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Forum Review

Reactive Oxygen and Nitrogen Species: Weapons of Neuronal Destruction in Models of Parkinson's Disease

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ABSTRACT

Parkinson's disease (PD) is a common neurodegenerative disease whose etiology and pathogenesis remain mainly unknown. To investigate its cause and, more particularly, its mechanism of neuronal death, numerous *in vivo* experimental models have been developed. Currently, both genetic and toxic models of PD are available, but the use of neurotoxins such as 6-hydroxydopamine, paraquat, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, and rotenone are still the most popular means for modeling the destruction of the nigrostriatal dopaminergic neurons seen in PD. These four neurotoxins, although distinct in their intimate cytotoxic mechanisms, kill dopaminergic neurons via a cascade of deleterious events that consistently involves oxidative stress. Herein, we review and compare the molecular mechanisms of 6-hydroxydopamine, paraquat, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, and rotenone, placing the emphasis of our discussion on how reactive oxygen and nitrogen species contribute to the neurotoxic properties of these four molecules. As the reader will discover, to achieve the above stated goal, we had to not only appraise recent findings, but also revisit earlier landmark studies to provide a comprehensive view on this topic. This approach also enabled us to describe how our understanding of the mechanism of actions of certain toxins has evolved over time, which is particularly striking in the case of the quatrogenarian neurotoxin, 6-hydroxydopamine. *Antioxid. Redox Signal.* 7, 685–693.

INTRODUCTION

Parkinson's disease (PD) affects ~1% of the population over the age of 50 in the United States alone, and it is the second most frequent neurodegenerative disorder after Alzheimer's disease (15). This common neurodegenerative disorder is essentially a sporadic disease, meaning that it presents itself with no apparent genetic linkage (15). Yet in rare instances, as in several other neurodegenerative diseases (70), PD can be inherited (16). Whether it is sporadic or familial, PD is a slow, progressive disease characterized mainly by resting tremor, slowness of movement (bradykinesia), stiffness (rigidity), and poor balance (postural instability) (25). Most, if not all, of these clinical abnormalities are attributed to the severe loss of the nigrostriatal dopaminergic neurons in the substantia nigra pars compacta (SNpc), which leads to a profound deficit in brain dopamine (15). Another pathological hallmark of PD is the eosinophilic intraneuronal proteinaceous inclusion called the Lewy body (27), whose pathogenic significance remains controversial.

There is no evidence that PD patients must be treated upon emergence of the clinical symptoms. However, at some point, the motor disability becomes so severe that treatment aimed at either replenishing dopaminergic stores in the brain (*e.g.*, levodopa) or stimulating dopamine receptors (*e.g.*, dopamine agonists), or both, is required to maintain the patient's autonomy and quality of life. Several of the approved drugs for PD are quite potent in alleviating symptoms, but their chronic administration often causes serious motor and psychiatric side effects (24).

Regardless of the nature of the etiologic factor that initially provokes neurodegeneration, two major hypotheses regarding

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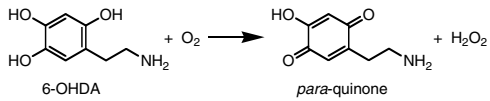
the pathogenesis of the disease have emerged from studies probing the functions of genes implicated in inherited forms of PD and from animal and cellular model systems of PD. One hypothesis postulates that inappropriate aggregation of proteins is instrumental in the death of SNpc dopaminergic neurons, whereas the other, which is the focus of this review, suggests that the offender is oxidative stress, including potentially toxic intermediates of oxidized dopamine. This latter hypothesis posits that the fine-tuned balance between the production and destruction of oxidants is altered in such a way that oxidative damage arises, leading to cellular dysfunction and, ultimately, to cell death. Unquestionably, support for the "oxidative stress hypothesis" of PD comes from descriptive investigations performed on fluids and tissue samples of PD patients (64). However, in our opinion, the most compelling evidence for a role of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the death of SNpc dopaminergic neurons in PD originates, not from human studies, but rather from investigations in animal models of PD generated by various neurotoxins. What these neurotoxins are and how they engender oxidative stress are the topics that we will discuss in this review. Conversely, how faithfully these neurotoxins model PD and how they should be used to achieve this goal will not be discussed. Readers interested in these latter aspects are encouraged to review other references (65, 66).

ROS-PRODUCING "PARKINSONIAN" NEUROTOXINS

Toxic models of PD are numerous, but thus far only a handful of such models have been thoroughly characterized with respect to their biochemical and molecular modes of action and neurodegenerative effects. Relatively well characterized models of PD include 6-hydroxydopamine (6-OHDA), paraquat, rotenone, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (15). In principle, these toxins all share the same function, namely, the killing of SNpc dopaminergic neurons by a process in which oxidative stress is instrumental. Yet, as discussed below and depending on the neurotoxin, the molecular basis of the generated oxidative stress is quite different, and in broad terms 6-OHDA toxicity is dependent on its oxidation. The toxic action of paraquat is due to its reduction–oxidation cycling, whereas, at least in part, inhibition of the mitochondrial electron transport chain is responsible for the neurotoxicity of both rotenone and MPTP.

THE 6-OHDA MODEL: A PATRIARCH STILL IN THE RACE

6-OHDA was introduced as a catecholaminergic toxin >30 years ago (46) and, ever since, it has remained an extensively tested model both *in vitro* and *in vivo*. The effects of 6-OHDA on both the central and peripheral catecholaminergic pathways in rodents and in a variety of cultured cell types have been reviewed elsewhere, as well as the molecular basis for its specificity (45, 46, 65). 6-OHDA can be administered to rodents via a variety of different routes, but its proper utilization *in vivo* and *in vitro* relies on one's knowledge of a series of technical points that have been discussed in detail (45, 46, 65). Because of the emphasis of this special *Forum* of *Antioxidants & Redox Signaling* on oxidative stress in PD and experimental models of the disease and as 6-OHDA is a prototypical "oxidative-stress neurotoxin," we will focus the discussion on the 6-OHDA-induced neurotoxic mechanism. From the outset, it can be said that most experts agree on the concept that 6-OHDA destroys catecholaminergic structures by a combined effect of ROS and quinones (10). This popular view is based on the evidence that 6-OHDA, once dissolved in an alkaline solution, readily oxidizes in the presence of oxygen, yielding, in a stoichiometric fashion, hydrogen peroxide (H_2O_2) and *para*-quinone (37, 72) as depicted by the following reaction:

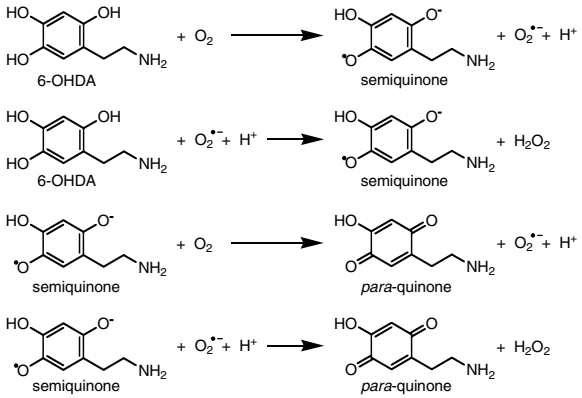


6-OHDA *para*-quinone

Although the chemical reaction that underlies 6-OHDA-induced neurotoxicity appears quite straightforward, it is in fact a remarkably complicated reaction that does not occur as a spontaneous oxidation by molecular oxygen. Still, molecular oxygen is mandatory for the reaction or, in anaerobic conditions, no conversion of 6-OHDA into quinones is detectable (30). If oxygen is necessary for the reaction, it is not, however, sufficient to drive 6-OHDA oxidation alone because desferrioxamine, a potent metal chelator, does inhibit the aerobic formation of 6-OHDA quinones to a dramatic extent (29–31, 80). This observation implies that 6-OHDA oxidation requires the presence of redox-capable transitional metals such as iron or copper to catalyze the transfer of electrons from 6-OHDA to molecular oxygen. It is now well accepted that even the presence of trace amounts of transitional metal contaminants, brought into the reaction mixture by the reagents and glassware, suffice to set this aerobic reaction in motion.

Aside from quinones, the oxidation of 6-OHDA also generates H_2O_2, as illustrated above. In biological systems, the production of H_2O_2 results from a two-electron reduction of oxygen. Thus, it can be surmised that during 6-OHDA oxidation a pair of electrons is transferred from 6-OHDA to molecular oxygen to produce H_2O_2. Yet it appears that the reaction of molecular oxygen with 6-OHDA is primarily a two-electron process only in the presence of excess oxygen, but it is a four-electron process in the presence of excess 6-OHDA (30). Accordingly, H_2O_2 is an end product of the reaction merely if 6-OHDA is limiting. Furthermore, even if the experimental conditions favor an overall exchange of a pair of electrons, the fact that oxygen has two unpaired electrons on its outermost orbital with a same spin quantum number makes it more likely that the reduction of oxygen proceeds by one electron at the time forming superoxide ($O_2^{\bullet -}$) and semiquinone radicals as the intermediary species. This interpretation is consistent with the demonstration that superoxide dismutase (SOD), by scavenging superoxide radicals, dramatically inhibits the oxidation of 6-OHDA (39). Subsequent studies have confirmed the production of superoxide radicals, and have moreover demonstrated that superoxide radicals generated by the first step of 6-OHDA oxidation are critical in propagating the oxidation of 6-OHDA (11, 30, 31, 80). As de-

tailed elsewhere (39), the progressive oxidation of 6-OHDA can be schematized as follows:



This shows that the oxidation of 2 moles of 6-OHDA leads to the formation of 2 moles of quinone and 2 moles of H_2O_2. In addition to the H_2O_2 and superoxide radicals, 6-OHDA oxidation is also associated with the production of hydroxyl radicals as demonstrated by using spin-trap 5,5-dimethyl-1-pyrroline-*N*-oxide (26) and methional as spin traps (11). In this system, hydroxyl radicals can arise from the Fenton reaction by which the breakdown of H_2O_2 is catalyzed by transitional metals such as iron.

The above studies indicate that 6-OHDA oxidation generates not only *para*-quinone and H_2O_2, but also the superoxide and hydroxyl radicals. As stressed by many authors throughout this *Forum*, ROS such as H_2O_2, superoxide radical, and hydroxyl radical can either directly or indirectly inflict an array of cellular oxidations that can ultimately lead to cell death. Given this, the reader may encounter no difficulty envisioning how ROS generated by the oxidation of 6-OHDA could contribute to the neurotoxicity of this compound. On the other hand, how the quinone of 6-OHDA may exert deleterious effects may be less obvious. Early on in the characterization of the 6-OHDA mode of action, it was recognized that *para*-quinone formed though the oxidation of 6-OHDA undergoes covalent binding with sulfhydryl and other biological macromolecules with nucleophilic centers (32, 72). Accordingly, *para*-quinone is thus likely to react with glutathione and protein amino acid residues such as cysteine, tyrosine, and lysine. The deleterious consequences of the *para*-quinone of 6-OHDA may thus range from depletion of vital antioxidants such as glutathione, whose concentration is diminished in PD (64), to inactivation of critical enzymes such as catechol-*O*-methyltransferase (4) and tyrosine hydroxylase (49) and, more importantly, to an accumulation of potentially neurotoxic α-synuclein protofibrils, a proposed key event in PD pathogenesis (12).

Although the above-cited studies would argue that both the produced ROS and *para*-quinone are probably equally instrumental in the 6-OHDA neurotoxic processes, available evidence appears to favor the view that ROS are the dominant noxious mediators. For example, the addition of ascorbic acid to tissue slices, which is known to recycle *para*-quinone into 6-OHDA with a net formation of H_2O_2 (38, 80), prevents the appearance of colored quinones, but enhances neurotoxicity (38).

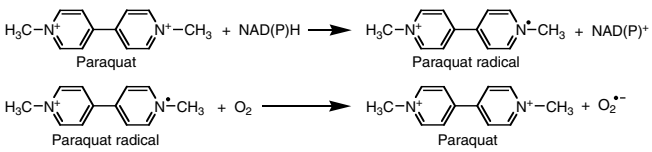
Finally, it should be emphasized that, like other monoamines, 6-OHDA can be metabolized by monoamine oxidase (MAO), a reaction that also generates ROS. This observation raises the possibility that the oxidative domination of 6-OHDA contributes to the neurotoxic process. Yet the finding that pretreatment with MAO inhibitors such as pargyline, rather than mitigating 6-OHDA toxicity, enhances it (45), argues against a MAO-dependent source of ROS as being contributive to the 6-OHDA neurotoxic process. It should also be stressed that, as long as the environmental conditions are favorable, oxidation of 6-OHDA can occur *in vivo* both intra- and extraneuronally. Consistent with this view is the demonstration that, in mesencephalic cultures, 6-OHDA toxicity is not restricted to dopaminergic neurons (55), and that several cell types devoid of transporters allowing 6-OHDA to be translocated inside the cell—such as C6 glioma, NIH-3T3, and CHO cells—can be damaged by this neurotoxin (3).

THE HERBICIDE PARAQUAT

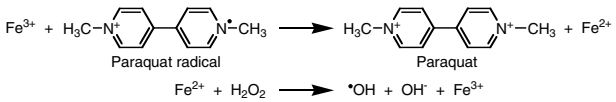
The potent herbicide paraquat, whose chemical name is *N*,*N*′-dimethyl-4-4′-bipyridinium ion, is another prototypic toxin known to exert deleterious effects through oxidative mechanisms. Structurally, paraquat comprises two pyridine rings, *i.e.*, aromatic rings in which one carbon atom is replaced by a nitrogen atom, joined covalently by their number-4 carbon and with a methyl group attached to each nitrogen. The overall biochemical reaction governing the neurotoxic mechanism of paraquat was reported by Bus and collaborators roughly 30 years ago (6, 7). According to these authors, paraquat undergoes a single electron, reduction-oxidation cycling with subsequent formation of superoxide radicals:

The first of the two steps of this biochemical reaction requires that paraquat go through a single-electron reduction to the blue-colored cation radical, paraquat$^{\bullet+}$ (28, 59). This initial step is not dependent on oxygen, as it can proceed under anaerobic conditions, but it does depend on the presence of diaphorase activity (28), *i.e.*, an enzyme that transfers an electron from a NAD(P)H molecule. Paraquat diaphorases are usually oxidoreductase enzymes containing flavin groups and using NADPH and, presumably, NADH as electron donors (9, 19, 22, 52, 77, 89). Relevant to the brain toxicity of paraquat, it should be noted that nitric oxide synthase (NOS) has been identified as one of the diaphorases capable of reacting with paraquat (19).

The second step of the paraquat toxic reaction is the reoxidation of this compound by oxygen that occurs through a transfer of a single electron from the paraquat radical to molecular oxygen, yielding oxidized paraquat (*i.e.*, the parent compound) and superoxide radicals. The actual reduction-oxidation cycling reaction of paraquat can thus be depicted as follow:



Of note, paraquat$^{\bullet+}$ is a powerful reducing radical capable not only of reacting with molecular oxygen to generate superoxide radicals as shown above, but also of reacting with transitional metals such as iron. Paraquat$^{\bullet+}$ can readily reduce iron(III) and most iron(III)chelates (9) into iron(II) or iron(II) chelates, which in turn could catalyze the formation of hydroxyl radicals via the Fenton reaction:



Presumably, whether paraquat$^{\bullet+}$ reacts with oxygen or iron depends on the concentration of oxygen. Accordingly, it can be speculated that in the brain the high oxygen content should favor the reaction of paraquat$^{\bullet+}$ with molecular oxygen over that with iron(III). Yet hydroxyl radicals can also be produced by an alternative mechanism in which paraquat$^{\bullet+}$ simply supplies the metal needed for the hydroxyl radical formation by reductively mobilizing iron from ferritin (83). Thus far, there is not an unequivocal demonstration that hydroxyl radicals are implicated in the deleterious effects caused by paraquat intoxication. Conversely, there are countless demonstrations that SOD overexpression and SOD mimetics confer resistance against paraquat (17, 18, 61), thus supporting the concept that superoxide radicals are pivotal in paraquat cytotoxicity.

Aside from oxidative stress, it has also been suggested that depletion of the intracellular stores of NAD(P)H, due to its increased oxidation during the reduction-oxidation cycling of paraquat stores, contributes to the overall cytotoxic process. Indeed, loss of NAD(P)H or change in the NAD(P)H over NAD(P)$^+$ ratio may have serious harmful consequences by impairing vital metabolic pathways such as fatty acid synthesis. Increased oxidation of glucose by the pentose phosphate shunt and inhibition of fatty acid synthesis in paraquat-intoxicated animals (79) are consistent with this view.

Most experimental studies of paraquat are related to its effects on the lungs, liver, and kidneys, probably because the toxicity induced by this herbicide in these organs is responsible for death after acute exposure. However, less known is the fact that significant damage to the brain is also seen in individuals who died from paraquat intoxication (33, 41) in spite of the fact that paraquat poorly crosses the blood–brain barrier (78). Thus far, the use of paraquat as a model of PD has been performed mainly in mice, perhaps because previous studies in rats have shown that oral administration of paraquat to these rodents was ineffective in damaging the brain (86) and that its intraventricular or intracerebral injection produces diffuse neurodegeneration (20, 53). Conversely, several authors have now reported reduced motor activity and dose-dependent losses of striatal dopaminergic nerve fibers and substantia nigra neuronal cell bodies in mice that received systemic injections of paraquat (5, 58, 82). Aside from killing dopaminergic neurons, paraquat induces α-synuclein up-regulation and aggregation (56).

Quite surprising is the finding that dopaminergic but not other subpopulations of neurons, such as GABAergic cells in the substantia nigra and the striatum, appeared to be affected by paraquat injection (58). Neuronal subpopulations expressing high activity of NADPH-diaphorase in the brain are apparently not the dopaminergic neurons (63). Given the mechanism of action of paraquat, it is thus fascinating to note that dopaminergic neurons are specifically affected in this model.

THE MITOCHONDRIAL POISONS MPTP AND ROTENONE

Both MPTP and rotenone are well-known mitochondrial toxins used to recapitulate hallmarks of PD in laboratory animals (15). Although MPTP has been regarded for the past 20 years as the parkinsonian toxin *par excellence*, rotenone has recently received major attention for its capacity to produce previously unattainable PD features such as intraneuronal proteinaceous inclusions in rats (2). However, studies about the detailed mechanisms by which rotenone kills dopaminergic neurons are thus far few, which is in striking contrast with the large body of literature available on this topic for MPTP. Because it can be assumed that these two neurotoxins share many of the key molecular mechanisms responsible for their neurotoxicity, we will focus the rest of our discussion on MPTP and only refer to rotenone whenever published data permit.

A distinct feature of MPTP is that this neurotoxin is highly lipophilic and thus readily capable of crossing the blood–brain barrier after its systemic administration (57). Yet, once in the brain, MPTP as such is unable to provoke any neurotoxicity unless it is converted to 1-methyl-4-phenylpyridinium (MPP$^+$) (40, 57). The activation of the protoxin MPTP is a two-step process: first, MPTP is oxidatively deaminated by enzyme MAO-B to form the intermediate, unstable compound 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP$^+$); and second, MPDP$^+$ spontaneously oxidizes, yielding MPP$^+$ (Fig. 1). This process takes place not in dopaminergic neurons, which are devoid of MAO-B, but primarily in glial cells and secon-

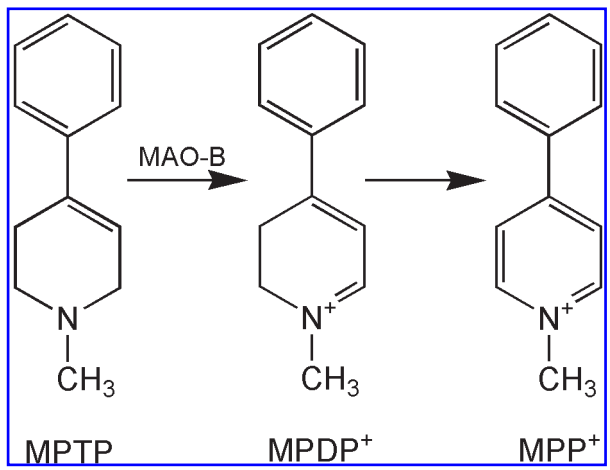


FIG. 1. Bioactivation of MPTP to MPP$^+$. The entire process of MPTP bioactivation takes place in serotonergic neurons and in astrocytes. The transformation of MPTP to MPDP$^+$ is catalyzed by MAO-B; the subsequent transformation of MPDP$^+$ to MPP$^+$ is spontaneous.

darily in serotonergic structures scarcely present in the vicinity of the nigrostriatal dopaminergic neurons. Once formed in these nondopaminergic cells, MPP^+ is released to the extracellular space, and through its binding to the plasma membrane dopamine transporter (44) it is translocated in dopaminergic neurons. Soon after its entry into dopaminergic neurons, MPP^+ participates in a variety of deleterious biochemical processes, among which many could generate oxidants. Although most of these oxidative reactions are taking place within the dopaminergic neuron itself, some meaningful reactive pathways originate from the surrounding glial cells. The current consensus in the field is that both the intrinsic and extrinsic oxidative stresses participate in the demise of nigrostriatal dopaminergic neurons in the MPTP model.

With respect to the intrinsic oxidative stress in the MPTP model, one of the main sources of the oxidant presumably emanates from the mitochondria. MPP^+, like rotenone, can accumulate within the mitochondria and bind to complex I of the electron transport chain (60). In doing so, MPP^+ interrupts the natural flow of electrons along this chain of cytochromes, which leads not only to an acute deficit in ATP formation, but also to an increased production of ROS, especially of superoxide (8, 35, 71). Because of the high amounts of Mn-SOD (SOD2) in the inner compartment of the mitochondria, it is likely that most, if not all, of the superoxide radicals produced by the blockade of complex I are immediately converted into H_2O_2. The latter, in contrast to the superoxide radical, could permeate through the mitochondrial membranes and thus can readily gain access to the cytosol. Accordingly, it is likely that mitochondrially generated superoxide may contribute to oxidative damage inside the mitochondria, whereas H_2O_2 may contribute to oxidative damage both inside and outside the mitochondria. These ROS may also engage in producing secondary and strong oxidants such as the hydroxyl radical, by reacting with an iron released from the destruction of mitochondrial aconitase (36), as well as with nitric oxide to generate peroxynitrite (43). Although there is little evidence that any of the reactive species cited above actually do inflict structural or functional mitochondrial damage in the MPTP model, the demonstration that transgenic mice with increased SOD2 activity are resistant to MPTP toxicity (47) argues that some type of MPP^+-mediated mitochondrial oxidative event has to be instrumental in the neurodegenerative process.

Presumably, ROS production can also occur in the MPTP model from the autooxidation of dopamine (54) resulting from an MPP^+-induced massive release of vesicular dopamine to the cytosol. Furthermore, the induction of cyclooxygenase-2 (COX-2) within the dopaminergic neurons after MPTP injection (42, 81) can also serve as a source of ROS. Indeed, via the peroxidase activity of COX-2, this enzyme can use catecholamines such as dopamine as an electron donor needed to catalyze the formation of dopamine-quinones. The latter may modify proteins by forming dopamine-cysteinyl adducts, which may have major consequences on the structure and function of modified proteins. In support of this scenario, we have found that, following MPTP injections to mice, contents of dopamine-cysteinyl in proteins increase markedly in a COX-2-dependent manner in affected brain regions (81).

The striking structural similarity between MPP^+ and paraquat (Fig. 2) has prompted several investigators to test the idea that MPP^+, like paraquat, could inflict oxidative stress via a reduction-oxidation cycling mechanism. Compared with paraquat, MPP^+ is an extremely stable species unlikely to undergo reduction–oxidation cycling (50). The reason paraquat is more reactive than MPP^+ relates to the double-positive charge on the paraquat, whereas MPP^+ has only one such charge (Fig. 2). For example, the one-electron reduction potential, which reflects the energy required to form the free radical, is -0.446 V for paraquat, well within the range of biological systems. In contrast, MPP^+ has a one-electron reduction potential of -1.18 V or greater, which is outside the range of known biological systems that might be involved in this reaction. Therefore, it seems unlikely that MPP^+ could participate in paraquat-like reduction–oxidation cycling unless an enzyme catalyzes it.

Although fierce discussions are still ongoing about which of these different sources of ROS, or combinations thereof, are implicated in MPTP neurotoxicity, there is compelling evidence that oxidative stress does play a critical role in the neurodegenerative process seen in this PD model. For instance, reduction of Cu/Zn-SOD (SOD1) activity by diethyl dithiocarbamate, which chelates copper and inhibits SOD1, or by genetic ablation of SOD1, potentiates MPTP-induced toxicity in mice (13, 90). The mirror opposite picture is found upon overexpressing human SOD1, in that transgenic mice with increased SOD1 activity are more resistant to MPTP (67). Although similar studies have not yet been done in rotenone, the toxicity of this other poison on dopaminergic cells appears also to implicate oxidative stress (74, 76).

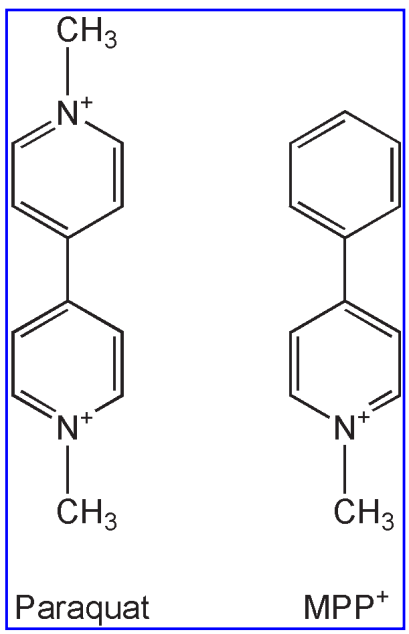


FIG. 2. Comparison of paraquat and MPP^+ chemical structures. Note the striking resemblance of the two compounds.

As already referred to above, ROS exert many or most of their toxic effects in the MPTP model in conjunction with other reactive species such as nitric oxide (1, 62, 69, 73) produced in the brain by both the neuronal and the inducible isoforms of the enzyme NOS (51, 68). A comprehensive review of the source and the role of nitric oxide in the MPTP model can be found in other references (63, 84).

Before discussing the extrinsic oxidative stress in the MPTP model, we should first emphasize the fact that the loss of dopaminergic neurons caused by both MPTP and rotenone is associated with a glial response (75, 85). Activation of microglia, which is quite pronounced in the MPTP and rotenone mouse models (14, 21, 48, 51, 75), reaches a maximum before the peak of dopaminergic neurodegeneration following the last MPTP injection (51). This observation has led to the idea that the MPTP- and rotenone-associated glial response may participate in the demise of dopaminergic neurons in these models. Studies showing that the blockade of microglial activation mitigates nigrostriatal damage caused by MPTP supports the notion that activated microglia participate in the neurodegenerative process (23, 87).

Activated microglial cells can produce a variety of noxious compounds, including ROS and RNS, proinflammatory cytokines, and prostaglandins. In many pathological settings, including MPTP injections, microglia activation involves the up-regulation of inducible NOS (21, 51) and the activation of NADPH oxidase (34). The former produces large amounts of nitric oxide in a calcium-independent manner, whereas the latter reduces oxygen to form superoxide radicals. Targeting inducible NOS by genetic interventions has shown that ablation of this enzyme, which reduces the production of nitric oxide, attenuates MPTP-induced neurotoxicity (21, 51). Similarly, mice defective in NADPH oxidase—and thus having reduced levels of extracellular superoxide—show less dopaminergic neuronal loss and protein oxidation than their wild-type littermates after MPTP injections (88). Further supporting the involvement of extracellular superoxide radicals in MPTP neurotoxicity is the finding that stereotaxic injection in the striatum of purified SOD1, which remains in the extracellular compartment, mitigates MPTP dopaminergic neurotoxicity on the infused side as compared with the noninfused side (88). Together, these findings indicate that the levels of extracellular nitric oxide and superoxide radicals are important components in the MPTP neurotoxic process.

CONCLUSIONS

This review summarized the molecular mechanisms underlying key neurotoxins used to model PD with a specific emphasis on oxidative stress. Whereas all four neurotoxins reviewed undoubtedly kill dopaminergic neurons, they all achieve this goal through different oxidative processes. By far the most complex of all appears to be that engendered by MPTP and, by analogy, probably by rotenone as well. If one is thus interested in the molecular biology behind dopaminergic neurotoxicity, it seems that MPTP, and by extension rotenone, may affect a greater variety of cellular pathways, perhaps making their study more appealing, but also more challenging. Nevertheless, whether the complexity of MPTP and rotenone oxidative processes more closely mimics the actual pathogenic cascade occurring in PD than the simpler oxidative processes engendered by 6-OHDA and paraquat is essentially unknown. Thus, if one is interested in testing new antioxidants for the treatment of PD, it may be necessary to preclinically ascertain the effectiveness of this putative neuroprotective intervention in more than one toxic model of PD.

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ABBREVIATIONS

COX-2, cyclooxygenase-2; H_2O_2, hydrogen peroxide; MAO, monoamine oxidase; $MPDP^+$, 1-methyl-4-phenyl-2,3-dihydropyridinium; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NOS, nitric oxide synthase; 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; RNS, reactive nitrogen species; ROS, reactive oxygen species; SNpc, substantia nigra pars compacta; SOD, superoxide dismutase; SOD1, Cu/Zn-superoxide dismutase; SOD2, Mn-superoxide dismutase.

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