Homocysteine and glutamate receptors in the neuronal dysfunction and cell death in levodopa therapy: are B-complex vitamins and herbal medicine the panacea?

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Abstract

Introduction
Levodopa treatment in Parkinson’s disease can lead to elevated homocysteine levels. Increased homocysteine levels may accelerate dopaminergic cell death in Parkinson’s disease through neurotoxic effects. Homocysteine has been found to induce neurological dysfunction via excitotoxic stress. Accordingly, homocysteine-induced toxicity is mediated by glutamate receptors. The neurological pathologies associated with hyperhomocysteaemia are hypothesised to be caused by oxidative stress, excitotoxicity via glutamate receptors, and DNA hypomethylation. Low vitamin B status combined with polymorphisms in pathway-specific gene mutations can lead to hyperhomocysteaemia. Consequently, lowering homocysteine level in the blood by B-complex vitamin may relieve Parkinson’s disease and motor complication associated with levodopa therapy. Alternatively, medicinal plants-derived antioxidants have been demonstrated to reduce lipid peroxidation and enhance activities and levels of endogenous antioxidants. Therefore, medicinal plants might be considered as important remedies to improve the pathological alterations associated with oxidative-stress-related diseases such as Parkinson’s disease.

and levodopa-induced dyskinesias. This review surveys the molecular mechanisms of levodopa-induced dyskinesias, and sheds the light into the potentials of dietary supplementations and traditional medicine as therapeutic strategies in synergy with levodopa therapy.

Conclusion

Novel approaches to improve the bioavailability of medicinal plants might be their use as an adjunctive therapy with levodopa or glutamate receptor antagonists or vitamin B supplementation. Pre-clinical and clinical investigations of this treatments regime need to be undertaken.

Introduction

The prevalence of Parkinson’s disease (PD) is dramatically increasing as people are living longer and currently the disease affects around 5 million people worldwide. PD is pathologically characterised by degeneration of nigrostriatal dopamine (DA) neurons, leading to bradykinesia, tremor and muscle rigidity. Since the 1960s being introduced as a strategy for replacement of the loss striatal DA, levodopa (L-DOPA) remains the gold standard for the PD management and is still the most effective symptomatic therapy. However, long-term L-DOPA replacement therapy is accompanied by abnormal involuntary movements, known as L-DOPA-induced dyskinesias (LID). These side effects are often more debilitating than the motor symptoms themselves. Effective treatment of LID in PD remains a significant unmet clinical need. Several hypotheses have been proposed to explain the development of LID. LID may emerge as a consequence of abnormal fluctuations in synaptic DA levels induced by L-DOPA treatment. Glutamate receptors hyperactivity was also suspected to induce LID. There is evidence of cross talk between glutamate receptors and L-DOPA via homocysteine (Hcy).

In clinical practice, L-DOPA is combined with DOPA decarboxylase inhibitors (DDI). This increases the plasma half-life of L-DOPA and the clinical effects of L-DOPA on motor behaviour of PD patients are improved. DDI action diverts L-DOPA metabolism towards catechol-O-methyltransferase enzyme and thus decreases the plasma half-life of L-DOPA. Consequently, an increased synthesis of the L-DOPA metabolite 3-O-methylidopa (3-OMD) occurs. 3-OMD accumulates in particular during repeated L-DOPA/DDI application. Moreover, the COMT enzymatic activity requires S-adenosylmethionine (SAM) as the methyl donor. Therefore O-methylation of L-DOPA to 3-OMD is associated with the conversion of SAM to S-adenosylhomocysteine (SAH), which can subsequently be transformed to Hcy. Hcy is an endogenous glutamate receptor agonist acting on N-methyl-D-aspartate (NMDA) receptors. In response to excitatory stimulations the brain cells produce homocysteic acid from Hcy. Homocysteic acid is an oxidative product that functions as an excitatory neurotransmitter by activating NMDA receptors. Folate functions as a cofactor for enzymes involved in amino acid metabolism and nucleic acid synthesis.

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Critical review

With vitamins B6 and B12, folate is member of B-complex vitamins involved in converting Hcy to the harmless amino acid metabolites methionine and cysteine\(^9\). PD is known as an age-related neurodegenerative disorder\(^20\). Ageing is associated with deficiencies in folate, vitamin B6 and vitamin B12\(^21\), resulting in hyperhomocysteinemia (HHcy). Thus, it is reasonable to think that the use the trio of vitamins B (folate, B6 and B12) as a nutritional supplement may have a therapeutic potential in the treatment of PD and preventing LID.

The cause of neuronal death in neurological disorders appears to be multifactorial. However, it is clear that the underlying factor in these diseases is increased oxidative stress. This is substantiated by the findings that the protein side chains are modified either directly by reactive oxygen species (ROS) or reactive nitrogen species (RNS), or indirectly, by the products of lipid peroxidation\(^22\). The antioxidant properties observed with some medicinal plants\(^23\) could be of interest in fighting neurotoxicity in PD and LID. In the present review, we will focus on the literature showing a prominent role of Hcy in the excitotoxicity and how vitamins B and medicinal plants may contribute to relieve this pathological condition.

Discussion

The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. Animal care was in accordance with the institutional guidelines.

Parkinson’s disease and L-DOPA-induced dyskinesias pathophysiology

The basal ganglia (BG) are subcortical structures implicated in various neurological disorders including PD\(^24\). An understanding of BG circuits is essential for unravelling the neural basis of motor control. It may also help to develop effective treatments for these disorders. The BG contains two parallel circuits, so-called direct and indirect circuits\(^2\). In the BG, a subtle balance between inhibition and excitation of the major output nuclei (striatum and pallidum) is necessary for normal motor control\(^2\). The direct and indirect inhibitory projections (GABAergic) from the striatum (caudate nucleus/putamen) to the globus pallidus internal (GPi)/substantia nigra pars reticulata (SNr) and excitatory projections (glutamatergic) from the subthalamic nucleus (STN) to the substantia nigra pars compacta (SNc) and GPi/SNr provide the functional balance\(^1\). An appropriate dopaminergic input from the SNc to the striatums plays a key role in maintaining this balance\(^2\). In PD, degeneration of DA nigral neurons within the SNc causes an asymmetry between the direct and indirect striatal output pathways. Subsequently, there is an increase of the overall excitatory drive in the BG, a disruption of voluntary motor control and the characteristic symptoms of the disease\(^25\).

The cellular mechanisms leading to dopaminergic neurons loss are not fully understood. However, it is known that excessive glutamatergic transmission\(^26\) and oxidative stress\(^27\) are implicated. Chronic treatment with L-DOPA induces changes of the neurochemistry in the BG\(^28\) and LID has been linked to abnormal glutamate transmission in the striatum\(^29\).

Glutamate and homocysteine toxicity

Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS) and normal brain function requires balanced glutamatergic neurotransmission. As a result of striatal dopaminergic denervation, the glutamatergic projections from the STN to the BG output nuclei become overactive with reduced regulation of glutamate receptors\(^26\).

The excessive excitation of glutamate receptors in the BG circuitry is toxic to any remaining dopaminergic neurons. Subsequently, there is a further loss of dopaminergic transmission and progression of PD symptoms\(^30\). Normalisation of motor function is initially seen with L-DOPA treatment. However, as the severity of PD increases, the worsening of dopaminergic degeneration leads to altered function of non-dopaminergic BG neurotransmitters, such as glutamate\(^3\), GABA\(^31\), and serotonin\(^32\). Abnormal glutamate signalling within the BG is evident in L-DOPA-induced motor-complicated states. Enhanced levels of striatal glutamate receptor have been observed in both PD patients with LID\(^3\) and in L-DOPA-treated parkinsonian primates\(^4,5\). Further evidence for a role for excessive glutamate transmission in PD and LID comes from the clinical use of amantadine, a non-selective antagonist of NMDA glutamate receptors, in the treatment of LID\(^33\).

But, glutamate receptors overtactivity induced-neurotoxicity also comes from their interaction with Hcy.

Figure 1 summarises the connection between vitamins B and L-DOPA in the metabolism of Hcy. Hcy is a homologue of the amino acid cysteine, differing by an additional methyl group. Hcy is formed as a result of the transformation of methionine to cysteine in all types of animal cells, including human cells\(^13\).

In the reaction catalysed by methionine adenosyltransferase (SAM synthetase), adenosine is transferred from adenosine triphosphate to methionine to form SAM. SAM is the major donor of methyl groups for various methylation reactions. When a methyl group (−CH\(_3\)) is transferred by SAM synthetase to individual acceptors, SAM is converted to SAH. Then, SAH is hydrolysed by a specific SAH hydrolase to adenosine and Hcy\(^34\).

Hcy is transported outside the cell and its concentration can be determined in plasma. In human cells, Hcy can be metabolised via two B-vitamins-dependent pathways
Homocysteine and reactive oxygen species
It is well known that oxidative stress plays a major role in the neurodegenerative process that underlies PD. In both idiopathic and genetic cases of PD, oxidative stress is thought to be the common underlying mechanism that leads to neuron death. As such, the substantia nigra of PD patients exhibit increased levels of oxidised lipids, proteins and DNA and decreased levels of reduced glutathione (GSH). Oxidative stress occurs when an imbalance is formed between production of ROS and cellular antioxidant activity.

Various experimental studies have also shown that L-DOPA may contribute to neuronal damage through the formation of free radicals. Indeed, an increased formation of hydroxyl radicals in blood cells of PD patients under treatment with L-DOPA has been reported. Compared with both untreated PD patients and healthy subjects, the formation of hydroxyl radicals was elevated in L-DOPA treated patients. L-amino acid decarboxylase enzyme converts L-DOPA into DA. The metabolism of the neurotransmitter DA is a source of oxidative stress. DA is normally stored in vesicles, re-methylation and transsulphuration. Alterations in transmethylation reactions that lead to HHcy have been suggested in the pathophysiology of neurodegenerative disorders such as PD. The processes of methyl-group transfer are involved in the metabolism of L-DOPA.

In PD treatment, repeated L-DOPA/DDI application, via the action of the enzyme COMT, induces an accumulation of 3-O-methyldopa (3-OMD). COMT uses SAM as the methyl donor. Therefore O-methylation of L-DOPA to 3-OMD is associated with the conversion of SAM to SAH, which can subsequently be transformed to Hcy. Because SAM is rapidly utilised to metabolise L-DOPA to 3-OMD, SAM and methionine are decreased and Hcy is increased in L-DOPA treatment. Thus, in PD patients treated with L-DOPA, plasma Hcy is higher than in controls and untreated PD patients. This further supports the idea that the drug, rather than the disease per se, promotes HHcy. An increased concentration of Hcy in human blood is a known risk factor in several diseases including PD and LID.

Studies have reported that Hcy induces NMDA receptor-mediated excitotoxicity, whereas several Hcy derivatives are selective agonists of group I metabotropic glutamate receptors (mGluRs). Hcy also mobilises intracellular calcium; this process also seems to be mediated by group I mGluRs. L-type calcium channels are key modulators of membrane excitability in striatal medium spiny neurons.

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excess cytosolic DA is oxidised both spontaneously and enzymatically to produce DA quinone. The DA quinone species are capable of covalently modifying cellular nucleophiles, including GSH and protein cysteiny1 residues, whose normal functions are important for cell survival. Higher levels of cysteyn1e-catechol derivatives are found in postmortem nigral tissues of PD patients compared with age-matched controls, suggesting cytotoxic nature of DA oxidation59. In animals, DA directly injected into the striatum caused selective toxicity to dopaminergic terminals that was proportional to the levels of DA oxidation and quinone-modified proteins60. Furthermore, DA quinone can cyclise to become the highly reactive aminochrome, whose redox cycling leads to generation of superoxide and depletion of cellular nicotinamide adenine dinucleotide phosphate-oxidase, and which ultimately polymerises to form neuromelanin50. Neurromelanin in turn can exacerbate the neurodegenerative process by triggering neuroinflammation52. Moreover, hydrogen peroxide is generated during DA metabolism by monoamine oxidase and is subsequently converted to the highly reactive hydroxyl radical in the presence of transition metal ions53, contributing to oxidative stress.

Because the adverse effects of Hcy are most likely related to its pro-oxidant properties54, a direct involvement of the amino acid in this phenomenon may be therefore hypothesised. Hcy itself can undergo auto-oxidation, thus causing the disruption of redox homeostasis and affecting the redox signalling pathways in vascular and neuronal cells54. Hcy induces neurological dysfunction by enhancing the production of ROS, and oxidative deactivation of nitric oxide. Moreover, Hcy causes brain lipid peroxidation by blocking NMDA receptor65.

Traditional explanations of the mechanism of Hcy neurotoxicity point to the key role of disturbances in methylation and re-methylation processes. SAM accumulated in cells in HHcy is a very strong competitive antagonist of many transferases56. Their prolonged suppression may inhibit the repair of damaged DNA chains and consequently lead to apoptotic cell death56. One of the hallmarks of the PD is the presence of intracellular inclusions Lewy bodies and Lewy neurons that are rich in a-synuclein (a-Syn), an abundant synaptic terminal protein57. Methionine oxidation is one of the potential causes of PD because it inhibits a-Syn fibrillation and leads to the formation of soluble oligomers58. Oligomerisation of a-Syn is believed to be an important step in its pathogenic toxicity59. In conclusion, Hcy appears to be a potent substance in disturbing DA turnover and causing dopaminergic neuronal damage in long-term exposure.

Potential beneficial effects of vitamins B

O-methylation of L-DOPA to 3-OMD is associated with the conversion of SAM to Hcy via SAH10. This may suggest that a combination of L-DOPA/DDI with an inhibitor of COMT may contribute to lower the Hcy concentrations. Thus, peripheral COMT inhibition may be a safer adjunct for L-DOPA/DDI therapy. But outcomes of the trials with COMT inhibitors, such as entacapone (EN) or tolcapone, showed contradictory results60. Conflicting data concerning the role of COMT inhibitors in preventing L-DOPA-induced HHcy suggest that this effect might be related to other factors such as the basal vitamin status and genetic background.

A strong negative correlation between blood Hcy level and concentrations of folate and vitamins B6 and B12 in body fluids has been reported61. There is a reciprocal causal relationship between HHcy and perturbations in methylation processes63. These metabolic disturbances have been suggested as important factors in the mechanisms of indirect Hcy neurotoxicity64. Hcy is elevated in cases of folic acid or vitamin B12 deficiency resulting from the impeded synthesis of methionine from Hcy which requires folic acid and vitamin B12 as cofactors65,66. Therefore, homocysteine could be induced in a folic acid deficient state.

Generally, vitamin supplementation and other non-genetic factors may amplify or mask phenotypic expression of genetic polymorphisms or genetic defects of enzymes involved in Hcy metabolism network. Otherwise, elevated Hcy is likely the product of variations in gene–nutrient interactions, for example, low B vitamin status combined with polymorphisms in pathway-specific genes. The most studied genetic variants contributing to HHcy is the C to T single nucleotide polymorphism at codon 677 of the 5,10-methylene-tetrahydrofolate reductase (MTHFR) gene67. The TT variant, codes for an enzyme, has a 50% reduced activity compared with the CC variant68. This polymorphism results in a drastic decrease in MTHFR enzyme activity. Consequently, there is a decrease in the production of 5-methyltetrahydrofolate (5-CH3-THF), the necessary substrate for Hcy conversion to methionine. By decreasing levels of 5-CH3-THF, the polymorphism could therefore result in accumulation of Hcy, leading to HHcy69. This may suggest that a combination with pyridoxine (vitamin B6) responsiveness70, and CBS-deficient siblings are always concordant for pyridoxine responsiveness70. To understand the factors that determine the plasma Hcy levels, it is necessary to appreciate in more detail, not only the molecular events underlying its production but also its removal. We mentioned above
that Hcy is formed from the metabolism of methionine and degraded either by its irreversible conversion to cysteine (transsulphuration) or by its reversible re-methylation to methionine, the metabolic precursor of SAM.

In the re-methylation pathway, Hcy may be re-methylated to methionine by methionine synthase, which uses 5-CH₃-THF (active form of folate) and vitamin B12 as cofactors. Tetrahydrofolate, a folic acid derivative, is converted to 5,10-methylenetetrahydrofolate (5,10-MTHF) by the enzyme methylenetetrahydrofolate dehydrogenase. The 5,10-MTHF is converted to 5-CH₃-THF by MTHFR. Ultimately, this substrate reacts with Hcy to form methionine and regenerates tetrahydrofolate. Alternatively, Hcy can be re-methylated by betaine-dependent Hcy methyltransferase. But betaine methyltransferase is not present in the brain and the brain is totally reliant on methionine synthase to metabolise Hcy and SAH. The transsulphuration leads to cysteine. This pathway of Hcy metabolism is catalysed by CBS and cystathionine γ-lyase, with pyridoxal-5 phosphate (active form of vitamin B6) as a cofactor. Interestingly, vitamins B supplementation decreases Hcy generation, as B12 and folic acid catalyse the irreversible degradation of Hcy to methionine and B6 the irreversible turnover of Hcy to cysteine.

The antioxidant properties of the extracts and metabolites tested partially support the wide use of these plants in traditional medicine. Based on investigations on their medicinal formulations, it is now evident that polyphenols play a pivotal role in mediating the therapeutic actions of these herbal products. Polyphenols are natural compounds with antioxidant properties, present in plants, vegetables and fruits. They represent secondary plant metabolites synthesised to defend against microbial attack, pests and harmful radiations, in addition to providing the plant with brilliant colours and fragrance. Natural polyphenols vary from simple molecules (phenolic acids) to complex polymeric forms (condensed tannins). The distinctive biological and medicinal properties of polyphenols are due to their unique structure with characteristic hydroxyl and phenolic groups. Phenolic compounds have been reported to inhibit 6-OHDA- and MPTP-induced apoptosis via the attenuation of oxidative stress.

Recently, an ethnomedical information on the use of medicinal plants for CNS disorders in the Sinai Peninsula region (Egypt) has been provided. More than 300 species were traditionally used in folk medicine in the Sinai Peninsula; 101 of these species belonging to 40 families were reported as useful in different CNS disorders. All different part of plants were used, leaves and aerial parts being the most frequent. Most of the remedies were prepared as infusion or decoction, while oral administration was the most common way to be used. The traditional use is supported by pharmacological studies for only a few of the studied species such as Acacia nilotica, Achillea fragrantissima, Ajuga iva or Mentha longifolia.

Oxidative stress is thought to be one of the factors that reduce cognitive and motor performance in neurodegenerative disease; oxidative defence mechanisms such as catalase and GSH decline, while oxidative damage molecules, such as hydroxyl radicals and peroxynitrite, increase. Thus, a high level of antioxidant activity has been linked with protection against neurodegenerative diseases. To overcome the adverse effects associated with allopathic medicine alternative medicines are preferred. According to their strong free radical scavenging activities, many species can be of interest in PD and LID treatment. Interestingly, it has been demonstrated that Mucuna pruriens endocard powder water extract significantly reduces parkinsonism in the parkinsonian primate at optimal doses similar to L-DOPA without inducing dyskinesias. Many other plants were reported to have L-DOPA. A systemic screening of these plants may provide an alternative to synthetic L-DOPA by opening new avenues for herbal cultivation and therapies. But before we reach that point, the safe use and protective effect of this natural L-DOPA in animal models should be established.

Experimental data from in vitro and in vivo models of PD have conclusively proved that curcumin targets multiple degenerative pathways in PD including oxidative/nitrosative stress, mitochondrial dysfunction, protein aggregation and restores neuronal function in the substantia nigra thereby restoring striatal DA levels. Furthermore, curcumin crosses the blood brain barrier and is non-toxic in humans even at high doses. Because it has a poor bioavailability, the therapeutic application of curcumin in PD and other diseases of the brain are limited.

**Conclusion**

Glutamate neurotoxicity is involved in PD and LID. Hcy directly and indirectly induces glutamate neurotoxicity. Hcy also generates ROS that is implicated in neurodegeneration. Because folate, vitamin B6 and B12 are capable to relieve Hcy toxicity,
their supplementation could help to prevent motor complication in PD treatment. A successful drug against PD should be able to restore DA levels, prevent neurodegeneration and LID in vivo. Considering the higher cost for producing synthetic drugs and the various side effects associated with their use, the need to search for alternative agents from medicinal plants used in traditional medicine is justified. This positive opinion is not, however, either a rubber cheque, or a blank cheque for one or another player because each metabolite generated can have its own undesirable effect. Novel approaches to improve the bioavailability of medicinal plants might be their use as an adjunctive therapy with L-DOPA or glutamate receptors antagonists or vitamin B supplementation. Preclinical and clinical investigations of this treatments regime need to be undertaken.

**Abbreviations list**

BG, basal ganglia; CBS, Cystathionine β-synthase; CNS, central nervous system; COMT, catechol-O-methyltransferase enzyme; DA, n nigrostriatal dopamine; DDI, DOPA decarboxylase inhibitors; Hcy, homocysteine; GPl, globus pallidus internal; HHcy, hyper-homocysteinemia; L-DOPA, levodopa; LID, L-DOPA-induced dyskinesias; mGlRs, metabotropic glutamate receptors; MTHFR, methylenetetrahydrofolate reductase; NMDA, N-methyl-D-aspartate; PD, Parkinson’s disease; ROS, reactive oxygen species; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SNC, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; α-Syn, α-synuclein; 3-OMD, 3-O-methyldopa; 5,10-MTHF, 5,10-methylenetetrahydrofolate.

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