


Role of homocysteine in the development and progression of Parkinson's disease

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Introduction

Parkinson's disease (PD), also known as tremor paralysis, is a common degenerative disease of the nervous system in middle-aged and elderly people. Pathologically, PD is characterized by the degenerative loss of dopaminergic neurons and the formation of Lewy bodies.¹ As an extrapyramidal disease, the typical symptoms of PD include resting tremor, muscle rigidity, and bradykinesia, associated with other nonmotor symptoms such as olfactory dysfunction, cognitive impairment, psychiatric symptoms, and autonomic dysfunction.² Once these psychiatric symptoms are manifested, they are often present persistently, increasing

Abstract

Homocysteine is an essential intermediate product of biochemical reactions that is present in various tissues of the human body. Homocysteine may be associated with the development and progression of Parkinson's disease. Plasma homocysteine levels in patients with Parkinson's disease are elevated compared to those of healthy individuals. High homocysteine drives PD development and progression while aggregating the clinical symptoms of PD patients. The relationship between PD and homocysteine involves multiple pathways, including nerve cell apoptosis, oxidative stress, and DNA damage. This is crucial for explaining how high homocysteine drives the PD procession. Elevated homocysteine level during PD development and progression offers a new strategy for the diagnosis and treatment of this disease.

the burden of care. Moreover, the psychiatric symptoms can even outcompete the motor symptoms and become the foremost factor affecting the quality of life and survival of patients. Hence, these psychiatric symptoms are important indications of poor prognosis in patients with PD.³ The prevalence of PD increases with increasing age, and 1% of the population aged over 60 years are affected by PD.⁴ Therefore, exploring the etiology and pathogenesis of PD is of enormous medical and social value for guiding the diagnosis, treatment, and prevention of the disease, and for improving the quality of life of the patients.

In recent years, some studies have shown that nearly 30% of the PD patients are associated with elevated

plasma Hcy levels.⁵ Based on a clinical trial, Licking et al.⁶ have shown that Hcy is closely related to PD development and progression, and high Hcy may be a major risk factor of PD. Hcy is a thio-containing amino acid generated through the demethylation of methionine. Methionine reacts with ATP to produce S-adenosyl-L-methionine which is then demethylase to form Hcy, without participating in protein synthesis.^{7–9} Hcy is mainly derived from methionine in food and it is present mainly in three forms in plasma. The most important form is albumin-bound Hcy (~70–80%), while 1% is free Hcy in a reduced form; the remaining portion is Hcy-cysteine disulfide.¹⁰ The total Hcy in plasma is the sum of the three forms, and the Hcy concentration usually refers to the total Hcy concentration.

The Hcy produced in the human body is mainly eliminated through the following three metabolic pathways. (1) Remethylation: Hcy is remethylated to methionine by 5,10-methylenetetrahydrofolate reductase and methionine synthetase, with vitamins B2 and B12 as cofactors.¹¹ (2) Transsulfuration, namely, the condensation of Hcy with serine: Hcy is first transformed into cystathionine, in which cystathionine β -synthase must be liberated and vitamin B6 is used as a cofactor; further, the cystathionine produced is metabolized into cysteine and α -ketobutyric acid which are excreted from the body.¹² (3) Release into the extracellular fluid, which is indicative of the balance between Hcy production and metabolism: at low concentrations of methionine, the cellular release of Hcy is mainly controlled by the activity of methionine synthase, while at high concentrations of methionine, cystathionine synthetase activity primarily determines Hcy release.¹³ Studies have shown that high concentrations of Hcy are closely linked to PD development and progression, and Hcy may become a feasible therapeutic target for cognitive decline in PD.¹⁴ Therefore, understanding the role of Hcy in PD development and progression is of great significance to revealing the pathogenesis of the disease.

Etiology of Hyperhomocysteinemia

Plasma Hcy concentrations can range from 5 to 15 $\mu\text{mol/L}$ in normal people,¹⁵ and elevated Hcy levels of 15 $\mu\text{mol/L}$ or higher are considered as hyperhomocysteinemia (Hhcy).^{7,8} Because plasma metabolism is regulated by many key enzymes, cofactors, and methyltetrahydrofolic acid as a substrate, the etiology of Hhcy is diverse, mainly including gene defects and nutritional deficiencies.¹⁶ Among the genetic causes of severe Hhcy and typical Hhcy hematuria (congenital Hhcy hematuria), homozygous C β S deficiency was found to be the most prevalent factor with an incidence of 1/100,000, which resulted in a 40-fold increase in the fasting plasma Hcy concentration compared

to the normal level.¹⁷ This defect is an autosomal recessive trait. So far, at least 60 C β S mutants have been reported, among which I278T and G307S are the most common ones.¹⁸ Other gene defects include homozygous deficiency of methylenetetrahydrofolate reductase (MTHFR), methionine synthetase deficiency, and functional impairment of methionine synthetase due to gene abnormality in vitamin B12 metabolism.^{19,20} The C677T point mutation in the coding region of the MTHFR gene is the most common gene defect associated with mild elevations of Hcy, and the activity of the resulting MTHFR variant is only half of the normal enzyme activity.²¹ A nutritional status analysis has revealed that folic acid, vitamin B12, and vitamin B6 are closely associated with Hcy metabolism; any nutritional deficiencies that could lead to a decrease in folic acid, vitamin B12, or vitamin B6 concentration would increase the risk of Hhcy.²² Reportedly, ~2/3 of Hhcy cases are caused by deficient blood concentrations of two or more vitamin cofactors.²²

Age and gender are also important factors related to Hhcy. Plasma Hcy concentrations gradually increase with increasing age, which may be attributed to the following three reasons²³: (1) the elderly are deficient in key cofactors including vitamin B16, vitamin B12, and folic acid, which results in decreased activities of amino acid metabolic enzymes; (2) the elderly often have renal hypofunction; and (3) the elderly often exhibit decreased cystathionase activity. Generally, plasma Hcy concentrations in men are higher than in women, and, the concentration levels increase with increasing age irrespective of gender. Additionally, Hcy concentrations may increase or remain constant in women after menopause.²⁴ The main pathway to clear Hcy in plasma is renal metabolism (~70%), rather than simple excretion with urine.^{25,26} Hence, renal hypofunction often results in Hhcy. Moreover, plasma levels of Hcy are affected by the effects of specific drugs,²⁷ while certain diseases may cause Hhcy. For example, plasma Hcy concentrations are increased in patients with severe scleroderma due to folic acid deficiency.²⁸ An increase in plasma Hcy concentrations has also been found among patients with lymphocytic leukemia,²⁹ breast cancer³⁰ ovarian cancer,³¹ hepatocellular carcinoma,³² perhaps due to the metabolic disorder of methionine in malignant cells.³² Furthermore, abnormal living habits such as staying up late, alcoholism, smoking, harsh environment, and high stress can lead to an increase in plasma Hcy concentrations.³³

Elevations of Homocysteine Levels in Parkinson's Disease

Epidemiological and clinical studies have shown that the elevation of plasma Hcy levels is a high-risk factor for

neurodegenerative diseases.³⁴ Kuhn et al.³⁵ reported that the plasma Hcy levels in PD patients were substantially higher than in the control group. High Hcy-induced loss of intracellular ATP was suggested to be a crucial factor leading to PD.³⁶ Duan et al.³⁷ noted that Hhcy contributed to the decrease in dopaminergic neurons in a rat PD model, while it enhanced the sensitivity of human dopaminergic neurons to rotenone and iron ions *in vitro*; these results suggest that Hhcy can destroy dopaminergic neurons through increasing their sensitivity to toxins, thereby driving the pathogenesis of PD and accelerating disease progression. O'Suilleabhain et al.³⁸ found that PD patients with elevated plasma Hcy levels were more depressed and cognitively impaired than the patients with nonelevated Hcy levels. Because an elevated Hcy level can usually be lowered by vitamin supplementation, these findings have potential therapeutic implications for ameliorating rates of clinical deterioration.³⁸

Role of Hhcy in Parkinson's Disease

Available studies show that the high concentrations of Hcy may drive PD development and progression through multiple pathways involving apoptosis, oxidative stress, mitochondrial dysfunction, and DNA damage in nerve cells. However, these molecular mechanisms still need to be further explored by *in vivo*, *in vitro*, and clinical studies.

Hhcy mediates nerve cell apoptosis

Hhcy mediates nerve cell apoptosis mainly through the promotion of energy consumption and damage of DNA strands. Streck et al.³⁶ injected Hcy into animals, which substantially reduced the production of tricarboxylic acid cycle products and the intake of glucose by negatively affecting succinate dehydrogenase and cytochrome C oxidase activities. In physiological conditions, Hcy is methylated into methionine to maintain low Hcy levels, and this reaction requires the participation of folic acid and vitamin B12.¹² Methionine plays a vital role in the metabolism of one-carbon units; it participates in many biosynthetic processes and is essential for the synthesis, repair, and methylation of DNA strands.³⁹ Deficiency of folic acid/vitamin B12 and/or excessive accumulation of Hcy can hinder the metabolic turnover of methionine, leading to decreased cytosine methylation in DNA and hence the breakage of DNA strands. Kruman⁴⁰ suggested that the mechanism underpinning neuronal DNA damage could be related to the interruption of DNA transmethylation. Therefore, folic acid deficiency and Hhcy can reduce DNA methylation, which in turn interferes with gene transcription and DNA replication, impairs DNA

repair, and thus leads to gene mutation and apoptosis.^{41,42} Additionally, Paul et al.⁴³ have shown that Hcy may induce oxidative stress in nerve cells, which produces reactive oxygen and nitrogen species, leading to apoptosis.

Hhcy can also induce nerve cell apoptosis via inhibition of mitochondrial activity. Both clinical and animal studies have demonstrated mitochondrial dysfunction in the striatum of patients with PD.^{44,45} Hcy-mediated oxidative stress inhibits the activity of mitochondrial complex I, leading to mitochondrial respiratory chain dysfunction.⁴⁶ Moreover, Hcy can enhance caspase activity and reduce the mitochondrial transmembrane potential; the resulting calcium influx causes calcium overload, eventually leading to apoptosis.⁴⁷ The reduction in mitochondrial complex I activity and the generation of reactive oxygen species may be a key mechanism of dopaminergic neuron apoptosis in PD.⁴⁸

Hhcy induces oxidative stress

Hhcy-induced oxidative stress may be associated with increased intracellular Ca^{2+} concentration and DNA damage.^{40,49} Previous experimental study showed a significant dose-effect relationship for Hcy and Ca^{2+} ; 0.5 mmol/L of Hcy markedly increased cytosolic Ca^{2+} concentrations, and the Ca^{2+} concentrations increased with increasing concentration of Hcy. In an *in vitro* culture of neuron cells, both Hcy treatment and folic acid deficiency resulted in an increase the intracellular Ca^{2+} concentrations, while this reaction was alleviated by adding a Ca^{2+} channel blocker. This result suggests that at least most of the increase in Ca^{2+} concentrations was caused by transmembrane Ca^{2+} inflow.⁴⁹ Additionally, Hcy can activate N-methyl-D-aspartate receptors (a subset of glutamic acid receptors) and boost the excitotoxicity of glutamic acid, leading to neuron degeneration. In contrast, N-methyl-D-aspartate receptor blockers can reduce Hcy-induced calcium influx, thereby reducing the toxic effect.^{40,50} Moreover, Refsum et al.⁵¹ have found that Hcy impairs glutathione peroxidase activity and reduces the levels of vitamins A, C, and E in tissues, thus inducing oxidative stress.

The direct toxic effects of Hhcy on neurons

Hhcy increases the sensitivity of the nervous system to the methylation of toxic substances in sensitive brain, which is a vital biochemical process. S-adenosylhomocysteine (SAH) is a potent methyltransferase inhibitor that can slow down the methylation process in brain tissue, which in turn causes damage and apoptosis of brain neurons.⁵² Hhcy can inhibit the decomposition of SAH and

thereby increase SAH levels in the brain. Kennedy et al.⁵³ showed that the SAH levels in the prefrontal cortex of PD patients with mild cognitive impairment were considerably higher than in normal individuals, while the inhibition of exogenous methyltransferase activity by brain tissue homogenates was 15% higher than in normal individuals; this result suggests that SAH may be an important binding point for Hhcy to cause cognitive impairment. Another study demonstrated that neurotoxicity was not induced by a direct infusion of free Hcy at a dose of 0.43 ng/ μ L (equivalent to threefold the peripheral plasma level) into the dorsal hippocampus of experimental animals; however, if kainic acid (a glutamate agonist that is highly toxic to the hippocampus) was coinjected, Hcy enhanced the neurotoxicity induced by kainic acid.⁴⁰ Duan et al.³⁷ also found a synergy between Hcy and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, a toxin widely used to induce PD-like changes in the nervous system of animals). Thus, Hcy *per se* may not be neurotoxic, but it increases the sensitivity of dopaminergic neurons to other toxic substances among patients with PD.

Hhcy impairs peripheral microcirculation

Hantatty et al.⁵⁴ conducted a trial involving 22 healthy adults and found that an increase in plasma Hcy concentrations, caused by oral administration of methionine or Hcy, led to vascular endothelial dysfunction. When Hcy enters the plasma, it oxidizes spontaneously to generate O₂ and H₂O₂. The reactive oxygen species generated by endothelial injury due to Hhcy can cause vascular endothelial cell damage. When metal ions such as iron and copper are present, hydroxyl radicals with high cytotoxicity can be produced, leading to apoptosis and loss of function in vascular endothelial cells.⁵⁵ The active thiol group of Hcy acts as a reducing group to break disulfide bonds in peptide chain (e.g., thrombomodulin, protein C, and certain molecules produced by endothelial cells, such as the Von Willebrand factor), thereby affecting the balance between coagulation and anticoagulation.⁵⁶ Moreover, Hcy inhibits glutathione peroxidase activity and intracellular glutathione mRNA expression, which in turn destroys the glutathione system and reduces the antioxidant function of endothelium.⁵⁷ Large amounts of Hcy also reduce the biological activity of NO produced by endothelial cells, thus weakening endothelium-dependent vasodilation. Additionally, Hcy redox receptor is present on human vascular smooth muscle, to which Hcy can bind, causing vascular smooth muscle hyperplasia and impairing vascular and endothelial function.⁵⁸ In summary, Hhcy affects microcirculation through endothelial

injury, which in turn leads to insufficient brain perfusion, causing cognitive impairment in PD.

Conclusion

Plasma Hcy concentrations are increased in patients with PD compared to healthy individuals. Hhcy drives PD development and progression while aggregating the clinical symptoms among PD patients. Thus, Hhcy may be a risk factor of PD. The relationship between PD and Hcy involves multiple pathways, including gene defects, apoptosis, oxidative stress, and DNA damage. Therefore, the detection and intervention of plasma Hcy levels in patients with PD can facilitate the retardation and control of PD progression, which has implications for improving the prognosis and the quality of life of the patients.

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Conflict of Interest

The authors declare no conflict of interest.

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