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REVIEW ARTICLE

The Etiopathogenesis of Parkinson Disease and Suggestions for Future Research. Part II

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INTRODUCTION

We are at a critical juncture in our knowledge of the etiology and pathogenesis of Parkinson disease (PD). It is clear that PD is not a single entity simply resulting from a dopaminergic deficit; rather it is most likely caused by a combination of genetic and environmental factors. Although there is extensive new information on the etiology and pathogenesis of PD, which may advance its treatment, new syntheses of this information are needed. The second part of this two-part, state-of-the-art review by leaders in PD research critically examines the research field to identify areas for which new knowledge and ideas might be helpful for treatment purposes. Topics reviewed in Part II are genetics, animal models, and oxidative stress.

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ROLE OF GENETICS

Current Knowledge

There have been 2 important approaches to genetic research studies in Parkinson disease (PD). The first focuses on rare families with parkinsonism apparently following Mendelian inheritance and using the classic methodology of linkage analysis and positional cloning. In the other, an attempt is made to evaluate the PD population as a whole, using association studies and nonparametric linkage methodology and trying to define risk alleles that contribute to the sporadic form of the disease. Over the past years, genetic research following the first approach has been highly effective in identifying the genes underlying monogenic PD, in particular *α-synuclein* (1) (*Park-1*) and *LRRK2 (Park-8)* (2, 3) in dominant families, as well as the recessive PD genes *parkin (Park-2)* (4), *PINK1 (Park-6)* (5), and *DJ-1 (Park-7)* (6). These mutations probably cause the disease in only a very small subset of families, with the exception of *LRRK2*, which is responsible for a significant proportion of familial PD (5.1%–18.7%) and a smaller portion of sporadic PD (1.5%–6.1%), although Ashkenazi Jews and Arabs have a higher frequency in the majority of populations studied. Nevertheless, these discoveries have been extremely fruitful, leading the way to some molecular pathways involved in nigral degeneration, which includes protein aggregation, defective proteasomal degradation, mitochondrial dysfunction, and oxidative stress (7). The findings that *LRRK2* encodes a protein from the family of mitogen-activated protein kinases and that mutations may lead to an increase of kinase activity (8) may translate relatively quickly into a novel treatment option involving kinase inhibition. However, the contribution of the mechanisms of these and other genes to idiopathic PD as a whole is still poorly defined.

To define this contribution is the aim of the second approach of genetic research in PD, using association studies or nonparametric linkage methodology. Unfortunately, these attempts have met with less success. Five total genome

screens have been performed in populations of approximately 200 to almost 400 affected sibling pair families, searching for regions of increased allele sharing between those affected, a method that should allow mapping of genes without knowledge of the underlying genetic parameters such as mode of inheritance and disease gene frequency (9–13). Each of these studies has provided several interesting areas of suggestive linkage, but so far none of them has lead to the discovery of a bona fide disease gene. One problem seems to be that only a few of the linkage peaks overlap among studies (10, 11, 13–15), suggesting that heterogeneity between populations may be more pronounced and more important than previously thought. In addition, some of these linkage regions are still very large (up to >100 cM) and contain hundreds of genes, and the manner of identifying causative variants is still poorly mapped out.

Progress in high-throughput genotyping technology has recently allowed genome-wide approaches not only in family samples (sib pairs, as described above) but also in large populations of patients with sporadic disease and control subjects using an association study design and very large panels of single nucleotide polymorphisms (SNPs). In the first very large study of this kind, Maraganore et al (16) genotyped almost 200,000 SNPs in several hundred samples, resulting in a large number of possibly associated genetic variants. However, none of the top 13 associated SNPs could be confirmed in a follow-up study (17). A similar study using almost 500,000 SNPs similarly did not identify unequivocal evidence for associations (18). Nevertheless, these results are likely to reflect inherent limitations of the study design, such as heterogeneity of the populations studied and still insufficient coverage of the genome, rather than lack of major genetic components to the etiology of the disease.

Studying association patterns of candidate genes in large, well-characterized series of patients and matched control subjects is another commonly used approach to identify genetic contributions to complex diseases. Unfortunately, again, despite a large number of studies, success has been limited. Association studies with candidate genes are particularly prone to produce spurious positive results if done poorly, and the great majority of the published associations in the area of PD have not been replicated. This may be due to the fact that the populations studied were too small to allow sufficient statistical power, that control subjects were not properly matched, or that the nature of the genetic contribution may in fact vary considerably between populations.

To overcome the limitations of these previous small sample studies, a large-scale international collaborative study pooled data from 2,692 PD cases and 2,652 control subjects to analyze the allele length variability in the dinucleotide repeat sequence (REP1) in the α-synuclein gene promoter. They found that the α-synuclein REP1 length variability is associated with PD but did not modify age at onset (19).

In fact, increasing and repeatedly confirmed evidence suggests that genetic variations in at least 2 of the genes that are known to cause monogenic parkinsonian syndromes, *α-synuclein* and MAP *tau*, modify the risk for idiopathic PD and progressive supranuclear palsy, respectively (20, 21). The underlying concept is that relatively common genetic variants (particularly SNPs) may alter the expression pattern of the gene (e.g. by changing binding sites for transcription factors) by altering splicing patterns or by influencing RNA stability or spatial distribution. As the encoded proteins seem to be crucial in the initiation of the neurodegenerative process, even small changes in protein homeostasis may push a cell across a critical threshold toward neurodegeneration. Although this variability probably still explains only a relatively small proportion of the total risk, it is very encouraging to realize that there seems to be a productive path of discovery that leads from well-defined, but rare, monogenic forms to idiopathic PD.

This hypothesis is supported by striking similarities among different neurodegenerative diseases that involve different proteins, including the prion protein, the amyloid precursor protein, α-synuclein, and tau. Although rare mutations cause monogenic disease in all cases, the risk for the sporadic disorders appears to be influenced, at least in part, by the expression levels of these proteins with their completely normal amino acid sequences. Extreme examples of this process are rare cases of *α-synuclein* multiplications (22). High concentrations of these potentially pathogenic proteins may favor rare stochastic events of initiation of the disease through the formation of pathogenic templates. After these templates have formed, other proteins may deposit on them and adopt the same conformation (23). This process appears to be highly efficient and less dependent on protein level, as suggested by seeding experiments. This theory of "permissive templating" suggests that disease propagation, once it has been initiated, may be a different process than disease initiation. Stopping the progression would require prevention of the spread of pathogenic templates between neurons, a process that admittedly is still completely enigmatic. Although the risk for disease initiation is modified by the concentration of the pathogenic protein, the process itself may be nothing more or less than a stochastic event or, in other words, "bad luck."

On the basis of these observations, a general framework of the molecular pathogenesis of PD, which is shared by several other common neurodegenerative diseases, is beginning to emerge: genetic variability sets the individual risk for the initiation of the disease process, which then spreads in a predetermined fashion (which may again be influenced by the individual genetic make-up) through susceptible neuronal populations.

Research Challenges

If this general scheme is accepted, a myriad of questions arise, which need to be addressed. It is clear that in addition to the shear level of protein expression, many other factors are likely to influence the risk for the disease-initiating event to occur, including intracellular distribution and processing of the respective proteins, oxidation stress, the state of cellular defense, and compensatory mechanisms. The genes encoding regulators that determine expression patterns of the major pathogenic proteins may be promising

candidates for further study. On the genetic level, it is therefore important to consider the suitable methodology to pin down these risk factors. There is increasing evidence that genetic risk factors may vary considerably between populations. If this is the case, it may be more promising to build up several small, but genetically well-characterized, populations for association studies rather than to pool them into huge repositories. In any case, much more effort will have to go into the characterization of patient populations if genetic variants conferring small increases in disease risk are to be detected.

Another important question is how other known risk factors, such as age and sex, or the more controversial ones, such as personality traits, lifestyle, or environmental insults, can be integrated into such a theory. For some it seems obvious: with increasing age, cellular defense mechanisms such as the activity of chaperones may decrease below a certain threshold that is needed to prevent disease initiation (24), a process that may be important in PD (25). For others, such as sex or personality, it is much less straightforward, but the epidemiologic observations may help in determining crucial questions to ask in future studies.

Finally, what is the mechanism of disease progression through time and space, and which genetic or nongenetic factors determine its course? The study of modifiers of the disease processes in more advanced cellular and animal models provides a large number of candidates that need to be tested for their relevance in humans.

Future Developments

The genetic tools for these tasks (i.e. increasingly dense marker maps, improved understanding of the genetic variability in different populations, and advanced computational capacities) are being developed at high speed. The costs for genotyping are decreasing, which will allow much more comprehensive studies. These will include analyses of gene-gene and gene-environment interactions, which will become more important as we discover an increasing number of factors in the degenerative process. What is urgently needed are large, well-characterized patient and control populations well beyond those that are available today and that include biographical, social, and environmental data.

ROLE OF ANIMAL MODELS

Current Knowledge

Until the discovery of genes causing familial forms of PD, animal models of the disease relied upon the administration of toxins to destroy nigrostriatal dopaminergic neurons. These include primarily, but not exclusively, 6-hydroxydopamine, injected into the brain, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, and paraquat, alone or in association with maneb (26). The 6-hydroxydopamine model has been used extensively primarily to examine the *consequences* of the loss of nigrostriatal dopaminergic neurons on downstream neuronal systems (27). The use of toxins for identifying *pathogenic* mechanisms of the disease is primarily based on the observation by Langston et al in 1983 (28) that the neurotoxin MPTP can kill dopaminergic neurons in humans, leading to severe parkinsonism. Although MPTP undoubtedly kills dopaminergic neurons in humans, it remains to be proven that the mechanism by which MPTP works is similar to the disease process occurring in idiopathic PD, the large majority of disease cases. The use of rotenone (which, like MPTP, is a mitochondrial complex 1 inhibitor) stems from evidence that mitochondrial dysfunction may be involved in sporadic PD (26). Similarly, paraquat and maneb represent classes of environmental agents that epidemiologic studies have implicated as risk factors for PD. These toxin-based models have provided some information on the mechanisms of cell death of dopaminergic neurons and have been useful in identifying symptomatic therapies and elucidating the neurophysiology of PD. However, they do not necessarily reproduce proven mechanisms of PD (29).

To reproduce more closely the pathogenic mechanisms of PD in animals, investigators have taken advantage of the identification of mutations that cause the disease in rare cases of familial PD, as described earlier in this review. In the case of mutations, the mechanistic link with PD is certain, but it must be established that these mechanisms are relevant to the much more frequent nonfamilial forms of PD. The underlying hypothesis is that mutations causing familial PD may trigger mechanisms that also play a role in sporadic PD. Several lines of evidence support this contention, as discussed extensively in other parts of this review. For example, mutations in *parkin* and *UCHL-1* affect proteasomal function, which has also been implicated in sporadic PD. PINK1 and DJ-1 are mitochondrial proteins. Finally, α-synuclein accumulates in central and peripheral neurons of patients with sporadic PD. Therefore, it appears that much can be learned by modeling the disease in animals expressing these recently discovered PD-causing mutations (30).

Because of its undisputed association with sporadic PD, many lines of mice were generated to overexpress either wild-type or mutant α-synuclein (31). Several of these mice show progressive motor deficits, abnormal dopamine function, and in some cases even reproduce nonmotor deficits observed in PD patients and delayed loss of striatal dopamine and/or tyrosine-hydroxylase-positive neurons in the substantia nigra pars compacta (32–34). The model generated by Lee et al (33) (transgenic mice expressing A53T α-synuclein under the prion promoter) presents a severe, rapidly progressive motor phenotype arising at approximately 10 to 15 months of age and frank cell death. However, the pathologic changes are particularly severe in spinal cord, brainstem, and cortex, with death of motor neurons, and not in nigrostriatal dopaminergic cells as in PD. Nevertheless, the model is useful to determine the mechanisms by which α-synuclein accumulation leads to neuronal loss in vivo. In particular, these mice show an accumulation of truncated α-synuclein amputated from its C terminus, which may increase the misfolding of the protein (8). This suggests a role for proteolytic processing in the pathologic process, similar to what has been described in Alzheimer disease.

Animal models of PD are not limited to mammals. Dopaminergic neurons can be killed with toxins in flies, fish, or worms, and the ability to express PD-causing mutations in flies has given rise to an exciting new generation of animal models that are amenable to rapid genetic manipulations (35, 36).

Research Challenges

There are few areas of PD research that elicit as much controversy as the animal models for the disease. Despite much effort, none of the available models fulfills all the criteria of a "perfect," model defined as a faithful reproduction of both the mechanisms and the outcome of the disease process that occurs in humans (30–34). Animal models of slowly progressive neurodegenerative diseases are needed not only to discover the mechanisms of the diseases but also to identify new therapeutic targets and test neuroprotective treatments. The use of animal models for preclinical drug testing is indeed valuable before clinical trials are performed in patients. For this purpose, it is important to have animal models displaying easily measurable phenotypes that reflect the desired outcome in patients. The validity of end point measures in animal models, however, cannot be ascertained until there is a recommended effective treatment. In PD, symptomatic treatments are available, with L-dopa or dopaminergic agonists as gold standards, but effect neuroprotective strategies do not exist as yet.

A major limitation of most traditional models based on the use of toxins to kill dopaminergic neurons is that cell loss is rapid and is for the most part restricted to dopaminergic neurons, a process that does not mimic the widespread pathologic alterations seen in PD. Indeed, pathologic hallmarks of PD such as the Lewy bodies are rarely reproduced in these models, and symptoms are usually limited to the equivalent of akinesia and the range of motor and nonmotor symptoms seen in patients with PD are not reproduced. Despite these limitations, toxin-based models have been, and continue to be, extremely useful to identify and test symptomatic treatments for PD, both pharmacologic and surgical. There is, however, a need for better designed preclinical studies, including the start of experimental treatments when degeneration has already progressed to a point at which functional deficits exist (29).

Genetic models are a new addition to our arsenal of animal models of PD. Genetic mouse models have led to some skepticism because, until now, only very few lines have demonstrated a loss of dopaminergic neurons within the lifetime of the animal (for one exception, see Reference 34). Whether or not animals lose nigrostriatal dopaminergic neurons, these models should help define mechanisms and pathogenic pathways. Because they exhibit strong evidence of neuronal dysfunction and pathologic features, these models are amenable to testing of novel neuroprotective therapies, although it remains uncertain whether the outcome of trials in mice will be predictive of efficacies and toxicities in humans. Advantages of genetic mouse models for testing disease-modifying strategies include the progressive nature of the phenotype, the ability to mimic extranigral symptoms and pathology, and the mechanistic relevance of PD-causing mutations to the disease.

Future Developments

Opinions diverge regarding the criteria for an acceptable model and the question that is asked is what aspect(s) of the disease should a model reproduce to be valuable? The answer to this question is that different models are most useful for different purposes. Investigators need to make educated choices when they select a model to answer their specific experimental questions. Additionally, there is great opportunity for the development of new models that more faithfully reproduce the disease phenotype.

The mouse models would gain in relevance if dopaminergic cell loss would occur more readily or be accelerated in the presence of an environmental insult, indicating a role for gene-environment interaction in PD. Results of such experiments with MPTP or paraquat have been ambiguous with groups reporting either no effect, worsening, or neuroprotection (37–39), although α-synuclein does seem to contribute to MPTP-induced toxicity (40). A major limitation of mouse models is the short lifespan of the animal and the usual resistance of laboratory strains, raised under optimal nutrition and in a pathogen-free environment, to neurodegeneration. Indeed, a lack of specific cell loss comparable to that observed in patients is not restricted to mouse models of PD but is a pervasive problem in attempts to model a number of chronic neurodegenerative diseases. Perhaps exposing different strains of mice kept on an antioxidant-poor diet to environmental toxins would uncover experimental conditions, leading to a progressive loss of dopaminergic neurons and help identify genetic susceptibility factors.

A lesson from mouse models is that neuronal dysfunction is likely to occur in PD long before cell loss (32). Therefore, an important challenge is to develop improved biomarkers, so that patients may be treated even before dopaminergic cell loss occurs. Indeed, neuroprotection should not be limited to preventing the death of neurons, which could be dysfunctional, but should focus on preventing or interrupting the pathologic process that leads to functional alterations and eventually to cell death. In other words, our focus should shift toward disease-modifying agents, rather than agents preventing neuronal death. Considering the progressive nature of PD and the presumably long duration of the preclinical phase, one may consider that the symptomatic phase of the disease represents a failure of homeostatic mechanisms rather than the primary effect of the etiologic factors, either unknown (sporadic PD) or known (PD-causing mutations). In that respect, it is remarkable that dopaminergic neurons of the ventral tegmental area are extraordinarily resistant to a variety of insults that lead to loss of the neighboring nigrostriatal dopaminergic neurons (41). Identifying the mechanisms for endogenous differences in vulnerability among different neuronal populations and eventually mouse strains, as well as protective factors emerging from epidemiologic and human genetic studies may provide useful cues for therapies. At the moment, one attractive avenue is the possibility to

lower the levels of α-synuclein to prevent its accumulation in affected neurons. More needs to be known about the normal function of α-synuclein to assess the safety of this approach, but a systematic use of knockout or silencing strategies in vivo, although expensive, could also identify novel targets for therapies.

In summary, whatever the limitation of current models of PD, the availability of a broad range of options already allows investigators to mimic diverse aspects of the disease. In the search for treatments that not only improve symptoms but also stop or perhaps even reverse the disease process, no stone should be left unturned. Even less than perfect genetic models offer glimpses of the disease process that could not have been suspected on the basis of the more classic models. Indeed, perhaps the most important aspect of these models is that they allow us to decipher the pathologic processes that take place in a brain exposed to a PD-causing insult *before* any nigrostriatal dopaminergic neurons are lost or before the disease begins elsewhere.

ROLE OF OXIDATIVE STRESS

Current Knowledge

The hypothesis that oxidative stress plays a key role in the pathogenesis of PD was been proposed and has been debated for at least 3 decades (42). Evidence in favor of this hypothesis includes 1) the seminal work of Graham et al (43) that emphasized the toxic potential of oxidative reactions involving dopamine and 2) observations on postmortem specimens of PD brains showing, for example, an increase in markers of lipid peroxidation, a loss of antioxidant defense mechanisms, and changes in iron biodisposition (44–46). The oxidative stress hypothesis of PD does not, however, have merely a "historical" value, because recent data from genetic studies as well as toxin-induced models have reignited interest in an imbalance between pro-oxidant and antioxidant events in the development of neurodegeneration and other pathologic changes (e.g. inclusion formation) in PD.

Genetic studies performed over the past few years have unraveled the molecular basis of familial forms of parkinsonism and, by doing so, have provided important clues about pathogenetic processes relevant also to sporadic PD. In 1996, Polymeropoulos et al (47) identified the first gene associated with familial parkinsonism that encodes for the protein α-synuclein. Shortly after this discovery, the likely role of α-synuclein in the pathogenesis of sporadic PD was indicated by the observation that this protein is a major component of Lewy bodies, the intraneuronal inclusions that are hallmarks of PD in human brain tissue (48, 49). Given the involvement of α-synuclein in familial and sporadic parkinsonism, it is noteworthy that evidence both in humans and experimental models strongly suggest a role of oxidative stress in α-synuclein-induced pathology. After staining human brain sections with antibodies against nitrated tyrosine residues of α-synuclein, Giasson et al (50) demonstrated a widespread accumulation of nitrated protein within Lewy bodies and Lewy neurites and concluded that this accumulation "provides evidence to directly link oxidative and nitrative damage to the onset and progression" of PD. Support in favor of a relationship among α-synuclein, oxidative stress, and PD stems also from in vitro studies showing that dopamine-dependent oxidative modifications of α-synuclein could facilitate the intraneuronal accumulation of toxic "protofibrils" and thus contribute to the demise of dopaminergic neurons seen in PD (51, 52).

Perhaps no clue derived from genetic studies links parkinsonism to oxidative stress more convincingly than the observation of a parkinsonian syndrome in families with mutations in the *DJ-1* gene (6). Even before its association to PD, *DJ-1* had been suggested to have an antioxidant function in the cellular response to oxidative stimuli (53). Subsequent work has revealed, for example, that posttranslational modifications of *DJ-1* at cysteine residues are important for its antioxidant properties, *DJ-1* is oxidatively damaged in the brains of PD patients and, in *Drosophila*, inhibition of *DJ-1* function through RNA interference leads to cellular accumulation of reactive oxygen species, enhanced vulnerability to oxidative stress, and degeneration of dopaminergic neurons (54–57).

As already mentioned, support in favor of a role of oxidative stress in the pathogenesis of PD has recently derived not only from genetic studies but also from work using toxin-induced PD models. Kaur et al (58) administered iron to newborn mice for a 1-week period (days 10 to 17 postpartum) and noted that, once these animals reached adult and old age, an increase in markers of oxidative damage was paralleled by a loss of nigrostriatal function and dopaminergic cell integrity. In another mouse model of selective nigrostriatal injury caused by exposures to the herbicide paraquat, both a temporal and causal relationship has been found between enhanced oxidative modifications in the nigrostriatal tissue and degeneration of nigral dopaminergic neurons (59, 60). Interestingly, new experimental paradigms also implicate oxidative stress in the interaction of PD-related toxins and genes. Meulener et al (61) showed evidence in a *Drosophila* model that *DJ-1* activity is specifically involved in protecting against oxidative injury caused by paraquat. Moreover, Wang et al (62) showed significant interactions between oxidative stress-inducing agents and parkin, another protein associated with familial parkinsonism. Addition of H_2O_2, paraquat, or iron to SY5Y neuroblastoma cells stably expressing FLAG-tagged parkin altered the solubility of this protein, thus compromising its protective function.

Research Challenges

Although a relationship between oxidative stress and PD has long been hypothesized and is strongly supported by recent findings, evidence of a direct role of oxidative stress in disease pathogenesis is still inconclusive. This may be explained by a number of considerations. Findings in animal models implicate oxidative processes in nigrostriatal degeneration and other pathologic features of PD. However, the extent to which these findings can be directly applied to PD patients remains unclear. Studies in human brains have suggested that oxidative changes (e.g. decreased glutathione levels) may be characteristic of PD. However, whether these

changes underlie the neurodegenerative process of PD or are mere consequences of it (oxidative reactions could occur in tissues "after" they have been damaged) is questionable (63, 64). Uncertainty concerning the role of oxidative stress is also based on the fact that antioxidant strategies have failed to yield convincing protection against PD, as exemplified by the disappointing outcome of a clinical trial that, over 10 years ago, assessed the neuroprotective action of tocopherol in PD (65). One could say that the ultimate proof of concept linking a specific mechanism (such as oxidative stress) to the pathogenesis of PD would be the development of therapeutic approaches that, by targeting that mechanism, result in neuroprotection.

Other research challenges concerning the role of oxidative stress in PD include 1) the elucidation of specific mechanisms by which oxidizing species could be generated during the neurodegenerative process and 2) the identification of critical neuronal targets that are impaired as a consequence of oxidative damage. Mitochondria have been suggested to represent a significant source of reactive oxygen species (ROS) that would contribute to neuronal demise in PD. This possibility is supported by findings with toxins, such as MPTP, that inhibit mitochondrial function and are capable of inducing PD-like pathology (26, 66). Similarly, mitochondrial abnormalities are likely to derive from mutations in PD-associated genes, such as *PINK1* and *DJ-1* (5, 67, 68). Thus, mitochondrial dysfunction and oxidative stress may underlie an important pathway of neurodegeneration common to sporadic and familial PD and should continue to be subjects of future investigation.

Besides mitochondria-mediated ROS formation, other mechanisms have been identified that could lead to the generation of oxidative species in PD. It is noteworthy that these oxidative pathways may not necessarily occur within neuronal cells as, for example, free radicals (permeable to cell membranes) could initially be formed extraneuronally as a consequence of microglial activation and inflammatory processes. An important role of microglia-mediated oxidative stress has been shown in a variety of experimental models of nigrostriatal degeneration, including mice treated with MPTP or paraquat and mice carrying the *weaver* mutation (69–71). The relationship between neuroinflammation, oxidative stress, and PD should continue to be studied not only to clarify mechanisms of ROS generation but also to further assess the potential use of anti-inflammatory agents for neuroprotection in PD (72).

Once oxidizing species are generated, important cellular components/functions could be targeted, and their impairment could ultimately lead to neuronal degeneration. A new potential target for toxic oxidative modifications has been highlighted by studies suggesting that dysfunction of protein degradation through the proteasomal system contributes to neurodegeneration in PD (73, 74). The issue of proteasomal impairment in PD has become somewhat controversial, because initial findings showing PD-like abnormalities in rats treated systemically with proteasomal inhibitors could not be replicated in other laboratories (75–77). These contrasting data relate specifically to the experimental use of proteasomal inhibitors to model PD in rodents and should not be interpreted as a "fatal blow" to the hypothesis of proteasomal dysfunction in PD. In fact, the links between oxidative modifications of proteins and cellular organelles and altered protein degradation will probably be a fruitful area of future investigation into PD pathogenesis.

Future Developments

Looking toward the future of research in this field, it is clear that many of our current uncertainties concerning the involvement of oxidative stress in PD can be resolved through 1) the use of advanced technical tools that will allow us to reassess long-standing issues and to address previously unanswered questions, 2) the development and use of new experimental models, and 3) more rigorous interpretation of the scientific data. This process of careful re-evaluation of the oxidative stress hypothesis is already underway as indicated by the new data and experimental models (some of which are reviewed above) that have been generated over the past few years. It is easy to anticipate that important insight into the relationship between oxidative injury, protein aggregation, inclusion formation, and neurodegeneration will be gained from both in vitro and in vivo studies using, for example, genetically manipulated cells in culture, invertebrate models (e.g. flies and *Caenorhabditis elegans*), transgenic mice, and animals challenged with pro-oxidant toxins. Advanced technical tools, such as the use of microdissection techniques, could be invaluable in identifying biochemical changes that occur within specific neuronal populations targeted by the degenerative process of PD, thus avoiding the confounding results of studies using multicellular tissue specimens. State-of-the-art approaches could also address mechanistic issues, such as the role of mitochondrial dysfunction as a primary event and as an important source of ROS in PD. Mitochondrial abnormalities in PD have been supported, for example, by studies in which mitochondria (and the contained mitochondrial DNA) are inserted into culturable human cell lines depleted of their own endogenous mitochondrial DNA (so called cybrids) (78, 79).

Examples of therapeutic approaches that could shed light upon the involvement of oxidative stress in PD are the administration of anti-inflammatory drugs and the use of coenzyme Q. The rationale for treatment with anti-inflammatory drugs stems, at least in part, from evidence of microglia-mediated oxidative injury in animal models of PD (40, 69). Minocycline, a second-generation semisynthetic tetracycline that possesses potent anti-inflammatory properties, has recently been recommended for Phase III clinical trials (72). If minocycline or other anti-inflammatory drugs are found to be effective as neuroprotective agents, these clinical trials will provide much needed confirmation that prevention/limitation of oxidative reactions (within and outside dopaminergic cells) represents a viable strategy for the development of antiparkinsonian drugs. Initial clinical findings also suggest that coenzyme Q may slow the rate of progression of idiopathic PD (80). The mechanism of action of coenzyme Q is likely to involve an antioxidant effect derived from the interception of aberrant electrons before they react with molecular oxygen and form ROS.

In summary, although a number of new hypotheses concerning the pathogenesis of PD have been proposed over the past few years, the potential role of oxidative stress in neuronal degeneration and formation of proteinaceous inclusions (2 pathologic features of PD) continue to draw a great deal of research attention. In fact, it is quite intriguing that many (if not all) of the new hypotheses (e.g. impairment of the proteasomal system) can be easily reconciled with involvement of oxidative reactions. It is also remarkable that recent findings from genetic studies further suggest that oxidative stress is implicated in the pathogenesis of familial cases of parkinsonism. Conclusive evidence linking oxidative stress to PD is still elusive. However, with the advent of new technical tools and experimental models and the integration of precious information from biochemical, pathologic and genetic studies, the involvement of oxidative damage in PD will probably cease to be just a hypothesis within the short foreseeable future. If so, our sustained efforts to unravel pathways and mechanisms of oxidative injury will also probably yield more specific and effective antioxidant strategies for neuroprotection in PD.

CONCLUSIONS

It is clear that PD is not a single entity. As with all disorders, the different phenotypes will be sorted out from complex combinations of genetics and modifiers with the help of a better understanding of cell biology processes, including oxidative stress. Animal models are certainly critical in this endeavor. Advances along these lines and those of protein misfolding and aggregation not specifically discussed in this review should be helpful for new approaches to therapy. We have probably reached the limit of symptomatic therapy, and our patients need therapy based on understanding of the etiology and pathogenesis of the disease.

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