Dopamine (DA) neuron degeneration will lead to significant decrease of DA content in SN, which contributes to onset of PD symptoms, including tremor, akinesia, bradykinesia and stiffness. So far PD is still incurable and L-DOPA replenish therapy can only transiently alleviate PD symptoms, but can not abrogate

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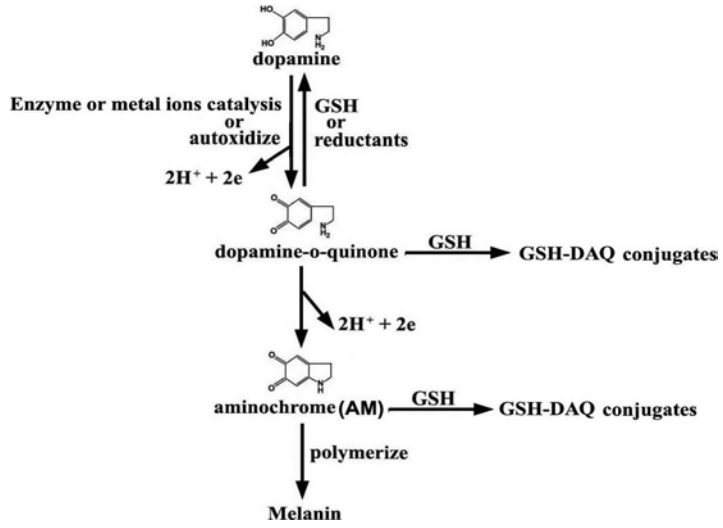

Figure 1: The process of DA oxidation.
In the absence of enzyme or metal ions, DA is apt to auto-oxidize in solutions, which can be effectively abrogated by reductants, especially by GSH. Enzyme (such as tyrosinase) or metal ions (such as iron species and Mn^{3+}) can mediate DA oxidation in solutions. The oxidation of DA will lead to formation of DA-*o*-quinone concomitant with desquamation of two protons and two electrons. The DA-o-quinone is highly reactive and can automatically cyclize to form AM accompanied by further desquamation of two protons and two electrons. The AM aggregates and forms melanin in the absence of conjugating chemicals. However in the presence of GSH, GSH can conjugate with DA-*o*-quinone or AM to form GSH-DAQ conjugates.

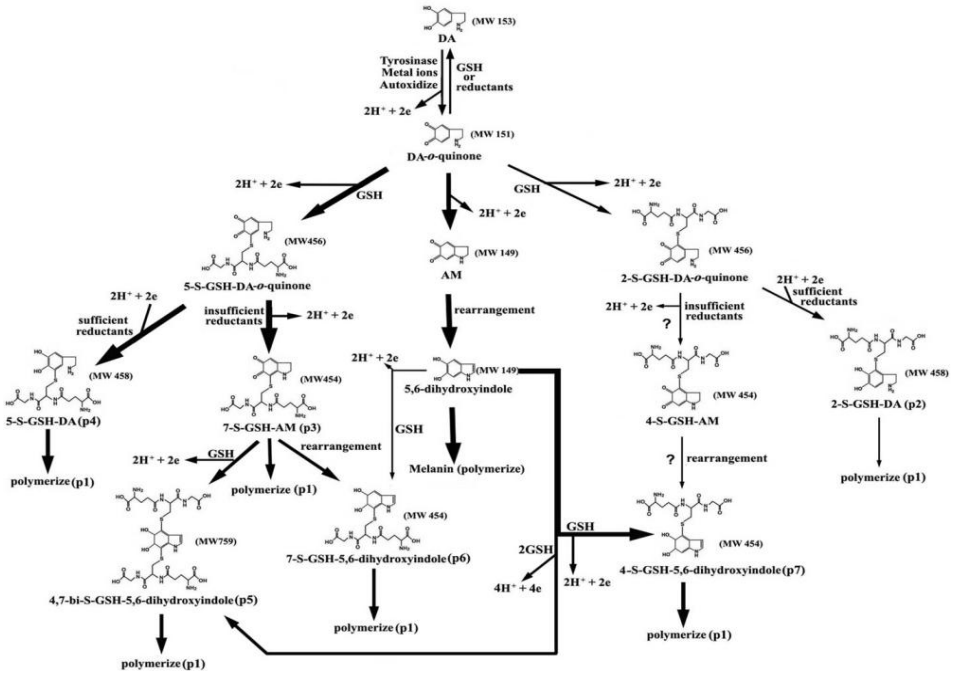

Figure 2: GSH-DAQ conjugates formation between GSH and DAQ.
Oxidation of DA can produce DA-*o*-quinone, which can cyclize spontaneously to form AM in the absence of GSH. In the presence of GSH, GSH can conjugate with DA-*o*-quinone to form intermediate GSH conjugates 5-S-GSH-DA-*o*-quinone (more) or 2-S-GSH-DA-*o*-quinone (less). The intermediate GSH conjugates were unstable and can be reduced to 5-S-GSH-DA (more) or 2-S-GSH-DA (less) by surrounding reductive agents. However if insufficient reductive force is present, the intermediate GSH conjugates can cyclize spontaneously to form reactive 7-S-GSH-AM (more) or 4-S-GSH-AM (less). The 4-S-GSH-AM has not been detected. The reactive cyclized GSH conjugates can spontaneously transform to non-reactive 7-S-GSH-5,6-dihydroxyindole (more) or 4-S-GSH-5,6-dihydroxyindole (less). The reactive 7-S-GSH-AM can further react with GSH to form 4,7-bi-S-GSH-5,6-dihydroxyindole. On the other hand, cyclized AM could react with GSH to form 4-S-GSH-5,6-dihydroxyindole (more) 4,7-bi-S-GSH-5,6-dihydroxyindole (middle) and 7-S-GSH-5,6-dihydroxyindole (less). All GSH conjugates, especially AM and GSH-AM, have inclination to polymerize in solutions.

progressive DA neurons degeneration in PD patient brains. Most PD cases are Sporadic PD (SPD) in nature and their exact pathogenesis is illusive. However, mutations in several genes can contribute to rare early-onset Familial form PD (FPD). So far more than 10 genes have been identified to be linked to FPD. These PD genes including α-Synuclein (α-syn), Parkin, Ubiquitin C-Terminal Hydrolase L-1

(UCHL1), PINK1, DJ-1, FBXO7, CHCHD2, COQ2 and LRRK2 [4-10]. While the detailed pathogenesis of PD is still unclear, a well-accepted notion has linked DA neuron degeneration in PD with aggravated oxidative stress in DA neurons [11]. DA can undergo oxidation to generate small molecular Reactive Oxygen Species (ROS) and highly reactive DA Quinones (DAQ) [12]. It is demonstrated that endogenous DA can be the culprit for up-regulated oxidative stress in DA neurons [13-15]. Furthermore accumulative evidence shows that DAQ play more significant pathological roles than ROS in DA neuron degeneration in PD [16,17]. Progress in this aspect should be vital to our understanding of PD pathogenesis and future improvement of our therapy against DA neuron degeneration in PD.

DA Metabolism and DA Derived Toxic Metabolites

DA is a neurotransmitter for DA neurons in SN, which is vital to human movement and motivated behaviour. Apart from this, it is also a central component of neuroendocrine axes and an intermediate in the formation of Norepinephrine (NE) and Epinephrine (EPI) [18]. In the presence of the DA-β-hydroxylase, DA is converted into NE and then modified into EPI by the phenylethanolamine-N-methyl-transferase [18]. DA itself is not toxic; however DA is unstable and its reactive metabolites can induce deleterious effects on DA neurons.

DA can be broken down into inactive metabolites by a set of enzymes-Monoamine Oxidase (MAO), Catechol-O-Methyl Transferase (COMT) and Aldehyde Dehydrogenase (ALDH), acting in sequence [19]. Different breakdown pathways exist but the main end-product is the Homovanillic Acid (HVA) with no known biological activity [20]. Briefly DA can be catabolized along two metabolic pathways: about 80% of DA is intraneuronal N-oxidized by MAOB to form 3,4-Dihydroxyphenylacetic Acid (DOPAC, an intermediate metabolite), while 20% of DA is extraneuronally O-methylated by COMT to 3-Methoxytyramine (3-MT, another intermediate metabolite) [21]. Large amounts of DA released into the synaptic cleft can be easily taken up by DA Transporter (DAT) and catabolized by MAOB which is located on mitochondrial membranes [22]. During DA oxidation the ROS can be generated, while O-methylation of DA is a safer process and 3-MT exhibits its own receptor activity [22]. It has been confirmed that increased MAOB level and activity is relevant to DA neuron degeneration in PD and MAOB inhibitors have been used clinically to up-regulate DA levels in brains of PD patients to alleviate PD symptoms [23,24].

Furthermore DA can undergo auto-oxidation or oxidation induced by enzyme or metal ions (manganese, iron and copper) to produce small molecular ROS as well as highly reactive DAQ [12]. The oxidation of DA commences from desquamation of two protons and two electrons from 2 hydroxyl groups of DA [12]. The desquamated two protons and electrons can be passed on to oxygen to form ROS [12]. The oxidized DA can form highly reactive DA-*o*-quinone after donating two electrons and two protons during oxidation [12]. The highly reactive DA-*o*-quinone is very short lived. It can be reversibly reduced back to DA by reductants or conjugate with other molecules such as sulfhydryl groups of proteins or form lower reactive cyclised DA quinone, the Aminochrome (AM), *via* internal cyclization in the absence of reductants or conjugation molecules [25]. During conjugation or internal cyclization reactions, protons and electrons can be further released, which can be used to generate more ROS. The detailed process of DA oxidation can be visualized in (Figure 1).

Evidence demonstrates that highly reactive DAQ, rather than small molecular ROS, play more vital pathological role in DA-related toxicity to DA neurons [26]. The small molecular weight ROS can induces reversible oxidative modification of macromolecules, whereas DAQ can conjugate with sulfhydryl groups of cysteine residues of proteins to induce irreversible modification of functional proteins [27-29]. Furthermore free DAQ or DAQ conjugated to proteins can undergo redox cycling to generate ROS [29-32]. Therefore DAQ can lead to DA neuron vulnerability *via* enhanced oxidative stress as well as covalent conjugation with function proteins, supporting the more vital pathological roles of DAQ than that of ROS. The DAQ modification of proteins can lead to protein misfold, inactivation aggregation, which will be toxic to DA neurons. Recent findings show that DAQ modified protein can accumulate in the SN of aged rats and is correlated with DA induced toxicity in human dopaminergic neurons [33].

DAQ can be linked to mitochondria impairment and inhibition of the Ubiquitin Proteasome System (UPS) in DA neurons [34]. We found that endogenous DA in dopaminergic cells could inhibit proteasome activity and further sensitize the proteasome to MG132 inhibition, leading to DA neurons vulnerability [15]. We have also demonstrated that DA induced irreversible proteasome inhibition *via* DAQ, rather than through small molecular weight ROS [16]. Other study demonstrated that DAQ was also relevant to mitochondria impairment [17]. The DAQ was found to conjugate with mitochondrial proteins to induce mitochondria injure [35]. Proteomic techniques were utilized in a previous study to identify proteins directly conjugated with DA from isolated rat brain mitochondria and human dopaminergic SH-SY5Y cells [35]. A subset of rat brain mitochondrial proteins were observed to be covalently modified by DA, including chaperonin, ubiquinol-cytochrome c reductase core protein 1, glucose regulated protein 75/mitochondrial HSP70/mortalin, mitofilin and mitochondrial creatine kinase [35]. The PD associated proteins ubiquitin carboxy-terminal hydrolase L1 and DJ-1 were also found to be covalently modified by DA in both brain mitochondrial preparations and SH-SY5Y cells [35]. Furthermore it was observed that DA induces mitochondrial membrane depolarization and a loss of phosphorylation capacity in brain mitochondria could be aggravated by accelerated DA oxidation [17]. The DA induced toxicity to mitochondria could only be weakly prevented by MAO inhibitor. Furthermore the DA induced toxic effect to mitochondria could be significantly prevented by GSH and N-Acetyl-Cysteine (NAC), but not by other ROS scavengers or metal chelators [17]. In another report, it was demonstrated that the generation of DAQ in isolated respiring mitochondria triggered the opening of the permeability transition pore most probably by inducing oxidation of NADH [36]. All these results imply the toxic roles of DAQ involved in DA induced mitochondrial impairment [17,35,36]. Therefore DAQ induced functional protein misfold, inactivation and aggregation as well as subsequent UPS inhibition and mitochondria impairment significantly account for selective DA neuron degeneration in PD.

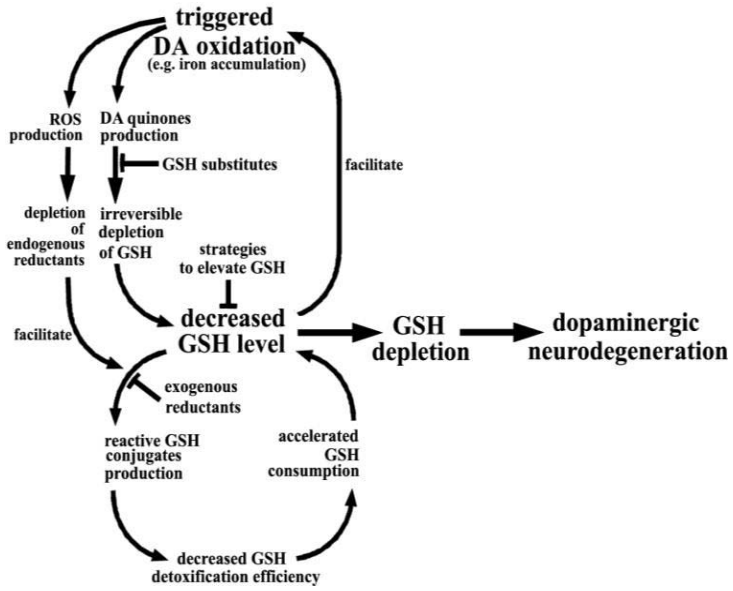

Figure 3: Two detrimental positive feedback loops may contribute to decreased GSH level and vulnerability of dopaminergic neuron.
Accelerated DA oxidation can be triggered by pathological factors, such as iron accumulation independent of surrounding reductive force. The accelerated DA oxidation can produce more DAQ which can conjugate with GSH, leading to irreversible depletion of GSH and finally decreased GSH level in DA neurons. The decreased GSH level in DA neurons can promote reactive GSH conjugates formation (7-S-GSH-AM) which can further react with GSH. Such situation will lead to impaired GSH detoxification capacity against toxic DAQ; accelerated GSH consumption and further aggravated down regulation of GSH level (refer to the lower detrimental positive feedback loop). On the other hand, the decreased GSH level will further favour DA oxidation and contribute to increased oxidative stress and DAQ generation (refer to the upper detrimental positive feedback loop). The ROS produced from DA oxidation will also deplete endogenous reductants and favour reactive GSH conjugates formation. These effects will finally converge and lead to an imbalance between DA induced toxicity and GSH protective capacity and even dopaminergic neuron degeneration. Strategies to elevate GSH level can abrogate these detrimental feedbacks. GSH substitutes with DAQ conjugating capacity may be used to conjugate with and detoxify DAQ, so as to preserve GSH and help elevate GSH level in DA neurons. Replenishing of exogenous reductants may help reduce the intermediate GSH conjugates and promote formation of non-reactive GSH conjugates. All these measures may take effects to help break down the potential vicious cycles, deleterious to DA neurons.

Protection against DAQ Toxicity by GSH and Other Sulfhydryl Groups Containing Chemicals

GSH is an important endogenous ROS scavenger and DAQ detoxifier [12,37]. In the absence of metal ions, GSH can inhibit DA auto-oxidation and tyrosine catalyzed DA oxidation [12]. We also found that GSH could inhibit DAQ internal cyclization and abrogate formation of Aminochrome (AM), a cyclised DAQ [12]. GSH can provide its sulfhydryl group to react with and detoxify DAQ [12]. GSH can react with DAQ to form 5-S-GSH-DA or 2-S-GSH-DA spontaneously or catalyzed by GSH transferase M2-2 [38]. We also showed that GSH could react with AM to form various conjugates including 4, 7-bi-S-GSH-5, 6-dihydroxyindole, 4-S-GSH-5, 6-dihydroxyindole and 7-S-GSH-5, 6-dihydroxyindole without enzymatic catalysis [12]. These functions of GSH can protect DA neurons from DA and DAQ induced toxicity. Therefore decreased GSH level may contribute to onset and development of DA neuron degeneration in PD. The post-mortem studies had shown increased GSH-DA conjugates in PD brains compared to normal controls, suggesting pathological roles of DAQ and active conjugation of DAQ with GSH in PD brains [39]. Furthermore post-mortem studies also showed that the GSH content in SN in early onset PD was found to be significantly decreased by ~50% when compared to age matched controls [40]. These findings indicate that impaired GSH protective capacity in DA neurons may be the important factor contributing to DA neuron degeneration in PD [40].

Our recent findings demonstrated that short-lived intermediate GSH-conjugate (5-S-GSH-DA-o-quinone and 2-S-GSH-DA-o-quinone) could be first formed when GSH reacted with DA derived DA-o-quinones [41]. These intermediate GSH-conjugates were unstable and could transform into reactive or non-reactive GSH-DAQ conjugates dependent on ambient reductive forces [41]. In the presence of sufficient reductive force, the intermediate GSH-DAQ conjugates could be reduced and transformed into non-reactive 5-S-GSH-DA and 2-S-GSH-DA [41]. However, under insufficient reductive forces, the intermediate GSH-DAQ conjugates could cyclize spontaneously to form reactive 7-S-GSH-aminochrome (7-S-GSH-AM) [41]. The 7-S-GSH-AM was still reactive and toxic, as it could further conjugate with another GSH to finally form non-reactive 4,7-bi-GSH-5, 6-dihydroindole in solutions [41]. We validated the toxicity of 7-S-GSH-AM and found that it could inhibit tyrosinase and proteasome activity rapidly in solutions [41]. The detailed GSH conjugation with DAQ is illustrated in (Figure 2). The DAQ can conjugate with cysteine residues of functional proteins. Based on our current findings, it can be speculated that DAQ conjugated with cysteine residues of proteins can further conjugate with other cysteine residue of the same protein or conjugate with cysteine residues of nearby proteins to form complicated crosslink. These conjugation and crosslink can significantly impair cell viability of DA neurons. Thus DAQ induced toxicity to cells may be more complicated and serious than we have expected.

Our findings showed that under impaired reductive force (such as

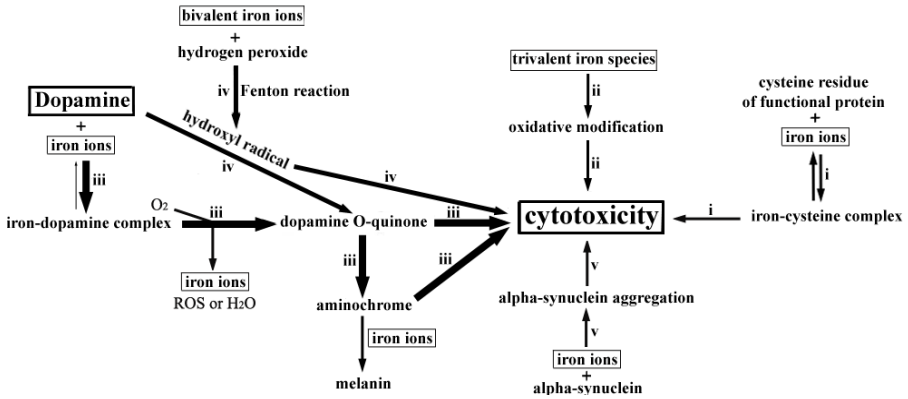

Figure 4: The toxic mechanisms of iron species relevant to DA neuron degeneration in PD.
i) Direct interaction with cysteine residues of functional proteins and influence their activity; ii) Leading to oxidative damage to cells by trivalent iron ions; iii) Catalysis of DA oxidation and production of ROS and highly reactive DA quinones; iv) Production of highly reactive ROS, the hydroxyl radicals, through Fenton reaction between bivalent iron ions and H_2O_2 (75-77); the hydroxyl radicals then could also oxidize DA leading to DA related cell impairment. v) Other toxic mechanisms that contribute to PD, such as induction of aggregation of the α-syn, which is a protein that accumulates in Lewy bodies in PD.

decreased GSH level) DAQ conjugated to GSH could still be reactive [41]. These findings implicate that insufficient ambient reductive force may significantly impair GSH detoxification capacity against DAQ induced toxicity and aggravate DAQ induced toxicity [41]. Based on our results two potential detrimental positive feedback loops have been proposed involving accelerated DA oxidation, increased GSH consumption and impaired GSH detoxification efficiency [41]. These detrimental positive feedback loops may play roles in DA relevant dopaminergic neuron degeneration in PD (Figure 3). These hypotheses can be supported by post-mortem evidence that decreased GSH concentration and increased GSH-DAQ conjugation in SN can be the early event in the development of PD [42]. We found that the cytotoxicity and proteasome inhibition induced by DA, AM and H_2O_2 could be abrogated by GSH, Ascorbic Acid (AA), Vitamin E, Superoxidase Dismutase (SOD) or Catalase (CAT) with different profiles [16]. However only GSH was the most potent to abrogate DA, AM or H_2O_2-induced cell toxicity and proteasome inhibition, as well as to reverse H_2O_2-induced proteosome inhibition [16]. These findings strengthen the key roles of GSH in protection of DA neurons against DA induced toxicity. *Vice versa*, factors to trigger or accelerate DA oxidation in dopaminergic neurons should be vital to DA neuron degeneration in PD.

Besides GSH, DAQ can readily conjugate with other sulphydryl groups containing chemicals including L-Cysteine (L-Cys) and NAC [10,12,43,44]. L-Cys can conjugate with DAQ to form 5-S-Cys and 2-S-Cys [45]. NAC contains sulfhydryl group and has been found to covalently react with DAQ with its thiol group to detoxify reactive DAQ [46]. Based on our findings, two protective mechanisms against DA-induced toxicity in dopaminergic neurons in SN have been proposed [12]. The first protective mechanism is to inhibit DA oxidation by reductants and ROS catalyzing enzymes (such as SOD and CAT); and the second is to eliminate or detoxify DA oxidative metabolites, especially highly reactive DAQ, in time [12]. The elimination of DA oxidative metabolites includes scavenging of ROS by reductants or ROS catalyzing enzymes as well as detoxification of DAQ by DAQ conjugating reagents, such as GSH, NAC and L-cys.

Iron Relevant DA Neuron Toxicity and Neuroprotective Roles of Iron Chelators

To date, accumulative evidence suggests that iron species are highly related to PD pathogenesis [47]. Studies show that there is a decrease in GSH level associated with an increase of iron level in the degenerating SN of PD patients [48]. In relevance to PD, elevated levels of selective iron species have been detected in the SN region of brain in both living and post-mortem PD patients [49,50]. The iron species in SN is found to be mainly Fe^{3+} ions rather than Fe^{2+} ions [51]. Our previous findings have shown that iron species can mediate DA oxidation to produce deleterious ROS and DAQ, leading to serious DA cell vulnerability [52]. We found that free iron ions (trivalent or bivalent) and iron ions in complex formation (potassium ferricyanide ($K_3Fe(CN)_6$)) could mediate DA oxidation and generate DAQ, leading to proteasome inhibition and even DA cell demise with different profiles [52]. The free iron ions could form complexes with DA and then induce continuous and intensive DA oxidation [52]. The iron species induced DA oxidation was found to be dependent on the dosage of iron ions [52]. It was also observed that there was no difference in the rate of DA oxidation mediated by either free ions Fe^{3+} or Fe^{2+} [52]. It was hypothesized that the electrons and protons donated by the DA under iron mediated oxidation was transferred to the oxygen in solution finally. This explained the continuous and iron ions-dosage dependency oxidation of DA. Moreover, our study also demonstrated the high-binding potential of the free iron ions to AM, facilitating the aggregation of AM to form melanin [52]. Other transition metal like copper sulphate was also shown to mediate DA oxidation [52].

However iron ions in complex form could not induce DA oxidation ($K_4Fe(CN)_6$) or only induce rapid but brief DA oxidation ($K_3Fe(CN)_6$) [52]. When iron species, in a stable complex with cyanide ions, were utilized to accelerate DA oxidization only the Fe^{3+} cyanide complex ($K_3Fe(CN)_6$) was able to catalyse DA oxidation for a limited duration [52]. Since $K_3Fe(CN)_6$ could be readily reduced to $K_4Fe(CN)_6$ during $K_3Fe(CN)_6$ induced DA oxidation, 2 molecules of $K_3Fe(CN)_6$ could help to oxidize 1 molecule of DA $K_3Fe(CN)_6$. This explained the lack of catalysis of DA oxidation by $K_4Fe(CN)_6$

and the limited capacity of $K_3Fe(CN)_6$ to catalyze DA oxidation. In comparison to free iron ions, iron cyanide ions complexes also had a lower binding affinity to AM, leading to less amount of melanin formation under $K_3Fe(CN)_6$ induced DA oxidation [52].

Deferoxamine (DFO), an iron chelator, could abrogate free iron mediated DA oxidation and subsequent cytotoxicity *via* abrogation of iron-DA complex formation [52]. However DFO did not disturb stable iron cyanide complex, therefore would not influence $K_3Fe(CN)_6$ induced DA oxidation [52]. We found that GSH could not disturb iron-DA complex formation; therefore GSH lost the capacity to inhibit DA oxidation mediated by free iron species [52]. However GSH could protect against iron mediated toxicity *via* detoxification of toxic DAQ produced from iron mediated DA oxidation [52]. Therefore GSH could protect DA neurons against iron induced proteasome inhibition and DA neuron degeneration [52]. Our findings implicated that iron chelators with Blood Brain Barrier (BBB) penetrating capacity might function well to protect against iron related progressive DA neuron degeneration in PD. Our findings also strengthened the protective roles of GSH against iron induced DA neuron degeneration. The toxic relevance between iron and DA had also been discussed by a recent update that neurodegeneration in PD brains might result from the potent redox couple formed between iron and DA itself [53]. The detailed iron species induced toxicity relevant to DA neuron degeneration in PD is illustrated in (Figure 4).

DA Dependent α-Syn Toxicity in PD

The α-syn (PARK1) gene is the first identified PD-related gene, the mutations of which are implicated in early onset FPD [54-56]. Furthermore the α-syn is also found to be relevant to SPD, as high immunoreactivity of α-syn is found in LBs in brains of SPD patients [52]. The α-syn immunoreactive LBs can also be found in brains of transgenic mice models overexpressing human WT and MT α-syn [57,58]. Previous studies had demonstrated that α-syn toxicity was DA relevant [10,59,60]. It was found that increased expression of either Wild-Type (WT) or mutant α-syn enhanced the cellular toxicity induced by the accumulation of intracellular DA [61]. Our findings showed that WT α-syn could be beneficial to dopaminergic neurons but overexpression of WT α-syn in the presence of DA made it a potential threat to DA cells [10]. Our conclusion was supported by recent findings that overexpression of WT α-syn was not toxic in non-dopaminergic human cortical neurons, but rather exhibited neuroprotective activity [61]. In contrast, mutant α-syn not only caused the loss of WT protective function but also the gain-of-toxicity which became more serious in the presence of DA and neurotoxins [10]. Furthermore we demonstrate that transient transfection and overexpression of human mutant A53T mutant α-syn could induce DA-dependent, non-apoptotic cell death in dopaminergic PC12 cells [10]. We concluded that auto-oxidation of endogenous DA aggravated non-apoptotic cell death induced by overexpression of human mutant A53T α-syn in PC12 cells [10]. NAC and L-Cys could potentially provide neuroprotection against DAQ induced toxicity; therefore they had neuroprotective function by recovering the cell toxicity caused by endogenous DA in the presence of mutant α-syn [10].

It was reported that DAQ could interact with α-syn to form unstructured adducts [62]. Furthermore DA could conjugate with α-syn to form a covalent adduct that slowed the conversion of protofibrils to fibrils [63]. Furthermore it was found that α-syn protofibrils could permeabilize synthetic vesicles and form pore-like assemblies on the surface of brain derived vesicles [64]. This finding suggested that cytosolic DA in dopaminergic neurons promoted the accumulation of toxic α-syn protofibrils, which might explain why these neurons are most vulnerable to degeneration in PD [64]. In A53T mutant α-syn β-sheet protofibrils could oligomerize at a higher rate [64]. We showed by *in vitro* study that PC12 cells expressing A53T mutant α-syn had elevated endogenous DA content and showed increased vulnerability to neurotoxins MPP+ and 6-OHDA (10). The α-syn protofibrils might increase the permeability of the membrane allowing leakage of DA from DA vesicles into cytoplasm, leading to increased DA oxidation in cytosol and subsequent deleterious impacts on cells [64]. Furthermore elevated levels of Tyrosine Hydroxylase (TH), the key enzyme for DA synthesis, were noted in α-syn mutant DA cells [10]. Thus the combined effects of up-regulated DA level and oxidation as well as toxicity induced by mutant α-syn protein could account for mutant α-syn induced DA neurons toxicity. However the toxicity could be partially nullified by α-methyltryptamine (α-MT, the TH inhibitor), which could down regulate the endogenous DA levels in cells [10]. This finding further supported the DA dependent toxicity of α-syn protein.

Mutant PINK1 Induced DA Dependent Vulnerability of DA Neurons

PINK1 (PARK6) is a serine-threonine kinase localized to the outer membrane of mitochondria as well as in cytosol [65]. The WT PINK1 is hypothesized to play a neuroprotective role in dopaminergic neurones and PINK1 mutations can contribute to autosomal recessive early onset FPD [66]. PINK1 is intimately involved with mitochondrial quality control to maintain mitochondria homeostasis [67,68]. Upon mitochondria damage, PINK1 can be accumulated into mitochondria and further recruit parkin to target impaired mitochondria for mitophagy clearance [67,68]. However PINK1 can also be localized to cytosol and play important roles in neuroprotection of cells [69,70]. Our previous studies had demonstrated that extra-mitochondrial PINK1 could regulate TH expression and DA content in dopaminergic cells in a PINK1 kinase activity dependent manner [71]. We have shown that overexpression of WT PINK1 down regulated the expression of TH and decreased DA content in dopaminergic neurons [71]. However overexpression of PD related mutant PINK1 could significantly up-regulate levels of TH and DA and rendered DA cells more vulnerable to neurotoxins induced challenges [71]. The capacity of PINK1 to modulate TH and DA levels had been strengthened by *in vivo* observations in PINK1 knockout mice models [72,73]. It was demonstrated that elevated iron species was detected in PINK1 mutations associated early onset FPD brains [74-77]. We verified that the elevated levels of TH and DA levels under PINK1 mutations cells made DA neurons more susceptible to iron mediated toxicity [71]. The toxicity could be alleviated by NAC and L-Cys [71]. These findings reiterated the iron mediated DAQ toxicity under PINK1 mutations and implicated the protective roles of DAQ detoxification agents against DA neuron degeneration in PINK1-linked FPD.

Conclusion

DA is an essential neurotransmitter that plays a vital role in DA neurons in SN. Pathological factors induced progressive DA neurons degeneration will decrease DA level in brain, leading to PD onset and development. However, accumulative evidence demonstrates that the progressive DA neuron degeneration in PD is DA dependent. The DA can undergo oxidation to produce deleterious reactive DAQ and ROS. Furthermore DAQ derived from DA oxidation play more significant roles than small molecular ROS in DA neuron degeneration in SPD as well as FPD. Small molecular weight ROS can induce reversible oxidative modification of macromolecules only. However DAQ can covalently and irreversibly conjugate to cysteine residues of proteins, leading to inactivation of functional proteins, protein misfold and formation of deleterious protein aggregates. Furthermore free DAQ or DAQ conjugated to proteins can undergo redox cycling to generate ROS to increase oxidative stress to DA neurons. However DAQ induced DA neurons vulnerability can be abrogated by DAQ detoxification agents such as NAC or GSH. Furthermore our findings also showed that iron species could mediate DA oxidation to generate toxic DAQ in DA neurons, indicating the DAQ relevant toxicity of iron species in DA neuron degeneration in PD. Therefore studies to search for new and potent DAQ detoxification agents with capacity to chelate deleterious iron ions in brains should be significant to improve our therapies against DA neuron degeneration in PD.

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