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Review

Paraquat exposure as an etiological factor of Parkinson's disease

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Abstract

Parkinson's disease (PD) is a multifactorial chronic progressive neurodegenerative disease influenced by age, and by genetic and environmental factors. The role of genetic predisposition in PD has been increasingly acknowledged and a number of relevant genes have been identified (e.g., genes encoding α-synuclein, parkin, and dardarin), while the search for environmental factors that influence the pathogenesis of PD has only recently begun to escalate. In recent years, the investigation on paraquat (PQ) toxicity has suggested that this herbicide might be an environmental factor contributing to this neurodegenerative disorder. Although the biochemical mechanism through which PQ causes neurodegeneration in PD is not yet fully understood, PQ-induced lipid peroxidation and consequent cell death of dopaminergic neurons can be responsible for the onset of the Parkinsonian syndrome, thus indicating that this herbicide may induce PD or influence its natural course. PQ has also been recently considered as an eligible candidate for inducing the Parkinsonian syndrome in laboratory animals, and can therefore constitute an alternative tool in suitable animal models for the study of PD. In the present review, the recent evidences linking PQ exposure with PD development are discussed, with the aim of encouraging new perspectives and further investigation on the involvement of environmental agents in PD.

Keywords: Parkinson's disease; Environmental factors; Paraquat; Neurotoxicity; Animal models

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Abbreviations: BBB, blood-brain barrier; CNS, Central Nervous System; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; DTCs, dithiocarbamates; ETC, electron transport chain; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; $HO^{\bullet}$, hydroxyl radical; HVA, homovanillic acid; LBs, Lewy bodies; MAO, monoamine oxidase; MB, maneb; MPP^+, 1-methyl-4-phenyl-2,3-dihypyridinium ion; MPPP, 1-methyl-4-phenyl-propion-oxypiperedine; MPTP, 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine; NMDA, *N*-methyl-D-aspartate; $^{\bullet}NO$, nitric oxide; NOS, nitric oxide synthase; $O_2^{\bullet-}$, superoxide radical; $ONOO^-$, peroxynitrite anion; PD, Parkinson's disease; PQ, paraquat; RNS, reactive nitrogen species; ROS, reactive oxygen species; SN, *substantia nigra*; SNpc, *substantia nigra pars compacta*; TH, tyrosine hydroxylase

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1. Introduction

Parkinson's disease (PD), first described by James Parkinson in 1817, is a chronic progressive neurodegenerative disease, affecting at least 1% of the population over the age of 55 (Rajput, 1992). It is the second most common neurodegenerative disorder after Alzheimer's disease, with new 5–24 cases per 100,000 population diagnosed every year (Rajput, 1992).

1.1. The pathology of Parkinson's disease

Fully developed PD comprises motor symptoms such as resting tremor on one or both sides of the body, rigidity, bradykinesia, hypokinesia, and postural reflex impairment (Marsden, 1994). The pathology of PD is not fully understood. In normal brains the number of nigral cells is reduced by 4.7–6% per decade between the fifth and the ninth decade of life (Gibb and Lees, 1991), but this loss is not sufficient to cause PD (McGeer et al., 1977). The common feature of PD is the degeneration of the neural connection between the *substantia nigra* (SN) and the striatum (Wooten, 1997), two essential brain regions in maintaining normal motor function (Fig. 1). The striatum receives its dopaminergic input from neurons of *substantia nigra pars compacta* (SNpc) *via* the nigrostriatal pathway (Moore et al., 1971). Progressive degeneration of the nigrostriatal dopaminergic pathway results in profound striatal dopamine (DA) deficiency (Albin et al., 1989; Crossman, 1989; DeLong, 1990; Greenamyre, 1993; Klockgether and Turski, 1989). By the time that the clinical manifestations of PD are fully developed, a large proportion (80%) of dopaminergic neurons in the SN are already lost, resulting in reduced synthesis and release of DA from the striatal nerve terminals (Lang and Lozano, 1998).

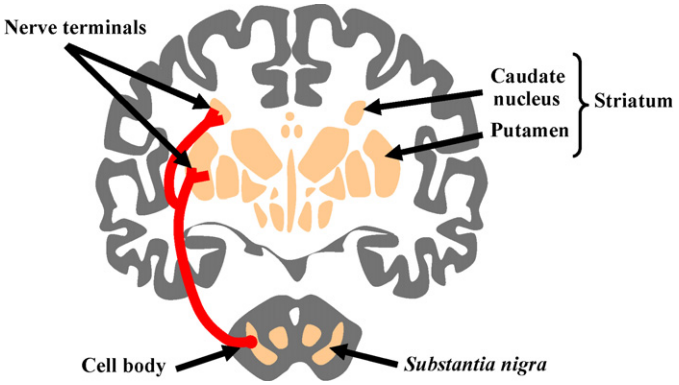

Fig. 1. Schematic diagram showing the nigrostriatal dopaminergic pathway. A cross-section of human brain shows the caudate and putamen, which constitute the striatum. A section through the midbrain shows the *substantia nigra*. Dopaminergic neurons (in red), whose cell bodies are located in the SN, send projections that terminate and release dopamine in the striatum. With the degeneration of the dopaminergic pathway, there is a progressive drop in dopamine release into the striatum. Striatal dopamine deficiency, in turn, results in complex changes in the brain's motor circuitry and causes the motor deficits characteristic of Parkinson's disease (for interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Besides the loss of SN neurons, another important pathological feature of PD is the presence of neuronal cytoplasmatic inclusions known as Lewy bodies (LBs) (Gibb and Lees, 1988; Marsden, 1994) in some surviving nigral dopaminergic neurons. In PD, LBs are present in the dopaminergic neurons of SN, as well as in other brain regions such as the cortex and magnocellular basal forebrain nuclei (Braak et al., 1995). A major component of the LBs is the α-synuclein protein (thioflavin S-positive staining), and LBs seem to derive from α-synuclein aggregation (Spillantini et al., 1997, 1998; Uversky, 2003). However, several other clinical syndromes are also associated with intracellular α-synuclein inclusions (synucleinopathies) (Mukaetova-Ladinska and McKeith, 2006).

1.2. Etiology of Parkinson's disease

Considerable evidence suggests a multifactorial etiology for PD, involving genetic and environmental factors. The contribution of genetic predisposition to PD has been investigated in twin studies (Piccini et al., 1999), case-control studies (Gasser, 1998, 2001; Sveinbjornsdottir et al., 2000), and in studies identifying mutations in genes encoding α-synuclein (Kruger et al., 1998; Polymeropoulos et al., 1997; Zarranz et al., 2004), parkin (Kitada et al., 1998), PINK1 (Valente et al., 2004), dardarin (Hernandez et al., 2005) and DJ-1 (Bonifati et al., 2003).

However, inheritance cannot fully explain all PD cases. In fact, a comprehensive study of over 19,000 white male twins showed that inheritance is not the cause of sporadic PD (Tanner et al., 1999). In addition, α-synuclein is found in all LBs, even in the majority of the idiopathic PD cases without α-synuclein mutations (Spillantini et al., 1997), thus indicating that additional mechanisms may lead to conformational changes and consequent protein aggregation.

Numerous environmental risk factors have been associated with the PD as causative agents, either in the modulation of the

disease onset and/or on its progression (Di Monte, 2001, 2003; Di Monte et al., 2002; McCormack et al., 2002; Tanner, 1989; Tanner and Ben-Shlomo, 1999). Several environmental agents are known to cause nigrostriatal damage, and may thus contribute to PD, namely: (i) metals (Altschuler, 1999; Good et al., 1992; Gorell et al., 1999; Hellenbrand et al., 1996; Hirsch et al., 1991; Tanner, 1989; Yasui et al., 1992), (ii) solvents (Davis and Adair, 1999; Hageman et al., 1999; Pezzoli et al., 1996; Seidler et al., 1996; Uitti et al., 1994), and (iii) carbon monoxide (Klawans et al., 1982). Additionally, data from epidemiological studies point to an association between increased PD risk and specific environmental factors such as rural residence (Liou et al., 1997; Marder et al., 1998; Morano et al., 1994), farming (Fall et al., 1999; Gorell et al., 1998; Liou et al., 1997; Semchuk et al., 1992), drinking water from wells (Marder et al., 1998; Morano et al., 1994), and exposure to agricultural chemicals, including paraquat (PQ) (Fall et al., 1999; Gorell et al., 1998; Liou et al., 1997; Semchuk et al., 1992, 1993; Vanacore et al., 2002).

Given the public health implications concerning risk factors for the development of PD, the study of the environmental factors involved in the etiology of PD has gained renewed interest of the scientific and medical community as well as of the regulatory governmental agencies. In the present review the recent evidence from epidemiological, clinical, and experimental work linking the widely used herbicide, PQ, to PD pathology is discussed.

2. Paraquat toxicity

2.1. Paraquat toxicity mechanism

The cellular toxicity of PQ is essentially due to its redox cycle (Fig. 2). Paraquat is reduced, mainly by NADPH-cytochrome P-450 reductase (Clejan and Cederbaum, 1989), NADPH-cytochrome *c* reductase (Fernandez et al., 1995), and the mitochondrial complex I also known as NADH: ubiquinone oxidoreductase (Fukushima et al., 1993; Yamada and Fukushima, 1993), to form a PQ monocation free radical ($PQ^{\bullet+}$). It is generally accepted that PQ uses cellular diaphorases, which are a class of enzymes that transfer electrons from NAD(P)H to small molecules, such as PQ (Aziz et al., 1994; Day et al., 1999; Dicker and Cederbaum, 1991; Liochev and Fridovich, 1994). The PQ monocation free radical is then rapidly reoxidized in the presence of oxygen generating the superoxide radical ($O_2^{\bullet-}$) (Busch et al., 1998; Dicker and Cederbaum, 1991). This then sets off the well-known cascade of reactions leading to the generation of other reactive oxygen species (ROS), mainly hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$) and the consequent cellular deleterious effects. Indeed, hydroxyl radicals (Busch et al., 1998; Youngman and Elstner, 1981) have been implicated in the initiation of membrane damage by lipid peroxidation during the exposure to PQ in vitro (Busch et al., 1998) and in vivo (Burk et al., 1980; Dicker and Cederbaum, 1991).

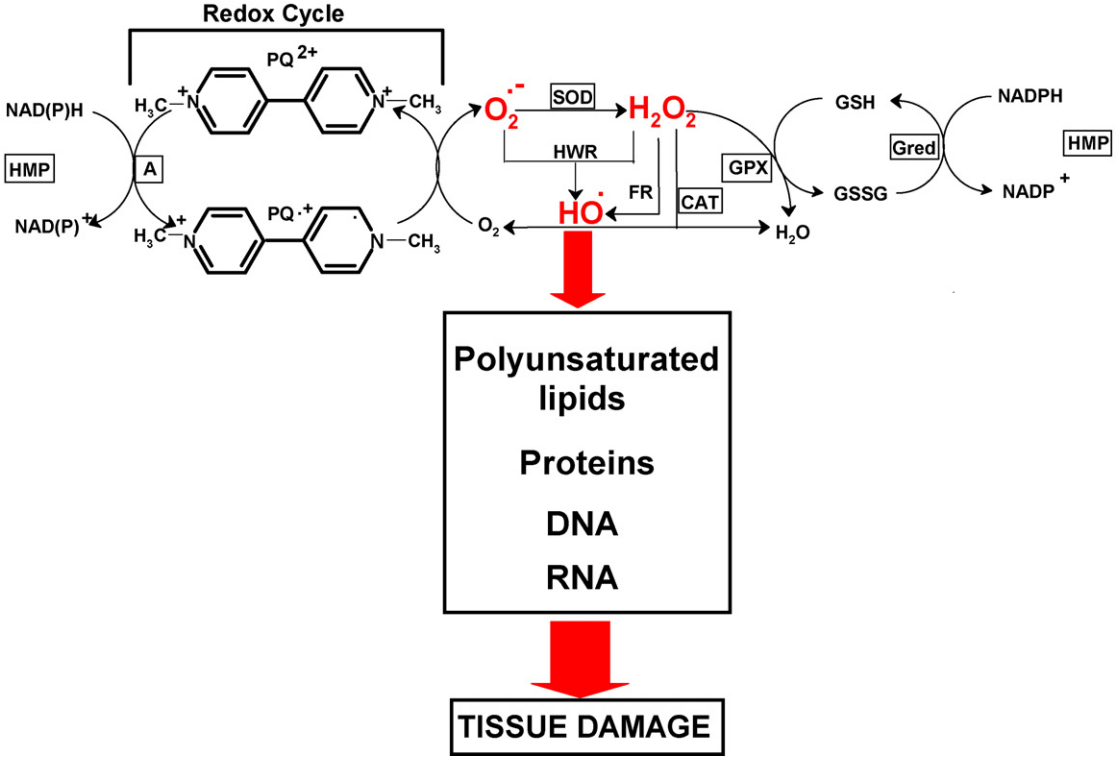

Fig. 2. Schematic representation of the mechanism of paraquat toxicity. A, cellular diaphorases; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; Gred, glutathione reductase; PQ^{2+}, paraquat; $PQ^{\bullet+}$, paraquat cation radical; HMP, hexose monophosphate pathway; FR, Fenton reaction; HWR, Haber-Weiss reaction.

2.2. Recent studies in the Central Nervous System

Studies of PQ toxicity have recently focused on its Central Nervous System (CNS) effects. Unlike the exposure to high levels of PQ that mainly produces pulmonary toxicity, chronic low levels, resulting from prolonged exposure to non-pneumotoxic doses, may produce damage to the basal ganglia and Parkinsonism. Toxic damage to the brain has been observed in patients who died from PQ poisoning (Grant et al., 1980; Hughes, 1988). Autopsy findings in cases of acute PQ poisoning showed cerebral damage with edema, haemorrhage and neural death. However, in these studies, the possibility that the observed tissue changes occurred either post-mortem or as a consequence of anoxia due to respiratory dysfunction could not be excluded.

3. Paraquat and Parkinson's disease—proposed mechanisms

3.1. Paraquat induces long-lasting dopamine overflow and reduction of dopamine synthesis

The excitotoxicity induced by *N*-methyl-D-aspartate (NMDA) receptor activation, associated to Ca^{2+} penetration into the cells by activation of non-NMDA receptors, is a central mechanism of neurodegeneration in several neurological diseases (Dugan and Choi, 1999). There is also increasing evidence that the excitotoxic injury plays a critical role in progressive degeneration of DA neurons in PD (Beal, 1998). In vivo studies on the mechanisms of PQ-induced toxicity in the striatum, indicated that PQ stimulates glutamate efflux initiating excitotoxicity mediated by reactive nitrogen species (RNS). After activation of nitric oxide synthase (NOS) containing neurons, evoked depolarization of NMDA receptor channels and Ca^{2+} penetration into the cells occur by activation of non-NMDA receptor channels (Shimizu et al., 2003a). It was also shown that the elevation of extracellular glutamate levels was PQ dose-dependent. This phenomenon was observed shortly after PQ administration. However, the mechanism by which PQ induces glutamate efflux is yet to be clarified. The influx of Ca^{2+} into the cells triggers the mobilization of Ca^{2+}-dependent intracellular processes including the activation of neuronal NOS. Nitric oxide ($^{\bullet}NO$) produced by NOS diffuses to dopaminergic terminals, where it is thought to play an important role in excitotoxicity, probably through the formation of the peroxynitrite anion ($ONOO^-$) upon reaction with $O_2^{\bullet -}$ produced by the redox-cycle of PQ (Figs. 2 and 3) (LaVoie and Hastings, 1999). Peroxynitrite, which is a lipid-permeable ion with a wider range of chemical targets than $^{\bullet}NO$, can oxidize proteins, lipids, RNA, and DNA. It inhibits the function of manganese superoxide dismutase, which can lead to increased $O_2^{\bullet -}$ and $ONOO^-$ formation.

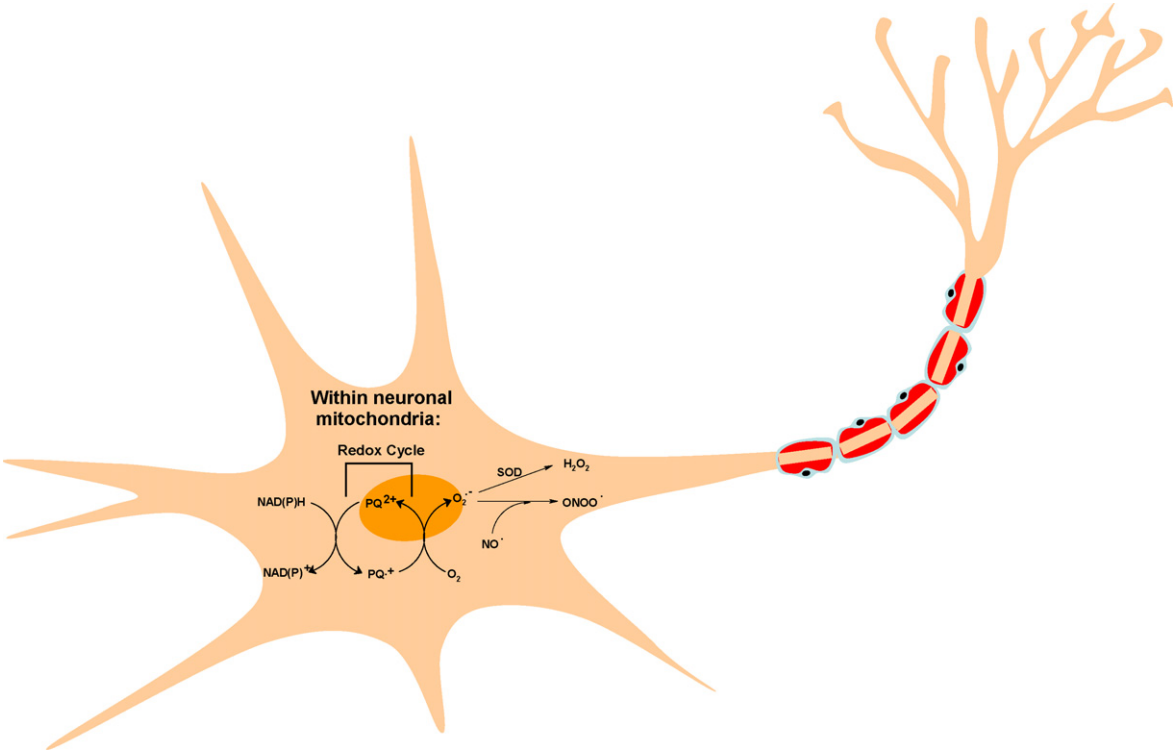

Fig. 3. Schematic representation of the events that occur in neuronal mitochondria. PQ can be converted into a radical (accepting one electron) from NADH via complex I in mitochondrial ETC (Fukushima et al., 1993), blocking mitochondrial electron flow (McCormack et al., 2002). By redox cycling with molecular oxygen, PQ leads to superoxide anions ($O_2^{\bullet -}$) formation in dopaminergic neurons. $O_2^{\bullet -}$ formation and lipid peroxidation inhibits complex I activity (Fukushima et al., 1994), and consequently affects mitochondrial function. $O_2^{\bullet -}$ easily reacts with nitric oxide ($^{\bullet}NO$) to generate peroxynitrite anion ($ONOO^-$), that can also be responsible for PQ-induced neurotoxicity. $ONOO^-$ is not only an oxidizing agent on its own but also degrades to form hydroxyl radicals, among other reactive species (Beckman et al., 1990). This neurotoxic event could cause a continuous and long-lasting overflow of dopamine.

Additionally, $ONOO^-$ is an effective inhibitor of enzymes in the mitochondrial respiratory chain, decreasing ATP synthesis. Secondly, $ONOO^-$ damages DNA strands and inhibits DNA ligase, which increases DNA strand breaks (Ebadi and Sharma, 2003). Noteworthy, long-lasting DA release (Shimizu et al., 2003a) and consequent death of the dopaminergic neurons was prevented by treatment with glutamate receptor antagonists, by a NOS inhibitor and by the monoamine oxidase inhibitor L-deprenyl, strongly suggesting that chronic exposure to low PQ doses leads to an increased vulnerability of dopaminergic neurons in the nigrostriatal DA system via the excitotoxic pathway (Shimizu et al., 2003b). A study in PC12 cells showed that the rate-limiting enzyme in DA synthesis, tyrosine hydroxylase (TH), is a selective target for nitration following exposure to $ONOO^-$ (Ara et al., 1998). Nitration of tyrosine residues in TH results in loss of enzymatic activity (Ara et al., 1998). In the mouse striatum, tyrosine nitration-mediated loss of TH activity parallels the decline in DA levels (Ara et al., 1998). These results indicate that tyrosine nitration induces TH inactivation and consequent DA synthesis impairment. Thus, glutamate-induced RNS-mediated cytotoxicity plays an important role in the toxic effect of PQ on dopaminergic terminals.

3.2. Paraquat inhibits the complex I of the mitochondrial electron transport chain

The mitochondrial complex I (located in the inner mitochondrial membrane and protruded into the matrix) is the first and the most complex of the three energy-transducing enzyme complexes of the mitochondrial electron transport chain (ETC). It is the point of entry for the major fraction of electrons that cross the respiratory chain. As a component of the ETC, complex I oxidizes NADH to NAD^+ and transfers electrons to ubiquinone. In addition, it translocates protons from the mitochondrial matrix to the intermembrane space, contributing to the electrochemical gradient required for ATP synthesis (Hatefi, 1985). Several authors suggest that PQ direct cytotoxicity is the consequence of a mitochondrial dysfunction (Blaszczynski et al., 1985; Hirai et al., 1985; Thakar and Hassan, 1988; Tomita, 1991). Once PQ is reduced to its radical $PQ^{\bullet+}$ (accepting one electron from NADH) via complex I in mitochondrial ETC (Fukushima et al., 1993), the consequent $O_2^{\bullet-}$ high production rate may inhibit the activity of the complex I (Fukushima et al., 1994) and thus causing mitochondrial dysfunction. This redox-cycling can also cause lipid peroxidation of the mitochondrial inner membrane (Yamada and Fukushima, 1993), and as a result, the target tissue may be damaged (Fukushima et al., 1994). Tawara et al. (1996) proposed that the involvement of PQ in the etiology of PD is based on the significantly lower activity of complex I. The electron flux through complex I regulates the mitochondrial transition pore permeability (a large Ca^{2+}-dependent pore in the inner mitochondrial membrane). Under pathological conditions, mitochondria de-energize and depolarize as a consequence of the opening of the transition pore, leading to apoptotic or necrotic cell death (Greenamyre et al., 2001).

Severe defects in complex I activity depress ATP synthesis, induce mitochondria depolarization, and favour Ca^{2+} deregulation. The combination of all these factors may cause the early onset and rapid progression of neurological diseases such as PD. On the other hand, subtle abnormalities of complex I might produce milder, late-onset disorders (Greenamyre et al., 2001). Supporting this hypothesis, non-familiar sporadic PD has been characterized by a 15–30% reduction of complex I activity (Schapira et al., 1990).

Importantly, dopaminergic neurons are particularly vulnerable to complex I inhibitors. Complex I activities in rat brain, lung and liver have all been shown to decrease with time, with a significant effect observed 2 h after PQ administration. It was therefore concluded that PQ decreases the mitochondrial complex I activity of the brain at an early stage after PQ exposure, even before respiratory dysfunction is observed (Tawara et al., 1996).

3.3. Paraquat markedly induces α-synuclein up-regulation and aggregation

The abundant presynaptic protein α-synuclein plays an important role in the formation of LBs inclusions involved in the pathogenesis of PD (Masliah et al., 2000). It has been hypothesized that pathological changes may arise from interactions of α-synuclein with toxic agents, a likely mechanism through which environmental risk factors could contribute to the pathogenesis of PD. Supporting this hypothesis, the in vitro incubation of recombinant α-synuclein in the presence of PQ resulted in increased protein fibrillation (Uversky et al., 2001, 2002) with clear concentration-dependent accelerating effects (Manning-Bog et al., 2002), probably due to the preferential binding of PQ to a partially folded α-synuclein intermediate. Accordingly, following in vivo PQ administration, α-synuclein-containing aggregates were observed in the rodent SN (Manning-Bog et al., 2002). This up-regulation followed a consistent pattern, with higher α-synuclein levels attained 2 days after each of three weekly PQ injections and with protein levels returning to control values by day 7 after PQ administration (Manning-Bog et al., 2002). The up-regulation of α-synuclein as a consequence of toxicant insult and the direct interaction between the protein and environmental agents are potential mechanisms leading to α-synuclein pathology in neurodegenerative disorders (Manning-Bog et al., 2002).

4. Permeability of blood-brain barrier to paraquat and putative uptake by the dopamine transporter

Another important feature of PQ toxicity is related to its ability to permeate the blood-brain barrier (BBB) into the CNS. Paraquat is a charged molecule, with a hydrophilic structure, low partition coefficient and does not readily cross membranes. Thus, it is unlikely that the passive entry of PQ across the BBB leads to a significant accumulation of the compound in the brain. In accordance, it was previously shown that the structurally related dopaminergic neurotoxin 1-methyl-4-phenyl-2,3-dihypyridinium ion (MPP^+) must be formed

intracerebrally by monoamine oxidase (MAO)-B in glia or non-dopaminergic neurons, since it cannot cross the BBB (Shimizu et al., 2001). Nevertheless, PQ does cross the BBB, with maximal brain levels evident after 24 h, as compared with 30 min in other tissues, following subcutaneous administration (Widdowson et al., 1996). In fact, PQ could be measured in the CNS after systemic injection in rodents (Corasaniti et al., 1998). It is well known that PQ-induced lung damage is initiated, at least partially by an energy-dependent accumulation into the lung through an uptake system shared by endogenous polyamines such as putrescine (Smith, 1982). However, the polyamine transporters are not expressed in the BBB (Shin et al., 1985). Recent studies suggest the involvement of an active uptake system, the BBB neutral amino acid transporter, in the transport of PQ into the CNS (McCormack and Di Monte, 2003; Shimizu et al., 2001), to the detriment of a possible dysfunction of BBB caused by PQ itself or by $PQ^{\bullet+}$. Brain accumulation and neurotoxicity of PQ in the mouse model was completely prevented by co-administration of simple amino acids, such as L-valine and L-phenylalanine, or levodopa, which are competitive substrates for the same BBB transporter (McCormack and Di Monte, 2003). Taken together, these findings suggest that active uptake across the BBB may be essential in the sequence of events that leads to toxin-induced nigrostriatal damage. Intake of specific dietary elements (e.g., amino acids) or therapeutic agents (e.g., levodopa) may also significantly modulate the effects of environmental xenobiotics such as PQ, by changing their rate of uptake into the brain.

Several studies have been performed to explain how PQ is taken into striatal cells. Similarly to the polyamine uptake system in the lung (Dinis-Oliveira et al., 2006b), this uptake seems to be sodium dependent (Shimizu et al., 2001). It was reported that PQ is rapidly taken up by nerve terminals isolated from mouse cerebral cortex, where it induces lipid peroxidation in a concentration-dependent manner in the presence of NAD(P)H and ferrous iron (Yang and Sun, 1998). Importantly, PQ also, in a concentration-dependent manner, reduces the number of dopaminergic neurons in cultured rat organotypic midbrain slices (Shimizu et al., 2003b). Since this damage is prevented by GBR-12909 (a selective inhibitor of DA transport), the involvement of the DA transporter in the PQ uptake into the striatal cells has been proposed. However, the transport of PQ through the DA transporter remains a controversial issue (Barlow et al., 2003).

5. The inherent susceptibility of dopaminergic neurons contributes to the paraquat-induced damage

Comparing to other neuronal cell types, dopaminergic cells are much more sensitive to oxidative injury due to the participation of DA in harmful oxidative reactions (Fitsanakis et al., 2002; Graham, 1978). The activity of MAO, which is involved in DA metabolism, produces H_2O_2 as a normal byproduct. Moreover, autoxidation of DA results in the formation of ROS (Lotharius and O'Malley, 2000). Nevertheless, the toxicological implications of the inherent vulnerability of the nigrostriatal DA system are still not fully understood. One critical feature of the mammalian SN in PD that may contribute to its susceptibility to ROS injury is the depletion of reduced glutathione (GSH) with no change in oxidized glutathione (GSSG) (Sian et al., 1994a). This appears to be due to the efflux of GSH mainly out of the glia promoted by γ-glutamyltranspeptidase, with a possible additional increased conversion of GSH to GSSG (which is itself transported out of the cells by γ-glutamyltranspeptidase), in response to increased intracellular levels of H_2O_2 (Sian et al., 1994b). Whether the lower level of GSH is a cause or a consequence of the pathogenic sequence leading to PD remains to be determined. Although speculative, the hypothesis that chronic low level PQ-induced redox cycling within the SN dopaminergic system may exceed the oxidative defenses of these cells and thus produce deleterious intracellular events, including the activation of the apoptotic cascade, remains a likely explanation for the association between PQ and PD.

6. Epidemiological studies

A study performed with 120 patients in Taiwan, where the herbicide PQ is commonly sprayed over rice fields, showed a strong association between PQ exposure and PD risk. The hazard increased by more than six times in individuals who had been exposed to PQ for more than 20 years (Liou et al., 1997). These observations were consistent with a dose-dependent effect and increased with duration of pesticide use in agricultural workers (Liou et al., 1997; Petrovitch et al., 2002). Occupational PQ exposure in other 57 cases also showed association with Parkinsonism in British Columbia (Hertzman et al., 1990). A door-to-door survey conducted in Taiwan to estimate the PD prevalence and incidence, indicated that the environmental factors may be more important than racial factors in the pathogenesis of PD (Chen et al., 2001). In a population-based case-control study in Calgary previous occupational herbicide use was the only significant predictor of PD risk after multivariate statistical analysis (Semchuk et al., 1992). Seidler et al. (1996) reported a significant association between PD and pesticide use but not between PD and other rural factors in Germany.

When considering environmental pesticide exposure, concern must be raised upon the effects of prolonged exposure to low levels of compounds, combination of agrochemicals, and the subtle cellular disruptions that may enhance the risk for developing major dysfunctions or disease. For example, the combined exposure to PQ and maneb (MB) targets the nigrostriatal DA system and induces locomotor impairment suggesting that this combination may be considered as a potential environmental risk factor for Parkinsonism (Thiruchelvam et al., 2000a,b, 2002).

7. Paraquat as a tool for animal models of Parkinson's disease

Animal models are an invaluable tool for studying the pathogenesis and therapeutic intervention strategies of human disease, including PD and in particular, toxicant-induced

Parkinsonism. Since PD does not develop spontaneously in animals, characteristic functional changes have to be mimicked by neurotoxic agents. Although an ideal model should reproduce the characteristic clinical and pathological features of PD (i.e., animals should develop progressive loss of dopaminergic neurons, show deposition of LB-like inclusions in the brain and some features of L-dopa-responsive movement disorder), up to now, no animal model was able to entirely reproduce all the features of the human disease. Albeit, these models are vital in the dissection of the many different molecular and biochemical pathways that are combined in the final clinical and pathological manifestations of PD (Orth and Tabrizi, 2003).

Presently, the MPTP model represents the best characterized PD animal model because it fulfils many of the criteria for the ideal model of this disease. The development of this model was based on the accidental discovery in the early 1980s, when a Parkinsonian syndrome in young drug addicts was linked to their unintentional MPTP intravenous self-administration when injecting 1-methyl-4-phenyl-propion-oxypiperedine (MPPP), also known as "synthetic heroin", that was contaminated with MPTP (Davis et al., 1979; Langston and Ballard, 1983). Subsequent work revealed that MPTP is not toxic on its own, but that it easily enters the brain where it is metabolized in the astrocytes by MAO-B into the active toxin MPP^+. This neurotoxin displaces DA from intracellular vesicles into the cytoplasm where auto-oxidation occurs leading to cellular damage (Lotharius and O'Malley, 2000). MPP^+ is selectively transported into the dopaminergic neurons through the DA transporter, accumulates in mitochondria and inhibits complex I (Greenamyre et al., 2001), thus acting as a selective complex I mitochondrial ETC toxicant that produces a Parkinsonian syndrome similar to the idiopathic PD in humans (Langston, 1996). In spite of the similarity between MPTP-induced PD in a number of species (mice, cats, and primates) and the sporadic PD in humans with respect to nigrostriatal dopaminergic degeneration and the characteristic behavioural changes (MPTP causes tremor, rigidity, akinesia, and postural instability, which are all successfully treated with L-dopa and DA agonists), some of the characteristic features of the disease differ, such as the lack of pronounced LB-related pathology (Forno et al., 1986; Langston et al., 1999). These differences suggest that the full complement of clinical (motor and cognitive) and pathological (nigrostriatal and extra-nigrostriatal) features of PD is unlikely to be mimicked by a single toxic insult but would rather involve multifactorial events, such as exposure to multiple toxicants, genetic factors, gene–toxicants interactions, and age-related effects.

The unfortunate accident linking MPTP exposure and PD provided a new insight into the possibility of interaction between other environmental agents and PD (Langston and Ballard, 1984; Tanner and Ben-Shlomo, 1999). If MPTP is capable of inducing neurochemical, pathological, and clinical features that resemble those of idiopathic PD (Di Monte et al., 2002), similar effects might be caused by other neurotoxicants.

Shortly after the discovery of the neurotoxicity of MPTP, the potential involvement of pesticides in PD pathology became

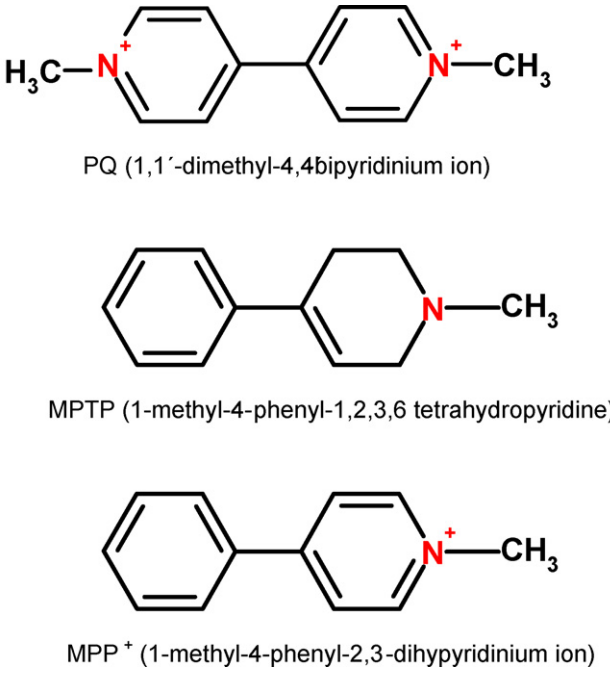

Fig. 4. Chemical structures of paraquat, MPTP and MPP^+.

obvious. Due to the close structural similarity between MPP^+ and PQ (Fig. 4), this widely used non-selective contact herbicide emerged as a putative risk factor of PD (Di Monte et al., 1986). Ironically, in the 1960s, MPP^+ itself had been tested as an herbicide under the commercial name of cyperquat (Di Monte, 2001). Several studies show that the exposure of mice to PQ may be a suitable experimental model to study the mechanisms involved in PD (Brooks et al., 1999; McCormack et al., 2002). However, as a candidate SN toxicant, PQ systemic delivery must produce the loss of SN dopaminergic neurons and the subsequent neurobehavioral syndrome with depletion of DA terminals within the striatum. Initial attempts to establish convincing evidence for the direct link between PQ exposure and PD failed. The first studies showed that striatal DA levels did not decrease after the systemic administration of PQ to animal models (Perry et al., 1986). Recently, this conclusion has been re-evaluated by using a stereological technique to count dopaminergic neurons in the SN of mice (McCormack et al., 2002). The authors found that systemic subchronic exposure to PQ induces dopaminergic neurons cell death in SNpc, as evaluated by the stereological counting of TH-immunoreactive and Nissl-stained neurons, without significant depletion of striatal DA. Furthermore, other investigators showed that PQ induced a neurobehavioral syndrome characterized by reduced ambulatory activity (Brooks et al., 1999), thus fulfilling the basic criteria for a neurotoxicant-induced model of PD (Brooks et al., 1999; McCormack et al., 2002). Given the propensity of PQ to stimulate lipid peroxidation, Brooks et al. (1999) proposed that the oxidative stress events due to the increased production of ROS might be the underlying mechanism responsible for SN toxicity. In spite of nigral degeneration (about 20–30% of neurons were lost), this effect was not accompanied by a significant DA depletion

or behavioural changes, a feature that distinguishes this model from the MPTP and rotenone models (McCormack et al., 2002). Thus, toxicant exposure may decrease the number of nigral neurons without triggering acute or major functional consequences. Whether the effects of PQ (e.g., DA depletion) become evident and progress over time or whether PQ-induced injury predisposes to damage from subsequent toxicant exposure remains to be determined.

Furthermore, exposure of mice to PQ also led to the formation of intraneuronal aggregates that were evidenced by anti-α-synuclein antibodies and thioflavin S staining (a dye that binds to amyloid fibrils) (Manning-Bog et al., 2002), another extremely important PD feature.

8. Two insults are more effective than one: paraquat + maneb

The hypothesis that a combination of environmental risk factors may result in more severe nigrostriatal injury is supported by several lines of experimental evidence. These observations are of special interest, since humans are likely to be exposed to a complex mixture of chemical agents in their residential and occupational environments. PQ is a member of only one class of agricultural chemicals known to have adverse effects in the nigrostriatal DA system. Complex mixtures of several pesticides are often used in overlapping geographical areas. Such is the case of the simultaneous use of PQ and diethyldithiocarbamates like the manganese ethylenebisdithiocarbamate [maneb (MB), a dithiocarbamate (DTC) fungicide]. In the US, the heavy use of these chemicals along the Pacific Coast, in the Northeast, the Plains states, the mid-Atlantic, the Southeast states, and also Texas, where these pesticides are used either separately or combined on the same crops (e.g. tomatoes), may be important for the etiological basis of PD (Thiruchelvam et al., 2000a). The extensive geographical overlap of the PQ + MB applications and PD prevalence suggests a possible correlation. This possibility has been corroborated by animal studies. In fact, the co-treatment of mice with PQ and MB resulted in potentiated neurotoxicity (Thiruchelvam et al., 2000a,b). The observed effects were, moreover, highly selective and irreversible for the nigrostriatal DA system, causing a reduction in motor activity and increased damage of both striatal terminals and nigral cell bodies. Additionally, it was shown that MB and other DTCs are able to alter the biodisposition of DA and PQ, resulting in a prolonged exposure to these ROS and RNS generating compounds (Barlow et al., 2003, 2005). Barlow et al. (2003, 2005) showed, in striatal synaptosomal vesicles, that some DTCs elicit an increase in DA accumulation, without altering its influx, but rather delaying the efflux out of the synaptosomes. The same DTCs also increased the lung and brain tissue content of [^{14}C]PQ in vivo. Thus, certain DTCs and other agents are capable of converting a non-toxic dose of PQ and other xenobiotics into a toxic dose through alterations in toxicokinetics (Thiruchelvam et al., 2000b). A common mechanism whereby selective DTCs might alter the kinetics of both [^{3}H]DA in vitro in synaptosomes and [^{14}C]PQ and DA in vivo seems to be via direct inhibition of an efflux transporter that transports both compounds out of the cells (Barlow et al., 2003, 2005). Direct action of some DTCs on the protein involved in this transport seems likely, given the rapid nature of the effect, as opposed to a slower mechanism such as altered transcription or translation. However, the identity of this efflux transporter is yet unknown. Efflux transporters are common in humans and other species, have wide tissue expression, and diverse substrates (Taylor, 2002). There are three large families of efflux transporters present in the brain and other organs (Taylor, 2002), each having several members: the multidrug resistance transporters (Ambudkar et al., 1999; Holland and Blight, 1999; Leslie et al., 2001), the monocarboxylic acid transporters and the organic ion transporters. Recently, we demonstrated that the induction of the synthesis de novo of membrane P-glycoprotein by dexamethasone decreases PQ lung accumulation and consequently its toxicity (Dinis-Oliveira et al., 2006a). On the other hand, verapamil, a competitive inhibitor of this transporter (Stein, 1997), when given 1 h before dexamethasone blocked these protective effects, causing an increase of PQ lung concentration and an aggravation in toxicity (Dinis-Oliveira et al., 2006a). In the light of our results, we hypothesize that this efflux impairment could similarly be a consequence of P-glycoprotein inhibition by some DTCs. Other interesting families of efflux transporters are the organic cation transporter family (OCT1–3) and the organic cation/carnitine family (OCTN1–3). Several transporters in these families have been shown to efflux DA, MPTP and MPP^{+}, and to be expressed in neurons (Busch et al., 1998; Wu et al., 1998; Zhang et al., 1997). Also, the fact that PQ is not metabolized in vivo suggests that it must be removed from cells by an active transport process.

Given the extreme importance of coexposure in epidemiological and toxicological studies of PD in human populations (Thiruchelvam et al., 2000a,b), the interactions of toxic chemicals are valuable models for the study of the potential mechanisms by which environmental exposures can cause PD. In such models, exposure to a single chemical may be insufficient to induce major effects, whereas multiple concurrent exposures, may preclude homeostatic deregulation with ensuing neuropathological changes by provoking changes at multiple target sites of the nigrostriatal DA system (Thiruchelvam et al., 2000a,b).

Accordingly, a model of PD in young adult C57BL/6 mice based on combined exposure to these pesticides was recently developed (Thiruchelvam et al., 2000a,b). To this end, C57BL/6 mice were injected intraperitoneally with either PQ at a dose of 5 or 10 mg/kg or MB at a dose of 15 or 30 mg/kg alone or in combination once a week for 4 weeks (Thiruchelvam et al., 2000a,b). Only the combined exposure to both chemicals produced a sustained decrease in motor activity immediately after the injections. Under the same conditions, the levels of DA and its metabolites [dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] and the efficiency of DA turnover (DOPAC/DA) were shown to increase immediately after injection. Furthermore, the reductions in TH immunoreactivity (i.e., the decrease in the number of DA neurons), measured 3

days after the last injection, were clearly observed only in animals with combined PQ + MB exposure. Finally, the exposure of mice to PQ + MB significantly reduced their locomotor activity (Thiruchelvam et al., 2000a,b). The finding that combined PQ + MB exposure targets the nigrostriatal DA system and induces locomotor impairment suggests that this combination may be considered as potential environmental risk factor for Parkinsonism (Thiruchelvam et al., 2000a,b). Additionally, studies using the PQ + MB model have shown a greater vulnerability of males to the combined treatment, which is consistent with observations from epidemiologic studies of PD (Wooten et al., 2004). These studies suggest that the greater incidence of the disease in males observed in epidemiologic studies may not only be due to a greater exposure to environmental risk factors such as pesticides but may also be related to gender-based physiologic differences. Other studies also indicate that both aging (Thiruchelvam et al., 2003) and overexpression of mutant human α-synuclein (Thiruchelvam et al., 2004) enhance the PD phenotype produced by PQ + MB. To investigate the influence of ageing the effects of PQ (10 mg/kg) and MB (30 mg/kg) alone and in combination were examined in C57BL/6 mice aged 6 weeks, 5 or 18 months old, and were evaluated 2 weeks and 3 months post-treatment (Thiruchelvam et al., 2003). The findings clearly demonstrated an age-related enhancement of sensitivity to combined PQ + MB. Additionally, some of the observed effects were not only permanent but also increased in magnitude over time. The first indication that ageing enhanced vulnerability was that the total number of treatments had to be abbreviated. Thiruchelvam et al. (2000a,b) in their initial study using 6-week-old mice, administered 12 PQ + MB treatments, while with the aged mice it was necessary to stop at six injections, since the 18 month PQ + MB treated mice did not recover the locomotor activity 24 h post-treatment (Thiruchelvam et al., 2003), an outcome that has been shown to accurately predict underlying dopaminergic changes, particularly dopaminergic cell loss (Thiruchelvam et al., 2000a,b). Both 5- and 18-month-old mice showed decreased DA 2 weeks after the last PQ + MB treatment that was still evident 3 months after the final treatment. For the 5- and 18-month-old mice groups, progressive reductions in the levels of DA metabolites and DA turnover (DOPAC/DA), between the second week and third month after treatment were most pronounced in the 18-month-old mice group injected with PQ + MB.

To evaluate the effect of the combined exposure to PQ + MB on the overexpression of mutant human α-synuclein, transgenic male mice expressing human wild-type α-synuclein (line hwα-SYN-5) and human doubly-mutated α-synuclein (A53T and A30P, line hm^{2}α-SYN-39) (6–7 months of age) were treated twice a week for 7 weeks, with a saline vehicle or combined PQ (5 mg/kg) + MB (15 mg/kg). Ten days after the last treatment, only the mice expressing the human doubly-mutated α-synuclein line exposed to combined PQ + MB showed a persistent reduction in locomotor activity (≈70% decrease) compared to the saline treatment (Thiruchelvam et al., 2004).

Thus, combined PQ + MB exposure may be a valuable tool for inducing PD, since it showed to induce selective, age-related, progressive and irreversible nigrostriatal dopaminergic system neurotoxicity (Thiruchelvam et al., 2003).

9. Concluding remarks

A number of clinical and experimental studies have increased the interest in the possibility that environmental chemicals, including PQ, may be related to the development of PD (Brooks et al., 1999; Corasaniti et al., 1998; Liou et al., 1996). PQ seems to be one of the most eligible herbicides that may contribute for the development of PD, given that the incidence and development of the disease and the extent of PQ exposure strongly correlate. (Brooks et al., 1999; Corasaniti et al., 1998; Liou et al., 1996, 1997; Morano et al., 1994). Furthermore, PQ administered systematically to experimental animals induces behavioural and biochemical changes that are compatible with PD symptoms, such as increased rigidity, akinesia, tremor and decreased DA concentration (Brooks et al., 1999; Lindquist et al., 1988). Since many human disorders do not arise spontaneously in animals, characteristic functional changes have to be mimicked by neurotoxic agents, and thus PQ can provide a good model to induce PD symptoms in experimental animals for the study of the pathogenesis and therapeutic intervention strategies in this neurodegenerative disease.

Despite the suggestive results of epidemiological investigations, some of the data are equivocal and more detailed information about the association between PQ exposure and risk for PD is needed (Koller, 1986). Inconsistencies between the results of different studies could be explained, at least partially, by the lack of biological markers for PD and the consequent variability in case definition and diagnostic accuracy. The development of such biomarkers of disease predisposition, occurrence, and progression in vivo (in contrast to studies of biomarkers in post-mortem brain specimens) is therefore critical in PD research. In the future, collection of more precise data about PQ use should ideally be corroborated by direct-exposure assessments. The effects of PQ exposure may also vary due to genetic differences among individuals. For example, the lack of significant DA depletion, even in the presence of significant nigral cell loss, and the increase in TH activity caused by PQ suggest that toxicant-induced nigrostriatal injury may remain relatively ‘‘silent’’ (McCormack et al., 2002). Several explanations may account for the lack of significant DA depletion after PQ treatment, including the possibility that, in contrast to other toxicants, this herbicide may preferentially target the dopaminergic cell bodies rather than its terminals or could be the result of compensatory mechanisms through which enhanced DA synthesis counteracts the effects of terminal damage and restore the tissue levels of the neurotransmitter (McCormack et al., 2002). The contribution of additional environmental and/or genetic risk factors may also be required for this ‘‘subclinical’’ toxic insult to develop into a complete pathological, neurochemical, and behavioural syndrome.

Further studies on the association between PQ and PD should include assessment of PQ exposure in humans and

testing of long-term effects in animal models. Also, the recurrent association of dopaminergic cell injury with specific mechanisms of neurotoxicity suggests that putative risk factors may be screened on the basis of their effects on mitochondrial complex I activity, ROS production, and α-synuclein aggregation. Moreover, it is also clear that interactive mechanisms, such as additive/synergistic/potentiation, probably underlie the effects of environmental agents on the pathogenesis of PD. The study of these complex events in human populations and the development of animal models of such toxic interactions is challenging and essential to the elucidation of the etiology of PD.

Finally, as stated by Di Monte (2003) it is important to emphasize a multidisciplinary approach for future investigations on the role of PD environmental risk factors. A cross-linked validation of clinical, epidemiological, and experimental evidence should lead to the formulation of tenable pathogenetic hypotheses, the identification of specific risk factors, and the design of effective preventive strategies. Although the effect of PQ exposure on the development of PD is still not comprehensively explained, supporting evidence is accumulating that this widely used herbicide can penetrate the CNS, producing lethal injury to SNpc neurons and a consequent neurobehavioral syndrome. The future investigations in this field can have dramatic implications in public health, as they may help in PD prevention via the elimination or reduction of specific exposure risk factors.

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References

Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. Trends Neurosci 1989;12:366–75.

Altschuler E. Aluminum-containing antacids as a cause of idiopathic Parkinson's disease. Med Hypotheses 1999;53:22–3.

Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. Annu Rev Pharmacol Toxicol 1999;39:361–98.

Ara J, Przedborski S, Naini AB, Jackson-Lewis V, Trifiletti RR, Horwitz J, et al. Inactivation of tyrosine hydroxylase by nitration following exposure to peroxynitrite and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Proc Natl Acad Sci USA 1998;95:7659–63.

Aziz SM, Lipke DW, Olson JW, Gillespie MN. Role of ATP and sodium in polyamine transport in bovine pulmonary artery smooth cells. Biochem Pharmacol 1994;48:1611–8.

Barlow BK, Thiruchelvam MJ, Bennice L, Cory-Slechta DA, Ballatori N, Richfield EK. Increased synaptosomal dopamine content and brain concentration of paraquat produced by selective dithiocarbamates. J Neurochem 2003;85:1075–86.

Barlow BK, Lee DW, Cory-Slechta DA, Opanashuk LA. Modulation of antioxidant defense systems by the environmental pesticide maneb in dopaminergic cells. Neurotoxicology 2005;26:63–75.

Beal MF. Excitotoxicity and nitric oxide in Parkinson's disease pathogenesis. Ann Neurol 1998;44:S110–4.

Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 1990;87:1620–4.

Blaszczynski M, Litwinska J, Zaborowska D, Bilinski T. The role of respiratory chain in paraquat toxicity in yeast. Acta Microbiol Pol 1985;34:243–54.

Bonifati V, Rizzu P, Baren MJv, Schaap O, Breedveld GJ, Krieger E, et al. Mutations in the DJ-1 gene associated with autosomal recessive early-onset Parkinsonism. Science 2003;299:256–9.

Braak H, Braak E, Yilmazer D, Schultz C, Vos RAd, Jansen EN. Nigral and extranigral pathology in Parkinson's disease. J Neural Transm Suppl 1995;46:15–31.

Brooks AI, Chadwick CA, Gelbard HA, Cory-Slechta DA, Federoff HJ. Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. Brain Res 1999;823:1–10.

Burk RF, Lawrence RA, Lane JM. Liver necrosis and lipid peroxidation in the rat as result of paraquat and diquat administration. Effect of selenium deficiency. J Clin Invest 1980;65:1024–31.

Busch AE, Karbach U, Miska D, Gorboulev V, Akhoundova A, Volk C, et al. Human neurons express the polyspecific cation transporter hOCT2, which translocates monoamine neurotransmitters, amantadine, and memantine. Mol Pharm 1998;54:342–52.

Chen RC, Chang SF, Su CL, Chen TH, Yen MF, Wu HM, et al. Prevalence, incidence, and mortality of PD: a door-to-door survey in Ilan county, Taiwan. Neurology 2001;57:1679–86.

Clejan L, Cederbaum AI. Synergistic interaction between NADPH-cytochrome P-450 reductase, paraquat and iron in the generation of active oxygen radicals. Biochem Pharmacol 1989;38:1779–86.

Corasaniti MT, Strongoli MC, Rotiroti D, Bagetta G, Nistico G. Paraquat: a useful tool for the in vivo study of mechanisms of neuronal cell death. Pharmacol Toxicol 1998;83:1–7.

Crossman AR. Neural mechanisms in disorders of movement. Comp Biochem Physiol A 1989;93:141–9.

Davis LE, Adair JC. Parkinsonism from methanol poisoning: benefit from treatment with anti-Parkinson drugs. Mov Disord 1999;14:520–2.

Davis GC, Williams AC, Markey SP, Ebert MH, Caine ED, Reichert CM, et al. Chronic Parkinsonism secondary to intravenous injection of meperidine analogues. Psychiatry Res 1979;1:249–54.

Day BJ, Patel M, Calavetta L, Chang LY, Stamler JS. A mechanism of paraquat toxicity involving nitric oxide synthase. Proc Natl Acad Sci USA 1999;96:12760–5.

DeLong MR. Primate models of movement disorders of basal ganglia origin. Trends Neurosci 1990;13:281–5.

Dicker E, Cederbaum AI. NADH-dependent generation of reactive oxygen species by microsomes in the presence of iron and redox cycling agents. Biochem Pharmacol 1991;42:529–35.

Di Monte DA. The role of environmental agents in Parkinson's disease. Clin Neurosci Res 2001;1:419–26.

Di Monte DA. The environment and Parkinson's disease: is the nigrostriatal system preferentially targeted by neurotoxins? Lancet Neurol 2003;2:531–8.

Di Monte DA, Sandy MS, Ekstrom G, Smith MT. Comparative-studies on the mechanisms of paraquat and 1-methyl-4-phenylpyridine (MPP+) cytotoxicity. Biochem Biophys Res Commun 1986;137:303–9.

Di Monte DA, Lavasani M, Manning-Bog AB. Environmental factors in Parkinson's disease. Neurotoxicology 2002;23:487–502.

Dinis-Oliveira RJ, Remião F, Duarte JA, Sanchez Navarro A, Bastos ML, Carvalho F. P-glycoprotein induction: an antidotal pathway for paraquat-induced lung toxicity. Free Radic Biol Med 2006a;41:1213–24.

Dinis-Oliveira RJ, Valle MJdJ, Bastos ML, Carvalho F, Sanchez Navarro A. Kinetics of paraquat in the isolated rat lung. Influence of sodium depletion. Xenobiotica 2006b;36:724–37.

Dugan L, Choi DW. Hypoxic-ischemic brain injury and oxidative stress. In: Siegel GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD, editors. Basic neurochemistry. Philadelphia, PA: Lippincott-Raven; 1999. p. 712–29.

Ebadi M, Sharma SK. Peroxynitrite and mitochondrial dysfunction in the pathogenesis of Parkinson's disease. Antioxid Redox Signal 2003;5:319–35.

Fall PA, Fredrikson M, Axelson O, Granerus AK. Nutritional and occupational factors influencing the risk of Parkinson's disease: a case-control study in southeastern Sweden. Mov Disord 1999;14:28–37.

Fernandez Y, Subirade I, Anglade F, Periquet A, Mitjavila S. Microsomal membrane peroxidation by an Fe3+/paraquat system. Consequences of phenobarbital induction. Biol Trace Elem Res 1995;47:9–15.

Fitsanakis V, Amarnath V, Moore J, Montine K, Zhang J, Montine T. Catalysis of catechol oxidation by metal-dithiocarbamate complexes in pesticides. Free Radic Biol Med 2002;33:1714–23.

Forno LS, Langston JW, DeLanney LE, Irwin I, Ricaurte GA. Locus ceruleus lesions and eosinophilic inclusions in MPTP-treated monkeys. Ann Neurol 1986;20:449–55.

Fukushima T, Yamada K, Isobe A, Shiwaku K, Yamane Y. Mechanism of cytotoxicity of paraquat. I. NADH oxidation and paraquat radical formation via complex I. Exp Toxicol Pathol 1993;45:345–9.

Fukushima T, Yamada K, Hojo N, Isobe A, Shiwaku K, Yamane Y. Mechanism of cytotoxicity of paraquat. III. The effects of acute paraquat exposure on the electron transport system in rat mitochondria. Exp Toxicol Pathol 1994;46:437–41.

Gasser T. Genetics of Parkinson's disease. Ann Neurol 1998;44:S53–7.

Gasser T. Genetics of Parkinson's disease. J Neurol 2001;248:833–40.

Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51: 745–52.

Gibb WR, Lees AJ. Anatomy, pigmentation, ventral and dorsal subpopulations of the *substantia nigra*, and differential cell death in Parkinson's disease. J Neurol Neurosurg Psychiatry 1991;54:388–96.

Good PF, Olanow CW, Perl DP. Neuromelanin-containing neurons of the *substantia nigra* accumulate iron and aluminum in Parkinson's disease: a LAMMA study. Brain Res 1992;593:343–6.

Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Richardson RJ. The risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. Neurology 1998;50:1346–50.

Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Kortsha GX, Brown GG, et al. Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease. Neurotoxicology 1999;20:239–47.

Graham DG. Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. Mol Pharmacol 1978;14:633–43.

Grant H, Lantos PL, Parkinson C. Cerebral damage in paraquat poisoning. Histopathology 1980;4:185–95.

Greenamyre JT. Glutamate–dopamine interactions in the basal ganglia: relationship to Parkinson's disease. J Neural Transm Gen Sect 1993;91:255–69.

Greenamyre JT, Sherer TB, Betarbet R, Panov AV, Complex I. Parkinson's disease. IUBMB Life 2001;52:135–41.

Hageman G, Hoek Jvd, Hout Mv, Laan Gvd, Steur EJ, Bruin Wd, et al. Parkinsonism, pyramidal signs, polyneuropathy, and cognitive decline after long-term occupational solvent exposure. J Neurol 1999;246:198–206.

Hatefi Y. The mitochondrial electron transport and oxidative phosphorylation system. Annu Rev Biochem 1985;54:1015–69.

Hellenbrand W, Boeing H, Robra BP, Seidler A, Vieregge P, Nischan P, et al. Parkinson's disease. II. A possible role for the past intake of specific nutrients. Results from a self-administered food-frequency questionnaire in a case-control study. Neurology 1996;47:644–50.

Hernandez D, Ruiz CP, Crawley A, Malkani R, Werner J, Gwinn-Hardy K, et al. The dardarin G 2019 S mutation is a common cause of Parkinson's disease but not other neurodegenerative diseases. Neurosci Lett 2005;389:137–9.

Hertzman C, Wiens M, Bowering D, Snow B, Calne D. Parkinson's disease: a case-control study of occupational and environmental risk factors. Am J Ind Med 1990;17:349–55.

Hirai K, Witschi H, Cote MG. Mitochondrial injury of pulmonary alveolar epithelial cells in acute paraquat intoxication. Exp Mol Pathol 1985;43:242–52.

Hirsch EC, Brandel JP, Galle P, Javoy-Agid F, Agid Y. Iron and aluminum increase in the *substantia nigra* of patients with Parkinson's disease: an X-ray microanalysis. J Neurochem 1991;56:446–51.

Holland IB, Blight MA. ABC-ATPases, adaptable energy generators fueling transmembrane movement of a variety of molecules in organisms from bacteria to humans. J Mol Biol 1999;293:381–99.

Hughes JT. Brain damage due to paraquat poisoning: a fatal case with neuropathological examination of the brain. Neurotoxicology 1988;9: 243–8.

Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, et al. Mutations in the parkin gene cause autosomal recessive juvenile Parkinsonism. Nature 1998;392:605–8.

Klawans HJ, Stein RW, Tanner CM, Goetz CG. A pure Parkinsonian syndrome following acute carbon monoxide intoxication. Arch Neurol 1982;39: 302–4.

Klockgether T, Turski L. Excitatory amino acids and the basal ganglia: implications for the therapy of Parkinson's disease. Trends Neurosci 1989;12:285–6.

Koller WC. Paraquat and Parkinson's disease. Neurology 1986;36:1147.

Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, et al. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinsons disease. Nat Genet 1998;18:106–8.

Lang AE, Lozano AM. Parkinsons disease-first of two parts. N Engl J Med 1998;339:1044–53.

Langston JW. The etiology of Parkinsons disease with emphasis on the MPTP story. Neurology 1996;47:S153–60.

Langston JW, Ballard PA. Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. N Engl J Med 1983;309:310.

Langston JW, Ballard P. Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): implications for treatment and the pathogenesis of Parkinson's disease. Can J Neurol Sci 1984;11:160–5.

Langston JW, Forno LS, Tetrud J, Reeves AG, Kaplan JA, Karluk D. Evidence of active nerve cell degeneration in the *substantia nigra* of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. Ann Neurol 1999;46:598–605.

LaVoie MJ, Hastings TG. Peroxynitrite- and nitrite-induced oxidation of dopamine: implications for nitric oxide in dopaminergic cell loss. J Neurochem 1999;73:2546–54.

Leslie EM, Deely RG, Cole SP. Toxicological relevance of the multidrug resistance protein 1, MRP1 (ABCC1) and related transporters. Toxicology 2001;167:3–23.

Lindquist NG, Larsson BS, Lyden-Sokolowski A. Autoradiography of [14C]paraquat or [14C]diquat in frogs and mice: accumulation in neuromelanin. Neurosci Lett 1988;93:1–6.

Liochev SI, Fridovich I. Paraquat diaphorases in *Escherichia coli*. Free Radic Biol Med 1994;16:555–9.

Liou HH, Chen RC, Tsai YF, Chen WP, Chang YC, Tsai MC. Effects of paraquat on the *substantia nigra* of the wistar rats: neurochemical, histological, and behavioral studies. Toxicol Appl Pharmacol 1996;137: 34–41.

Liou HH, Tsai MC, Chen CJ, Jeng JS, Chang YC, Chen SY, et al. Environmental risk factors and Parkinsons disease: a case-control study in Taiwan. Neurology 1997;48:1583–8.

Lotharius J, O'Malley KL. The parkinsonism-inducing drug 1-methyl-4-phenylpyridinium triggers intracellular dopamine oxidation. A novel mechanism of toxicity. J Biol Chem 2000;275:38581–8.

Manning-Bog AB, McCormack AL, Li J, Uversky VN, Fink AL, Di Monte DA. The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha-synuclein. J Biol Chem 2002;277: 1641–4.

Marder K, Logroscino G, Alfaro B, Mejia H, Halim A, Louis E, et al. Environmental risk factors for Parkinsons disease in an urban multiethnic community. Neurology 1998;50:279–81.

Marsden CD. Parkinson's disease. J Neurol Neurosurg Psychiatry 1994;57: 672–81.

Masliah E, Rockenstein E, Veinbergs I, Mallory M, Hashimoto M, Takeda A, et al. Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. Science 2000;287: 1265–9.

McCormack AL, Di Monte DA. Effects of L-dopa and other amino acids against paraquat-induced nigrostriatal degeneration. J Neurochem 2003;85:82–6.

McCormack AL, Thiruchelvam M, Manning-Bog AB, Thiffault C, Langston JW, Cory-Slechta DA, et al. Environmental risk factors and Parkinson's disease: selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. Neurobiol Dis 2002;10:119–27.

McGeer PL, McGeer EG, Suzuki JS. Aging and extrapyramidal function. Arch Neurol 1977;34:33–5.

Moore RY, Bhatnagar RK, Heller A. Anatomical and chemical studies of a nigro-neostriatal projection in the cat. Brain Res 1971;30:119–35.

Morano A, Jimenez-Jimenez FJ, Molina JA, Antolin MA. Risk-factors for Parkinsons disease: case-control study in the province of Caceres, Spain. Acta Neurol Scand 1994;89:164–70.

Mukaetova-Ladinska EB, McKeith IG. Pathophysiology of synuclein aggregation in Lewy body disease. Mech Ageing Dev 2006;127:188–202.

Orth M, Tabrizi SJ. Models of Parkinsons disease. Mov Disord 2003;18:729–37.

Perry TL, Yong VW, Wall RA, Jones K. Paraquat and two endogenous analogues of the neurotoxic substance *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine do not damage dopaminergic nigrostriatal neurons in the mouse. Neurosci Lett 1986;69:285–9.

Petrovitch H, Ross GW, Abbot RD, Sanderson WT, Sharp DS, Tanner CM, et al. Plantation work and risk of Parkinson's disease in a population-based longitudinal study. Arch Neurol 2002;59:1787–92.

Pezzoli G, Strada O, Silani V, Zecchinelli A, Perbellini L, Javoy-Agid F, et al. Clinical and pathological features in hydrocarbon-induced Parkinsonism. Ann Neurol 1996;40:922–5.

Piccini P, Burn DJ, Ceravolo R, Maraganore D, Brooks DJ. The role of inheritance in sporadic Parkinsons disease: evidence from a longitudinal study of dopaminergic function in twins. Ann Neurol 1999;45:577–82.

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 1997;276:2045–7.

Rajput AH. Frequency and cause of Parkinson's disease. Can J Neurol Sci 1992;19:103–7.

Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD. Mitochondrial complex I deficiency in Parkinson's disease. J Neurochem 1990;54:823–7.

Seidler A, Hellenbrand W, Robra BP, Vieregge P, Nischan P, Joerg J, et al. Possible environmental, occupational, and other etiologic factors for Parkinsons disease: a case-control study in Germany. Neurology 1996;46:1275–84.

Semchuk KM, Love EJ, Lee RG. Parkinson's disease and exposure to agricultural work and pesticide chemicals. Neurology 1992;42:1328–35.

Semchuk KM, Love EJ, Lee RG. Parkinson's disease: a test of the multifactorial etiologic hypothesis. Neurology 1993;43:1173–80.

Shimizu K, Ohtaki K, Matsubara K, Aoyama K, Uezono T, Saito O, et al. Carrier-mediated processes in blood-brain barrier penetration and neural uptake of paraquat. Brain Res 2001;906:135–42.

Shimizu K, Matsubara K, Ohtaki K, Fujimaru S, Saito O, Shiono H. Paraquat induces long-lasting dopamine overflow through the excitotoxic pathway in the striatum of freely moving rats.. Brain Res 2003a;976:243–52.

Shimizu K, Matsubara K, Ohtaki K, Shiono H. Paraquat leads to dopaminergic neural vulnerability in organotypic midbrain culture. Neurosci Res 2003b;46:523–32.

Shin WW, Fong WF, Pang SF, Wong PC. Limited blood-brain barrier transport of polyamines. J Neurochem 1985;44:1056–9.

Sian J, Dexter DT, Lees AJ, Daniel S, Agid Y, Javoy-Agid F, et al. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann Neurol 1994a;36:348–55.

Sian J, Dexter DT, Lees AJ, Daniel S, Jenner P, Marsden CD. Glutathione-related enzymes in brain in Parkinson's disease. Ann Neurol 1994b;36:356–61.

Smith LL. The identification of an accumulation system for diamines and polyamines into the lung and its relevance to paraquat toxicity. Arch Toxicol Suppl 1982;5:1–14.

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature 1997;388:839–40.

Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. α-Synuclein in filamentous inclusions of Lewy bodies from Parkinsons disease and dementia with Lewy bodies. Proc Natl Acad Sci USA 1998;95:6469–73.

Stein WD. Kinetics of the multidrug transporter (P-glycoprotein) and its reversal. Physiol Rev 1997;77:545–90.

Sveinbjornsdottir S, Hicks AA, Jonsson T, Petursson H, Gugmundsson G, Frigge ML, et al. Familial aggregation of Parkinsons disease in Iceland. N Engl J Med 2000;343:1765–70.

Tanner CM. The role of environmental toxins in the etiology of Parkinson's disease. Trends Neurosci 1989;12:49–54.

Tanner CM, Ben-Shlomo Y. Epidemiology of Parkinson's disease. Adv Neurol 1999;80:153–9.

Tanner CM, Ottman R, Goldman SM, Ellenberg J, Chan P, Mayeux R, et al. Parkinson disease in twins: an etiologic study. J Am Med Assoc 1999;281:341–6.

Tawara T, Fukushima T, Hojo N, Isobe A, Shiwaku K, Setogawa T, et al. Effects of paraquat on mitochondrial electron transport system and catecholamine contents in rat brain. Arch Toxicol 1996;70:585–9.

Taylor EM. The impact of efflux transporters in the brain on the development of drugs for CNS disorders. Clin Pharmacokinet 2002;41:81–92.

Thakar JH, Hassan MN. Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), cyperquat (MPP+) and paraquat on isolated mitochondria from rat striatum, cortex and liver. Life Sci 1988;43:143–9.

Thiruchelvam M, Brockel BJ, Richfield EK, Baggs RB, Cory-Slechta DA. Potentiated and preferential effects of combined paraquat and maneb on nigrostriatal dopamine systems: environmental risk factors for Parkinson's disease? Brain Res 2000a;873:225–34.

Thiruchelvam M, Richfield EK, Baggs RB, Tank AW, Cory-Slechta DA. The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: implications for Parkinson's disease. J Neurosci 2000b;20:9207–14.

Thiruchelvam M, Richfield EK, Goodman BM, Baggs RB, Cory-Slechta DA. Developmental exposure to the pesticides paraquat and maneb and the Parkinson's disease phenotype. Neurotoxicology 2002;23:621–33.

Thiruchelvam M, McCormack A, Richfield EK, Baggs RB, Tank AW, Di Monte DA, et al. Age-related irreversible progressive nigrostriatal dopaminergic neurotoxicity in the paraquat and maneb model of the Parkinson's disease phenotype. Eur J Neurosci 2003;18:589–600.

Thiruchelvam MJ, Powers JM, Cory-Slechta DA, Richfield EK. Risk factors for dopaminergic neuron loss in human alpha-synuclein transgenic mice. Eur J Neurosci 2004;19:845–54.

Tomita M. Comparison of one-electron reduction activity against the bipyridylium herbicides, paraquat and diquat, in microsomal and mitochondrial fractions of liver, lung and kidney (in vitro). Biochem Pharmacol 1991;42:303–9.

Uitti RJ, Snow BJ, Shinotoh H, Vingerhoets FJ, Hayward M, Hashimoto S, et al. Parkinsonism induced by solvent abuse. Ann Neurol 1994;35:616–9.

Uversky VN. A protein-chameleon: conformational plasticity of α-synuclein, a disordered protein involved in neurodegenerative disorders. J Biomol Struct Dyn 2003;21:211–34.

Uversky VN, Li J, Fink AL. Pesticides directly accelerate the rate of alpha-synuclein fibril formation: a possible factor in Parkinsons disease. FEBS Lett 2001;500:105–8.

Uversky VN, Li J, Bower K, Fink AL. Synergistic effects of pesticides and metals on the fibrillation of α-synuclein: implications for Parkinsons disease. Neurotoxicology 2002;23:527–36.

Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 2004;304:1158–60.

Vanacore N, Nappo A, Gentile M, Brustolin A, Palange S, Liberati A, et al. Evaluation of risk of Parkinsons disease in a cohort of licensed pesticide users. Neurol Sci 2002;23(Suppl 2):S119–20.

Widdowson PS, Farnworth MJ, Simpson MG, Lock EA. Influence of age on the passage of paraquat through the blood-brain barrier in rats: a distribution and pathological examination. Hum Exp Toxicol 1996;15:231–6.

Wooten GF. Neurochemistry and neuropharmacology of Parkinson's disease. In: Watts RL, Koller W, editors. Movement disorders: neurologic principles and practice. New York: McGraw-Hill; 1997. p. 153–60.

Wooten GF, Currie LJ, Bovbjerg VE, Lee JK, Patrie J. Are men at greater risk for Parkinson's disease than women? J Neurol Neurosurg Psychiatry 2004;75:637–9.

Wu X, Kekuda R, Huang W, Fei YJ, Leibach FH, Chen J, et al. Identity of the organic cation transporter OCT3 as the extraneuronal monoamine transporter (uptake2) and evidence for the expression of the transporter in the brain. J Biol Chem 1998;273:32776–8.

Yamada K, Fukushima T. Mechanism of cytotoxicity of paraquat. II. Organ specificity of paraquat-stimulated lipid peroxidation in the inner membrane of mitochondria. Exp Toxicol Pathol 1993;45:375–80.

Yang W, Sun AY. Paraquat-induced free radical reaction in mouse brain microsomes. Neurochem Res 1998;23:47–53.

Yasui M, Kihira T, Ota K. Calcium, magnesium and aluminum concentrations in Parkinsons disease. Neurotoxicology 1992;13:593–600.

Youngman RJ, Elstner EF. Oxygen species in paraquat toxicity: the crypto-OH radical. FEBS Lett 1981;129:265–8.

Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, et al. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol 2004;55:164–73.

Zhang L, Dresser MJ, Gray AT, Yost SC, Terashita S, Giacomini KM. Cloning and functional expression of a human liver organic cation transporter. Mol Pharmacol 1997;51:913–21.