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Mini-Review

OXIDATIVE STRESS AND PARKINSON DISEASE

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ABSTRACT
The cause of Parkinson disease is not known at present, and there are many theories as to why the loss of nerve cells that characterises the condition occurs. In recent years, there has been some interest in role of free-radical damage, particularly the damage caused by highly reactive molecules in Parkinson disease and other conditions.
In early stages of Parkinsonism, there appears to be a compensatory increase in the number of dopamine receptors to accommodate the initial loss of dopamine neurons. As the disease progress, the number of dopamine receptors decreases, apparently due to concomitant degeneration of dopamine target sites on striatal neurons. The loss of dopaminergic neurons in Parkinson's disease results in enhanced metabolism of dopamine, augmenting the formation of H_2O_2, thus leading to generation of highly neurotoxic radicals ($OH^{\bullet}$).
It has been supposed that degeneration in Parkinson's disease could be a result from the oxidative stress due to dysregulation of dopamine metabolism with consequent free radical formation, depletion of reduced glutathione, a high level of total iron with reduced level of ferritin and deficiency of mitochondrial complex I.

Key words: Parkinson disease, free radicals, oxidative stress.

INTRODUCTION

Parkinson's disease (PD) is considered one of the major neurological disorders of the population over 65 years of age and about 3% of the population over the age of 65 have PD. There are convincing evidences that the oxidative stress and reactive oxygen species (ROS) play an important role in the aetiology and/or progression of a number of human diseases (1). Parkinson disease is a progressive neurodegenerative disorder affecting primarily the dopamine neurons that arise in the midbrain (mesencephalon) and project to the putamen and caudate regions (the striatum) of the brain, areas concerned with the control of motor movements (2). Parkinson disease produces bradykinesia, muscular rigidity, rest tremor and loss of postural balance. Unaffected by disease are dopamin neurons that arise in the region of the midbrain and project to cortical and limbic regions (3). As its name implies, this region is normally heavily pigmented; the pigment is visible to the unaided eye as a brown-to-black region of the mesencephalon. The pigment is an insoluble polymer that is related to melanin of skin, and has been termed neuromelanin (4). The principal cytoskeletal pathology of Parkinson disease is the Lewy body, which, in 85-100% of cases occur in many aminergic and other subcortical nuclei, spinal cord, sympathetic ganglia, and less frequently in cerebral cortex, myenteric plexuses, and adrenal medulla (5-9). Lewy bodies are abnormal intracytoplasmic neuronal inclusions that are considered to be a major anatomic hallmark of Parkinson's disease, although they are seen in pigmented brain stem nuclei in various disorders and in normal ageing brain. Lewy bodies occur in two characteristic forms -the classical, or subcortical (brain stem) type consists of a single or multiple round or oval eosinophilic structures with a central *core* surrounded by a less dense peripheral zone and an outermost pale halo that is sharply demarcated from the neuronal cytoplasm. The cortical type is not

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sharply delineated from the cytoplasm, and the outline of the central part is rather indistinct (10). Lewy bodies are composed of intermediary type filaments 8-10 nM in diameter, admixed with vesicular and granular materials. Immunocytochemical studies have shown that the Lewy bodies react with antisera raised against neurofilaments, phosphorylated and non-phosphorylated epitopes of neurofilament proteins, tubulin, and various microtubule-associated polypeptides (10). In Parkinson's disease, in addition to degeneration of the nigrostriatal dopaminergic pathway, a variety of other neuronal systems are involved, causing multiple neuromediator dysfunctions that account for the complex patterns of functional deficits. This degeneration affects the dopaminergic mesocorticolimbic system, the noradrenergic locus ceruleus (oral parts) and motor vagal nucleus, the serotonergic raphe nuclei, the cholinergic nucleus basalis of Meynert, pedunculopontine nucleus, Westphal-Edinger nucleus, and many peptidergic brainstem nuclei containing cholecystokinin, Metenkephalin, substance P, somatostatin, and neuropeptide Y (11-12).

Ethiology of Parkinson disease

Parkinson disease is characterized by the loss of dopaminergic neurons of the substantia nigra and the deposition of intracellular inclusion bodies. The principal protein component of these deposits is α-synuclein (13), which is ubiquitously expressed in the brain; mutations of α-synuclein (A30P and A53T) contribute to familial forms of the disease (14). A characteristic feature of the neurons within the substantia nigra is the age-dependent accumulation of neuromelanin (15). In PD, these neuromelanin-containing cells are most likely to be lost (16). Neuromelanin is a dark brown pigment that accumulates metal ions, particularly iron. Although, the composition of neuromelanin has not been rigorously characterized, it is known that it consists primarily of the products of dopamine redox chemistry (17-18). Dopamine is an essential neurotransmitter, but as it is a catechol it is also a good metal chelator, and a potential electron donor (that is, a metal reductant). Dopamine coordinates metals such as Cu^{2+} and Fe^{3+} (19), reduces the oxidation state of the metal, and subsequently engenders production of H_2O_2, setting up conditions for Fenton chemistry (20). The causes for degeneration of dopamine neurons are not well understood. However, it can be assumed that interactions between external toxins (which arise from environmental, dietary and lifestyle factors), internal toxins arising from normal metabolism and the genetic (nuclear genes) and epigenetic (mitochondria, membranes, and proteins) components of neurons occur continuously.

The environmental hypothesis posits that PD-related neurodegeneration results from exposure to a dopaminergic neurotoxin. Theoretically, the progressive neurodegeneration of PD could be produced by chronic neurotoxin exposure or by limited exposure initiating a down self-perpetuating cascade of deleterious events. The finding that people intoxicated with MPTP develop a syndrome nearly identical to PD (21) is a prototypic example of how an exogenous toxin can mimic the clinical and pathological features of PD. Paraquat is structurally similar to 1-methyl-4-phenylpyridinium (MPP^+), the active metabolite of MPTP, and has been used as herbicide (22).

Another possibility, which does not fit neatly into a genetic or environmental category, is that an endogenous toxin may be responsible for PD neurodegeneration. Distortions of normal metabolism might create toxic substances because of environmental exposures or inherited differences in metabolic pathways. One source of endogenous toxins may be the normal metabolism of dopamine, which generates harmful reactive oxygen species (ROS) (4).

Free radicals formation

There is a growing body of evidence that nigral neurons may be damaged by cytotoxic substances known as free radicals (23-28). Free radicals are generated under normal and pathological conditions. Due to the presence of an unpaired electron, free radicals are highly unstable and tend to react with cellular elements (29). Free radicals are thought to be produced locally within the basal ganglia and to lead to progressive damage to and death of substantia nigra neurons in susceptible individuals (25-26, 28).The most common cellular free radicals are hydroxyl radical ($OH^{\bullet}$), superoxide radical ($O_2^{-\bullet}$), and nitric oxide ($NO^{\bullet}$). Other molecules, such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) are not free radicals but can lead to the generation of free radicals through various chemical reactions. Free radicals and other

oxygen-derived species (H_2O_2, $ONOO^-$) are constantly generated *in vivo*, both by "*accidents of chemistry*" and for specific metabolic purposes. The reactivity of different free radicals varies, but some can cause severe damage to biological molecules, especially to DNA, lipids, and proteins (30).

Superoxide

Superoxide radical ($O_2^{-\bullet}$) is formed by reduction of oxygen molecule with one electron. In aqueous solution it is a weak oxidant and acts mainly on ascorbic acid and thiol compounds. Superoxide radical is a very strong reducing agent and can reduce certain iron complexes, such as cytochrome C. In vivo, it is decomposed by SOD to hydrogen peroxide and oxygen:

$$O_2^{-\bullet} + O_2^{-\bullet} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Hydroxyl radical

Particularly, the most reactive hydroxyl radical, when generated in excess, causes cellular damage leading to cell death (31). Hydroxyl radical is generated *via* the Fenton reaction [1] from hydrogen peroxide in the presence of ferrous ions or *via* the Heber-Weiss reaction [2] from hydrogen peroxide and superoxide radical (32).

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad [1]$$

$$H_2O_2 + {}^{\bullet}O_2^- \longrightarrow O_2 + OH^- + {}^{\bullet}OH \quad [2]$$

Hydroxyl radical reacts at a diffusion-controlled rate with almost all molecules in living cells (33). Hence, when $OH^{\bullet}$ is formed in vivo, it damages whatever it is generated next to, as $OH^{\bullet}$ cannot migrate any significant distance within the cell (30). Free radicals are known to be produced metabolically in living organisms. [2]

Nitric oxide

The free radical $NO^{\bullet}$ is synthesized from amino acid L-arginine by vascular endothelial cells, phagocytes, certain cells in the brain and other cell's types. Nitric oxide is a vasodilator agent and possibly an important neurotransmitter (34). The $NO^{\bullet}$ contains an unpaired electron and is paramagnetic, it rapidly reacts with $O_2^{-\bullet}$ to form peroxynitrite anion ($ONOO^-$) in high yield (35).

$$NO + O_2^{-\bullet} \longrightarrow ONOO^-$$

$$ONOO^- + H^+ \longrightarrow {}^{\bullet}OH + NO_2^{\bullet}$$

Peroxynitrite anion is very strong oxidizer and can damage many biomolecules. It decomposed spontaneously yielding hydroxyl radicals (36).

Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is formed in two ways: ***indirectly*** through superoxide anion dismutation, and ***directly*** in some oxidative reactions associated with the transfer of two electrons to the oxygen.

Hydrogen peroxide is a relatively stable in water and appears as a weak oxidiser and reductant. It is readily diffuses through cell membranes and in the presence of ions with variable valency it is formed the highly toxic for the cell –hydroxyl radicals (31). Hydrogen peroxide is converted by the glutathione peroxidase enzyme to form water and oxygen, thus preventing the accumulation of precursor to free –radical biosynthesis (30).

Oxidative stress

Oxidative stress defined as an imbalance between biochemical processes leading to production of reactive oxygen species (ROS) and the cellular antioxidant cascade, causes molecular damage that can lead to a critical failure of biological functions and ultimately cell death (37). In Parkinson disease, oxidative stress induced by free radicals damages neuronal membrane lipids, proteins and other components of brain tissue. There are several potential sources of the increased free radical production in Parkinson disease, including mitochondrial dysfunction, increased free iron levels and increased dopamine metabolism (38- 39).

Oxidative stress and nigral neuronal death in Parkinson's disease

Unsaturated lipids are particularly susceptible to oxidative modification and lipid peroxidation is sensitive marker of oxidative stress. Lipid peroxidation is the result of attack by radicals on double bond of unsaturated fatty acid and arachidonic acid, to generate highly reactive lipid peroxyl radicals that initiate a chain reaction of further attacks on other unsaturated fatty acid (20). Oxidative stress is implicated as a major factor for nigral neuronal cell death. Metabolic failure in antioxidant

mechanisms could hypothetically facilitate the chemical processes that lead to lipid peroxidation (40). A number of data have shown that catecholamines and particularly dopamine are an important source of free radicals in the brain (41) they are formed primarily in redox reactions. At rate they have been removed by cell antioxidant defense system, but when rate obtained free radicals exceeds the antioxidant capacity of the system and cell falls into a state of oxidative stress (42).

Perhaps the best-characterized biologic damage caused by $^{\bullet}OH$ is its ability to stimulate the free radical chain reaction known as lipid peroxidation. This occurs when the $^{\bullet}OH$ is generated close to membranes and attacks the fatty acid side chains of the membrane phospholipids. It preferentially attacks polyunsaturated fatty acid side chains, such as arachidonic acid. The $^{\bullet}OH$ abstracts an atom of hydrogen from one of the carbon atoms in the side chain and combines with it to form water:

This reaction removes the •OH, but leaves behind a carbon-centered radical in the membrane. Carbon-centered radicals formed from polyunsaturated fatty acid side chains usually undergo molecular rearrangement to give conjugated diene structures, which can have various fates. Thus, if two such radicals collided in the membrane, cross-linking of fatty acid side chains could occur as the two electrons joined to form a covalent bond. Reaction with membrane proteins is also a possibility. However, under physiologic conditions, the most likely fate of carbon-centered radicals is to combine with oxygen, creating yet another radical, the peroxyl radical (31). Hence one •OH can result in conversion of many hundred fatty acid side-chains into lipid hydroperoxides. Transition metal ions also affect lipid peroxidation by decomposing peroxides. When transition metal ions are added to lipid systems already containing peroxides, their main action is to decompose these peroxides into peroxyl and alkoxyl radicals that in turn abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (31). Formed in membrane of nerve cell lipid hydroperoxides alter its fluidity, which prerequisite for the introduction of various ions as Ca^{2+} in neurons that significantly disrupts normal functioning (31). Except polyunsaturated fatty acids of membrane lipids the free radicals attack proteins. This leads to distortions of its tertiary and quaternary structure and a number of enzymes in the neurons lose their activity. Neurofilaments and proteins constituting the cytoskeleton of the nerve cell are particularly rich in lysine residues. In the oxidative stress the amino groups of these residues react with aldehydes derived from lipid oxidation, and lose their electric charge. As a result, prejudice both the native conformation of a single protein molecule and the protein interactions with the environment. In the recent years has been found that ROS can cause DNA damage (20).

In cases where the balance between ROS generation and antioxidant activity is disturbed, oxidative stress occurs and complicates the underlying disease. The main target substrates for free oxygen radical activity are polyunsaturated fatty acids in membrane phospholipids, the modification of which result in disorganization of cell framework and function. The end product of these reactions is malondialdehyde (MDA). It is excreted in urine, blood, and other fluids and therefore serves as a marker of lipid peroxidation and the presence of oxidative stress respectively. Deactivation and removal of ROS depends on the activity of antioxidant defensive systems including the following enzymes –superoxide dismutase (SOD), Catalase (CAT).

Oxidative stress in Parkinson disease

Support for oxidative stress mechanisms in dopaminergic degeneration in the *substantia nigra* in Parkinson's disease (12) comes from a growing body of evidence, indicating that this region has a high propensity for oxidative stress and is also deficient in protective mechanisms (43). It has been found:

- Increased oxidation of dopamine and formation of nevromelanin
- Increased iron concentration and low concentration of ferritin
- Decreased production of reduced glutathione and increased amount of oxidized glutathione

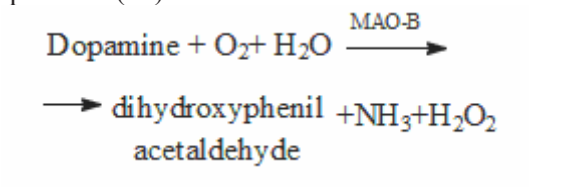
Described changes in the *substance nigra* are specific only for Parkinson's disease and not

found in other neurodegenerative diseases also associated with degeneration of dopaminergic neurons.

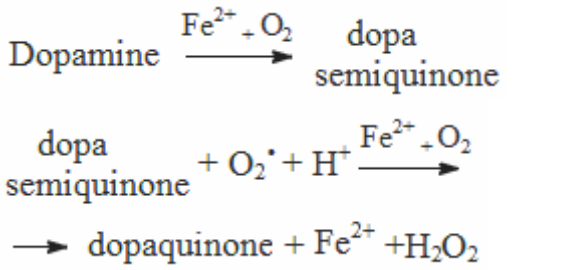
As markers of oxidative damage in the substantia nigra considered changes in the amount of dopamine and its metabolites malondialdehyde and 4-hydroxy-nonenal-modified proteins.

Increased oxidation of dopamine and formation of neuromelanin

A number of data have shown that catecholamines and particularly dopamine are an important source of free radicals in the brain (44). As long as dopamine is stored in synaptic vesicles, it is stable. However, when it is in excess in cytosol, then it is easily metabolized by monoamine oxidase (MAO) to produce hydrogen peroxide or by autoxidation to form quinones (45).



or subjected to autoxidation:

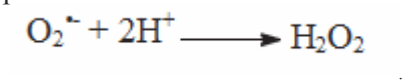


Autoxidation of dopamine or L-dopa via quinone formation generates free radicals such as superoxide radical and hydrogen peroxide. Moreover, dopamine and L-dopa quinone are easily oxidized to aminochromes and finally polymerize to form melanin (45). Thus, L-dopa therapy leading to high brain concentration of dopamine may potentially contribute to progression of oxidative damage of dopaminergic cells in patients with Parkinson disease (39). In patients with PD metabolism of dopamine is greatly enhanced and in saved dopaminergic neurons tyrosinehydrolase activity is increased. This probably represents a compensatory mechanism to fill the dopamine shortage. The data show that in PD first is damaged the dopaminergic neurons containing the largest quantities of neuromelanin.

Dopamine is a precursor of concentrated mainly in dopaminergic neurons neuromelanin. Neuromelanin is the dark pigment present in pigment-bearing neurons of four deep brain nuclei. These are the substantia nigra- Pars Compacta part, the locus coeruleus (blue spot), the dorsal motor nucleus of the vagus nerve (cranial nerve X), and the median raphe nucleus of the pons (20).

Although, the functional nature of neuromelanin is unknown in the brain, the pigment is made from oxyradical metabolites of monoamine neurotransmitters including dopamine and norepinephrine.

Neuromelanin can also be seen as a kind of free radicals, which is able to catalyze the dismutation of superoxide radical to hydrogen peroxide:



In addition, neuromelanin can bind free radicals, $O_2^{\bullet-}$, $^{\bullet}OH$ and some metal ions, including iron ions (30- 31). Synthetic melanins are produced by incubating dopamine with Cu^{2+} and Fe^{3+} (46). The purpose (if any) of neuromelanin is unknown, but it has been postulated that it protects against dopamine induced redox-associated toxicity (47- 48). At low iron concentrations, melanins are known to have antioxidant properties, but at higher metal loads melanins are prooxidant (49). Another postulated role for neuromelanin is as an iron-storage molecule. Double et al. (46) have shown that neuromelanin isolated from human substantia nigra has both high- and low-affinity Fe^{3+}-binding sites, and that the iron bound to neuromelanin is redox active (50). The oxidative stress associated with PD could be the result of a breakdown in the regulation of dopamine (neuromelanin) / iron biochemistry (20).

Increased iron concentration and low concentration of ferritin

Iron may contribute to free radical production. It is found in high concentrations in several parts of the basal ganglia including the substantia nigra, the globus pallidus, and the putamen (51-53), and may therefore increase the vulnerability of nigral dopaminergic neurons to toxic oxygen radicals, especially in the substantia nigra of parkinsonian patients where iron content is increased (51-52, 54).

Iron is important for developing the activity of tyrosinehydroxylase and monoamineoxidase. The tyrosinehydroxylase enzyme catalyzes the conversion of L-tyrosine to L-dihydroxyphenylalanine, and the monoamineoxidase - catalyzes the oxidative degradation of dopamine.

The translocation of iron across the blood-brain barrier is mediated by specific transferrin receptors located on brain microvasculature. In the cells a large part of iron joins to ferritin, other formed chelate compounds with phosphate groups of membrane components and some included in iron micronutrient enzymes.

Ferritin serves to store iron (Fe^{3+}) in a non-toxic form, to deposit it in a safe form, and to transport it to areas where it is required. By linking the iron from some biochemical reactions the ferritin limited its ability to stimulate oxidative processes.

Iron mediate oxidative damage to cellular components through the one-electron transfer called the Fenton reaction, which leads to production of the unstable hydroxyl radical (OH•) that will oxidize nucleic acid, protein, carbohydrate, and lipid, whichever is proximate (55-56).

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\bullet}OH + OH^{-}$$

Lipids are oxidized, initiating the chain reaction of lipid peroxidation. Iron ions are also dangerous in other ways for example lipid peroxidation results in the accumulation of lipid hydroperoxides (LOOH). These can be degraded in the presence of iron ions for example:

$$LOOH + Fe^{2+} \longrightarrow Fe^{3+} + LO^{\bullet} + OH^{-}$$
$$LOOH + Fe^{3+} \longrightarrow Fe^{2+} + LOO^{\bullet} + H^{-}$$

The resulting alkoxyl (LO•) and peroxyl (LOO•) radicals can damaged membrane proteins to propagate lipid peroxidation. End – products of metal ion induced LOOH decomposition are numerous and include highly cytotoxic carbonyl compounds such as malondialdehyde and the unsaturated C_9 aldehyde 4-hydroxy-2-transnonenal (57) Furthermore, Mash et al. (58) found that given the iron dependency of both synthetic and degradative enzyme activities, dopaminergic neurons may express transferrin receptors on their cell surface to facilitate the uptake of iron bound to transferrin. If the intracellular iron pool is regulated by receptor-mediated transferrin uptake, then an up-regulation of transferrin receptor number may play a role in the pathogenesis of nigral cell damage in Parkinson's disease. Early in the disease process, surviving dopaminergic neurons may increase the number of transferrin receptors in order to meet the increased metabolic demand associated with compensatory changes in dopamine synthesis and turnover. The uptake of ferrotransferrin by dopaminergic neurons may result in a progressive elevation in the cellular iron load that exceeds the regulatory capacity for increased ferritin expression in the ageing brain (59). It has been shown that increased iron stimulate the formation of free radicals and changes in the ratio of Fe^{2+} / Fe^{3+} confirmed the presence of oxidative stress.

Decreased production of reduced glutathione and increased amount of oxidized glutathione in PD

Antioxidant protection of the brain is provided by SOD, catalase and glutathione peroxidase. Glutathione peroxidase, one of the most potent enzymes that protects against oxygen toxicity by scavenging H_2O_2 generated by cellular metabolism, is detected exclusively in glial cells of the midbrain.

$$H_2O_2 + GSH \xrightarrow{GSHPx} GS\text{-}SG + 2H_2O$$

Under normal conditions, there is a balance between the rate of formation and decomposition of H_2O_2, which prevents the occurrence of oxidative stress. It is assumed that the amount of reduced glutathione (GSH) is the limiting factor for the removal of H_2O_2 and other obtained by membrane oxidation phospholipid peroxides. The recovery of reduced glutathione is performed by reduction of oxidized glutathione (GS-SG) by glutathione reductase (GS-SGRd).

The level of reduced glutathione in substantia nigra is decreased (60-62). The depletion of reduced glutathione in the substantia nigra in Parkinson's disease could be the result of neuronal loss. As a matter of fact, the positive correlation has been found to exist between the extent of neuronal loss and depletion of glutathione (63). A decrease in the availability of reduced glutathione would impair the capacity of neurons to detoxify hydrogen peroxide and increase the risk of free radical

formation and lipid peroxidation. Indeed, the nigra contains increased levels of malondialdehyde and hydroperoxides (50-51). An increase in the activity of mitochondrial superoxide dismutase in the substantia nigra in Parkinson's disease (64) may indicate a compensatory mechanism to nullify the augmented oxidative stress.

In the substantia nigra of patients deceased by Parkinson disease the quantity of reduced glutathione is decreased, and of oxidized glutathione – is increased. It seems to be specific to substantia nigra and is not observed in other tissues by increasing oxidative metabolism. The rapid consumption of reduced glutathione in the brain establishes a dangerous condition for increasing amounts of H_2O_2 and the occurrence of oxidative stress.

CONCLUSION

Parkinson disease is the second most common neurodegenerative disorders. Equally strong evidence has implicated oxidative stress in the pathogenesis of Parkinson disease. The brain— an organ that requires high metal ion concentrations to maintain many of its functions— has a poor capacity to get through with oxidative stress, and demonstrates little regenerative capacity. There are several reasons why the brain and the nerves are especially vulnerable to oxidative stress.

- Relative to its size, the brain experiences an increased rate of oxidative activity, which creates a significant number of free radicals.
- The normal activity which various chemicals create to establish nerve conduction is a major producer of free radicals. The brain and nerve tissue contain relatively low level of antioxidants.
- In addition, those brain regions that are rich in catecholamines are exceptionally vulnerable to free radical generation. The catecholamine adrenaline, noradrenalin, and dopamine can spontaneously break down (auto-oxidise) to free radicals, or can be metabolized to radicals by the endogenous enzymes such as MAO (monoamine oxidases). One such region of the brain is the substantia nigra (SN), where a connection has been established between antioxidant depletion (including GSH) and tissue degeneration

REFERENCES

1. Dalle-Donne, I., Scaloni, Giustarini, A., Cavarra, D., Tell, E., Lungarella, G., Proteins as biomarkers of oxidative stress in diseases: the contribution of redox proteomics, *Mass Spectrom Rev,* 24: 55-99, 2005.
2. Hornykiewicz, O., Kish, J., Biochemical pathophysiology of Parkinson disease, *Adv Neurol*, 45: 19-34, 1986.
3. Lozoncy, F., Davidson, M. and Davis, K., The dopamine hypothesis of schizophrenia in Psycholopharmacology: A second generation of progress, *Raven Press*, pp. 715-726, 1987.
4. Cohen, G., Oxidative stress and Parkinson's disease, In Reactive Oxygen Species in Biological Systems: an Interdisciplinary Approach, ed. *Gilbert D. L. & Colton*, pp. 593—608, 1999.
5. Jellinger, K., An overview of morphological changes in Parkinson's disease, *Adv Neurol*, 45, 1-16, 1986.
6. Jellinger, K., The pathology of Parkinsonism, In: Movement disorders, *Marsden and S Fahn*, pp.124-165, 1987.
7. Hornykiewicz O., Neurochemical pathology and the etiology of Parkinson's disease: basic facts and hypothetical possibilities, *Mt Sinai J Med*, 55: 11-20, 1988.
8. Forno, L., The Lewy body in Parkinson's disease, *Adv Neurol*, 45: 35-43, 1986.
9. Louis, D., Goldman, E., Powers, M. and Fahn, S., Parkinsonian features of eight pathologically diagnosed cases of diffuse Lewy body disease, *Mov. Disord*, 10: 188-194, 1995.
10. Jellinger, K., Neuropathological substrate of dementia in Parkinson's disease, Ann of Neurol, 28:723-724, 1990.
11. De Erausquin, A., Costa, E. and Hanbauer I, Calcium homeostasis, free radical formation and trophic factor dependence mechanisms in Parkinson's disease, Pharmacol, 46: 467-482, 1994.
12. Jenner, P., Dexter, T., Sian, J., Schapira, H., Marsden, C.D., Oxidative stress as a cause of nigral cell death in Parkinson's disease and incidental Lewy body disease, *Ann Neurol*, 32(Suppl): S82–S87, 1992.
13. Spillantini, M., Schmidt, M., Lee, M., Trojanowski, Q., Jakes, R., Goedert, M., Alpha -synuclein in Lewy bodies, *Nature*, 388: 839–840, 1997.
14. Gasser, T., Genetics of Parkinson's disease, *J Neurol*, 248: 833–840, 2001.

15. Marsden, D., Neuromelanin and Parkinson's disease, *J Neural*, 19 (Suppl): 121–141, 1983.
16. Hirsch, E., Graybiel, M. and Agid, A., Melanised dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease, *Nature*, 334: 345–348, 1988.
17. Wakamatsu, K., Fujikawa, K., Zucca, A., Zecca, L and Ito, S., The structure of neuromelanin as studied by chemical degradative methods, *J Neurochem,* 86: 1015–1023, 2003.
18. Zecca, L., The neuromelanin of human substantia nigra: structure, synthesis and molecular behaviour, *J Neural*, 65(Suppl): 145–155, 2003.
19. Gerard, C., Chehhal, H. & Hugel, R. P., Complexes of iron (III) with ligands of biological interest: dopamine and 8-hydroxyquinoline-5-sulfonic acid, *Polyhedron,* 13: 591–597, 1994.
20. Barnham, K., Masters, C and Bush, A., Neurodegenerative diseases and oxidative stress, *Nature*, 3: 205-214, 2004.
21. Langston, W., Ballard, P., Tetrud, W., Irwin, I., Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis, *Science*, 219(4587): 979-80, 1983.
22. Dauer, W., Przedborski, S., Parkinson's disease: mechanisms and models, *Neurol*, 39(6): 889-909, 2003.
23. Ebadi, M., Srinivasan, R., Baxi, D., Oxidative stress and antioxidant therapy in Parkinson's disease. *Prog Nrurohiol*, 48:1-19, 1996.
24. Evans, H., Free radicals in brain metabolism and pathology, *Br Med Bulb*, 49: 577-587, 1993.
25. Hirsch, C., Does oxidative stress participate in newe cell death in Parkinson's disease? *Eur Neurol*, 33(suppl): 52-59, 1993.
26. Jenner, P, Olanow, W., Oxidative stress and the pathogenesis of Parkinson's disease, *Neurology*, 47(suppl 3):161-170, 1996.
27. Olanow, W., An introduction to the free radical hypothesis in Parkinson's disease, *Ann Neurol*, 32(suppl): S2-SI, l992.
28. Simonian, A., Coyle, T., Oxidative stress in neurodegenerative diseases, *Toxicol*, 36: 83-106, 1996.
29. Halliwell, B., Reactive oxygen species in living systems: source, biochemistry and role in human disease, *Am J Med*, 91: 14-21, 1991.
30. Halliwell, B., Free radicals and Antioxidants: A personal view, *Nutrition*, 8 (I): 253-265, 1994.
31. Halliwell, B., Oxidative stress and neurodegeneration: where are we now? *J Neurochem*, 97: 1634–1658, 2006.
32. Halliwell, B, Gutteridge, C: Free Radicals in Biology and Medicine, *Oxford Univ Press*, 2006.
33. Von Sonntag, C., The chemical basis of radiation biology, *Taylor and Francis*, 1987.
34. Moncada, S., Higgs, A., The L – arginine-nitric oxide pathway, *N Engl J Med*, 329: 2002-11, 1993.
35. Blough, V. and Zafiriou, C., Reaction of superoxide with nitric oxide to form peroxonitrite in alkaline aqueous solution, *Inorg Chem*, 24: 3504-3505, (1985).
36. Beckman, S., Beckman, W., Chen, J., Marshall, P. and Freeman B., Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide, *Proc Nati Acad Sci* ,87: 1620-1624, 1990.
37. Sayre, M., Smith, A. and Perry, G., Chemistry and Biochemistry of Oxidative Stress in Neurodegenerative Disease, *Current Med Chem*, 8: 721-738, 2001.
38. Moore, J., West, B., Dawson, L., Dawson, M., Molecular pathophysiology of Parkinson's disease, *Annu Rev Neurosci*, 2005, 28, 57–87.
39. Gołembiowska, K., Dziubina, A., Kowalska, M., Kamiñska, K., Paradoxical effects of adenosine receptor ligands on hydroxyl radical generation by L-DOPA in the rat striatum, *Pharmacol Rep*, 60: 319-330, 2008.
40. Sanyal, J., Bandyopadhyay, S., Banerjee, T., Mukherjee, S., Chakraborty, D., Ray, B., Rao, V., Plasma levels of lipid peroxides in patients with Parkinson's disease, *Eur Rev Med and Pharmacol Sci*, 13: 129-132, 2009.
41. Cadet, L., Brannock, C.,Free radicals and the pathobiology of brain dopamine systems, *Neurochem Int,* 32: 117–131, 1998
42. Yoshikawa, T., Free radicals and their scavengers in Parkinson disease, *Eur Neurol*, 33(suppl): 60-68, 1993.

43. Dickson, W., Linking selective vulnerability to cell death mechanisms in Parkinson's disease, *Am J Pathol*, 170: 16–9, 2007.
44. Lovell, A., Xie, C and Markesbery, R., Acrolein, a product of lipid peroxidation, inhibits glucose and glutamate uptake in primary neuronal cultures, *Free Radic Biol Med*, 29: 714–720, 2000.
45. Asanuma, M., Miyazaki, I., Ogawa, N., Dopamine- or L-DOPA-induced neurotoxicity: the role of dopamine quinone formation and tyrosinase in a model of Parkinson's disease, *Neurotox Res*, 5: 165–176, 2003.
46. Double, L., Gerlach, M., Schunemann, V., Iron-binding characteristics of neuromelanin of the human substantia nigra, *Biochem Pharmacol*, 66: 489–494, 2003.
47. Smythies, J., On the function of neuromelanin. *Biol Sci*, 263: 487–489, 1996.
48. Sulzer, D., Bogulavsky, J., Larsen, K., Behr, G., Karatekin, E., Kleinman, M., Turro, N., Krantzi, D., Edwardsi, R., Greene, L. and Zecca, L., Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles, *Proc Natl Acad Sci,* 97: 11869–11874, 2000.
49. Ben-Shachar, D., Riederer, P. and Youdim, B., Iron-melanin interaction and lipid peroxidation: implications for Parkinson's disease, *J Neurochem* 57: 1609–1614, 1991.
50. Faucheux, A. Martin, M., Beaumont, C., Hauw, J., Agidl, Y., Hirsch, E., Neuromelanin associated redox-active iron is increased in the substantia nigra of patients with Parkinson's disease, *J Neurochem*, 86: 1142–1148, 2003.
51. Dexter, T., Carter, J., Wells, R, Javoy-Agid, F., Agid, Y., Lees, A., Jenner, P. and Marsden D., Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease, *J.Neurochem*, 52: 380 -389, 1989.
52. Dexter, T., Wells, R., Lees, J., Agid, F., Agid, Y., Jenner, P. and Marsden, D., Increased nigra iron content and alterations in other metal ions occurring in brain in Parkinson's disease, *J Neurochem*, 52: 1830-1836, 1989.
53. Earle, M., Studies on Parkinson's disease including x-ray fluorescent spectroscopy of formalin fixed tissue, *J Neuropath Exp Neurol*, 27: 1-14, 1968.
54. Riederer, P., Rausch, D., Schmidt, B., Kruzik, P., Konradi, C., Sofia, E., Danielczyk, W., Fisher, M and Ogris, E., Biochemical fundamentals of Parkinson's disease, *Mr Sinai J Med*, 55:21-28, 1988.
55. Gutteridge, M., Halliwell, B., Iron toxicity and oxygen radicals, *Baillieres Clin Haematol* 2: 195-256, 1989.
56. Halliwell, B., Gutteridge, M., Oxygen toxicity, oxygen radicals, transition metals and disease, *Biochem J*, 219: 1-14, 1984.
57. Halliwell, B., Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment, *Drugs Aging*, 18: 685–716, 2001.
58. Mash, C., Singer, J., Pablo, J., Basile, M., Bruce, J. and Weiner, J., Iron storage and transport markers in Parkinson's disease and MPTP treated mice, *In: Iron in Central Nervous System Disorders*, pp. 103-116, 1993.
59. Ebadi, M., Srinivasan, S. and Baxi, M., Oxidative stress and antioxidant therapy in Parkinson's disease, *Progr in Neurobiol*, 48: 1-19, 1996.
60. Perry, L., Godin, V. and Hansen, S. Parkinson's disease: A disorder due to nigral glutathione deficiency? *Neurosci Lett*, 33: 305-310, 1982.
61. Perry, L. and Young, W., Idiopathic Parkinson's disease, progressive supranuclear palsy and glutathione metabolism in the substantia nigra of patients, *Neurosci Lett*, 67: 269-274, 1986.
62. Sofic, E., Lange, W., Jellinger, K. and Riederer, P., Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease, *Neurosci Lett,* 142: 128-130, 1992.
63. Riederer, P., Sofic, E., Rauseh, D., Schmidt, B., Reynolds, P., Jellinger, K and Youdim, H, Transition metals, ferritin, glutathione, and ascorbic acid in Parkinsonian brain, *J Neurochem*, 52: 515-520, 1989.
64. Saggu, H., Cooksey, J. and Dexter, D., A selective increase in particulate superoxide dismutase activity in Parkinsonian-substantia nigra, *J Neurochem*, 53: 692-697, 1989.