REVIEWS

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Parkinson's Disease as a Consequence of Impaired Redox Homeostasis in the Brain*

Choroba Parkinsona jako rezultat zaburzenia homeostazy *red-ox* mózgu

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Abstract

Parkinson's disease (PD) is one of the most frequent neurodegenerative disorders of advanced age. Clinically, PD is characterized by akinesia, resting tremor, and rigidity. A characteristic feature of PD is loss of pigmented neurons in the substantia nigra. A review of available data on PD shows that multiple factors are involved in maintaining the redox state of the brain, and impairment of any of these components may result in PD. This includes changes in the neuromelanin level, iron level, mitochondrial function, dopamine and tyrosine metabolism, and inflammation. This mini-review presents some new aspects of the relationship between impairment of brain redox homeostasis and PD (**Adv Clin Exp Med 2006, 15, 4, 705–709**).

Key words: Parkinson's disease, free radicals, neuromelanin, mitochondria, iron.

Streszczenie

Choroba Parkinsona (PD) jest jedną z najczęstszych chorób degeneracyjnych mózgu zaawansowanego wieku. Klinicznie PD charakteryzuje się akinezją, drżeniem spoczynkowym i sztywnością. Charakterystyczną cechą PD jest utrata barwnika neuronów w obrębie *substantia nigra*. Z dostępnych danych na temat PD wynika, że za utrzymanie równowagi *red-ox* w mózgu odpowiedzialnych jest wiele czynników, a zaburzenie któregokolwiek z nich może spowodować rozwój PD. Zalicza się do nich: neuromelaninę, stężenie żelaza, funkcję mitochondriów, metabolizm dopaminy i tyrozyny oraz procesy zapalne. Ta praca poglądowa ukazuje niektóre nowe aspekty związku między zaburzeniem homeostazy *red-ox* a PD (**Adv Clin Exp Med 2006, 15, 4, 705–709**).

Słowa kluczowe: choroba Parkinsona, wolne rodniki, neuromelanina, mitochondria, żelazo.

Parkinson's disease is a progressive neurodegenerative disorder affecting about 2% of the population above 65 years [1]. The clinical presentation of PD includes bradykinesia, resting tremors, rigidity, and later loss of postural reflexes [2]. Histopathological findings in PD include progressive dysfunction and degeneration of the nigrostriatal dopaminergic neurons. The loss of nigral neurons (about 5% cell loss per year) correlates with both the duration of the illness and the severity of motor dysfunctions [1].

In the pre-clinical stage of PD, some striatal compensatory phenomena occur such as increased neuronal activity [3] or sensitization of dopaminergic receptors. PD is therefore not clinically obvious until 50–70% of the dopaminergic neurons are lost. Hence PD is diagnosed in morphologically and biochemically advanced stages of the disease and it is difficult to establish the time of onset and exact cause of PD. Some clinical data indicate that oxidative stress may play an important role in the etiology of PD. In the cerebro-

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spinal fluid and peripheral blood samples of PD patients, malondialdehyde content was elevated and the activities of antioxidative enzymes (glutathione reductase, Cu, and Zn superoxide-dismutase) were increased. This indicates the presence of a chronic oxidative stress state in the brain of PD patients [4].

Neuromelanin in Parkinson's Disease

Neuromelanin (NM) is a true melanin, containing bound metal ions *in situ* [5]. NM is an insoluble pigment found in the neurons of specific brain regions [5], e.g. the substantia nigra (SN) and locus coeruleus [6]. It appears in humans at 2–3 years of age and accumulates with aging. Because tyrosinase is not present in the brain tissue, other mechanisms are suspected to be involved in NM formation. NM accumulates normally through the autoxidation of catecholamines and binds tightly to the redox-active metal ions, processes which would accelerate under conditions of intracellular or extracellular oxidative stress [7]. An inverse relationship was observed between the percentage of surviving neurons in PD and the amount of neuromelanin they contain, suggesting that the vulnerability of the dopaminergic neurons is related to their NM content. The NM level in PD subjects was less than 50% of that of controls.

The function of NM remains unclear, but at physiological pH, NM is an efficient antioxidant [8] and hence has a cytoprotective function in the sequestration of redox-active metal ions under normal conditions [5]. It is well known that iron levels are increased in the substantia nigra of PD patients, and the absence of a simultaneous increase in neuronal ferritin suggests that iron may be redox active and thus able to catalyze the Fenton reaction in the presence of H_2O. Increased tissue iron may saturate the iron-chelating sites on NM, and NM may thus cause increased, rather than decreased, production of reactive oxygen species (ROS). One possible trigger for this mechanism is suggested by the increased nigral iron content in postmortem PD brains and the correlation of this disease with urban living, where exposure to heavy metal ions is high, i.e. saturation of neuromelanin with redox-active metal ions. PD may therefore be a form of accelerated aging in the substantia nigra associated with environmental toxins in which neuromelanin has an active central role. Synthetic neuromelanin showed its toxic properties in dopaminergic cell cultures [9], but other data failed to support the hypothesis of neurotoxicity of melanin as a cause of PD.

Dopamine and Parkinson's Disease

The etiology of neuronal death in PD is still unclear, but several lines of evidence support the involvement of dopamine-induced apoptotic striatal neuron death [10]. Apoptosis has been reported in *post mortem* nigral tissue of parkinsonic patients. A possible mechanism of dopamine-related toxicity may involve the oxidation of dopamine (DA) [11], the formation of reactive oxygen species (ROS), inhibition of mitochondrial respiration, lipid peroxidation, and neuronal death. The levels of DA, 3,4-dihydroxyphenylacetic acid, and 3,4-dihydroxyphenylalanine decreased with the degree of depigmentation and degeneration in the putamen, nucleus caudatus, and substantia nigra. Thus depigmentation and degeneration of dopaminergic SN neurons seem to be correlated to enhanced rates of autoxidation, possibly due to an impaired antioxidant capacity.

DA neurotoxicity is enhanced under the conditions induced by cyanide and involves both ROS and nitric oxide-mediated oxidative stress as an initiator of apoptosis [12]. DA during *in vitro* oxidation induced cross-linking of membrane proteins in the mitochondrial-synaptosomal fraction of rat brain. The process was inhibited at low glutathione (GSH) level, but was not affected by the presence of scavengers, metal chelators, or catalase. This indicates that dopamine-induced protein damage is related rather to quinones than to ROS formation [11]. DA has a neurotoxic potential in the substantia nigra, and it is counterbalanced by the cytoprotective status of these neurons at any particular time. In contrast, in the target field of the substantia nigra, namely the neostriatum, DA has a neuroprotective role [13]. An increased DA turnover, observed in PD, may not only reduce the intermediate symptoms of the disease, but also contribute to its progression.

The Role of Mitochondria in Parkinson's Disease

Mitochondria are the major source of ROS, which appear to be released into both the matrix and intermembrane space [14]. Neurodegeneration may be caused by disrupted mitochondrial function and/or an excessive production of ROS. The main mitochondrial defect observed in PD concerns complex I (nicotinamide adenine dinucleotide coenzyme Q reductase) of the mitochondrial respiratory chain [15]. Disturbed mitochondrial function results in uncoupling of the respiratory chain,

excessive ROS formation, outflow of matrix calcium and GSH, change in the mitochondrial transmembrane potential, release of intermembrane proteins, and necrosis with or without caspases activation and activation of endonuclease [16].

The signaling pathway leading to apoptosis via mitochondria is triggered by the binding of proteins of the Bcl-2 family. This interaction forms large pores in the mitochondrial membrane through which cytochrome c is released. Premkumar and Simantov showed that the mitochondrial voltage-dependent anion channel (VDAC) is involved in DA-induced apoptosis, but whether the VDAC plays a role in mitochondrial dysfunction in Parkinson's disease is still worth examination. Abnormal accumulation of presynaptic protein alpha-sunuclein, which has recently been implicated in PD etiology, could lead to mitochondrial alterations that may result in oxidative stress [14].

Glutathione and Parkinson's Disease

Oxidative stress has been implicated in playing a major role in the neuronal death in PD. One of the indices of oxidative stress is GSH depletion. In neural cells, redox maintains a balance between the level of ROS and thiol buffers such as GSH, which protect cells from oxidative stress. The increase in ROS formation, exceeding the compensatory actions of the level of thiol buffers, may result in the activation of signaling pathways and the expression of genes that induce apoptosis in affected neural cells [17]. An early event following GSH depletion is phospholipase A2-dependend release of arachidonic acid, which can cause damage to the GSH-depleted cells through its metabolism by lipoxygenase. The generation of superoxide radicals seems to play an important role in the toxic events that follow GSH depletion [18]. GSH depletion has been shown to effect mitochondrial function, probably via selective inhibition of mitochondrial complex I.

GSH and glutathione-dependent enzymes represent the major mechanism and a multifaceted detoxification system of endogenous antioxidant. GSH-biosynthesis, glutathione peroxidases, glutathione S-transferases, and glutathione S-conjugate efflux pumps protect the neural cells against an excess of ROS [19]. The antioxidant responsive element (ARE) was found recently in the gene promotors inducible by ROS. Recently it was concluded that Bcl-2, and antiapoptotic protein located in the outer mitochondrial membrane, affects the cellular level of ROS, which can include either their overproduction or an endogenous antioxidant pathway [20], but its mode of action is still uncertain.

Iron and Parkinson's Disease

Several studies implicate iron in the pathomechanism of PD. One of the defining characteristics of neurodegeneation, including the cases of PD, is abnormal elevation of iron. The high concentration of iron in the melanin/glycidic-lipid matrix of neuromelanin suggests that most of the iron is chelated by NM [22]. A relatively selective lesion of DA-neurons, similar to PD, following injection of iron into rats' brains was described by Sengstock. These observations indicate that Fe(II)-mediated generation of ROS, via the Fenton reaction, might be a contributing factor in the etiology of PD. Moreover, production of DA from phenyloalanine by tyrosine hydroxylase is facilitated by Fe(II) [23].

Ascorbic acid serves as an electron donor for dopamine beta-hydroxylase in chromaffin vesicles and probably for peptide amidating monooxygenase in neurohypophyseal secretory vesicles. It appears that the semidehydroascorbate that is produced is reduced by cytochrome b561 to regenerate intravesicular ascorbate. Cytochrome b561, a transmembrane protein, is reduced in turn by an extravesicular electron donor, probably cytosolic ascorbic acid [24]. The human gene product stromal cell-derived receptor 2 is a homologue of cytochrome b-561 and duodenal cytochrome b and is thus predicted to be active as a ferric reductase. This protein also contains a domain homologous to the N-terminal regulatory region of dopamine beta-hydroxylase. These findings from sequence analysis lead to the prediction that stromal cell-derived receptor 2 is a catecholamine-regulated ferric reductase active in the brain. Dysfunction of cytochrome b-561 or stromal cell-derived receptor 2, therefore, might predispose individuals to abnormal accumulation of Fe(III) and/or generation of cytotoxic free radicals as a consequence of a rapid cycling between Fe(III) and Fe(II) [25]. Generation of ROS might result in neurodegeneration and thus cause PD.

Tyrosine and Parkinson's Disease

The DA content in the brain is directly related to DA synthesis from tyrosine. It is clear that oxidation of tyrosine (leading to $TyrO^{\bullet}$ formation) cannot be omitted as a possible cause of PD. Adduction of nitric oxide to $TyrO^{\bullet}$ results in TyrONO and 3-nitrotyrosine (3-NT) formation [26]. 3-NT may act as a promoter of repetitive redox cycling by its reduction to the corresponding nitroanion ra-

dical, which can be oxidized by molecular O_2 and regenerate to maternal 3-NT and superoxide anion radical [27].

The mechanism of oxidation and nitration of proteins (including enzymes) still remains unclear, but recent experimental data suggest involvement of tyrosine radical (TyrO$^\bullet$) [26]. The nitration reaction with TyrO$^\bullet$ involvement might result in DA synthesis depletion by inactivation of tyrosine hydroxylase [28]. Scavenging free radicals, such as NO$^\bullet$, $NO_2^\bullet$, and $CO_3^{\bullet-}$, and $ONOO^-$ may also be depleted by reducing superoxide radical anion formation and reductive stress.

Inflammation in Parkinson's Disease

Recent findings suggest that inflammatory processes are associated with neurodegeneration, including PD. In the MPTP model of PD, an immune reaction was shown in regions of impaired neurons as infiltration of CD4- and CD8-positive cells in the substantia nigra and MHC class I and II antigen expression on microglia [29]. Treatment with an anti-inflammatory drug (dexomethasone) resulted in a decrease in the inflammatory reaction and thus neuronal impairment [29]. Sriram proved that the proinflammatory cytokine TNF-α is an obligatory component of DA-neuron degeneration, and because TNF-α is synthesized predominantly by microglia and astrocytes, these findings support the hypothesis of an inflammatory origin of PD. Inflammation-induced dopaminergic neurodegeneration may be nitric-oxide mediated.

An *in vitro* model of nigral injury in which lipopolysaccharide-induced microglial activation leads to injury of DA cell lines suggested that only nitric oxide and H_2O_2 appear to mediate the microgila-induced DA injury [29]. The postmortem study of PD brains revealed a significantly higher percentage of DA neurons displaying caspase-8 activation. But its activation in the MPTP PD model showed that activation of caspase-8 precedes and is not the consequence of cell death. In an *in vitro* study, co-treating DA cell cultures with MPTP and caspase-8 inhibitors did not result in neuroprotection, but instead seemed to trigger a switch from apoptosis to necrosis. This effect is probably a consequence of ATP depletion, and the same mechanism may be in effect in PD [31].

Parkinson's disease is a neurodegenerative process in which *post-mortem* studies have provided evidence that many connected factors are implicated in its etiology. Moreover, disturbances in an individual factor immediately results in dysfunction of the others. Thus it is unlikely that the single components (e.g. oxidative stress, reductive stress, tyrosine stress etc) are a part of the primary causative factor contributing to PD. Rather they appear to form a cascade initiated by an unknown factor. However, the identification of this cascade of elements provides valuable information for understanding the pathogenesis of PD.

References

[1] Hughes AJ, Daniel SE, Blankson S, Lees AJ: A clinicopathologic study of 100 cases of Parkinson's disease. Arch Neurol 1993, 50, 140–148.

[2] Blum D, Torch S, Lamberg N, Nisson MF, Benabid AL, Sadoul R, Verna JM: Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. Neurobiology 2001, 65, 135–172.

[3] Agid Y: Aging, disease and nerve death. Bull Acad Natl Med 1995, 179, 1193–1203.

[4] Ilic TV, Jovanovic M, Jovicic A, Tomovic M: Oxidative stress indicators are elevated in de novo Parkinson's disease patients. Funct Neurol 1999, 14, 141–147.

[5] Enochs WS, Sarna T, Zecca L, Riley PA, Swartz HM: The roles of neuromelanin, binding of metal ions, and oxidative cytotoxicity in the pathogenesis of Parkinson's disease: a hypothesis. J Neural Transm Park Dis Dement Sect 1994, 7, 83–100.

[6] Fornstedt B, Brun A, Rosengren E, Carlsson A: The apparent autoxidation rate of catechols in dopamine-rich regions of human brains increases with the degree of depigmentation of substantia nigra. J Neural Transm Park Dis Dement Sect 1989, 1, 279–295.

[7] Fahn S, Cohen G: The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. Ann Neurol 1992, 32, 804–812.

[8] Korytkowski W, Sama T, Zaremba M: Antioxidant action of neuromelanin: the mechanism of inhibitory effect on lipid peroxidation. Arch Biochem Biophys 1995, 319, 142–148.

[9] **Nguyen A, Gille G, Moldzio R, Hunh S-T, Rausch W-D:** Synthetic neuromelanin is toxic to dopaminergic cell cultures. J Neural Transm 2002, 109, 651–661.

[10] Cheng N, Maeda T, Kume T, Kaneko S, Kochiyama H, Akaike A, Goshima Y, Misu Y: Differential neurotoxicity induced by L-dopa and dopamine in cultured striatal neurons. Brain Res 1996, 743, 278–283.

[11] Kochman A, Segura-Aguillar H, Metodiewa D: Metabolism of dopamine and its derivative in model systems: involvement of superoxide and reduced glutathione. Zjazd Streszczenia (XXXVII Zjazd PTBioch Toruń 10–14 IX 2001) 2001, No: P-14B-49.

[12] Jones DC, Gunasekar PG, Borowitz JL, Isom GE: Dopamine-induced apoptosis is mediated by oxidative stress and is enhanced by cyanide in differentiated PC12 cells. J Neurochem 2000, 76, 2296–2304.
[13] Kostrzewa RM, Brus R, Kostrzewa JP: Insidious dopamine: provocateur or protective agent in Parkinson's disease? Pol J Pharmacol 2001, 53, 165–166.
[14] Lenaz G: The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. JUBMB Life 2001, 52, 159–164.
[15] Hattori N, Tanaka M, Ozawa T, Mizuno Y: Immunohistochemical studies on complexes I, II, III and IV of mitochondria in Parkinson's disease. Ann Neurol 1991, 30, 563–571.
[16] Beal MF: Aging, energy, and oxidative stress in neurodegenerative diseases. Ann Neurol 1995, 38, 357–366.
[17] Beal MF, HymanBT, Koroshetz W: Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases? Trends Neurosci 1993, 16, 125–131.
[18] Davis W Jr, Ronai Z, Tew KD: Cellular thiols and reactive oxygen species in drug-induced apoptosis. J Pharmacol Exp Ther 2001, 296, 1–6.
[19] Mytilineou C, Kramer B, Yabut J: Glutathione depletion and oxidative stress. Parkinsonism Relat Disord 2002, 8, 385.
[20] Segura-Aguilar J, Metodiewa D, Baez S: The possible role of one-electron reduction of aminochrome in the neurodegenerative process of dopaminergic system. Neurotoxicity Res 2001, 3, 157–166.
[21] Winterbourn CC, Metodiewa D: Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide. Free Rad Biol Med 1997, 27, 322–328.
[22] Hayes JD, McLellan LI: Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defense against oxidative stress. Free Rad Biol Med 1999, 31, 273–300.
[23] Hochman A, Sternin H, Gorodin S, Korsmeyer S, Ziv I, Melamed E, Offen D: Enhanced oxidative stress and altered antioxidants in brains of Bcl-2-deficient mice. J Neurochem 1998, 71, 741–748.
[24] Aime S, Bergamasco B, Bigliano D: EPR investigations of the iron domain in neuromelanin. Biochim Biophys Acta Mol Basis Dis 1997,1361, 49–58.
[25] Jellinger KA, Stadelmann CH: Mechanisms of cell death in neurodegenerative disorders. J Neural Transm 2000, 59, 95–114.
[26] Njus D, Kelley PM, Harnadek GJ, Pacquing YV: Mechanism of ascorbic acid regeneration mediated by cytochrome b561. Ann N Y Acad Sci 1987,493, 108–119.
[27] Ponting CP: Domain homologues of dopamine beta-hydroxylase and ferric reductase: roles for iron metabolism in neurodegenerative disorders? Hum Mol Genet 2001, 10, 1853–1858.
[28] Goldstein S, Czapski G, Lind J, Merényi G: Tyrosine nitration by simultaneous generation of $NO^{\bullet}$ and $O_2^{\bullet-}$ under physiological conditions. How the radicals do the job. J Biol Chem 2000, 275, 3031–3036.
[29] Krainev AG, Williams TD, Bigelow DJ: Enzymatic reduction of 3-nitrotyrosine generates superoxide. Chem Res Toxicol 1998, 11, 495–502.
[30] Santos CXC, Bonini MG, Augusto O: Role of the carbon radical anion in tyrosine nitration and hydroxylation by peroxynitrite. Arch Biochem Biophys 2000, 377, 146–152.
[31] Kurkowska-Jastrzebska I, Wronska A, Kohutnicka M, Czlonkowski A, Czlonkowska A: MHC class II positive microglia and lymphocytic infiltration are present in the substantia nigra and striatum in mouse model of Parkinson's disease. Acta Neurobiol Exp 1999, 59, 1–8.
[32] Le W, Rowe D, Xie W, Ortiz I, He Y, Appel SH: Microglial activation and dopaminergic cell injury: an in vitro model relevant to Parkinson's disease. J Neurosci 2001, 21, 8447–8455.
[33] Hartmann A, Troadec JD, Hunot S, Kikly K, Faucheux BA, Mouatt-Prigent A, Ruberg M, Agid Y, Hirsch EC: Caspase-8 is an effector in apoptotic death of dopaminergic neurons in Parkinson's disease, but pathway inhibition results in neuronal necrosis. J Neurosci 2001, 21, 2247–2255.

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