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Artículo revisión

Parkinson's disease: molecular aspects and prospective neuroprotective and restorative therapies

Hebert Luis Hernández-Montiel

ENFERMEDAD DE PARKINSON: ASPECTOS MOLECULARES TERAPIA RESTAURATIVA Y NEUROPROTECCIÓN

RESUMEN

La enfermedad de Parkinson (EP) es el trastorno neurodegenerativo del movimiento más común y está caracterizado por la pérdida progresiva de las neuronas dopaminérgicas en la sustancia negra, pars compacta. El principal obstáculo para el desarrollo de terapias de neuroprotección y restauración es el conocimiento limitado de los eventos moleculares que provocan esta neurodegeneración. Para poder desarrollar terapias que prevengan o protejan de EP, es necesario identificar la cascada de eventos deletéreos que provocan la disfunción y muerte de las neuronas dopaminérgicas. Esta revisión resume los últimos hallazgos en el estudio de esta enfermedad.

Palabras clave: enfermedad de Parkinson, disfunción mitocondrial, neurodegeneración, terapias restaurativas y de neuroprotección.

ABSTRACT

Parkinson´s disease (PD) is the most common neurodegenerative movement disorder and is characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta. The main obstacle to developing neuroprotective and restorative therapies is a limited understanding of the key molecular events that provoke neurodegeneration. In order to establish causal or protective treatments for PD, it is necessary to identify the cascade of deleterious events that lead to the dysfunction and death of dopaminergic neurons. This review summarizes most recent insights gained from many studies in this disorder.

Key words: Parkinson´s disease, mitochondrial dysfunction, neurodegeneration, neuroprotective and restorative therapies.

Parkinson's disease (PD) is a high incidence affliction in persons of advanced age. It is characterized by the death of dopaminergic neurons of the substantia nigra of the mesencephalon. Due to the nature of the disease, once the pathological process has been initiated, the therapeutic measures known to date are not effective in reestablishing the survival of the dopaminergic neurons. The best therapeutic measure would be the early identification of the sick subjects and the development of neuroprotective and neurorestorative therapies in order to avoid the progress of neuronal death.

The present work reviews the physiopathological aspects involved in Parkinson's disease including its genetic and environmental aspects. It also makes reference to new therapies that currently are being developed. All of these focus in the development of new therapies that will reduce the progression of the disease in carrier patients and avoid the development to of new cases through neuroprotective measures.

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Laboratorio de Neurobiología y Bioingeniería Celular de la Facultad de Medicina de la Universidad Autónoma de Querétaro. Correspondencia: Hebert Luis Hernández. Laboratorio de Neurobiología y Bioingeniería Celular de la Facultad de Medicina de la Universidad Autónoma de Querétaro. Clavel # 200, Prados de la Capilla, Santiago de Querétaro, 76170, México. E-mail: heberth2003@yahoo.com.mx

Background

Parkinson's disease (PD) is the second most common neurodegenerative illness after Alzheimer's disease (AD) and the first with movement disorders. With a prevalence that increases with age and affects approximately one percent of the population over 65 years old, patients with PD show clinical symptoms that include bradikynesia, trembling in rest, rigidity and posture inestability[1]. While normal population shows a loss of around 4.4 % of substantia nigra cells, patients with PD loose ten times more[2]. A significant loss (70-90%) of dopaminergic neurons (DA) of the substantia nigra *pars compacta* (SNc) and the presence of intracytoplasmic inclusions know as bodies of Lewy in some of the remaining dopaminergic neurons, are pathognomonics of PD[3]. Ninety percent of PD is of the sporadic type and in the remaining ten percent mutations in different genes that cause the familial PD are found. By the time that symptoms initiate, dopamine in the striate has diminished by 80% and 60% of *pars compacta* dopaminergic mesencephalic neurons have been lost (figure 1). Neurodegeneration and bodies of Lewy are also found in noradrenergic neurons of the *locus ceruleus*, raphe (serotonergic) and cholinergic of the Meynert basal and dorsal motor nucleus of vagus, as well as in the cerebral cortex (especially in the cingulate and entorhinal cortices), olfactory bulb and autonomous nervous system[4,5].

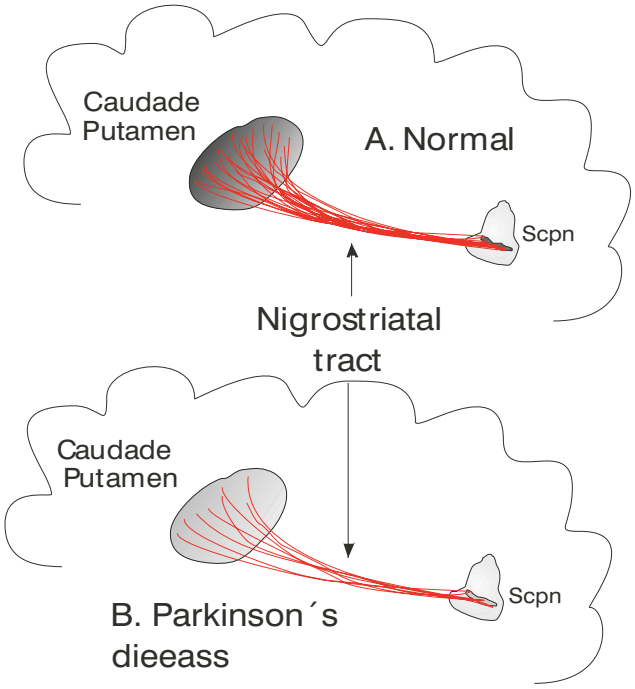


Figure 1. Neuropathology of Parkinson´s disease. (a) Schematic representation of the normal nigrostriatal pathway. Composed of dopaminergic neurons whose cell bodies are located in the substantia nigra *pars compacta* (SNpc). These neurons project to the basal ganglia and synapse in the striatum (putamen and caudate nucleus). The picture represents the normal projection and relation among its components. (b) Schematic representation of the diseased nigrostriatal pathway in Parkinson´s disease. The nigrostriatal pathway degenerates, with a marked loss of dopaminergic neurons that project to the putamen and a much more modest loss of those that project to the caudate. There is depigmentation of the SNpc due to the marked loss of dopaminergic neurons. A syntomatic patient has a decrease of 60-80% of the dopaminergic neurons in the substantia nigra *pars compacta.*

Oxidative damage, mitochondrial dysfunction and inflammation

All aerobic organisms are constantly exposed to oxidative stress[6]. Oxidative phosphorylation involves the transfer of electrons, which could provoke the generation of free radicals, regardless of the existent species that have one or more unpaired electrons. Many free radicals are unstable reactive species that can extract an electron from neighbor molecules to complete their own orbital. This causes the oxidation of neighboring molecules, critical biological molecules that include DNA, proteins and membrane lipids, subject to oxidative damage.

Mitochondria are the most important source of free radicals, generating superoxide radicals to ubiquinone and NADH dehydrogenase (complex I)[7]. Mitochondria are organelles whose internal membrane and matrix contain multiple enzymes and the last one contains copies of a different mitochondrial genome, mtDNA. The superoxide produced in the cells by means of oxidative reactions is usually converted to H2O2, by superoxide dismutase (SOD). This superoxide can react with nitric oxide to form peroxynitrite (ONOO-)[8], a reaction that takes place with a speed three times faster than the range of superoxide dismutation by dismutase peroxide. Generation of peroxynitrite depends on the concentration of superoxide and nitric oxide in the cell and it may exist in an active form similar to hydroxyl radical. Physiological pH can diffuse many cellular diameters, causing damage due to the oxidation of lipids, proteins and DNA. It can also react with Cu, Zn SOD to form nitronium ion, which can nitrate the residues of tyrosine[9]. The produced 3-nitrotyrosine is an excellent biochemical marker for oxidative damage through peroxynitrite.

A decrease in glutathione antioxidant has consistently been found in PD patients. It has been determined that this depletion, possibly consequence

of its over-utilization in oxidative stress reactions, could play an important role in nigra degeneration in all disorders with nigrostriatal dopamine deficiency such as progressive supranuclear paralysis and multiple systemic atrophy[1].

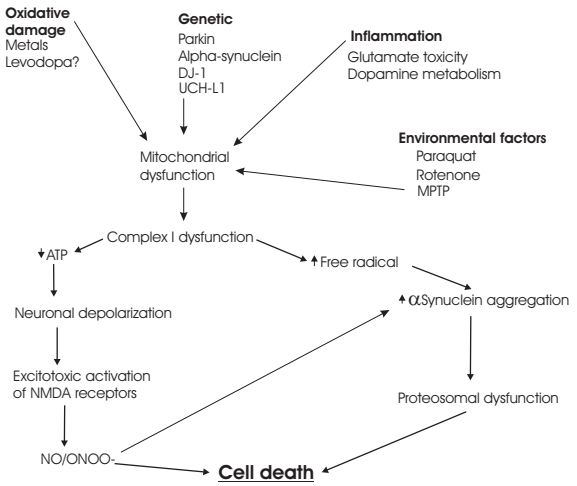
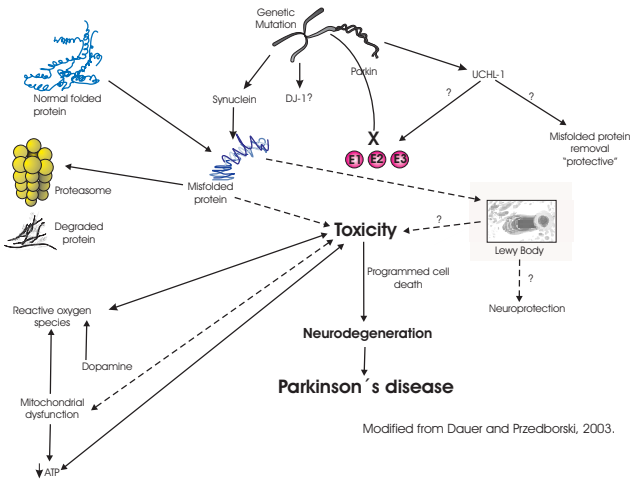


Figure 2. Complex I deficiency may be central to sporadic PD. Dysfunction of complex I leads to increased oxidative stress, free radical formation, and reduction in adenosine triphosphate (ATP) formation. The complex deficiency can be multifactorial and maybe present in other diseases that contribute to the dysfunction. Slow and chronic complex I deficiency leads to accumulation and aggregation of -synuclein, which leads to dysfunction of the proteasome and contributes to cell death. This status can be induced by environmental factors and chronic inflammation. ATP decrease leads to membrane depolarization and contributes to excitotoxic neuronal injury and further free radical-mediated injury involving peroxynitrite (ONOO-) and nitric oxide (NO) and a feedforward cycle of increasing oxidative stress and injury. Genetic factors maybe the cause of cellular death; Parkin appears to be a multipurpose neuroprotective agent that may allow the cell to more readily handle proteosomal impairment. Loss of Parkin function may decrease the cell´s ability to deal with proteosomal dysfunction. Familial-associated mutations in α-synuclein bypass complex I deficiency, but they promote α-synuclein accumulation and aggregation. DJ-1 may function as a chaperone, and its absence may also decrease the cells ability to deal with proteosomal dysfunction, and may cause cellular death.

Recent evidence suggest that the inhibition of mitochondrial complex I could be the main cause of sporadic PD cases and that this inhibition causes the aggregation of α-synuclein, which contributes to DA neuron death. This enzymatic abnormality is only found in substantia nigra *pars compacta* which causes a reduction in respiratory chain activity. Up until now no genetic abnormality of the nuclear or mitochondrial genome or an endo or exotoxine that explains the incidence of sporadic PD cases has been identified (figure 2). Regardless of this, the increase of the incidence of PD with age is linked to the fact that aging provokes deletions above 5% of all mitochondrial genomic molecules in the brain[11]. Many of the known routes of neuronal death are involved with complex I: excitotoxicity, oxygen reactive species, apoptosis dependent and independent of caspases, necrosis and damage due to inflammation. Observations suggest that the inhibition of complex I creates an oxidative environment that causes the aggregation of α-synuclein, with subsequent DA neuronal death. Biochemical evidence of respiratory chain complex I defects in the substantia nigra of patients with PD[12], along with the finding that the potent toxin 1-phenyl-4-methyl-1,2,3,4-tetra-hydropyridine (MPTP) causes acute Parkinsonism[13,14], raises the possibility that mitochondrial factors participate in PD pathogenesis. Studies in genomic transplant have shown that the effect of complex I is determined by mitochondrial DNA[15-17]. It is not known if this effect is caused by mutations in mitochondrial DNA or if the functional polymorphism makes the cell susceptible to damage by external agents[18].



Figue 3. Mechanisms of neurodegeneration in Parkinson´s disease. The accumulation of misfolded proteins is likely to be a key event in PD neurodegeneration. Pathogenic mutations may directly induce abnormal protein conformations (as believe to be the case with α–synuclein) or damage the ability of the cellular machinery to detect and degrade misfolded proteins (Parkin, UCH-L1); the role of DJ-1 remains to be identified. Oxidative damage, linked to mitochondrial dysfunction and abnormal dopamine metabolism, may also promote misfolded protein conformations. It remains unclear whether misfolded proteins directly cause toxicity or damage cells via the formation of protein aggregates (Lewy body). Oxidative stress, energy crisis (ATP depletion) and the activation of the programmed cell death machinery are also believed to be factors that trigger the death of dopaminergic neurons in Parkinson´s disease.

On the other hand, the accumulation and aggregation of α-synuclein could contribute in great part to the death of dopaminergic neurons and oligodendrocytes due to damage in protein control

and detoxification[19-21]. These inclusions could cause cellular damage by obstructing normal cellular traffic and altering the morphology, as well as trapping cellular components, which eventually causes neuronal death[22]. *Pos mortem* studies in humans indicate that species reactive to oxygen are important in the pathogenesis of sporadic PD[23,24]. Damage to mitochondrial complex I releases stress activity due to free radicals and makes neurons vulnerable to glutamate excitotoxicity.

Genetic and environmental factors associated with Parkinson's disease

Alteration in α-synuclein, Parkin and UCH-L1 (Ubiquitin C-terminal hydrolase-L1), members of the Ubiquitin-Proteosomal, suggest that the affectation to this system could intervene in the disease[18]. The mutant gene of α-synuclein is involved in the pathogenesis of autonomic dominating PD. The deletions in Parkin gene have been identified as the primary cause of rare forms of juvenile autosomic recessive PD[25]. The proteins ubiquitinized by this system are recognized by the proteasome subunit 26S and are a target for degradation[22,26]. The alteration of this system alters protein processing capacity, causing abnormal protein aggregation and contributes to its accumulation in the bodies of Lewy. The protein DJ-1, whose function is unknown, is altered in some cases of autosomic recessive PD (figure 3)[27].

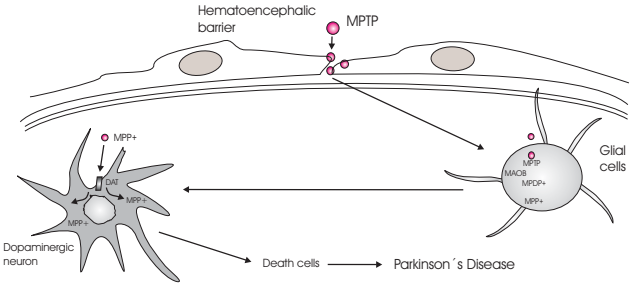


Figure 4. Schematic representation of MPTP metabolism in the brain. After systemic administration, MPTP crosses the blood-brain barrier. Once in the brain, MPTP is converted to MPDP+ by MAO-B within nondopaminergic cells, such as glial cells and serotonergic neurons (not shown), and then to MPP+ by an unknown mechanism (?). Thereafter, MPP+ is released, again by an unknown mechanism(?), into the extracellular space. MPP+ is concentrated into dopaminergic neurons via the dopaminergic transporter (DAT). The increase MPP+ into cell body can follow one of three routes: cytosolic enzymes, mitochondria and synaptic vesicles. Last two can produce death cell of dopaminergic neurons in substantia nigra pars compacta.

Epidemiological studies suggest that environmental toxic agents that inhibit complex I are also involved in PD; for instance MPTP, paraquat and rotenone[28-31]. Exposure to the herbicide paraquat has been implicated as a risk factor for PD. The exact mechanism is still unknown, but it apparently induces the sequential phosphorylation of c-jun N-terminal kinase (JNK) and c-jun, and the activation of capase-3 and causes sequential dopaminergic neuron death *in vivo* as well as *in vitro*[32]. Another herbicide, rotenone, is also capable of provoking PD. Even though epidemiological studies suggest that pesticides play an important role in the pathogenesis of the disease, it is clear that a particular genetic disposition exists, potentially associates with the organism's capacity to metabolize dopamine related neurotoxins (figure 2)[33].

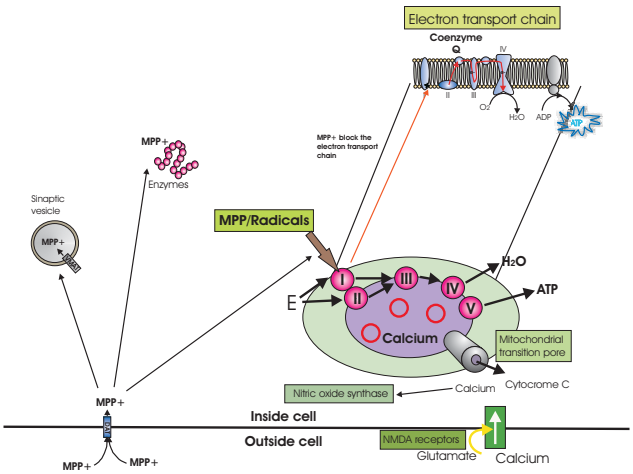


Figure 5. Schematic representation of MPP+ intracellular pathways. Inside dopaminergic neurons, MPP+ can follow one of three routes: interaction with cytosolic enzymes (toxic), sequestration into synaptic vesicles via the vesicular monoamine transporters and concentration into mitochondria through an active process (toxic). Within the mitochondria, MPP+ blocks complex I (X), which interrupts the transfer of electrons from complex I to ubiquinone (Q). This perturbation enhances the production of reactive oxygen species (not shown) and decreases the ATP synthesis. This decrease on ATP production diminishes metabolism in dopaminergic neurons.

Dopamine toxicity and inflammation

Dopamine metabolism is, on its own, a source for the production of toxic oxide radicals due to auto-oxidation and enzymatic oxidation, a mechanism closely linked to 6-hydroxydopamine toxicity. To date, the hypothesis that the chemically reactive quinone/semiquinone, intermediate products of dopamine, are highly neurotoxic and potential genotoxic has been proposed. This very important consideration suggests that treatment with levodopa hastens the progression of PD[34].

Catecholamine biosynthesis is regulated by tyrosine hydroxylase (TH), likewise the activity of this enzyme is regulated by tetrahydrobiopterine (BH4) cofactor, which in turn is regulated by the activity of GTP cyclohydrolase I (GCH). So, GCH activity indirectly regulates the activity of catecholamine levels. TH activity in nigrostriatal dopaminergic neurons is more sensitive to a decrease in BH4 and the mutation of GCH results in activity reduction of GCH, BH4, TH and dopamine, causing a recessive inherited deficiency of GCH or dominating inherited progressive dystonia, also called dopa responsive dystonia. Even though, this aspect does not seem altered PD and juvenile PD patients[35].

The area of the brain where substantia nigra is found shows high microglia density. When this is activated increases the expression of cellular surface markers such as complex I macrophage antigen (MAC-1) and this produces a variety of pro-inflammatory cytokines as well as oxygen reactive species[36]. A number of cytokines including interleukine-1 (IL-1) IL-6 and tumoral necrosis factor (TFN-α) contribute to the inflammatory process[37]. Recent studies show that (TFN-α) -a is increased by 366% in the striate and by 432% in the cerebrospinal fluid of patients with PD[38-40]. Some investigations suggest that intense glial secondary reaction to lesions by MPTP is not directly correlated to neurodegeneration intensity[41]. Cyclooxygenase 2 (COX-2) inflammatory route has been implicated in neurodegeneration. Cytokines and lipoid polysaccharides express and regulate COX-2 in glial cells[42] catalyzing the formation of prostaglandins, which promote the reduction of hydroperoxide levels, resulting in the production of free radicals (figure 3)[43]. Nevertheless, when excitotoxic lesions, synaptic excitation, neuronal death due to apoptosis and cerebral ischemia appear, COX-2 is chiefly expressed in neurons[44-46].

Glutamate toxicity

Indirect evidence suggests the participation of glutamatergic mechanisms in the pathogenesis of the disease (figure 2). Glutamate, the major exciting neurotransmitter of the central nervous system of vertebrates, is known for its neurotoxic activity when expressed in the synapses. Two general mechanisms protect neurons from this toxicity: firstly the rapid removal by membrane proteins that carry it, known as exciting amino acid transporters and secondly by metabolism and recycling of glutamate by synaptic astrocytes via synthetase glutamine, a reaction that requires ATP. When extracellular levels of glutamate are high (0.5-1.0 mM), glutamate metabolism suffers a change and enters ATP-generating oxidative deamination cycle, where glutamate dehydrogenase plays a part. This enzyme requires ADP for its activity and becomes functional when cellular energy levels are low. It has been found that one of glutamate transports, neuron specific EAAT3, is found in high concentrations in mesencephalon dopaminergic neurons. It has been found, through immunocytochemical studies, that dopaminergic cells are intensely tinted by glutamate dehydrogenase enzyme. These data suggest that glutamate could play a part in the physiopathology of PD[47]. At present is known that neuron exposure to glutamate causes mitochondrial depolarization associated to flow increase inside the mitochondria. Additionally activity of glutamate receptors, NMDA, induces a rapid flow of mitochondrial Ca^{2+}, reduction in O^2 and complexes I, II/III and IV respiratory chain activity inhibition (figure 5)[48].

Oxidative stress and metals

Superoxide dismutase activity (Mn-SOD and/or Cu/Zn-SOD) controls O^{2-} levels, producing hydrogen peroxide (H_2O_2). Similarly to O^{2-}, H_2O_2 is not very reactive, nevertheless when met with Fe^2 or Cu+, forms a highly reactive short-life hydroxyl radical through the Fenton-Haber Weiss reaction[49]. Lipid peroxidation and related oxidative protein modifications of the membrane, take place in neurodegenerative illnesses. The oxidative stress associated with the membrane is promoted by redox activity metals, mainly iron and copper. The levels of iron are increased in neuronal vulnerable populations in AD and PD. Dietary and pharmacological manipulations of iron and copper modify the course of the disease in AD and PD mice models, which suggest the role played by these metals in the pathogenesis of the disease[50]. The increase of iron content in human SNC and neuromelanine in the presence of iron, play an important role in the generation of free radicals (figure 2)[51]. Desferol, an iron chelator has shown to be a potent neuroprotector in PD models with 6-hydroxydopamine[52].

MPTP and its relation with Parkinson's disease

Much of the current interest in the association between neurodegeneration and mitochondrial dysfunction by oxidative dysfunction/damage comes from the studies of parkinsonism induced by MPTP, mitochondrial dysfunction that also occurs in PD.

MPTP, identified as the PD causing agent in humans and currently used in research to induce PD, inhibits mitochondrial complex I and replicates the characteristics of sporadic PD[53]. The first indication of this dysfunction comes from the observation that humans exposed to the MPTP molecule, develop PD phenotype. Later it was demonstrated that MPTP follows a conversion to a derivative, 1-metil-4-phenilpiridine (MPP+), by monoamine oxidase in glial cells (figure 4). MPP+ is taken by the cells that process dopamine to the capture sites and highly concentrated within the negatively charged mitochondrial matrices. Inside the mitochondria, MPP+ inhibits enzymatic complex I of the electron transport chain (ETC), which causes specific degeneration of catecholaminergic neurons of the substantia nigra and *locus ceruleus*, thus reducing ATP production in the mitochondria (figure 5)[54]. This reaction results in a range of clinical alterations and histopathology reminiscent of sporadic PD[55-57].

There exists a decrease of about 30 to 40% in complex I activity in the substantia nigra in PD patients[58-62]. Defects localized in mitochondrial DNA for complex I of the platelet of patients with PD are transferable to cellular lines with lacking mitochondria. These effects are associated with the production of free radicals, the increase of MPP+ susceptibility and damage to calcium mitochondrial buffer.

Relatively late in the apoptosis process, the activation of endonucleases results in the fragmentation of cellular DNA, clearly show in agarosa gel as a DNA smears[63]. Specifically, 3'OH terminal extremes resulting of DNA fragmentation with the TUNEL technique (terminal deoxynucleotidyl transferase-mediated dUTP-X 3'nick end-labeling) can be detected. *In vivo*, the apoptotic nucleus was detected in mice after five days treatment with MPTP (5x30 mg/kg)[64], while it could not be detected after a day of exposure to MPTP[65,66].

The development of an inadequate mitochondrial mechanism can alter a great variety of cellular processes and homeostatic mechanisms. The lack of electron transport in ETC may result in ATP depletion, the formation of oxygen reactive species or free radicals, calcium exit with mitochondrial depolarization and the formation of transition pores with channels through both mitochondrial membranes. Mitochondrial transition pores provide a cytoplasmic passage to molecules that are usually concentrated inside the mitochondria, such as cytochrome C (figure 5). This last one can activate signal cascades of programmed cellular death through its contribution in cysteine activation in aspartate sequestering enzymatic pathway (caspases)[67] Caspases are a family of cysteine protease with a specific substrate for aspartic acid. It has been demonstrated that there is activation of caspase-3 after the application of MPP+ in cerebellum neurons[68]. The activation of this enzymatic cascade results in the self ingestion of cellular content (figure 6)[14]. External ligands interact with death receptors, activating the initiator caspase in, which can cleave Bid. tBid translocates to the mitochondria and induces the release of cytochrome c (cyt c) to the cytosol, linking the death receptor pathway with the mitochondrial pathway. Activation of caspase-2 may also induce Bid cleavage and signal the mitochondria to release cyt c, implicating the mitochondria as amplifiers to the caspase cascade. Within the cytosol, cyt c forms the apoptosome (in the presence Apaf-1, dATP and procaspase-9) activating caspase-9. The effectors caspases -3 and -7, wich can be activated by caspases -8 or -9 are required for the activity of the endonuclease DFF40/CAD, responsible for genomic DNA fragmentation. In addition, Smac/Diablo (released from the mitochondria) binds to IAPs, potentiating caspase activation. AIF is also released to the cytosol and translocates to the nucleus, inducing the fragmentation of DNA and *killing* the cells in a caspase-independent manner. By other way, in the presence of an apoptotic stimulus (e. g, Ca2+-calmodulin), calcineurin dephosphorylates Bad, which is translocated to the mitochondria, where it interacts with Bcl-2 or BclXL. Under these conditions, the release of cytochrome c (cyt c) to the cytosol occurs, triggering the apoptotic machinery through the activation of the caspase cascade. Activation of the receptors for neurotrophins (Trk receptors) activates the PI3K/Akt or MAPK signaling pathways, responsible for phosphorylation of Bad. Phosphoarylated Bad at Ser112 and Ser136 is sequestrated by the cytosolic protein 14-3-3, avoiding its translocation to the mitochondria[69].

Role of neurokinin in Parkinson's disease

In mammals, neurokinins are a group of neuropeptides that include substantia P (neuorkinin-1, NK-1), substantia K (NK-2) and neuromedine (NK-3). Their biological effects as neurotransmitters, neuromodulators or factors similar to neurotrophic molecules are mediated by three different neurokinin receptors namely: SP receptor (NK-1R), NK-2R and NK-3R. Several research lines have shown that neurokinins are involved in PD pathogenesis. The decrease of substantia P and immunoreactivity to substantia P has been found in substantia nigra and striatal tissue in

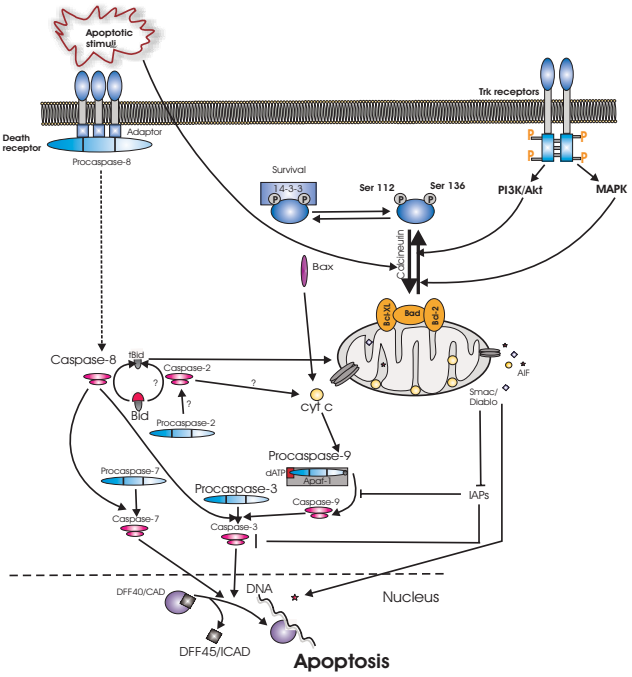


Figure 6. Schematic overview of the death receptor, the mitochondrial apoptotic pathways and the regulation of apoptosis mediateal by Bad phosphosrylation/dephosphorylation.

animals with Parkinson as well as in *pos mortem* analysis of patients with PD. Neurokinins 1 and 3 are abundantly found in cholinergic and dopaminergic neurons of the basal ganglia, indicating that these neurons are physiologically regulated by neurokinins. The administration of agonist of receptors NKs in Parkinson models in mice treated with MPTP creates a modulation of motor activity. These constitute molecules that are associated to the functioning and survival of neurons of the basal ganglia, particularly dopaminergic neurons[70].

Neuroprotective and restorative therapies

Nowadays studies in the development of effective therapeutic strategies that could have a protective effect against the deterioration and death of DA neurons are been undertaken[71,72]. It is very important to understand that, in order to develop neuroprotective treatments, is necessary an early diagnosis of all subjects. In this way, neuroprotective therapy could have a particularly positive effect if preclinical patients or during the early stages of the disease. Positron emission tomography (PET) and simple photon emission tomography (SPECT) images are useful tools for preclinical diagnosis of the disease[73,74], but their application as detection methods among the general population is not possible. Study groups of patients with early symptoms associated with PD are being formed. Sense of smell alterations, light neurocognitive dysfunction, visual and motor control abnormalities and to a lesser degree, personality and temper disorders, have been suggested as markers that anticipate or accompany early alterations of the disease[75].

Treatment with levodopa promotes the lesion

Oxidation studies in patients with PD suggest the influence of levodopa treatment (LD) as having a pro-oxidative effect. Numerous *in vitro* studies have shown an increase in oxidation and subsequent secondary neurotoxicity with LD treatment. Dopamine metabolism and LD catalyzed with MAO-B produce toxic free radicals[76] that, *in vitro*, can initiate the disruption of cellular membrane through lipid peroxidation[77,78] and promote the death of dopaminergic and other neurons in cultures[79-81]. Due to these mechanisms, LD could be toxic to dopaminergic neurons and accelerate neurodegeneration in PD[82,83]. In the presence of high oxidative stress, high levels of poliprotein oxidation in plasma and cerebrospinal fluid, a decrease in plasmatic levels of sulphidryl-protein groups and levels α-tocoferol in cerebrospinal have been observed fluid in patients with PD compared this patients with other neurological diseases and their controls. Treatment with LD does not change significantly lipoprotein oxidation in plasma but it does increment auto-oxidation and diminishes levels of plasmatic antioxidants with relevance to ubiquinol-10, an effect that was not observed when dopamine agonist were used[84].

Antioxidant therapies

Ubiquinol-10 is the reduced form of the Q10 coenzyme and acts as an antioxidant, resulting in oxidation of ubiquinol-10. No changes has been reported with[85] or reduced[86] Q10 coenzyme in patients with PD. In other study the relation between ubiquinol-10 and ubiquinol-10/coenzyme Q was less in patients with PD not treated with LD. This could imply a deficit of ubiquinol-10 in patients with PD treated with LD. These data provide the bases for therapy with supplementation of Q10 in patients that receive treatment with LD[84]. Recent studies show that there no exists correlation between values of seric Q10 coenzyme and risk for PD[85,87].

Other antioxidants have been used, such as

nicotinamide adenine dinucleotide (NADH) necessary of the generation of ATP by the mitochondria, with partial results. The administration of glutathione has not reported positive results due to the complete hydrolysis of the compound in the gastrointestinal tract[88].

Non dopaminergic therapies

It was recently reported that dextromethorphan (DM), a widely used antitusive agent, attenuates *in vitro* dopaminergic neurodegeneration induced by an endotoxine. In mesencephalic cultures treated with MPTP, DM significantly reduces superoxide free radicals in extra cellular media as well as intracellular reactive oxygen species. The neuroprotective effect of DM was observed in uncultivated animals but not in mutant animals deficient in NADPH oxidase, indicating that this enzyme is a critical mediator for DM neuroprotective activity[89,90].

Another agent used is bupidine, an antagonist of adenosine A2 receptors. This drug experimentally increases cerebral levels of norepinephrine, serotonine and histamine, without affecting affinity of receptors to serotonine, norepinephrine, gamma-aminobutyric acid and endorphins; but it increases affinity of the receptor to NMDA and sigma receptors[91]. It was recently found an increment in the synthesis of receptors of A2A adenosine in neurons of the striato-pallidal system, a change related to the onset of dyskinesia after a long term therapy with levodopa[92].

Modafinil, a medication used in sleeping alterations has shown to have a protective effect against harmful effects caused by the administration of MPTP; it inhibits the liberation of GABA in the striate and glutathione levels and GABA in the substantia nigra. Part of this protective effect is due to GABA nigrostriatal modulation and modulation of the liberation of norepinephrine and serotonine in the striate[93].

MPTP induces apotheosis through many signals, one of them by means of kinase c-jun N-terminal. At present research is being done on a blocker called SP600125, a reversible inhibitor that competes with ATP to join kinase and has great selectivity for JNK[94].

Therapy with antiinflamatories

Another therapy under investigation is the use of antiinflamatories[36], of which meloxicam, and inhibitor of COX2 has shown positive results in animals[37,95] but not in humans[96]. This could indicate that experimental models do not exactly reflect neurodegenerative processes or that neuronal death comprises a cascade of processes not solved by a single therapy.

Neurotrophic factors and restorative therapies

Due to their neuroprotective therapies, neurotrophic factors show great potential as therapeutic agents in neurodegenerative illnesses. The glial cell derived neurotrophic factor (GDNF) is one of the most potent promoters of mesencephalic dopaminergic neuron survival[97,98], additionally brain derived neurotrophic factor (BDNF) and glial cell derived factor have similar effects[99]. The intracellular signal cascade is mediated by a multicomponent receptor system that comprises GDNFa (a component of the receptor of the GDNF family) and tyrosine synaptic receptor, abundantly found in the substantia nigra[100]. Their direct administration into the substantia nigra produces partial functional and biochemical restoration after treatment with MPTP[101-103]. Depending on the experimental design, GNDF has neuroprotective and restorative effects[104, 105].

BDNF promotes survival of the main sort of affected neurons in AD and PD, such as hippocampal, neocortical, cholinergic, anterior brain basal neurons and dopaminergic neurons of the substantia nigra[106, 107].

Antioxidant vitamin therapies

Oxidative stress is an important mechanism for cellular death in PD, therefore vitamins C, E and A, important antioxidant agents, have been associated with physiopathological mechanisms of the disease. The determination of these vitamins in groups of PD patients showed that their levels are similar to those found in healthy patients[108]. Vitamin E supplement studies were found that they can be protected from striatal damage caused by 6-hydroxydopamine[109]. *In vitro* studies showed that vitamin E protects neurons against glutamate cause neurotoxic effects[110]. Vitamins E and C supplement increase levodopa doses intervals[111].

A balanced diet that includes adequate amounts of fruit and vegetables in addition supplements of S-adenosine metionine, vitamins C, B6, B12 and pholate have been suggested[34,111]. In a study by VanItallie in 2003[112], the use of a hyperketogenic diet is suggested, due to the fact that ketones are efficiently used by the mitochondria for the generation of ATP and could protect vulnerable neurons against damage by free radicals.

It is known that cerebrospinal fluid in PD pa-

tients has low levels of free thiamine[113]. Cocarboxylase or pyrophosphate thiamine (PPT) is a coenzyme derived of thiamine, involved in most respiratory processes and reactions of the intermediate metabolism of the cells[114]. It is found in multi enzymatic complexes and takes part in pyruvate descarboxylase, transketolase, α-oxoacid descarboxylase, cytochro-mes, acetolacetate synthetase and transketolase[115,116]. PPT is also involved in the synthesis of ATP, therefore its deficiency causes the inhibition of the energetic metabolism[117-119] as well as damage due to hypoxia[120,121]. Because of its effects in the reduction of anaerobic metabolism and the reduction of free radical formation, the administration of PPT could diminish physiopathological events that promote lesions in the substantia nigra.

CONCLUSION

Parkinson´s Disease does not seem to be a pathological entity caused by a single triggering factor, characteristically not all dopaminergic systems of the central nervous system are affected, but also parts of other systems that involved neurotransmitters different to dopamine.

The combination of antiapoptotic therapies with restorative therapies seems promising. Interestingly in humans, the pre symptomatic phase can last around 5 years since the onset of symptoms, according to functional image analysis and pathological studies. The changes in therapeutics should focus on the identification of pre symptomatic stages or initial symptoms and application of early therapy. It is probable that the best therapy for patients in the early stages of the disease is a combination of the therapies previously analyzed.

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