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Parkinson's : Keep Plugging the Meter

by

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Abbreviations:
CAT (catalase)
DA (dopamine, 3-hydroxytyramine)
FAD (flavin adenine dinucleotide)
GPx (glutathione peroxidase)
GR (glutathione reductase)
MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)
PD (Parkinson's Disease)
RNS (reactive nitrogen species)
CNS (central nervous system)
dopa (3,4-dihydroxyphenylalanine)
FMN (flavin mononucleotide)
L-DOPA (levodopa)
MRI (magnetic resonance imaging)
NOS (nitric oxide synthetase)
ROS (reactive oxygen species)

Abstract

Recent research has shown that active oxygen species, active nitrogen species, and free radicals are correlated to various disease states. Information is accumulating to support the hypothesis that oxidative stress involving damage to DNA, proteins, and lipids may contribute to Parkinson's disease. The purpose of this report is, first, to give a background on the manifestation of this disease state on the whole organism level; second, to consider the natural prooxidant and antioxidant capabilities of the cell; third, to look at some of the evidence of damage in Parkinson's and relate that evidence to oxidative stress; and fourth, to present future courses of action suggested by the literature which are both directly and indirectly related to free radical processes.

Introduction

In 1817, James Parkinson described the shaking palsy now referred to as Parkinson's disease [1]. Additional observations have indicated that Parkinson's is a movement disorder associated with multiple moter control deficits [1]. Initial presentation tends to be the occurrence of a rhythmic temor in resting limbs, usually in a foot or hand, after the age of 50 [1]. This slowly progressive movement disorder also includes bradykinesia, akinesia, and muscle rigidity [1]. While multiple secondary symptoms appear in various combinations, these are the primary indicators of Parkinson's in a clinical setting.

Epidemiological surveys show that around 10% of individuals over the age of 65 have detectable symptoms of Parkinson's [1]. People of all races and ethnic groups are afflicted with the disease; though some differences are seen in the rate of occurrence, differences in specific criteria for diagnosis can make direct comparisons difficult [2]. Of the on average 50,000 people diagnosed in the United States every year, 10% are early onset (<50 years old) cases [2]. Only 1% of people over 65 in the U.S. are diagnosed; however, the total number with a current diagnosis is close to 1.5 million [2]. Risk factors correlating to Parkinson's in order of highest to lowest risk include a family history of Parkinson's, head trauma, and the occupational use of herbicides [3]. More specific causative factors are not clear at this time, although there is ample evidence suggesting a free radical role in both initiation and progression. Most clues to Parkinson's mechanisms have resulted from post-mortem examinations and models of Parkinson's created through exposure to the drug, MPTP, or its derivatives.

Parkinson's disease can significantly detract from the productivity and quality of life experienced by both those who have it and the other people in their lives, especially in nations like the U.S. with large aging populations. The qualitative and quantitative aspects of both

preventative and clinical therapy development are essential to the improvement of Parkinson's patient's outcomes, and an understanding of the basic science involved in pathogenesis is an invaluable part of that development.

Normal oxidative stress processes due to ROS, RNS, and free radicals

Oxidative stress is a naturally occurring phenomenon in aerobic biological systems [1]. A healthy body's machinery attempts to maintain a constant redox equilibrium where the overall oxidative and reductive reactions deep each other in check. Over the course of a lifetime, it is believed that the accumulation of damage caused by imbalances between prooxidants (ROS and RNS) and antioxidants leads to aging and age related diseases. These imbalances are collectively referred to as oxidative stress and result in damaged molecules including nuclear DNA, mitochondrial DNA, proteins, and lipids within the cellular milieu. It is important to note that free radicals are not synonymous with ROS and RNS; there are non-radical ROS and RNS (H_2O_2 and $ONOO^-$) as well as free radicals, which are not ROS or RNS (iron and other metals). Free radicals by definition must simply have at least one unpaired electron.

The direct cause of oxidative stress can be traced to one of two things [1]. The first is an overproduction of ROS, RNS, and/or free radicals. The second is an underproduction of antioxidants. Some of the most biologically relevant redox reactions of interest when discussing these imbalances within mammalian cells are listed below:

$$O_2 + \text{L-arginine} \xrightarrow{\text{NOS's}} \text{L-citruline} + {}^{\bullet}NO \quad (1)$$

$$O_2 + e^- \rightarrow O_2^{\bullet-} \quad (2)$$

$$NO^{\bullet} + O_2^{\bullet-} \rightarrow ONOO^- + H^+ \rightarrow {}^{\bullet}NO_2 + {}^{\bullet}OH \quad (3)$$

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH \quad (4)$$

$$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2 \quad (5)$$

$$H_2O_2 + O_2^{\bullet-} \rightarrow OH^- + OH^{\bullet} + O_2 \quad (6)$$

$$2H^+ + O_2^{\bullet-} + O_2^{\bullet-} \xrightarrow{SOD} H_2O_2 + O_2 \quad (7)$$

$$2GSH + H_2O_2 \underset{GR}{\overset{GPx}{\rightleftarrows}} 2H_2O + GSSG \quad (8)$$

Free radical damage can be repairable, as in most DNA damage; require replacement, as in most protein damage; create potentially lethal structural damage, as in lipid peroxidation; and create long term propagative damage, as in non-repairable DNA mutations. The above list is by no means meant to be a comprehensive one and cofactors have been excluded for simplicity. Reactions (1), (2), (3), (4), and (6) all produce free radicals. The synthesis of the nitric oxide free radical ($^{\bullet}NO$) requires four cofactors (FAD, FMN, tetrahydrobiopterin, and haem) to work with NOS enzymes in a five electron oxidation with electrons supplied by NADPH [1]. Nitric oxide's rapid reactivity upon production actually protects the cell from a great deal of damage as long as compounds which can cage it safely are near its production site. Some of nitric oxide's physiological roles include neurotrasmission, neuromodulation, synaptic plasticity , smooth muscle control as in the regulation of blood pressure, ingibition of platelet aggregation, antibiotic defense, and lung vasodialation. The production of superoxide in reaction (2) can occur via enzymatic catalysis, autooxidation of biologically prevalent molecules in the presence of oxygen, the delocalization of electrons when oxygen is bound to iron centered haems, and as a byproduct of electron leakage in mitochondrial energy production. Mitochondrial leakage is the most significant of these sources of superoxide. Superoxide is detoxified by a dismutation reaction catalyzed by superoxide dismutase in reaction (7). Reaction (3) gives an example of radical propagation through a non-radical RNS ($ONOO^-$). Reaction (4), the Fenton reaction, combined

with reaction (5) to produce the superoxide-assisted Fenton reaction, also known as the net iron catalyzed Haber-Weiss reaction, (6). This is a dangerous reaction cycle because of the especially high reactivity of hydroxyl radicals ($^{\bullet}OH$). The GPx enzyme and GSH in reaction (7) protect against iron catalyzed oxidative stress by removing the majority of the hydrogen peroxide substrate. Metal chealators are also capable of acting as antioxidants by sequestering reactive metal species. It is thought that H_2O_2 must be kept available in low concentrations for signaling purposes within the cell. Hydrogen peroxide in the brain is primarily scavenged by glutathione peroxidase in the brain; little to no catalase or peroxidase activity occurs there [4]. Unless they are quickly sequestered in large proteins capable of delocalizing radical charges, nitric oxide and hydroxyl radicals will certainly cause damage to cellular structures. The complexity of the interplay between these reactions *in vivo* is significant. However, it is not within the scope of this report to explore these reactions in detail. More detailed explanations can be found in the references.

The relationship between changes in Parkinson's and redox imbalances

While free radicals are implicated in almost all disease processes, they are not always thought to be a part of the "causative" factors. Reperfusion injuries after heart operations involve free radicals, but free radicals certainly did not crack open the patient's chest. Still, a growing body of evidence indicates that free radicals may be causative factors and/or part of the disease progression in Parkinson's. The development of therapies for treatment and prevention can be more accurately guided by an understanding of the disease mechanisms underlying changes witnessed in pathogenesis; some of those changes will be presented here in combination with an oxidative stress interpretation.

The locus of Parkinson's disease symptoms is within the CNS. The degeneration and death of subpopulations of dopaminergic mesencephalic neurons, particularly neurons in the substantia nigra, directly correlates to the clinical recognition of Parkinson's [4]. All diagnosed and examined cases, from a literature review by this report's author, had lowered densities of dopaminergic cells in the SN. Amounts of dopamine found postmortem are significantly decreased in the substantia nigra of Parkinson's patients following the loss of dopaminergic neurons, Figure1 [5]. The presence of Lewy bodies is associated with pathogenesis [6]; an example and description of these cytosolic structures can be seen in Figure 2 [7].

(A) (B)

Figure 1 [5]. The loss of dopaminergic neurons in the substantia nigra of a Parkinson's patient (B) versus a control (A). The bright dots are melanized dopaminergic neurons and clearly show a marked disappearance when comparing (A) with (B). It is thought that controlled cell death, or apoptosis may be initiated as a cellular response to excessive oxidative stresses.

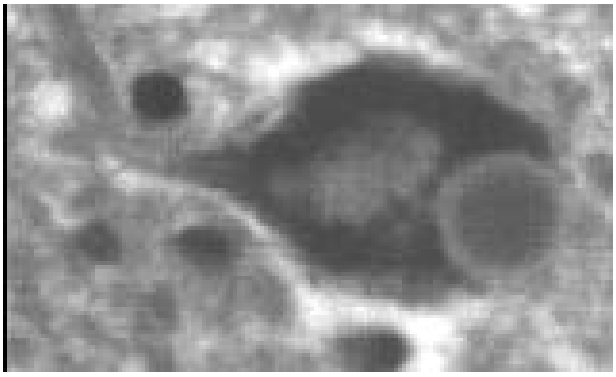

Figure 2 [7]. A classic brainstem Lewy body is shown here in the substantia nigra (SN). Lewy body (LB) inclusions occur in two other pigmented brain stem locations in addition to the SN (the locus ceruleus nucleus and the basalis of Meynert). The LB in this dopaminergic neuron has a halo around it inside the cytoplasm. EM studies and biochemical evidence show that non-membrane bound and abnormally phosphorylated neurofilaments make up the majority of the LB structure . Extracellular LB are visible after neuronal cell death. A direct free radical mechanism for this formation is not apparent, however, it is associated with the changes seen in Figure 3 and Table 1.

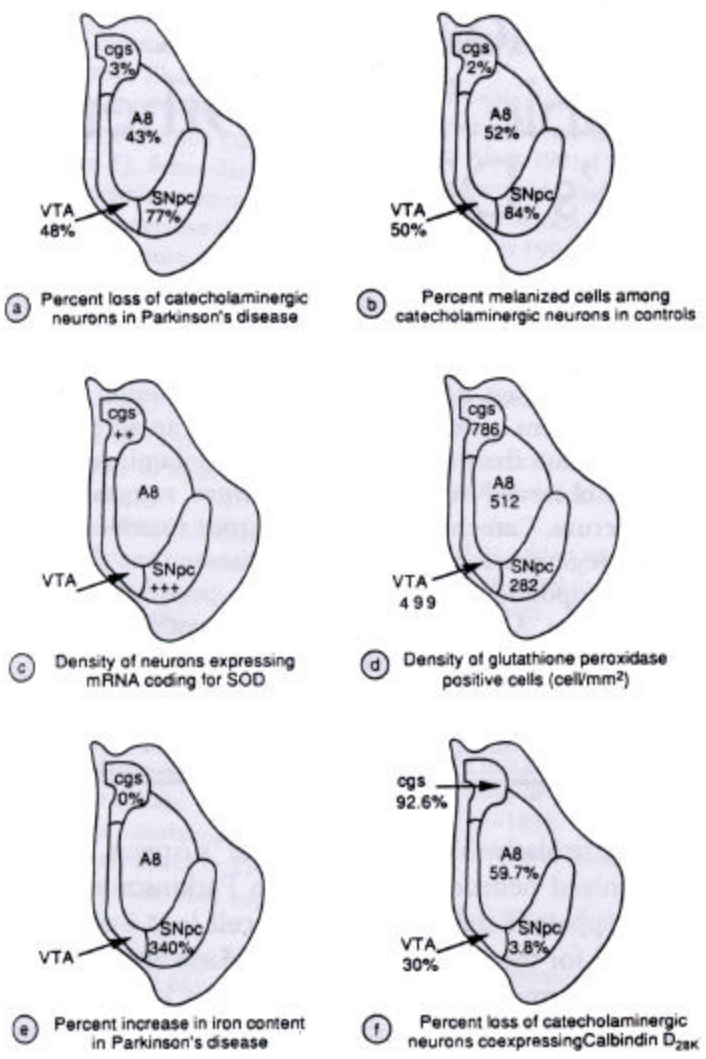

Figure 3 [8]. Cellular neurotoxicity and several substance's distributions in the mesencephalic region of Parkinson's patients. 8A = catecholaminergic cell group 8A, cgs = central gray substance, SNpc = substantia nigra pars compacta, and VTA = ventral tegmental area.

Correlative factors relating to dopaminergic neurotoxicity in Parkinson's are visible in Figure 3 [8]. Figure 3A shows the sensitivity or toxicity of the catacholaminergic (primarily dopaminergic) cells when compared with normal controls. The role of free radicals in this death has already been mentioned. Figure 3B shows a higher percent of melanin-containing neurons in the areas most effected by cell death. This indiciates the likelihood that neuromelanin is either part of the toxic effect or a response to it.

Neuromelanin, the black pigment origin of the nigra in substantia nigra, is one of the many metal binding proteins involved in regulating metal concentrations in the biological milieu [1]; it is found in a higher concentration in those areas most effected by neurodegeneration within a PD patient's brain. Melanin's ability to bind reactive metal ions and particularly neuromelanin's ability to bind iron may contribute to endogenous antioxidant defenses in the substantia nitgra [1]. On the other hand, if the iron is still involved in redox reactions when it is bound to melanin, then oxidative damage from highly reactive and site-specific hydroxyl radical products is likely to occur; this requires the presence of a hydrogen peroxide co-substrate source [1] and, therefore, a second antioxidant protection in the case of too much free iron is provided with the removal of hydrogen peroxide previously described. It is not clear which of these two options is occurring. Changes in the environment, particularly redox changes, may allow both to occur. Additionally, it is significant to note that hydrogen peroxide itself is not particularly toxic; an overabundance of it's free radical products via iron catalysis is considered to be the immediate source of danger to the cell.

Total iron content has been shown to increase in Parkinson's patients via detection and measurement in MRI studies, and is currently used to differentiate between primary Parkinson's and Parkinson's associated diseases [9]. Iron increases are centered in the SN and striatum of patients, thus allowing for a noninvasive clarification of the diagnosis as primary Parkinson's before treatment begins.

The issue of total iron content is a different one than the concentrations of active (generally unbound) and inactive (generally bound) forms. While neural tissue measurements *in vivo* have not been done, one group has done experiments testing bound and unbound iron in the blood (Logms6no G). The issue of total iron content is a different one than the concentrations of

active (generally unbound) and inactive (generally bound) forms. While neural tissue measurements in vivo have not been done, one group has done experiments testing bound and unbound iron in the blood [10]. The circulating concentrations of receptors for the iron transporting protein transferrin remained unchanged and dietary intake of iron was not significantly different in PD patients and controls. Circulating concentrations of iron, ferritin (where most intracellular iron is stored in mammals), and transferrin were substantially lower in Parkinson's patients. Iron transferrin saturation and binding capacity were also significantly lower. Ferritin, transferrin, and total iron binding capacity changes were the same as in postmortem studies. Reductions in serum iron concentrations and the transferrin saturation were surprisingly different from the examination of PD brains after death. Defects in the synthesis of iron-chelating liver proteins as well as defects in brain specific iron-chealators are suggested by these results. In such a scenario, more iron would enter the brain and result in less systemic iron. Iron accumulation in the brain could be especially destructive in localizations such as the SN where overproduction of neuromelanin might lead to protein aggregation. Protein aggregation can lower the activity of many proteins. This would support the possibility of higher levels of free radical producing iron than normal.

Figure 3C implies a cellular response to oxidative stress through the upregulation of SOD enzyme production which would decrease superoxide but increase hydrogen peroxide [8]. That would be okay if hydrogen peroxide removal upregulated too; however, as Figure 3D illustrates, there are lower concentrations of GPx containing cells in the same areas of the mesencephalon where there are upregulations in SOD. If these observations hold true for in vivo hydrogen peroxide coupled with free iron, a huge excess of free radical oxidative stress would be created. Figure 3E is an additional observation of elevated iron in dopaminergic areas of postmortem PD

patient's brains and Figure 3F suggests that calbindin D28K may have a neuroprotective effect in Parkinson's. This may be achieved through buffering intracellular calcium levels whose fluctuation could be a secondary cause of damage resulting from oxidative stress. Table I shows additional evidence of oxidative stress in Parkinson's disease.

Table 1 [8].

Evidence consistent with oxidative stress in Parkinson's disease (PD)

Observation	Comments
A. Direct evidence (measurement of increased oxidative damage or other biomarkers of ROS/RNS production)	
Increased lipid peroxidation	Measured in SN as elevated TBARS and (more convincingly) as HNE–protein adducts, and increased peroxides (by HPLC/chemiluminescence assays). No changes in SN vitamin E reported or any beneficial effect of administration of vitamin E in PD. HNE is neurotoxic: it can cause neuronal apoptosis.
Increased oxidative DNA damage	Rises in 8-OHdG reported in mitochondria and 'total DNA'; little or no rise in other DNA base damage oxidation or deamination products, suggesting rise is not due to $OH^{\bullet}$ or $ONOO^{-}$ attack on DNA.
Increased protein damage	Rises in carbonyls observed in SN but also in several other brain regions, including those unaffected in PD.
Increased nitrotyrosine	Core of Lewy bodies in parkinsonian SN stains with antibodies against nitrotyrosine
B. Indirect evidence (evidence suggestive of a response to oxidative stress)	
Fall in GSH, no marked rise in GSSG	Decreases about 40% in SN, not in other brain regions. Lowering GSH by buthionine sulphoximine does not produce PD in animals but renders them more susceptible to neurotoxins.
Increased iron content	SN zona compacta (but not other brain regions) have higher iron levels in PD, apparently with unaltered ferritin. 'Free' iron is neurotoxic but it is uncertain whether these extra iron deposits are 'catalytic' or not. No increase in Cu or Mn detected. Source of excess Fe unknown but increased expression of receptors for lactoferrin has been reported on neurones and microvessels in SN. Increased Zn also reported in substantia nigra.

Changes in SOD	SOD activity is elevated but it is unclear if CuZnSOD, MnSOD or both rise.
Changes in enzymes of glutathione metabolism	Small decreases in catalase and GSHPx reported in SN and other brain regions by some (not all) scientists. γ-Glutamyl transpeptidase (involved in degradation and cellular translocation of GSH; Chapter 3) elevated. No marked changes in glutathione reductase or γ-glutamylcysteine synthase (usually the rate-limiting enzyme in GSH synthesis).
C. Other evidence (consistent with the concept)	
Defects in mitochondrial function	See text. Coenzyme Q_{10} levels reported as decreased in platelet mitochondria in PD.
Increased glycoxidation	Formation of AGE products requires both glycation and oxidation.
Up-regulation of haem oxygenase I	—
Activation of NF-κB	Could be explained by oxidative stress.

SN, substantia nigra.

Current therapies and treatments

L-dopa variants are the primary treatment for Parkinson's symptoms at this time. Essentially this is just a chemical replacement therapy and does not address the underlying cause of the disease. In cases where L-dopa is not very useful, surgery is an option. Selective ablation in the nigrostriatal pathway can sometimes relieve symptoms and the implantation of stimulatory electrodes is being tested. Human trials involving the injection of dopamine producing fetal cells are currently underway as well. In the area of prevention, some suggestions for physical exercises and antioxidant consumption are made; but those regimines are not currently accepted treatments and no standards have been created.

One significant step forward in the delay of symptom progression is selegiline [3]. MAO-B is known to be inhibited by segeline (L-deprenyl). This could be particularly useful as a protective measure against neurotoxins similar to MPTP, which is initially metabolized by MAO-B and results in the production of free radicals. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) can be a contaminant in synthetic heroin and has been known to produce

Parkinson's like symptoms for over 20 years [1, 11]. While most Parkinson's patients have not been exposed to MPTP, it is a useful model for the mechanisms involved in primary Parkinson's. One of the products of MPTP metabotlism is MPP^+; it interferes with aerobic energy production at the mitochondrial complex I, near or directly on the rotenone binding site [1]. This interference can result in increased electron leakage and abnormally high free radical production. Segeline also enables cells to increase their ability to protect against free radicals via non-MAO-B related mechanisms [11]. Human studies have been conducted and they were correlated with some delay of symptomatic onset.

Experimental, Diagnostic, and Therapeutic Directions

There are many aspects to the pathogenesis of Parkinson's and the vast amount of literature that has been created in the attempt to elucidate mechanisms of action is very promising. Some directions for further study derived from previous experiments include the development of screening procedures, giving combination antioxidant therapy, blocking dopamine reuptake, giving neuron-specific growth factors, and the institution of a repetitive task regimine.

MRI's can be successfully utilized in the differentiation between primary Parkinson's and other neurodegenerative diseases which can be mistaken for PD; this is accomplished through the recognition of abnormally high iron level patterns in the brain [9]. This technique has not yet been employed in screening for presymtomatic Parkinson's. In order to test the accuracy of prescreening, the use of subsymptomatic doses of MPTP might be used on rats in multiple doses until symptoms occurred. MRI's could be used to track the process, although the current accuracy of MRI technology may not be able to track iron accumulation in brains the size of a rat's. Families in which a high incidence of early onset Parkinson's occurred could be scanned to

detect abnormal iron accumulation in combination with tests for the predisposing gene for early onset PD. This might not be useful if current therapeutic treatments interfere with iron accumulation. Yet another option would be to test a subpopulation of head trauma victims and/or people who have been exposed to a large amount of pesticides to follow the possible development of PD. All of these efforts would be geared toward the verification of a presymptomatic identification process, in order to make therapeutic options available at an earlier point in the disease pathogenesis.

The delivery of tocopherol (vitamin E) has been shown to correlate with less severe pathogenesis in human PD patients [3]. The efficiency of tocopherol protection is increased through coupling with vitamin C (ascorbate), although most *in vivo* studies surprisingly do not take advantage of this method for recycling tocopherol. The potential for much higher levels of protection against the free radical damage associated with PD progression could be significant. Although the tocopherol radical formed in tocopherol's antioxidant reaction is not as highly reactive as the species it detoxifies, it is still a radical and ascorbate further detoxifies that radical. A study can be conducted with PD patients at different stages of their disease who consume tocopherol alone or tocopherol plus ascorbate. Amounts taken per day should remain consistent and a control group choosing not to take either antioxidant should be used if possible. Dietary habits, physical activities, and other medications etc. will need to be noted in order to at least partially account for variances due to other factors.

Mazinol is known to be a dopamine receptor blocker on the presynaptic terminus and has been shown to prevent MPTP induced Parkinsonism [3]. By blocking the reuptake receptor mazinol keeps dopanine in the cleft; rather than increasing the levels of dopamine it simply degrades more slowly. An experiment testing whether or not mazinol has any significant

reversal effect on symptoms would be the next step. This would simply entail the treatment of an MPTP animal model with varying amounts of mazinol to determine if there was any effect on symptoms and compare any side effects with those of L-Dopa to determine if it might be a more beneficial method of maintaining dopamine stimulation levels in the nigrostriatal terminus. After that, a combination of mazinol and L-dopa might be attempted. Some of dopamine's side effects are likely to be due to the known production of free radicals during its metabolism. Antioxidants appropriate for the removal of these radical byproducts could greatly diminish the negative side of effects of both mazinol and L-dopa treatments.

The existence of brain-derived neurotrophic factors is known [3]. The administration of these factors to patients might optimize neuron survival and it is possible that these trophic factors stimulate the production of active antioxidant proteins. Animal model trials have been promising; however, the next step before thinking about treating patients in this way would be to develope and test a slow progressing animal model of PD. This would be especially important due to the suspicion of an increased cancer risk associated with abnormally high growth factor concentrations.

Potentially, engaging neurons used in movements effected by Parkinson's could stimulate the formation of additional dentritic connections; these connections may be capable of endogenously amplifying each neuron's effectiveness [12, 13] while decreasing the side effects associated with drug delivery. An experimental analysis of this possibility could involve a rat study and a human study. The rat study would entail a repetitive food retrieval task requiring balance. An analysis of brain structures for the parameters measured in Figure 3 would be performed after examining the circulating levels of iron and proteins measured by Logroseino et al. Levels of MNSOD (specific to the mitochondria), CuZnSOD (specific to the cytosol), and

ECSOD (extracellular SOD) would also be measured. The rat study would allow for an improved characterization of free radical disease processes and possible insights into future drug and gene therapies. The concurrent human trial would take a balance oriented repetitive exercise such as Tai Chi and select a combination of exercises; the exercise set would be the same for all participants. A control group choosing not to engage in exercise would be compared with groups practicing once a week, three times a week, and once a day. The progression of symptoms would be compared between groups. This human study could give preliminary results which might be used to recommend specific physical activities to Parkinson's patients as part of their efforts to delay symptom progression.

Summary

Parkinson's disease effects a significant portion of the aging population. Symptomatic onset can be physically, mentally, and emotionally difficult; however, there are many promising avenues of progress opening up in both the research and treatment of the disease. The oxidative stress hypothesis of initiation and progression in Parkinson's has offered many clues to pathogenesis and appears to be leading to more.

Glossary

Akinesia: defined as the slow initiation of movement [1]

Basal ganglia: "origionally, all of the large masses of graymatter at thebase of the cerbral hemisphere; currently, the corpus striatum (caudate and lentiform nuclei) and cell groups associated with the corpus striatum, such as the subthalamic nucleus and substantia nigra [14]"

Bradykinesia: defined as slow overall movement [1]

Dopa: "3.4-dihydroxyphenlalanine; an intermediate in the catabolism of phenylalanine and tyrosine, and in the biosynthesis of norepinephrine, epinephrine, and melanin; the L form, levadopa, is biologically active [14]"

Dopamine: "3-hydroxytyramine; decarboxylated dopa; an intermediate in tyrosine metabolism and precursor of norepinephrine an epinephrine; its presence in the central nervous system and localizationoin the basla ganlia (caudate and lentiform nuclei) suggest that dopamine may haveoher functions [14]"

Dopaminergic: describes dopamine neurotransmitter producing cells [1]

Melanin: "pigments, found throughout the animal kingdom…formed by oxidation and polymerization of tyrosine…one can regar melanins as large free radicals…eumelanins are 'radical sinks' for $O_2^{\cdot -}$ and $RO_2^{\cdot}$ [1]"

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