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Oxidative Stress in Parkinson's Disease: A Mechanism of Pathogenic and Therapeutic Significance

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Abstract

Parkinson disease (PD) is a common adult-onset neurodegenerative disorder. Typically, PD is a sporadic neurological disorder and over time, affected patients see their disability growing and their quality of life declining. Oxidative stress has been hypothesized to be linked to both the initiation and the progression of PD. Pre-clinical findings, from both *in vitro* and *in vivo* experimental models of PD, suggest that the neurodegenerative process starts with otherwise healthy neurons being hit by some etiological factors, which sets into motion a cascade of deleterious events. In these models, initial molecular alterations in degenerating dopaminergic neurons include increased formation of reactive oxygen species presumably originating from both inside and outside of the mitochondria. In the MPTP mouse model of PD, time-course experiments suggest that oxidative stress is an early event which may directly kill some of the dopaminergic neurons. In this model, it seems that oxidative stress may play a greater role in the demise of dopaminergic neurons indirectly by activating intracellular cell death-related molecular pathways. As the neurodegenerative process evolves in the MPTP mouse model, indices of neuroinflammation develop such as microglial activation. The latter increases the level of oxidative stress to which the neighboring compromised neurons are subjected to, thereby promoting their demise. However, these experimental studies have also shown that oxidative stress is not the sole deleterious factor implicated in the death of dopaminergic neurons. Should a similar multifactorial cascade underlie dopaminergic neuron degeneration in PD, then the optimal therapy for this disease may have to rely on a cocktail of agents, each targeting a different critical component of this hypothesized pathogenic cascade. If correct, this may be a reason why neuroprotective trials using a single agent such as an antioxidant have thus far generated disappointing results.

Keywords

Parkinson disease; Oxidative stress; reactive oxygen species

INTRODUCTION

For several decades now, there has been a tremendous infatuation with the idea that oxidative stress causes or contributes to the pathobiology of a variety of disorders of the central nervous system (CNS). Among the long list of neurological diseases linked to oxidative stress, not only will one find all sorts of acute disorders, but also, almost invariably, all of the prominent neurodegenerative disorders including Parkinson's disease

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(PD). The appeal for this view stems from at least three situations. First, there is ample evidence that oxidants, generated by normal and abnormal biological processes, have the capacity to inflict serious damage to biological elements of vital importance to the wellbeing of cells such as neurons. Second, the biology of oxygen and nitrogen reactive species (ROS; RNS) has been well studied and is often better understood than many of the other key molecular pathways considered in connection to mechanisms of neurodegeneration. Third, many natural and synthetic antioxidants are available and could be readily used to treat PD.

As we will discuss in this chapter, it is not the clues found in PD tissue samples or in experimental models of PD in support of the occurrence of an oxidative stress that are missing. Instead, what makes this question still debated so avidly after all of these years is our uncertainty about whether oxidative stress actually plays any real role in the neurodegenerative process of PD; is our lack of understanding about the mechanisms leading to and the sites of origin of the presumed ROS and RNS; and is our perplexity as to why have clinical trials with antioxidants consistently failed to show any significant benefit in PD. In this chapter, we will thus try to address some of these outstanding questions in an attempt to rationally determine whether oxidative stress in PD is a myth or a reality.

PARKINSON'S DISEASE: A DISEASE OR A SYNDROME

By now, many are familiar with PD and are aware that it is the second most common disorder of the aging brain after Alzheimer's disease.1 PD is clinically characterized by the tetrad of motor manifestations of tremor, rigidity, slowness of voluntary movements, and poor balance.2 PD arises essentially as a sporadic condition, i.e. in absence of any evidence of genetic linkage, but, in a handful of cases, it is inherited.1 These rare occurrences can be due to a variety of genetic defects, such as mutations in alpha-synuclein,3 parkin,4 DJ1,5 PINK1,6 and LRRK2,7›8 which are transmitted as either dominant or recessive Mendalian traits.9

That being said, clinically, both the sporadic and the familial forms of PD are almost indistinguishable and they share the same basic biochemical hallmark, that is, a profound deficit in brain dopamine. The reason for this reduction in brain dopamine is due to the fact that all of the ascending dopaminergic pathways in the CNS do degenerate, albeit to variable degrees.10 For instance, among these pathways, it is the nigrostriatal pathway - which is composed of dopaminergic neurons residing in the ventral midbrain nucleus called the substantia nigra and which projects upward to the striatum - that is consistently the most severely affected. It is important to stress, however, that PD neurodegeneration is not restricted to the dopaminergic systems as widespread neuronal loss can be detected in other catecholaminergic and non-catecholaminergic nuclei.11 Thus, if the main motor manifestations of PD are likely linked to neurodegeneration in the dopaminergic systems, non-motor manifestations of PD such as pain, cognitive impairments, and constipation are more likely linked to neurodegeneration in these non-dopaminergic pathways. Two other prominent features of PD neuropathologies include the presence of intraneuronal proteinaceous inclusions called Lewy bodies found within many of the spared neurons in nearly all affected brain areas12 and gliosis.13 Not only are there more than 40 distinct entities that can share the clinical features of PD,1 but even within the cases labeled as PD, there is some degree of clinical and neuropathological heterogeneity. This is particularly true in the genetic cases of PD which, as indicated above, can be provoked by different gene alterations such as LRRK2 and in which the neuropathology, aside from the loss of dopaminergic neurons in the substantia nigra, can be quite pleomorphic.7 This fact raises the possibility that perhaps PD is not a single neurological entity (i.e. a disease), but rather a collection of distinct neurological disorders sharing a similar clinical phenotype. While this, if confirmed, may complicate investigations geared toward unraveling the neurobiology of

PD, most researchers agree that the pathogenic cascade may, in fact, consist of common mechanisms. In this case, one may propose that similar molecular machinery are recruited, regardless of the nature of the initiating pathological factor. It is with this premise in mind that we will pursue our discussion of oxidative stress, i.e. a common pathogenic factor contributing to the demise of dopaminergic neurons in all forms and variants of PD.

MARKERS OF OXIDATIVE STRESS IN PD POSTMORTEM TISSUES

A number of studies have searched for indices of oxidative stress in autopsy materials obtained from PD patients. These markers come in different flavors since ROS and RNS can damage virtually all biological macromolecules.

Some of the oxidant-mediated alterations in cellular components are stable modifications and can thus be readily detected and quantified. Therefore, the demonstration that molecular factors, critical to cellular function or survival, or both, are structurally modified by oxidative stress may provide important clues about how ROS and RNS could contribute to the pathogenesis of PD. The brain is rich in phospholipids and polyunsaturated free fatty acids (PUFAs), both of which are highly susceptible to oxidants. Following oxidant-mediated damage to phospholipids and PUFAs, plasma and intracellular protein/lipid bilayer membranes can be profoundly affected. In PD, the concentration of PUFAs in the substantia nigra is decreased, while that of malondialdehyde, a marker of lipid oxidation, is increased.14 Additional evidence of lipid oxidation in PD is provided by the demonstration of an increase in 4-hydroxy-2-nonenal, a lipophilic product of the peroxidation of membrane-bound arachidonic acid.15 Both wild-type and mutant α-synuclein form amyloid fibrils resembling those seen in Lewy bodies16,17 as well as non-fibrillary oligomers16, termed protofibrils. Since the two known pathogenic α-synuclein mutations promote the formation of protofibrils18, it has been proposed that protofibrils may be the toxic species of α-synuclein. In connection to this view, it is interesting to mention that the incubation of alpha-synuclein with 4-hydroxy-2-nonenal results in its covalent modification in which each molecule of the protein incorporates up to six molecules of 4-hydroxy-2-nonenal.19 More importantly, the authors of this study have also demonstrated that this irreversible modification of alpha-synuclein inhibits its normal fibrillation, perhaps by allowing the modified protein to adopt the above-mentioned protofibril conformation and causes a marked neurotoxicity to cultured dopaminergic neurons.19 Levels of markers of oxidative damage to proteins, such as carbonyl modifications of soluble proteins, are also significantly increased in postmortem samples of substantia nigra in PD brains as compared to controls.20,21 While levels of both free and bound nitrotyrosine, an faithful indicator of the involvement of RNS, have been shown to be elevated in damaged areas of the MPTP mouse model of PD,22,23 we are not aware of such results for similar investigations in PD brains *per se*. However, nitration and nitrosylation of proteins and especially of alpha-synuclein and parkin in PD have been documented.24–26 These particular findings have led to a series of quite interesting and provocative studies on the biology of nitrated alpha-synuclein with respect to its normal capacity to bind to lipid vesicles or to form fibrils.27–29 DNA also does not escape oxidant attack as, for example, deoxyguanosine is converted to 8-hydroxydeoxyguanosine (8-OHdG)30 by ROS. Like other cited markers of oxidative damage, 8-OHdG is markedly increased in postmortem samples of substantia nigra from PD brains.31,32 Worth mentioning, is the fact that the content of common deletions in mitochondrial DNA, which can be caused by oxidative stress, was also reported to be strikingly elevated within the few spared dopaminergic neurons in the PD substantia nigra.33 However, when looking at the body of literature reviewed above, one must remember that many of the ROS and RNS abnormalities documented in PD tissues could be non-specific features of dying cells and proof that any of these is instrumental in the actual neurodegenerative process of PD is still lacking.

Several researchers have demonstrated that pathological features of PD can also be detected outside of the CNS, raising the possibility that PD may, in fact, be a generalized disease. One direct consequence of this concept has been to prompt scientists to scrutinize non-CNS tissues, including blood and other body fluids, from PD patients for hints of problems in oxidative metabolism. Several of these studies have reported significant alterations in the measured parameters in PD blood and CSF.[32,34-39] Even if oxidative stress turns out not to contribute to the neurodegenerative process of PD *per se*, it remains worthy to determine whether these systemic alterations could perhaps be used as biomarkers for early diagnostic purposes as well as to monitor disease progression or response to treatment.

INCREASED PRODUCTION OR DECREASED ELIMINATION

In all of our cells, including neurons, there is a necessary fine-tuned balance between the extent of the production of and the removal of oxidants, and it is this balance that keep ROS and RNS constantly at low, non-toxic levels. In PD, several studies have attempted to determine whether the disease process is associated with a higher production of or a lower detoxification of oxidants. Among the ROS-scavenging enzymes, superoxide dismutase (SOD) is often regarded as the first line of defense against a ROS upswing. Its analysis in PD brains[40-42] has revealed no changes in activity of its cytosolic isoform (i.e., SOD1) and increased activity in its mitochondrial isoform (i.e., SOD2). The latter observation is quite interesting since SOD2 is highly inducible in response to an excess of ROS. Therefore, the reported increases in SOD2 in PD suggests that the mitochondrial compartment in PD is the site of elevated ROS production. In contrast to SOD, both catalase and glutathione peroxidase activities are reduced in PD brains,[43,44] although to a smaller degree, casting doubt about whether such minute changes bear any significance here. The status of antioxidant small molecules has also been assessed in PD brains. There is no indication that levels of vitamin E are abnormal in PD tissues[45] or that vitamin E supplements modifies PD progression.[46] Conversely, the levels of both ubiquinone[47] and glutathione[48] have been reported to be low in PD tissues. Importantly, ubiquinone supplements are well tolerated and seem to increase the activity of complex I of the mitochondrial electron transport chain in PD patients,[49] and to attenuate MPTP toxicity in mice.[50] More recently, ubiquinone - also called coenzyme Q10 - has been tested for neuroprotection in PD patients.[51,52] These clinical trials have unfortunately generated a rather confusing picture, in that some groups have found that the administration of ubiquinone provided significant benefits to PD patients whereas other groups have found coenzyme Q10 to be ineffective.[51,52]

EXPERIMENTAL MODELS OF PD

In aggregate, the different results discussed above make a compelling argument for the concept that oxidative stress does occur in the brains of PD patients. However, we must remain cautious about the pathogenic significance of the findings cited above as all represent non-mechanistic, descriptive data; sometimes providing, at best, correlative information. None of these elegant studies have thus far succeeded in ruling out the possibility mentioned above that the oxidative stress indices found in PD brains are anything other than the non-specific expression of dying neurons. More distressing in terms of the potential importance of this large body of work is the fact that the data were invariably generated from post-mortem tissues from end-stage PD, meaning that the studied samples were essentially devoid of dopaminergic neurons. Furthermore, at this stage of the disease, virtually all PD patients are chronically treated with a battery of drugs such as L-DOPA which, like dopamine, can readily auto-oxidize giving rise to ROS. Although this fact does not imply that the chronic use of L-DOPA hastens the progression of PD,[53] it does raise the concern that many of the aforementioned observations may reflect artifacts due to the treatments received by PD patients before their death. To address these important issues, many

researchers have used experimental models of PD which provide a dynamic setting allowing both longitudinal and functional studies.

Models of PD come in a variety of forms that can be divided into two broad categories: genetic and toxic. Readers interested in the question of experimental models of PD may find detailed descriptions in the following reviews.[1,54-57] While, thus far, most of the work dealing with the question of oxidative stress have been generated using toxic models such as those produced by 6-hydroxydopamine, MPTP, and to a lesser extent, rotenone, interesting data have also begun to emerge from genetic models, mainly from knockout DJ1 mice[58] and PINK1 drosophila.[59]

Irrespective of the model used, most of the generated data are consistent with the notion that, if there were a pathogenic oxidative stress in PD, oxidants could emanate from the inside of the mitochondria, the outside of the mitochondria, or even the outside of the cell (i.e. extracellular space) and that none of these distinct sources are mutually exclusive.

MITOCHONDRIA, A MAIN SOURCE OF OXIDANTS

The vast majority of oxygen consumption by the cell occurs in mitochondria via the electron transport chain. In a normal situation, small amounts of molecular oxygen in the mitochondria, rather than being converted to water, are reduced to ROS such as superoxide radicals. Thanks to the arsenal of antioxidants including SOD2 inside of the mitochondria, the basal levels of ROS byproducts of mitochondrial respiration are minimal. However, in the case of a mitochondrial respiratory defect - as some believe to exist in PD - it may be envisioned that the amount of ROS generated by the electron transport chain increases dramatically, thereby overwhelming the protective mechanisms. The idea that a reduction in electron transport chain activity is linked to an increase in ROS production has been confirmed experimentally by many researchers. For instance, mitochondria can be purified from mouse or rat brain and incubated in a cell free system with different inhibitors of complex I such as the active metabolite of MPTP, MPP^+ or the pesticide, rotenone.[60-62] Consistently, these experiments have shown that ROS production, as evidenced, for example, by the fluorescent signal generated by ROS-sensitive probes such as AmplexRed, rises proportionally to the degree of complex I inhibition.[63] While more ROS inside of the mitochondria may be easily regarded as deleterious, we must examine whether oxidative damage, in at least one mitochondrial biological system, can be detected before this view can be accepted. In connection to this important issue, the work of Liang and Patel is quite significant.[64] Indeed, in this study, the authors have assessed the status of the mitochondrial enzyme, aconitase. This enzyme contains iron-sulfur clusters which are essential to aconitase catalytic function and which are quite susceptible to ROS. Supporting the idea that increased mitochondrial ROS is harmful, this study shows that, subsequent to complex I inhibition, aconitase is inactivated due to oxidation of its iron-sulfur clusters.[64] Furthermore, we have reported that a marked increase in peroxidation of the mitochondrial phospholipid cardiolipin occurs upon complex I inhibition.[63] These two observations leave little doubt that increased oxidant production inside of the mitochondria may cause meaningful pathological alterations, at least in experimental models of PD.

EXTRA-MITOCHONDRIAL ROS PRODUCTION

Aside from the mitochondria, alterations taking place in the cytoplasm may also contribute to the oxidative stress situation. By means of a toxic insult such as that caused by MPTP or malonate, it has been shown that soon after the initiation of the insult, a huge efflux of dopamine can be detected by microdialysis at the level of the striatum in rodents.[65-67] This led to the idea that, in response to an energy crisis or through an effect of a false-transmitter, or both, toxic agents such as those cited above, contribute to a collapse of synaptic vesicle

capacity to sequester dopamine. As soon as dopamine is no longer in the acidic, stabilizing environment of the synaptic vesicles, it enters the cytoplasm, where the physiological pH promotes its auto-oxidation and the ensuing production of ROS. While this event has been known to occur in cell free systems for some time, it has only been elegantly demonstrated recently in cells by Lotharius and O'Malley using ventral midbrain cultures.68 Cytoplasmic dopamine can also be converted to reactive quinone by enzymatic processes via cyclooxygenase69 or to 3,4-dihydroxyphenylacetic acid by oxidative deamination via monoamine oxidase through a reaction that generates ROS as by-products.70 These point to dopamine as possibly being the main culprit in the demise of dopaminergic neurons. As tantalizing as this view is, it remains speculative, and experimental attempts to demonstrate the *in vivo* significance of dopamine in the neurodegenerative process underlying the demise of dopaminergic neurons have generated conflicting results. For instance, it was shown that neurons containing the dark pigment neuromelanin - which is a byproduct of the synthesis of dopamine and other monoamines - are those that are degenerating to a greater extent in PD.71 Furthermore, dopamine can catalyze the oxidative modification of those proteins relevant to PD such as parkin and alpha-synuclein.72,73 Conversely, one of the most severe blows to the idea that dopamine contributes to the death process of dopaminergic neurons comes from a study performed in mutant mice deficient in tyrosine hydroxylase.74 These knockout mice, which cannot produce dopamine, were unexpectedly found to be as sensitive to MPTP and other dopaminergic neurotoxins as their wild-type counterparts.75,76

EXTRACELLULAR OXIDATIVE STRESS

Glial cells are mainly quiescent in normal brains, but, upon activation they can engage in the production of a host of cytotoxic molecules including ROS and RNS.77,78 The question of neuroinflammation in PD has been extensively reviewed recently and interested readers can refer to the following reviews for in-depth information about this topic.13,79-81 However, here, we will limit our discussion to the role of activated glial cells in inflicting oxidative damage to neighboring neurons.

As mentioned in the introduction, activation of glial cells has been well documented in all affected regions of PD brains.13 Identical observations have been made in both genetic and toxic models of PD.13 Although it seems well established that the glial response in all of these settings is secondary to the ongoing neurodegenerative process, the potential role played by neuroinflammation should not be neglected. Indeed, in neurodegenerative disorders such as PD, neurons are believed to die over time by an asynchronous process,82 i.e. not all at the same time. This implies that the very first neurons that succumb to the disease process might modify the surrounding microenvironment. This pathological change, even if subtle, can readily be perceived by the presence of glial cells in the vicinity, which, in turn, readily respond by altering their phenotype; that is, they shift from a dormant to an activated phenotype. In this context, it must be noted that astrocytes, and to a much greater extent, microglia, once activated, can produce ROS and RNS,83,84 hence subjecting neighboring compromised dopaminergic neurons to additional stresses. Although speculative, it has been proposed that glial activation detected in affected regions of PD brains could contribute to the progression of the disease by fueling the neurodegenerative process.

Over the past few years, growing evidence in a variety of experimental models of PD indicate that neuroinflammation could exacerbate dopaminergic neurodegeneration. If, in the context of this chapter, we only focus on the oxidative stress aspects of neuroinflammation, then two key enzymes must be discussed, namely inducible nitric oxide synthase (iNOS) and NADPH-oxidase. iNOS is absent in the normal brain but it is present in glial cells in diseased tissues such as the substantia nigra in PD.85 Similarly, mice injected with MPTP

quickly develop a robust microglial activation accompanied by iNOS upregulation. From a functional point of view, knockout mice deficient in iNOS exhibit a small, but significant attenuation of MPTP sensitivity compared to their wild-type counterparts.86 The lower susceptibility of these mutant mice to MPTP is associated with smaller levels of the oxidative modification of protein such as nitrotyrosine in the substantia nigra.86 A very similar situation is seen in the case of NADPH-oxidase. This inflammatory enzyme is upregulated and activated in PD post-mortem tissues and in the degenerating brain areas of mice injected with MPTP.87 Upon inactivation of this enzymatic complex by abrogation of its catalytic subunit, $gp91^{phox}$, MPTP dopaminergic neurotoxicity is attenuated in mice.87 These two studies suggest that ROS and RNS, produced by activated glial cells, damage proteins which, in turn, exert deleterious effects on neighboring dopaminergic neurons. While it is tempting to suggest that the above proposed scenario occurs by a toxic accumulation of oxidatively damaged macromolecules, it can also be hypothesized that the extracellular-mediated oxidative stress may hasten dopaminergic neuron death through impairment of key neuronal survival pathways. Relevant to this view are our findings that insulin growth factor receptor-1 can be oxidized by an inflammation-related event88 and that the transduction activity of the survival signaling Akt pathway is impaired in SH-SY5Y neuroblastoma cells following exposure to conditioned medium from activated microglial cells.88 Because both the insulin growth factor and the Akt pathways are important to the survival of dopaminergic neurons, oxidation of some of their molecular intermediates could promote dopaminergic neurodegeneration.

OXIDANTS AS VERSATILE SIGNALING MOLECULES

Conventionally, oxidants have been regarded as reactive factors which, by damaging vital macromolecules, contribute to the death of cells. In this context, we were quite surprised to find that, on meta-analysis, the time-course of MPTP-induced dopaminergic neurodegeneration did not fit nicely with the time course of oxidative events that we and others have reported. In fact, we found that the oxidative alterations of, for example tyrosine hydroxylase89 or alpha-synuclein,90 precede the peak of dopaminergic neuron degeneration.91 This led us and several other researchers in the field to believe that the described oxidative stress in the MPTP model of PD, regardless of whether it emanates intracellularly, extracellularly, or both, may be responsible for some, but perhaps not all of the dopaminergic neuron degeneration. If this assertion is proven correct, it may then be plausible that rather than directly killing dopaminergic neurons, oxidants may be key signal molecules which activate death-related pathways. For instance, while hydrogen peroxide can kill cells by direct toxic actions, it also modulates a range of intracellular signaling pathways including transcriptional factors such as NFκB and protein kinases, such as c-Jun N-terminal kinases (JNKs).92,93 Thus, neuronal death could arise from impaired control of these pathways rather than from the direct toxic effects of ROS or RNS. Consistent with this view is the demonstration that activated JNKs contribute to MPTP-induced neuronal death in mice.94 Also worth discussing are the striking observations that MPTP injection to mice stimulates CDK5/p35-dependent phosphorylation of peroxiredoxin-2 (Prx2) and reduction of Prx2 activity.95 This observation is quite relevant to the topic of oxidative stress in PD since Prx2 is an ubiquitous cysteine-containing antioxidant protein that reduces hydrogen peroxide to water.93 Furthermore, these authors showed that the loss of activity of Prx2 leads to a rise in the intracellular concentration of ROS and a greater loss of dopaminergic neurons.95 This suggests that CDK5, by altering Prx2 activity, can modulate dopaminergic neuron survival. Collectively, the data reported by Qu et al. provide a tantalizing new pathogenic mechanism for neurodegeneration in the MPTP model and in PD that centers on a functional coupling between CDK5 and Prx2. The cell death protein Bax, through the recruitment of the mitochondrial-dependent death pathway, was found to play a key role in the death of dopaminergic neurons in the MPTP mouse model.63,96,97 This Bax activation

appears to rely on both its transcriptional induction by the tumor suppressor p53 and its translocation into the mitochondria via a JNK-dependent phosphorylation of the BH3-ony protein Bim.96 Given the aforementioned findings, it can be speculated that the CDK5-dependent inactivation of Prx2 acts in concert with Bax and with the mitochondrial-dependent cell-death pathway to kill dopaminergic neurons. Perhaps, the pathogenic concept of aberrant kinase activation, combined with impaired peroxiredoxin activity should be examined beyond CDK5 and Prx2. Another interesting molecular pathway relevant to PD that can be engaged by oxidative stress is DJ-1. Mutations in DJ-1 have been linked to familial forms of PD.9 Although the actual function of DJ-1 remains uncertain, it has been shown that wild-type DJ-1 overexpression protects against a range of insults linked to oxidative stress.98¯101 This antioxidant property could be related to the supposition that DJ-1 operates as an atypical peroxiredoxin58 in which its cysteine residue at position 106 can be converted to cystein sulfonic acid by ROS.102. Alternatively, DJ-1 was also reported to upregulate glutathione synthesis during oxidative stress103 and to stabilize the antioxidant transcriptional master regulator Nrf2.104

CONCLUSION

In this chapter, we have reviewed the topic of oxidative stress in PD. We have reminded the reader that, indeed, a long list of markers of oxidative stress is elevated in the tissues obtained at autopsy from PD patients. Thanks to the use of experimental models, we have discussed the potential significance of these otherwise descriptive, correlative human data. And, we have shown that oxidants may reflect true pathogenic events as, in experimental models of PD, oxidative insults have been associated with dopaminergic neural death. Should the data generated from the experimental models of PD faithfully mirror the situation occurring in PD *per se*, ROS and RNS could originate not only from inside of dopaminergic neurons, but from outside of these neurons as well. This latter notion emphasizes the importance of taking into account neuroinflammation as a key factor in the overall neurodegenerative process of a disease such as PD, even if its actual pathogenic role remains to the established.

Another important take-home message from this chapter resides in the fact that we have tried to emphasize the multifactorial nature of the neurodegenerative cascade underlying dopaminergic neuronal death in experimental models of PD. From a therapeutic point of view, if a similar situation occurs in PD, then researchers and clinicians alike may wish to consider that the best therapy for PD may in fact relies on a combination of several different agents, each targeting specifically one aspect of the degenerative machinery.

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