Oxidative Stress and Parkinson's Disease: An Update

Satinder Kaur[1,2], Kanwalpuneet Kaur[2], Kamaldeep Kaur[2], R.K. Dhawan[2], Sonali Bajaj[1], Nisha Sharma[1], Vaishali Koul[1], Shobit Bansal[1], Nishith Kesarwani[1], Neha Morya[1], Neha Sharma[1], Navneet Khurana[*,1]

[1]School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India- 144411

[2]Department of Pharmacology, Khalsa College of Pharmacy, Amritsar, Punjab, India- 143001

Abstract: Parkinson's disease is a neurodegenerative motor disorder characterized by three cardinal symptoms i.e. tremors, bradykinesia and rigidity of muscles. The pathogenesis of PD has remained unknown largely. It is a multifactorial disease which involves neuroinflammation, oxidative stress, environmental risk factor, mitochondrial dysfunction and protein aggregation as the possible causative factors. The pathological hallmarks of the disease is the dopaminergic neurodegeneration in the SNc and the presence of Lewy bodies, the cytoplasmic proteins aggregates in the DA cells. Of the multiple factors thought to be involved in the pathogenesis of PD, most attention has been given to oxidative stress in PD due to the potential that dopamine is metabolized oxidatively to ROS and H_2O_2. Deficiency of glutathione, increased iron and increased turnover of DA in the SNc give rise to oxidative stress and the resultant cell death. Neuroinflammation also has an important role in the PD pathogenesis. The microglial cells of CNS get activated in response to immunological injury, and give rise to production of various of substances of proinflammation and neurotoxic factors production of various of substances of proinflammation and neurotoxic factors. The agents for providing a good neuroprotection in PD could be those that inhibit the pathogenic factors, such as antioxidants, anti-inflammatory drugs.

Key words: Parkinson's disease, Oxidative stress, Neuroinflammation.

Introduction

In the year 1817, a monograph was published by James Parkinson in which he described an entity that was bearing his name subsequently. The clinical features of the disease were described in the monograph. Parkinson described the symptoms in detail and he also discussed that the disorder goes on worsening progressively. Later on, after seventy years, Charcot suggested that *Parkinson's disease* (PD) be the name of the disorder (Gowers, 1893; Goetz, 1987).

PD is a motor disorder and has been characterized by three cardinal symptoms i.e. tremors, bradykinesia and rigidity of muscles. Basically the features of PD are pathlogically related to the progressive degradation of production of dopamine in substantia nigra (Kim et al., 2012).

Pathogenesis of Parkinson's disease

The pathogenesis of PD has remained unknown largely. It is a multifactorial disease which involves neuroinflammation, oxidative stress, environmental risk factor, mitochondrial dysfunction and protein aggregation as the possible causative factors.

Pathologically, the disease hallmarks are the neurodegeneration (dopaminergic neurons) in the SNc and the presence of Lewy bodies, the cytoplasmic proteins aggregates in the DA cells that are remaining (Dauer and Przedborski, 2003). Lewy bodies have also been found in various other neurodegenerative disorders also (Shults, 2006) and the primary component of Lewy bodies has been found to be the protein alpha-synuclein. The oxidative stress -induced by dopamine and the generation of free radicals are able to cause mutation in protein alpha- synuclein which leads to degeneration of DA neurons (Lotharius and Brundin, 2002). The impaired storage of the neurotransmitter (NT) DA arising from the alpha- synuclein mutations could lead to the decreased storage of DA vesicles and increased release of DA into synapse. The oxidative stress and the dysfunction of metabolism could be promoted by this labile NT's breakdown, DA in the cytoplasm that could, in turn, lead to the destruction of DA neurons.

Oxidative stress

In the biochemical processes occurring in the body, the imbalance is termed as an Oxidative stress which leads to the production of antioxidants and ROS (reactive oxygen species) resulting in damage to the macromolecular such as DNA, lipids and proteins. They also activate the pathways of apoptosis (Betarbet *et al*., 2005; Loh *et al*., 2006; Mariani *et al*., 2005; Mattson, 2006) and redox signaling dysfunction. The reactive oxygen species include the radicals such as alkoxyl, hydroxyl, peroxyl, hydrogen peroxide and superoxide anions (Fruehauf and Meyskens, 2007; Massaad and Klann, 2011). The ROS are derived from the major sources such as uncontrolled ARA (arachidonic acid) cascade, NADPH oxidase and respiratory chain of mitochondria (Farooqui, 2014). The molecular oxygen has been utilized by these processes and the ROS are produced that include H_2O_2 and $O^{2-}\bullet$ (superoxide anion). SOD (superoxide dismutase) coverts the superoxide to H_2O_2 (hydrogen peroxide) rapidly and H_2O_2 in turn is converted by catalase to H_2O (Inoue *et al*., 2003). In addition, the consumption of ATP in the brain at high levels are able to cause greater oxidative metabolism as in the formation of oxygen free radicals, electrons might be leaked.

Most attention has been given to oxidative stress in PD due to the potential that dopamine is metabolized oxidatively to ROS and H_2O_2 (Halliwell & Gutteridge 1985; Olanow1990, 1993). In the SNc, the oxidative stress and the resultant cell death can occur under the following conditions such as (i) GSH (glutathione) deficiency and thereby diminished H_2O_2 clearing capacity of brain (*ii*) reactive iron is increased that can promote the formation of OHq (*iii*) increased turnover of DA and thereby the excess formation of peroxide. Indeed, the PD patient's brain's postmortem studies have demonstrated the reduced GSH, elevated iron, and oxidative damage to DNA, proteins and lipids indicating the SNc oxidant stress state (Jenner & Olanow 1996).

The postmortem PD patient's brain studies have provided the strong evidence for enhanced oxidant stress in DA neurons of SNc (Berg *et al*., 2001). Most of the post mortem PD studies have found the impaired antioxidant status such as increased ROS, reduced GSH, increased levels of iron and enhanced DA oxidation (Jenner and Olanow et al., 1998; Castellani *et al*, 2002) that have been found to be associated with oxidant stress which as well has been reported in the post mortem PD studies. Proteins and lipids of neuronal membrane and other components of brain tissue have been reported to be damaged by free radicals induced oxidative stress. Increased peroxidation of lipids levels have been found in SNc, as suggested by increased MDA levels and decreased unsaturated and polyunsaturated fatty acids levels (Dexter *et al.*, 1989).

Oxidative Stress' sources

Many sources have been reported by various studieswhich potentially increase the production of free radical in PD, these include elevated levels of free iron, dysfunction of mitochondria, enhanced metabolism of dopamine and microglial NADPH oxidase.

Catecholamines

A large number of studies have provided the evidence that dopamine and other catecholamines are importantly involved in the free radicals production in brain. It has been reported that dopamine has found to be remained stable when it is stored in the vesicles in the synapse. Impaired DA in vescicles, that may be due to oxidative stress or increased protofibrils of alpha synuclein lead to elevated DA levels in brain. It is easily metabolized by an enzyme present on the outer membrane of mitochondria i.e. MAO (monoamine oxidase). The products of metabolism are 3,4- dihydroxyphenylacetic acid and hydrogen peroxide. DA can also undergo autoxidation and form superoxide and quinones. DA metabolism has found to be enhanced greatly in PD patients (Betarbet *et al.*, 2005; Cardoso *et al.*, 2005; Greenamyre *et al.*, 2001; Mariani *et al.*, 2005; Maguire-Zeiss *et al.*, 2005).

$$\text{Dopamine} + O_2 + H_2O \longrightarrow \text{Dihydroxyphenil acetaldehyde} + NH_3 + H_2O_2$$

Autoxidation of DA or Levodopa (drug of choice for PD treatment) results in quinone formation and generates free radicals such as hydrogen peroxide and superoxide radical which may interact with transition metal ion such as Fe^{2+}. DA and Levodopa quinone are converted aminochromes by oxidation easily and melanin is formed finally by polymerization (Asanuma *et al*., 2003). Thus, treatment with levodopa leads to higher concentrations of dopamine in brain which may fascilitate the progressive damage to the DA neurons by oxidative stress potentially in PD patients (Gołembiowska K., 2008). As discussed earlier, DA metabolism is enhanced greatly in PD patients and the activity of tyrosine hydrolase has found to be increased in DA neurons that are saved. To recover the shortage of DA, this mechanism is probably represented as a compensation.

Contribution of Iron

It has been demonstrated by numerous studies by the use of various of analytical methods that in PD patients, the levels of iron are increased in the SNc (Dexter et al 1989a, Olanow & Youdim 1996). LAMMA (Laser microprobe) studies have indicated that in the dopaminergic neurons, the iron gets accumulated

primarily within the neuromelanin granules (Good et al 1992). The specific antibodies of ferritin neuronal isoforms have not shown any evidence of a increase in compensation (Connor et al 1995) which indicates that the iron can be in a reactive form and unbound. Iron infusion into the SNc of rats/ mice induces a parkinson's model that has been characterized by dopamine loss from striatum (progressive and concentration dependent), behavioral changes and SNc neurodegeneration (Sengstock et al 1993, 1994). It is not known that how the iron gets accumulated within the substantia nigra in parkinson's. The receptors lactoferrin have been reported to be increased on SNc neurons in parkinson's patients and might be due to this, the iron gets accumulated preferentially within these cells (Faucheux et al 1995). The accumulation of iron is primary or secondary in PD is not clear. The accumulation of iron can be seen in the areas affected in a various other conditions of neurodegeneration also. Furthermore, it has been observed that in SNc, the iron levels are increased following the 6-OHDA (6-hydroxydopamine) lesions and MPTP administration (Temlett et al 1994, Oestreicher et al 1994). So, these reports indicate that the accumulation of iron can be secondary to degeneration of cells resulting from a various causes. However, the potential importance of iron in PD is not negated by this as the contribution of iron to cell death can still be there even if its accumulation is secondary to some other cause.

So, as iron is present in higher levels in different parts of the basal ganglia such as SNc, putamen and globus pallidus (Dexter et al.,1989;Earle, 1968), therefore it may make the of DA neurons of SNc more vulnerable to oxidative free radicalstoxicity in the SNc of PD patients especially where levels of iron is elevated (Dexter *et al*.,1989; Riederer, 1988). The damage by oxidative stress is mediated to the components of cell by iron through the transfer of 1 electron by the reaction called as Fenton reaction. The unstable OH• (hydroxyl) radical is produced by this rection which then oxidizes any of the protein, nucleic acid, lipid and carbohydrate that is proximate (Gutteridge *et al*., 1989; Halliwell *et al*., 1984).

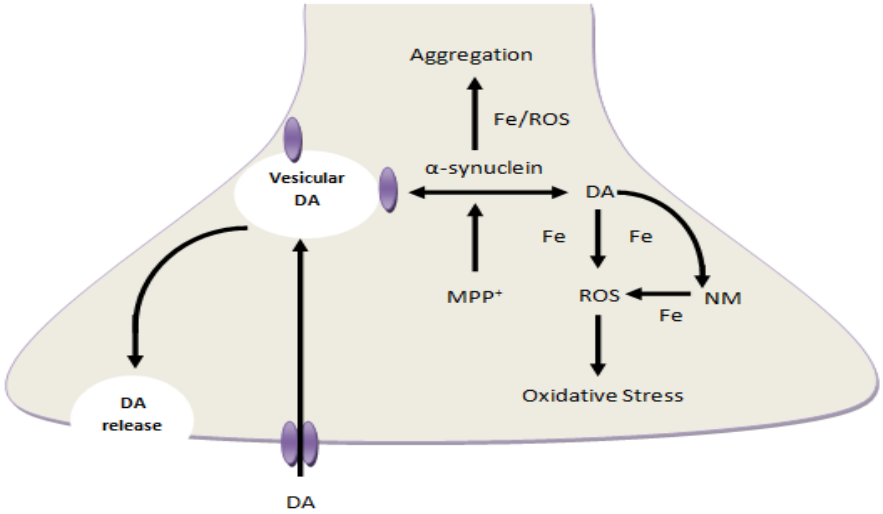

Figure 1: Oxidative stress mechanism in PD

(1) DA neurons are degenerated in SNc in PD. Neuromelanin and Fe^{2+} are contained in these, that resulted from the oxidation of DA, in a DAQ and ROS generating process. Neuromelanin binds with Fe^{2+} that facilitates the hydroxyl l radical formation by the Fenton reaction that highly toxic.

(2) Parkinson's familial form may be caused by mutations in α-synuclein or by its multiplication. Some other proteins like PINK1 DJ-1, LRRK2 and parkin may also be involved in causing PD.

(3) α-synuclein is found in LN (Lewy neuritis) and LN as a component in aggregated form.

(4) MPTP is a mitochondrial toxins which by MAO-B is converted into MPP^+ and inhibits the ETC(mitochondrial electron transportchain) complex I.

Contribution of Dysfunctioning mitochondria

Mitochondria are the most important intracellular organelles for energy generation and have been related with ROS generation (Kim *et al*., 2006). In physiological state, respiration chain in mitochondria consumes a predominant amount of oxygen (Sherratt, 1991). As the most commonly used PD model, MPTP is selectively detrimental to DA neurons in SN. MPTP, by MAO-B in glial cells, is converted to active molecule MPP^+, by glial monoamine oxidase B (MAO-B) and transported by DAT into neurons, to cause the inhibition of complex I in the mitochondrial electron transport chain (Kopin, 1987). DA neurons have insufficient energy and generate free radicals such as superoxide, leading to neuronal death in the SN. In post-mortem idiopathic PD brains, complex I deficiency in the SN mitochondria has been identified. Neurotoxin MPTP, rotenone and paraquat induce DA neuron loss through the inhibition of complex I. Somatic mutations of mtDNA have been related to PD by showing that SN neurons from PD brains have a higher deletion of mtDNA. Moreover, a variety of PD genes have been associated with mitochondrial dysfunction in different ways (Mortiboys *et al*., 2007; Reichmann and Janetzky, 2000; Schapira, 2007). The mice that are α- synuclein knocked out are resistant to mitochondrial toxins. α-synuclein overexpression in transgenic mice (transgenic) enhances neurotoxicity of MPTP with mitochondrial dysfunction (Lotharius and Brundin, 2002). Parkin, DJ-1 and PINK1 associate with mitochondria in direct or indirect pathways, including protection from apoptosis or reduction of oxidative stress (Harley *et al*., 1993).

In the respiratory chain of mt (mitochondrial)- complex I in the PD patient's SNc, the activity has been found to be decreased by 30%– 40% (Schapira et al 1990). No other regions of brain have found to be affected. The MSA (multiple system atrophy) patients also have an extensive neurodegeneration in substantia nigra and levodopa exposure as well, however, they are not found to have the same mitochondrial defect. In PD patients, the defect in complex I has been reported in their muscle and platelets, but consistency of results is less, in muscle especially (DiMauro 1993). So, the reduced activity of mt- complex I as a PD causing factor remained a mystery. No detection of toxins like MPTP has been there. No abnormality specifically in the mt- complex I subunits and in the nuclear or mitochondrial genes encoding for the same has been detected. Recently, a defect in the mt- complex I has been detected in mtDNA carried by cybrids that have been derived from the platelets of PD patients (Swerdlow et al 1996). It has been indicated that the defect is present in the genome that of mitochondria and is transferrable through a number of ways. The factors which could be responsible for such defect are: following oxidant stress, o toxic or a mutation that is inherited, although in PD, disease-related mutations in mtDNA have not been confirmed and both complexes of mitochondria I and IV have been found to be affected typically by oxidative stress. A defect in mt- complex I could have a contribution PD neurodegeneration by decreasing the synthesis of ATP

(Adenosine- triphosphate). Cellular ATP can be depleted by the MPPC or MPTP induced inhibition of complex I, studied in synaptosomes of mouse brain (Scotcher et al 1990). Studies on animals models have indicated that a 40% decrease (or lesser) in activity of complex I has not found tocompromise the cellular levels of ATP (Davey & Clark 1996). A reduction in the immunostaining of α- ketoglutarate dehydrogenase (α-KGD) in PD has also been repoted (Mizuno et al 1994). The metabolism of cellular energy would be affected adversely by the combination of both the decreased activity of complex I and α-KGD more likely than by an enzyme defect alone. A defect in mit- complex I defect could also damage cell through the generation of free radicals at this site directly or by increasing respiration by a compensatory mechanism at complex II. The coenzyme Q, which is a redox component (that accepts electrons from mit- complex I or mit- complex II) of respiratory chain of the mitochondria and the scavengers of free radicals can attenuate the toxicity induced by MPTP (Schulz et al 1995a). A defect in mit- complex I can have contribution to the apoptosis development as well. Numerous evidences have suggested that the decrease in the membrane potential of mitochondria resulting from the proton pump impairement leading to permeability transition pore opening in mitochondria and the releasing the small proteins of mitochondria signalling for apoptosis onset. As the main site for pumping of protons is the mit- complex I, it is quite possible that the defect in complex I may make the neurons more vulnerabile leading to apoptosis in PD.

Contribution of microglia

Microglia are the cells which are like macrophage and produce ROS in large amounts consequently through the NADPH oxidase of phagocytes. The microglia- produced ROS have been proposed to be involved in the defense of host and debris removal from the central nervous system (Lavigne *et al.,* 2001; Haslund-Vinding et al. 2017). The activated microglia- generated oxidative stress, however has been thought to have participation in parkinson's pathogenesis (Chun et al. 2001; Onyou, 2013). In the various PD models such as exposure to rotenone, MPTP, infection and Paraquat, the microglial cells in activated form have found to release nitric oxide and superoxide in the SN (Gao *et al.,* 2002; Delgado, 2003; Gao *et al.,* 2003a; Gao *et al.,* 2003b; Wu *et al.,* 2003; Thomas *et al.,* 2004; Scheller *et al.,* 2005; Block *et al.,* 2006). Microglial cells in activated form have been known to be involved in the expression of several enzymes such as myeloperoxidase iNOS, COX-2, NADPH oxidas and iNOS which are responsible for the processes neuroinflammation that is oxidative stress mediated coinciding with the changes in neurochemicals such as a decreased synthesis of dopamine (Wu *et al.,* 2003; Scheller *et al.,* 2005) and thus give contribution in PD progression and also promote the neurotoxins induced neurotoxicity (Gao *et al.,* 2002; Casarejos *et al.,* 2006).

Brain damage caused by oxidative stress

The oxidative damage has been reported by many studies in the PD patients' brains. In the SNc, increased MDA (malondialdehyde) levels and increased lipid hydroperoxide levels (the products of lipid peroxidation products) have been reported to be found however the same has not been found in the cerebellum of parkinson's patients (Dexter et al 1989b, 1994b). In the DA neurons that are surviving, the increased 4-hydroxynonenal staining has been observed. The 4-hydroxynonenal is a lipid peroxidation

product having protein altering capacity and thereby fascilitating the cellular toxicity (Yoritaka et al 1996). In addition, the elevated 8-hydroxy-2-deoxyguanosine and protein carbonyls levels reflect the oxidative damage to DNA and proteins respectively. These have been observed in the SNc and in the number of other regions of brain of PD patients (Sanchez- Ramos et al 1994, Alam et al 1997a, Alam et al 1997b). So, it has been suggested by these results that, the oxidative damage is widespread in PD. As the treatment with levodopa is received by most of parkinson's patients, the contribution of oxidative metabolites to the oxidative damage in postmortem detections seems uncertain. The neurodegeneration of cultured DA neuronshas been observed to be induced by levodopa (Mytilineou et al 1993, Walkinshaw & Waters 1995) but was not found to be toxic to the DA neurons in normal humans and animals. The mechanisms of defense are impaired in PD, therefore there may be a different situation. In the 6-hydroxydopamine pretreated rodents, the lipid peroxidation and neurodegeneration can indeed be augmented by Levodopa (Ogawa et al 1994). In PD, the oxidative damage that is occurring, is occurring as a primary event or is occuring secondary to some drugs, postmortem events or an alternate physiology remains undetermined.

Role played by glutathione

When one or more of the antioxidant defense mechanisms that are occurring naturally get defected, PD neurodegeneration could be lead (Jenner & Olanow 1996). There have not been detected any basic defects in glutathione peroxidase, catalase, alpha- tocopherol or ascorbic acid levels. The activity of Mn-SOD is increased that has been found to be increased consistently with an adaptive elevation in the enzyme's inducible form. The GSH (reduced glutathione) has been found to be decreased selectively in parkinson's patients in SNc and this finding has taken great attention (Sofic et al 1992, Sian et al 1994a). No GSH levels reduction has been detected in PD in the areas of brain other than SNc and in degenerative disorder other than PD. Reduced levels of GSH may cause impairment of clearance of H_2O_2 and promotion of the formation of OHq in the presence of elevated iron levels particularly. In PD, the cause of reduction in the levels of GSH is not known. The defects have not been found in the enzymes which are mainly involved in the synthesis of glutathione. The enzyme, glutamyltranspeptidase levels have been reported to be increased significantly. This enzyme has been found to be responsible for the metabolism and translocation of the oxidized form of glutathione and precursors of glutathione respectively (Sian et al 1994b). The levels of glutamyltranspeptidase may be increased either compensatory for the removal of the oxidized form of glutathione that is highly toxic or as an attempt for recruiting the precursors of glutathione into the cell for the replenishment of decreased GSH levels by surviving cells. A comparable GSH defect, as that found in parkinsons, has also been reported in the autopsy reports in the nigral region of patients having incidental Lewy bodies and might having preclinical PD (Dexter et al 1994a). However, no changes were detected in oxidative stress markers, mit- complex I and iron suggesting the reduced levels of GSH in PD as an initial biochemical defectin the disease. A selective alpha- glutamylcysteine synthetase inhibitor, buthionine sulphoximine has been demonstrated to induce the reduction in the levels of GSH and has also been found to be toxic to the cultured DA neurons (Mytilineou et al 1998). Buthionine sulphoximine administration in rats at the doses sufficient to decline the GSH levels to almost half, parallel to the loss occuring in PD, has not been found to affect the TH (tyrosine hydroxylase)–positive cells number in the substantia nigra (Toffa et al

1997). The depletion of GSH to this level, however, fascilitates the degeneration of neurons which is observed in rodents when they are treated with neurotoxins like MPPC or 6-hydroxydopamine (Pileblad et al 1989, Wullner et al 1996). This has been suggested by these reports that the decreased levels of GSH may not themselves damage the DA neurons but may increase their vulnerability to get damaged by other neurotoxins.

Role played by neuroinflammation

Neuroinflammation has been reported as a causative factor in various disorders. Neuroinflammation has been known as a condition or a process by which brain is responsive to various injuries, diseases and infections (Schmidt *et al*., 2005; Taupin, 2008). In neuroinflammatory process, two important immune cells are involved:

i. Microglial cells

ii. Macrophages, monocytes and lymphocytes

Neuroinflammation has been reported for its interference with the BBB (blood brain barrier) and thereby allowing the hematopoietic cells to leave the circulation and contact with the site of injury (Lossinsky and Shivers, 2004; Taupin, 2008). Responding to injuries, immune cells cause the removal of cellular dump and synthesis and release of factors like transforming growth factors, ROS, NO, interlukins, glutamate, chemokines and cytokines (Taupin, 2008). These factors have been found to play both good and bad roles inside the cell. So, playing bad role, they cause injury to neurons (Stoll *et al.*, 2002; Loane and Byrnes 2010). Mature astrocytes have also been reported to have an important participation in neuronal injury (Taupin, 2008). The activation of mature astrocytes has been thought to be compulsary in the initiation of immune response, repair of BBB and attenuation of further neurodegeneration (Lossinsky and Shivers, 2004; Taupin, 2008).

Neuroinflammation has now been well reported for its involvement in many neurogenic diseases and disorders such as PD, multiple sclerosis, HD, amyotrophic lateral sclerosis and AD (Hensley *et al.*, 2006; Klegeris *et al.*, 2007). The above mentioned immune cells in the activated or over activated form have been found to be involved in the neuroinflammatory process, releasing the mediators of proinflammation and would consequently causing the reduction in neuroprotection and neurone repairing mechanisms thereby increasing neurodegeneration resulting in neurodegenerative diseases (Bonifati and Kishore, 2007; Donnelly and Popovich, 2008).

It has been reported that neuroinflammation has an important role in the PD pathogenesis. The microglial cells of CNS get activated in response to immunological injury, and give rise to production of various of substances of proinflammation and neurotoxic factors production of various of substances of proinflammation and neurotoxic factors such as superoxide, NO, eicosanoids, TNF-α and interleukin-1β (Mogi *et al.*, 1994a, Mogi *et al.*, 1994b, Boka *et al.*, 1994, Liu *et al.*, 2002; Liu and Hong, 2003). It has been reported that in SNc, the microglial cells get concentrated markedly (Lawson *et al.*, 1990; Kim *et al.*, 2000). Upon activation, they get transformed into amoeboid round and big bodies having thick short processes from

striated bodies. The microglial cells in the activated form have been found to share many surface molecules with Mac- 1 (macrophage antigen complex-1) (Flaris *et al.,* 1993). In the SNc of PD brains, activated microglia has been found to over express the markers of neuroinflammation including increased ferritin (Mirza *et al.,* 2000), complement 3 receptor (Banati *et al.,* 1998; Mirza *et al.,* 2000), cyclooxygenase-2 (COX- 2) (Knott *et al.,* 2000), inducible nitric oxide synthase (iNOS) (Hunot *et al.,* 1996; Knott *et al.,* 2000), Ig (immunoglobin) E receptor CD23 of low affinity, IF (interferon)- γ (Hunot *et al.,* 1999), TNF- α) (Boka *et al.,* 1994; Hunot *et al.,* 1999), EMB11 macrophage marker (Banati *et al.,* 1998) and the major histocompatibility (MHC) II complex HLA-DR (McGeer *et al.,* 1988b).

The increased NADPH oxidase and inducible nitric oxide synthetase (iNOS) expression result in the generation and release of superoxide anion and NO free radicals (Etienne and Stéphane, 2009) respectively. The NO and superoxide anion free radicals, in turn, might undergo reaction and cause the generation of peroxynitrite that has been found to be really reactive and can induce oxidative protein damage in DA neurons. H_2O_2 might be produced by dismutation of superoxide anion radicals and in turn the hydroxyl radicals which are greatly reactive can be produced in the presence of higher free ferrous iron concentrations, by the H_2O_2, thereby can damage the neuronal cell oxidatively. Myeloperoxidase is expressed by reactive astrocytes in higher concentrations, which from H and Cl, produces hypochlorous (HOCl). HOCl can cause oxidative damage to DA neurons directly e.g. through conversion of amine to chloramine. It may also elevate the levels of damaging hydroxide radicals when it reacts with superoxide anion radicals. Myeloperoxidase can produce the free radical, reactive NO (RNO), by catalyzing NO_2, RNO in turn might also add to oxidative protein damage. Other than these neurodestruction mechanisms, COX- 2 expression in the damaged DA neurons may also give rise to oxidant stress associated with inflammation that can cause further damage.

Conclusion and future prospects

For the control of PD, the thing which is most desirable is the elimination of primary pathology. Various effective therapeutic strategies might be devised to provide neuroprotection, on the current huge knowledge regarding the pathology, pathogenesis, and cell death mechanisms in PD. However, the effectiveness seems to be unlikely because of the probable involvement of different genetic and environmental factors' contribution to PD development. The agents for providing a good neuroprotection could be those that inhibit the pathogenic factors, such as antioxidants, anti-inflammatory drugs, trophic factors, inhibiting rise in free cytosolic calcium, bioenergetics and the excitotoxicity interfering agents. Most of the focus has been on antioxidants.

Furthermore, it is essential to understand the inter- relationship of these different mechanisms to design the effective treatments to control PD in future.

References

1. Alam ZI, Daniel SE, Lees AJ, Marsden CD, Jenner P, Halliwell B. 1997a. A generalized increase in protein carbonyls in the brain in Parkinson's but not Incidental Lewy Body disease. *J. Neurochem.* 69:1326–29

2. Alam ZI, Jenner A, Daniel SE, Lees AJ, Cairns N, et al. 1997b. Oxidative DNA damage in the parkinsonian brain; a selective increasein 8-hydroxyguanine in substantia nigra? *J. Neurochem.* 69:1196–203

3. Asanuma, M., Miyazaki, I., Ogawa.N.(2003).Dopamine or L-dopa neurotoxicity: the role of dopamine quinine formation and tyrosinase in a model of Parkinson's disease. Neurotox Res ,5: 165–76.

4. Banati R. B., Daniel S. E. and Blunt S. B. (1998) Glial pathology but absence of apoptotic nigral neurons in long-standing Parkinson's disease. Mov. Disord. 13, 221–227.

5. Berg D, Gerlach M, Youdim MB, Double KL, Zecca L, Riederer P and Becker G (2001). Brain iron pathways and their relevance to parkinson's disease. J. Neurochem, 79: 225-236.

6. Betarbet, R., Sherer, T. B., Greenamyre, J. T.(2005). Ubiquitin-proteasome system and Parkinson's diseases. Experimental Neurology, 191:S17-27.

7. Block ML, Li G, Qin L, Wu X, Pei Z, Wang T, Wilson B, Yang J, Hong JS. Potent regulation of microglia-derived oxidative stress and dopaminergic neuron survival: substance P vs. dynorphin. FASEB Journal. 2006;20:251–8.

8. Boka G, Anglade P, Wallach D, Javoy-Agid F, Agid Y, Hirsch EC. Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. Neurosci Lett. 1994 May 19;172(1-2):151-4.

9. Bonifati DM, Kishore U. Role of complement in neurodegeneration and neuroinflammation. Mol Immunol. 2007;44:999–1010.

10. Cardoso, S. M., Moreira, P. I., Agostinho, P., Pereira, C., Oliveira, C. R. (2005). Neurodegenerative pathways in Parkinson's disease: therapeutic strategies. Current Drug Targets - CNS & Neurological Disorders, 4 :405-19.

11. Casarejos MJ, Menendez J, Solano RM, Rodriguez-Navarro JA, Garcia de Yebenes J, Mena MA. Susceptibility of rotenone is increased in neurons from parkin null mice and is reduced by minocycline. Journal of Neurochemistry. 2006;97:934–46.

12. Castellani, R. J., Perry, G., Siedlak, S. L., Nunomura, A., Shimohama, S.,Zhang, J., Montine, T., Sayre, L. M., and Smith, M. A. (2002). Hydrox-ynonenal adducts indicate a role for lipid peroxidation in neocortical and brainstem Lewy bodies in humans. Neurosci. Lett, 319:25–28

13. Chun HS, Gibson GE, DeGiorgio LA, Zhang H, Kidd VJ and Son JH. Dopaminergic cell death induced by MPP(+), oxidant and specific neurotoxicants shares the common molecular mechanism. J Neurochem. 76(4):1010-21; 2001.

14. Connor JR, Snyder BS, Arosio P, et al. 1995. Quantitative analysis of isoferritins in select regions of aged, Parkinsonian, and Alzheimer diseased brains. *J. Neurochem.* 65:717–24

15. Dauer , W., Przedborski, S.(2003). Parkinson's disease: Mecha-nisms and models. Neuron, 39:889 – 909.

16. Davey GP, Clark JB. 1996. Threshold effects and control of oxidative phosphorylation in non-synaptic rat brain mitochondria. *J. Neurochem.* 66:1617–24

17. Delgado M, Ganea D. Neuroprotective effect of vasoactive intestinal peptide (VIP) in a mouse model of Parkinson's disease by blocking microglial activation. FASEB Journal. 2003;17:944–6.

18. Dexter DT, Carter CJ, Wells FR, et al. 1989b. Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J. Neurochem.* 52:381–89

19. Dexter DT, Sian J, Rose S, et al. 1994. Indices of oxidative stress and mitochondrial function in individuals with incidental Lewy body disease. *Ann. Neurol.* 35:38–44

20. Dexter DT,Wells FR, Lees AJ, et al. 1989a. Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. *J. Neurochem.* 52:1830–36

21. DiMauro S. 1993. Mitochondrial involvement in Parkinson's disease: the controversy continues. *Neurology* 43:2170–72

22. Earle,K. M.(1968). Studies on Parkinson's disease including X-ray fluorescent spectroscopy of formalin fixed brain tissue. J. Neuro-pathol. Exp. Neurol. 27: 1–14.

23. Etienne C Hirsch and Stéphane Hunot. Neuroinflammation in Parkinson's disease: a target for neuroprotection? Neurology; 8 (4): 382-397.

24. Farooqui ,T.(2014). Oxidative stress and age-related olfactory memory impairment in the honeybee Apis mellifera. Genetics of aging 5: 1-4.

25. Faucheux B, Nillesse N, Damier P, et al. 1995. Expression of lactoferrin receptors is increased in the mesencephalon of patients with Parkinson's disease. *Proc. Natl. Acad. Sci. USA* 92:9603–7

26. Flaris NA, Densmore TL, Molleston MC, Hickey WF.Characterization of microglia and macrophages in the central nervous system of rats: definition of the differential expression of molecules using standard and novel monoclonal antibodies in normal CNS and in four models of parenchymal reaction. Glia. 1993 Jan;7(1):34-40

27. Fruehauf, J.P., Meyskens ,F.L. (2007) Reactive Oxygen Species: A Breath of Life or Death? clinical cancer research 13: 789–794.

28. Gao HM, Hong JS, Zhang W, Liu B. Distinct role for microglia in rotenone-induced degeneration of dopaminergic neurons. Journal of Neuroscience. 2002;22:782–90.

29. Gao HM, Liu B, Hong JS. Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopamingeric neurons. Journal of Neuroscience. 2003a;23:6181–7.

30. Gao HM, Liu B, Zhang W, Hong JS. Critical role of microglial NADPH oxidase-derived free radicals in the in vitro MPTP model of Parkinson's disease. FASEB Journal. 2003b;17:1954–6.

31. Goetz, C.G. 1987. Charcot, the Clinician: the Tuesday Lessons. Excerpts from Nine Case Presentations on General Neurology Delivered at the Salpetriere Hospital in FAHN: CLINICAL DESCRIPTION OF PARKINSON'S DISEASE 131887-88 by Jean-Martin Charcot. Translated with commentary: pp. 123-124. Raven Press. New York.

32. Golembiowska, K., Dziubina, A., Kowalska, M., Kaminska, K.(2009). Effect of adenosine A(2A) receptor antagonists on L-DOPA-induced hydroxyl radical formation in rat striatum. Neurotox Res, 15:155–66.

33. Good P, Olanow CW, Perl DP. 1992. Neuromelanin- containing neurons of the substantia nigra accumulate iron and aluminum in Parkinson's disease: A LAMMA study. *Brain Res.* 593:343–46

34. Gowers, W.R. 1893. A Manual of Diseases of the Nervous System, Vol. II, 2nd edit.: p. 644. Blakiston. Philadelphia.

35. Greenamyre, J. T., Sherer, T. B., Betarbet, R., Panov, A. V.(2001). Complex I and Parkinson's disease. IUBMB Life, 52: 135-41.

36. Gutteridge, M., Halliwell, B.(1989). Iron toxicity and oxygen radicals. Baillieres Clin Haematol 2: 195-256.

37. Halliwell B, Gutteridge J. 1985. Oxygen radicals and the nervous system. *Trends Neurosci.* 8:22–29

38. Halliwell, B., Gutteridge, M.(1984). Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J, 219: 1-14.

39. Harley, A., Cooper, J. M., Schapira, A. H., Iron induced oxidative stress and mitochondrial dysfunction: relevance to Parkinson's disease, *Brain Res.* 627:349-353, 1993.

40. Haslund-Vinding J, McBean G, Jaquet V, Vilhardt F. NADPH oxidases in oxidant production by microglia: activating receptors, pharmacology and association with disease. Br J Pharmacol. 2017 Jun;174(12):1733-1749.

41. Hensley K, Mhatre M, Mou S et al. On the relation of oxidative stress to neuroinflammation: lessons learned from the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. Antioxid Redox Signal. 2006;8:2075–87.

42. Hunot S, Bernard V, Faucheux B, Boissiere F, Leguern E, et al. 1996. Glial cell line-derived neurotrophic factor (GDNF) gene expression in the human brain: a post mortem in situ hybridization study with special reference to Parkinson's disease. *J. Neural Transm.* 103: 1043–52

43. Hunot S, Brugg B, Ricard D, et al. 1999. Nuclear translocation of NF-·B is increased in dopaminergic neurons of patients with Parkinson disease. *Proc. Natl. Acad. Sci. USA* 94:7531–36

44. Inoue, M., Sato, E.F., Nishikawa, M., Hiramoto, K., Kashiwagi, A. and Utsumi, K.(2004). Free radical theory of mitochondria-dependent apoptosis and metamorphosis. Redox Reports, 9: 1-11.

45. Jenner P, Olanow CW. 1996. Oxidative stress and the pathogenesis of Parkinson's disease. *Neurology* 47:161–70

46. Jenner P, Olanow CW. 1998. Understanding cell death in Parkinson's disease. *Ann. Neurol.*44 (3): S72-84.

47. Kim TH, Cho KH, Jung S W, and Myeong SL (2012),Herbal Medicines for Parkinson's Disease: A Systematic Review of Randomized Controlled Trials.PLoS One.; 7(5): e35695.

48. Kim YS, Joh TH. Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. Exp Mol Med. 2006 Aug 31;38(4):333-47.

49. Klegeris A, McGeer PL. R-(−)-Deprenyl inhibits monocytic THP-1 cell neurotoxicity independently of monoamine oxidase inhibition. Exp Neurol. 2000;166:458–464.

50. Knott, C., Stern, G., and Wilkin, G. P. (2000). Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. *Mol. Cell Neurosci*. 16, 724–739.

51. Kopin, I.J.(1987). MPTP: an industrial chemical and contaminant of illicit narcotics stimulates a new era in research on Parkinson's disease. Environ Health Perspect, 75:45-51.

52. Lavigne MC, Malech HL, Holland SM, Leto TL (2001) Genetic requirement of p47phox for superoxide production by murine microglia. FASEB J 15: 285–287.

53. Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience. 1990;39:151–70.

54. Liu B., Gao H. M., Wang J.-Y., Jeohn G.-H., Cooper C. L. and Hong J. S. (2002) Role of nitric oxide in inflammation-mediated neurodegeneration. Ann. NY Acad. Sci. 962, 700–715.

55. Loh, K. P., Huang, S. H., De Silva, R., Tan, B. K., Zhu, Y. Z. (2006). Oxidative stress: apoptosis in neuronal injury. Current Alzheimer Research, 3:327-37.

56. Lossinsky AS, Shivers RR. Structural pathways for macromolecular and cellular transport across the blood-brain barrier during inflammatory conditions. Histol Histopathol. 2004;19:535–64.

57. Lotharius, J. & Brundin, P. (2002). Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein. Nat Rev Neuroscience, 3: 932–942.

58. Maguire-Zeiss, K. A., Short, D. W., Federoff, H. J.(2005). Synuclein, dopamine and oxidative stress: co-conspirators in Parkinson's disease? Brain Research. Molecular Brain Research, 134: 18-23.

59. Mariani, E., Polidori, M. C., Cherubini, A., Mecocci, P.(2005). Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview. Journal of Chromatography B: Analytical Technologies in the Biomedical & Life Science,. 827: 65-75.

60. Massaad, C. A., and Klann, E. (2011). Reactive oxygen species in the regulation of synaptic plasticity and memory. Antioxid Redox Signal, 14: 2013–2054.

61. Mattson, M. P.(2006). Neuronal life-and-death signaling, apoptosis, and neurodegenerative disorders. Antioxidants & Redox Signaling. 8: 1997-2006.

62. McGeer PL, Itagaki S, McGeer EG. 1988. Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. *Acta Neuropathol.* 76:550–57

63. Mirza, B., Hadberg, H., Thomsen, P., and Moos, T. (2000). The absence of reactive astrocytosis is indicative of a unique inflammatory process in Parkinson's disease. *Neuroscience* 95, 425–432.

64. Mizuno Y, Matuda S, Yoshino H, et al. 1994. An immunohistochemical study on α-ketoglutarate dehydrogenase complex in Parkinson's disease. *Ann. Neurol.* 35:204 10

65. Mogi M, Harada M, Kondo T, Riederer P, Inagaki H, Minami M, Nagatsu T. Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients. Neurosci Lett. 1994a Oct 24;180(2):147-50.

66. Mogi M, Harada M, Riederer P, Narabayashi H, Fujita K, Nagatsu T. Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. Neurosci Lett. 1994b Jan 3;165(1-2):208-10.

67. Mortiboys, H.J., Schaefer, J., Reichmann, H., Jackson, S.(2007). Mitochondrial dysfunction in Parkinson's disease--revisited. Neurol Neurochir Pol, 41:150-159.

68. Mytilineou C, Han S-K, Cohen G. 1993. Toxic and protective effects of L-DOPA on mesencephalic cell cultures. *J. Neurochem.* 61: 1470–78

69. Mytilineou C, Leonardi EK, Radcliffe P, Heinonen EH, Han SK, et al. 1998. Deprenyl and desmethylselegiline protect mesencephalic neurons from toxicity induced by glutathione depletion. *J. Pharm. Exp. Ther.* 284:700–6

70. Oestreicher E, Sengstock GJ, Riederer P, Olanow CW, Dunn AJ, Arendash G. 1994. Degeneration of nigrostriatal dopaminergic neurons increases iron within the substantia nigra: a histochemical and neurochemical study. *Brain Res.* 660:8–18

71. Ogawa N, Asanuma M, Kondo Y, et al. 1994. Differential effects of chronic L-DOPA treatment on lipid peroxidation in the mouse brain with or without pretreatment with 6-hydroxydopamine. *Neurosci. Lett.* 171:55–58

72. Olanow CW,Youdim MHB.1996. Iron and neurodegeneration: prospects for neuroprotection. in *Neurodegeneration and Neuroprotection in Parkinson's Disease*, ed.CW

73. Olanow CW. 1990. Oxidation reactions in Parkinson's disease. *Neurology* 40:32–37

74. Olanow CW. 1993. A radical hypothesis for neurodegeneration. *Trends Neurosci.* 16: 439– 44

75. Onyou Hwang. Role of Oxidative Stress in Parkinson's Disease. Exp Neurobiol. 2013 Mar; 22(1): 11–17.

76. Pileblad E, Magnusson T, Fornstedt B. 1989. Reduction of brain glutathione by L-buthionine sulfoximine potentiates the dopaminedepleting action of 6-hydroxydopamine in rat striatum. *J. Neurochem.* 52:978–80

77. Reichmann, H., Janetzky, B., (2000). Mitochondrial dysfunction--apathogenetic factor in Parkinson's disease. J Neurol, 247: 63-68.

78. Riederer, P., Rausch, W.D., Schmidt, B., Kruzik, P., Konradi, C., Sofia E., Danielczyk, W., Fisher ,M. and Ogris, E. (1988). Biochemical fundamentals of Parkinson's disease. Mr. Sinai J. Me, 55: 21-28

79. Sanchez-Ramos J, Overvik E, Ames BN. 1994.Amarker of oxyradical-mediated DNA damage (8-hydroxy-20deoxyguanosine) is increased in nigro-striatum of Parkinson's disease brain. *Neurodegeneration* 3:197–204

80. Schapira AHV, Cooper JM, Dexter D, et al. 1990. Mitochondrial complex I deficiency in Parkinson's disease. *J. Neurochem.* 54:823– 27

81. Schapira, A.H. (2007). Mitochondrial dysfunction in Parkinson's disease. Cell Death Differ, 14: 1261-1266.

82. Scheller C, Sopper S, Jenuwein M, Neuen-Jacob E, Tatschner T, Grunblatt E, ter Meulen V, Riederer P, Koutsilieri E. Early impairment in dopaminergic neurotransmission in brains of SIV-infected rhesus monkeys due to microglia activation. Journal of Neurochemistry. 2005;95:377–87.

83. Schmidt OI, Heyde CE, Ertel W, Stahel PF. Closed head injury--an inflammatory disease? Brain Res. Brain Res Rev. 2005;48:388–99.

84. Schulz JB, Henshaw DR, Matthews RT, Beal MF. 1995a. Coenzyme Q10 and nicotinamide and a free radical spin trap protect against MPTP toxicity. *Exp. Neurol.* 132:279–83

85. Schulz JB, Matthews RT, Muqit MMK, Browne SE, Beal MF. 1995b. Inhibition of neuronal nitric oxide synthase by 7-nitroindazole protects against MPTP-induced neurotoxicity in mice. *J. Neurochem.* 64:936–39

86. Scotcher KP, Irwin I, DeLanney LE, et al. 1990. Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 1-methyl-4-phenylpyridinium ion on ATP levels of mouse synaptosomes. *J. Neurochem.* 54:1295–301

87. Sengstock G, Olanow CW, Dunn AJ, Barone S, Arendash G. 1994. Progressive changes in striatal dopaminergic markers, nigral volume, and rotational behavior following iron infusion into the rat substantia nigra. *Exp. Neurol.* 130:82–94

88. Sengstock GJ, Olanow CW, Dunn AJ, Arendash GW. 1993. Infusion of iron into the rat substantia nigra: nigral pathology and dose-dependent loss of striatal dopaminergic markers. *J. Neurosci. Res.* 35:67–82

89. Sherratt, H.S. (1991). Mitochondria: structure and function. Rev Neurol (Paris), 147: 417-430.

90. Shults, C. W. (2006). Lewy bodies. Proceedings of the National Academy of Sciences USA, 103(6):1661-1668.

91. Sian J, Dexter DT, Lees AJ, Daniel S, Jenner P, Marsden CD. 1994b. Glutathione-related enzymes in brain in Parkinson's disease. *Ann. Neurol.* 36:356–61

92. Sian J, Dexter DT, Lees AJ, et al. 1994a. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. *Ann. Neurol.* 36:348– 55

93. Sofic E, Lange KW, Jellinger K, Riederer P. 1992. Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. *Neurosci. Lett.* 142:128–30

94. Stoll G, Jander S, Schroeter M. Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. Adv Exp Med Biol. 2002;513:87–113.

95. Swerdlow RH, Parks JK, Miller SW, Tuttle JB, Trimmer PA, et al. 1996. Origin and functional consequences of the complex I defect in Parkinson's disease. *Ann. Neurol.* 40:663– 71

96. Taupin P, Adult neurogenesis, neuroinflammation and therapeutic potential of adult neural stem cells. Int J Med Sci. 2008; 5(3): 127–132.

97. Temlett JA, Landsberg JP, Watt F, Grime GW. 1994. Increased iron in the substantia nigra compacta of the MPTP-lesioned hemiparkinsonian African green monkey: evidence from proton microprobe elemental microanalysis. *J. Neurochem.* 62:134–46

98. Thomas DM, Walker PD, Benjamins JA, Geddes TJ, Kuhn DM. Methamphetamine neurotoxicity in dopamine nerve endings of the striatum is associated with microglial activation. Journal of Pharmacology and Experimental Therapeutics. 2004;311:1–7.

99. Toffa S, Kunikowska GM, Zeng B-Y, Jenner P, Marsden CD. 1997. Chronic glutathione depletion in rat brain does not cause nigrostriatal pathway degeneration. *J. Neural Transm.* 104:67–75

100. W¨ullner U, L¨oschmann P-A, Schulz JB, et al. 1996. Glutathione depletion potentiates mPTP and MPPC toxicity in nigral dopaminergic neurones. *NeuroReport* 7:921–23

101. Walkinshaw G, Waters CM. Induction of apoptosis in catecholaminergic PC12 cells by L-DOPA. Implications for the treatment of Parkinson's disease. J Clin Invest. 1995 Jun;95(6):2458-64.

102. Wu DC, Teismann P, Tieu K, Vila M, Jackson-Lewis V, Ischiropoulos H, Przedborski S. NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. PNAS. 2003;100:6145–50.

103. Yoritaka A, Hattori N, Uchida K, Tanaka M, Stadtman ER, Mizuno Y. 1996. Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson's disease. *Proc. Natl. Acad. Sci. USA* 93:2696–713