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REVIEW

ENDOGENOUS RISK FACTORS IN PARKINSON'S DISEASE: DOPAMINE AND TETRAHYDROISOQUINOLINES[1]

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Endogenous risk factors in Parkinson's disease: dopamine and tetrahydroisoquinolines. L. ANTKIEWICZ-MICHALUK. Pol. J. Pharmacol., 2002, 54, 567–572.

The cause of chronic nigral cell death in Parkinson's disease (PD) and the underlying mechanisms remain elusive. The selective action of exogenous and endogenous neurotoxic substances can provide partial explanation of these processes. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is an exogenous neurotoxin producing parkinsonism in humans, monkeys and various animals as the result of MAO_B-catalyzed conversion of it to the 1-methyl-4-phenyl-pyridinium ion (MPP^+), which selectively kills the nigrostriatal dopaminergic neurons. On the other hand, various isoquinoline derivatives were found in the brain, and they are considered to be the endogenous neurotoxins with neurochemical properties similar to those of MPTP, which cause PD. Among them, 1,2,3,4-tetrahydroisoquinoline (TIQ), 1-benzyl-TIQ, and 1-methyl-5,6-dihydroxy-TIQ (salsolinol) have the most potent neurotoxic action. Since PD is a slowly progressing neurodegenerative disease, it has been suggested that it could be connected with excitotoxicity and apoptosis. Therapeutic strategies should focused on the search for the drugs exhibiting antiapoptotic potential such as: antioxidants, MAO_B inhibitors, dopaminergic drugs and free radical scavengers.

Key words: *Parkinson's disease, dopamine and apoptosis, MPTP, endogenous neurotoxins, tetrahydroisoquinoline derivatives, neuroprotection*

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INTRODUCTION

A cause of idiopathic Parkinson's disease is unknown. In 70% of parkinsonian patients, the disease begins between 55–70 years of age, and its incidence is similar in men and women. While human life span is steadily increasing, incidence and prevalence of Parkinson's disease in the population are also on the rise. This disease is associated with focal atrophy of melanin-containing dopaminergic neurons in the substantia nigra pars compacta (SN_c). Parkinson's disease was the first central nervous system (CNS) disorder, whose biochemical basis was described [11]. Since DA is transported to the striatum through dopaminergic neuronal pathways, degeneration process of the SN leads to strong decreases in dopamine level in this structure, dropping even by 90%. Although substantial DA losses in the extrapyramidal system nuclei are undoubtedly the main cause of manifestation of clinical symptoms of idiopathic parkinsonism, the studies of many authors have also pointed to degenerative changes in pigment cells, melanocytes, in the locus coeruleus (LC), where clusters of noradrenergic cells are localized [2, 8]. Noradrenergic system damage is believed to be associated with depressive signs and aggravation of dementing symptoms in the patients suffering from Parkinson's disease. Moreover, it was experimentally proved that LC lesion inhibited dopamine synthesis in the striatum, while the stimulation of this structure enhanced the activity of dopaminergic neurons. However motor disturbances in Parkinson's disease are caused principally by dopaminergic neuron losses in the structures of extrapyramidal system, it can also be expected on the basis of the reported data that the noradrenergic structure, LC, is indispensable for proper function of dopaminergic neurons.

The studies of recent years shed new light on a possible cause of a number of serious neurodegenerative diseases, including Alzheimer's, Parkinson's, Huntington's diseases and even epilepsy. It appears that all these disorders are underlain by excitotoxicity. Excitotoxicity is defined as nervous cell death due to excessive Ca^{2+} influx into the cells. In many cases, excitotoxicity results in programmed cell death, i.e. apoptosis. Hence, Parkinson's disease can be connected with apoptosis induced by still unknown exogenous or endogenous factors [1, 13].

APOPTOSIS

In general, two types of cell death can be distinguished: apoptosis and necrosis. Necrosis consists in mechanical cell damage, which is always accompanied by inflammation.

Apoptosis in its ideal form occurs without inflammatory reaction and involves triggering suicidal death program in the cell. Apoptosis can be a physiological process or can be induced by many factors. Physiological phenomenon of programmed cell death has been known since early 1970s, and recently has been given much attention. It is believed that such program critically requires the stimulation of cellular biochemical machinery, whose effector elements are hydrolytic enzymes decomposing macromolecules. A cell dying an apoptotic death is characterized by certain morphological, biochemical and genetic features, which distinguish it from normal or necrotic cell. Chromatin of an apoptotic cell undergoes marginalization and fragmentation. The cell shrinks due to water loss and sometimes disintegrates into apoptotic bodies. Apoptosis very often requires the activation of genes and *de novo* protein synthesis. Apoptosis can be induced by very diverse factors acting *via* different mechanisms. It means that there are many routes leading to the morphological changes characteristic of apoptotic cell.

Apoptosis-inducing factors

— Disturbance in Ca^{2+} homeostasis;
— Neurotransmitters (DA, excitatory amino acids);
— Neurotoxic factors (e.g. 6-OHDA, MPTP, THIQ);
— Free radicals (H_2O_2, $^{\bullet}OH$, $NO^{\bullet}$);
— Damage to mitochondria.

Ca^{2+} as an element of life and death

Implication of Ca^{2+} in apoptosis is indisputable. It participates in all stages of apoptosis, although its excess can also lead to necrosis. It is well known that Ca^{2+} plays physiological role in the cell as an intracellular regulator of many physiological processes (release of neurotransmitters, hormones, activation of enzymes, etc.), but its overflow in the cell produces irreversible damages, thereby causing cell death. The Ca^{2+} concentration outside nervous cells is many times higher (in millimolar range) than inside (from nano- to low micromolar levels). Therefore, Ca^{2+} level in the cell is strictly regulated by voltage-dependent calcium channels and iono-

tropic (NMDA, KA) receptors, transporting this ion inside, and by cell compartmentalization, allowing the cell to store its excess in mitochondria, endoplasmic reticulum and nucleus. Disturbance of calcium homeostasis causes uncontrolled Ca^{2+} outflow from cellular compartments and its inflow from extracellular space inside the cell. It has been even suggested that apoptotic death of most of perishing neurons occurs due to the loss of their ability to regulate intracellular calcium level. The hypothesis of "calcium-related cell death" postulates that Ca^{2+} activates transcription of so-called "cell death genes" in dying neurons. It seems that all abovementioned apoptosis-inducing factors effectuate serious disturbances of cellular Ca^{2+} homeostasis.

Dopamine as a possible neurotoxic factor

As well enzymatic DA oxidation catalyzed by monoamine oxidize B (MAO_B) as spontaneous DA oxidation cause the formation of hydrogen peroxide (H_2O_2), a dangerous substance, which is a source of the most toxic free radical, hydroxyl radical ($^{\bullet}OH$). The enzymatic reaction of DA oxidation is represented by the following scheme:

$$DA + O_2 + H_2O \xrightarrow{MAO_B} \text{3,4-dihydroxyphenylacetaldehyde (DOPAC)} + NH_3 + \mathbf{H_2O_2}$$

Thus, DA oxidation is accompanied by the generation of H_2O_2, which is required for hydroxyl free radical formation ($^{\bullet}OH$). Free radical $^{\bullet}OH$ is produced in Fenton reaction:

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

Hydroxyl ion OH^- produced also in this reaction is incomparably less toxic (10^{14}) than hydroxyl radical. Iron plays an important role in Fenton reaction. It can derive from neuromelanin or ferritin. It should be emphasized that in the course of Parkinson's disease, exactly neuromelanin-containing dopaminergic neurons are those which die [6, 7], that suggests that Fe^{2+}, essential for Fenton reaction to occur, can be released from this compound by unknown toxic factors. Thus, DA metabolism leads to the formation of toxic hydroxyl radical, which poses a serious threat to nervous cells, causing their damage and death in the process of apoptosis. Experimental studies in rats with the lesioned SN neurons have shown increased levels of DA metabolites, DOPAC and HVA in the surviving nerve endings in the striatum, if damage level exceeded 60–80%. Experimental neuronal lesions in rats resemble situation in Parkinson's disease, in which classical symptoms, such as passive tremor and rigidity appear when 70–80% of the striatal neurons have been lost. Development of Parkinson's disease in humans is similar, namely dopaminergic system defends itself against the consequences of deficit of this neurotransmitter by increasing DA turnover. Postmortem studies on the brains of parkinsonian patients demonstrated specific, manifold augmentation of DA metabolism in the structures of the extrapyramidal system, but not in the limbic system [2]. The elevated DA turnover in Parkinson's disease causes the increased formation of H_2O_2, and thereby the raised concentration of neurotoxic $^{\bullet}OH$ radical. Cohen postulated that the increased release, uptake and presynaptic turnover of DA could contribute to progressive destruction of the nigrostriatal neurons, observed in Parkinson's disease [9]. Another characteristic change, accompanying Parkinson's disease, which can also lead to the increased production of H_2O_2, and, consequently, to the induction of apoptosis, is the inhibition of mitochondrial complex I in the SN neurons [18]. The suppression of normal function of complex I in Parkinson's disease is thought to occur only in mitochondria of the cells of the substantia nigra pars compacta (SN_C), and has not been observed in other structures (nucleus accumbens, putamen, globus pallidus) or in other degenerative diseases which symptomatology is similar to Parkinson's disease, e.g. multiple system atrophy.

Tetrahydroisoquinoline derivatives as possible endogenous neurotoxins

Parkinsonism has recently been suggested in world literature to be underlain by neurodegenerative processes induced by endogenous neurotoxic substances [14, 17]. These compounds belong to the isoquinoline group, and their structure closely resembles known exogenous toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP was proved to provoke in humans and animals the symptoms characteristic of Parkinson's disease with neurodegeneration of dopaminergic cells in the nigrostriatal pathway. Neurotoxic role of MPTP is critically dependent on its metabolite MPP^+, formed in glial cells in the reaction catalyzed by MAO_B. It is specifically taken up by dopaminergic

neurons of the nigrostriatal pathway, subsequently binding to neuromelanin present in DA cells of SN_c. MPP^+ accumulation in these cells leads to their death. Other MPP^+-triggered mechanisms deadly for DA cells in SN_c involve: enhanced DA release from nerve endings, iron liberation from neuromelanin, generation of free radicals ($^{\bullet}OH$), and inhibition of mitochondrial complex I, which leads to apoptosis [10, 12]. Neurotoxic action of MPTP is completely blocked by MAO_B inhibitors, e.g. selegiline. Endogenous tetrahydroisoquinolines resemble exogenous MPTP in terms of their chemical structure and capacity to form neurotoxic ions. They are produced in the CNS by condensation of catecholamines with acetaldehyde in Picket-Spengler reaction. In particular, it was shown that DA condensation with acetaldehyde yielded salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline). Our research indicated that salsolinol level in the cerebrospinal fluid of the patients suffering from Parkinson's disease was well correlated with a degree of motor impairment and advancement of the disease [3]. Condensation of other amines leads to the formation of various tetrahydroisoquinolines in the brain. Their methylation in the reaction catalyzed by N-methyltransferase and oxidation with MAO_B participation leads, similarly as in the case of MPTP, to the formation of N-methylisoquinoline ions, exhibiting neurotoxic properties, which, however are much weaker. Biologically active tetrahydroisoquinolines inspired wide interest, when they were noticed to provoke in experimental animal studies functional symptoms, such as muscular rigidity and biochemical changes (decreased DA level in SN) characteristic of Parkinson's disease. Tetrahydroisoquinolines, many of which were found in the brain, are strong complex I inhibitors after their transformation into N-methylisoquinoline ions, thereby being able to cause hypofunction of mitochondria observed in Parkinson's disease [16]. Experimental studies indicated that isoquinolines produced from catecholamines (e.g. N-methylsalsolinol) could initiate apoptic process in dopaminergic neurons by the generation of free radicals, particularly the most toxic hydroxyl radical ($^{\bullet}OH$). *In vitro* investigations demonstrated that antioxidants, such as vitamin C and vitamin E inhibited $^{\bullet}OH$ radical generation by N-methylsalsolinol [15]. It should be emphasized that neurotoxic effects and inhibitory influence on the activity of mitochondria are attributed mostly to N-methylisoquinoline ions, whose formation, contrary to MPTP, requires initial N-methylation, catalyzed by N-methyltransferase, in addition to MAO_B action. N-methyltransferase activity in the CNS is relatively low, and was detected only in certain structures (the highest its levels were found in the SN and LC). Limited availability of this enzyme can explain much lower neurotoxicity of tetrahydroisoquinolines [5] in comparison with strongly neurotoxic related compound, MPTP, which can be transformed into neurotoxic MPP^+ ion by the action of only one enzyme MAO_B widespread in the CNS [12]. Other isoquinolines, e.g. 1-methyl-1,2,3,4-tetrahydroisoquinoline (1Me-TIQ) can exhibit neuroprotective activity, since it acts as free radical scavenger [19]. 1Me-TIQ can restore disturbed activity of the enzymes involved in DA metabolizm and implicated also in tetrahydroisoquinoline transformations, thus preventing the formation of neurotoxic products of these reactions [4]. Neuroprotective action of 1Me-TIQ was shown by experimental studies to be based on two mechanisms: scavenging hydroxyl radical and enhancing the synthesis of neurotropic factors, NGF and BDNF [20].

ATTEMPTS TO CASUALLY TREAT PARKINSON'S DISEASE – NEUROPROTECTION

Even though exact pathomechanism underlying degeneration of the substantia nigra cells in Parkinson's disease is not clear, free radicals and mitochondrial dysfunction have been suggested to play a crucial role in this process, being able to induce apoptotic process. Therapeutic strategies based on symptomatic treatment of this disease do not stop dopaminergic nervous cell death, therefore, current endeavors to halt or slow progression of the disease are focused on the search for the drugs exhibiting antiapoptotic potential:

— Antioxidants (glutathione GSH, vitamin E, vitamin C, β-carotene, coenzyme Q);
— MAO_B inhibitors (selegiline, budipine), which suppress the formation of H_2O_2 and increase the level of endogenous neuroprotective substance 1Me-TIQ;
— Dopaminergic drugs (bromocriptine, lisuride, apomorphine, pramipexole);

— Inhibitors of glutamatergic neuron activity, such as NMDA receptor antagonists (amantadine, budipine);
— Free radical scavengers (acetylsalicylic acid, apomorphine, bromocriptine).

It appears that in neurodegenerative diseases, special attention should be concentrated on proper diet, rich in antioxidant compounds and vitamins, aiding the function of the only powerful free radical scavenger present in the CNS, glutathione (GSH). Maintenance of the balance between free radical formation and neutralization (scavenging) is crucial for nervous and glial cells. In the cells, where a true war in being waged with toxic weapons, glutathione is the most important physiological antioxidant. It is a sulfur-containing tripeptide, composed of three amino acids: cysteine, glutamic acid and glycine, which are produced in the organism. Reduced glutathione (GSH) is a key element in H_2O_2 neutralization in fats and glutathione cycle itself. The organism is not able to absorb glutathione. It has to be produced in the cells. Therefore, the elevation of glutathione level requires supplying the organism with components necessary for its synthesis. Glycine and glutamic acid are easily available, but inadequate supply of cysteine may limit glutathione production. In addition, vitamin C was shown to help to preserve high glutathione level. Vitamin C, E and β-carotene are strong antioxidants as well, protecting neurons from toxic action of many factors. Hence, keeping proper diet from early youth can significantly prevent the central neuron damage by different toxic substances.

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