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N-acetyl-cysteine in the treatment of Parkinson's disease. What are we waiting for?

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ABSTRACT

Parkinson's disease is an age-related neurodegenerative disorder that is ameliorated with levodopa. However, long-term use of this drug is limited by motor complications, postural instability and dementia resulting in the progression of the disease. Insights into the organization of the basal ganglia and knowledge of the mechanisms responsible for cell death in Parkinson's disease has permitted the development of putative neuro-protective drugs that might slow the disease progression. Although no drug has yet been established to alter the rate of disease progression, recent publications have confirmed previous results and hypotheses about the probable role of thiolic antioxidants on Parkinson's disease, demonstrating a significant reduction of dopaminergic neuronal degeneration in α-synuclein over expressing mice treated with oral *N*-acetyl-cysteine. This thiolic antioxidant is a modified form of the natural amino acid cysteine, which is the precursor of the most potent intracellular antioxidant glutathione. Besides, increasing evidence has been accumulated in the last 10 years about the beneficial effects of this thiolic antioxidant in experimental and pathologic states of the nervous system, including against neurotoxic substances. The present paper put forward the existing rationale evidence for the use of *N*-acetyl-cysteine alone or in combination with levodopa in the clinical management of this neurodegenerative disorder.

Introduction

Parkinson's disease (PD) is a very common neurodegenerative disorder caused by idiopathic degeneration of dopamine-producing cells in the pars compacta of the substantia nigra located in the midbrain [1]. This specific neuro-degeneration leads to clinical signs including tremor at rest, rigidity on passive movement, bradykinesia, and hypokinesia. Postural instability, orthostatic hypotension, and dementia are invariably developed with the progression of the disease. Understanding the pathogenesis of the disease has been advanced in the last decade, being necessary to improve and found new therapeutic strategies based on scientist evidence.

Levodopa (L-dopa) is the main pharmacological treatment for PD, but its use is limited by the development of motor fluctuations and drug-induced dyskinesias. Dopamine agonists are also used, either alone or in combination with L-dopa, acting directly on dopamine receptors and mimicking endogenous dopamine functions. Monoamine oxidase B (MAO-B) inhibitors act increasing dopamine levels in the basal ganglia by inhibiting dopamine catabolism. Catechol O-methyl transferase (COMT) inhibitors also inhibit the catabolism of dopamine, thereby extending the half-life of L-dopa. However, all PD patients ultimately require L-dopa for control their symptoms.

There are numerous unanswered questions regarding the therapeutic management of PD. The present paper put forward the existing rationale evidence for the use of *N*-acetyl-cysteine (NAC) alone or in combination with L-dopa in the medical management of this disorder (Fig. 1).

Etiologic factors in Parkinson's disease

The pathogenesis of PD seems to be multi-factorial including environmental factors that act on genetically susceptible individuals as they age [2,3]. A broad spectrum of both genetic and environmental factors have been suggested as contributing to the initiation and progression of PD, but aging is the single most important risk factor for this disorder and undoubtedly contributes to PD progression through its accumulative oxidative damage, decrease in antioxidant ability and impairment of mitochondrial bioenergetic capacity in the brain [4–10].

Many studies have examined the impact of environmental agents on the risk of PD. In fact, PD patients show abnormalities of oxidative phosphorylation that impair their mitochondrial energy metabolism increasing reactive oxygen species (ROS) generation, which closely resembles that attributable to 1-methyl, 4-phenyl, 1,2,3,6-tetrahydropyridine (MPTP) [11–13] but this impairment is apparently constitutive in origin [14,15]. Schapira and co-workers were the first to report that mitochondrial Complex I activity was selectively reduced in the substantia nigra of patients with PD [16]. The Complex I impairment is worse in more

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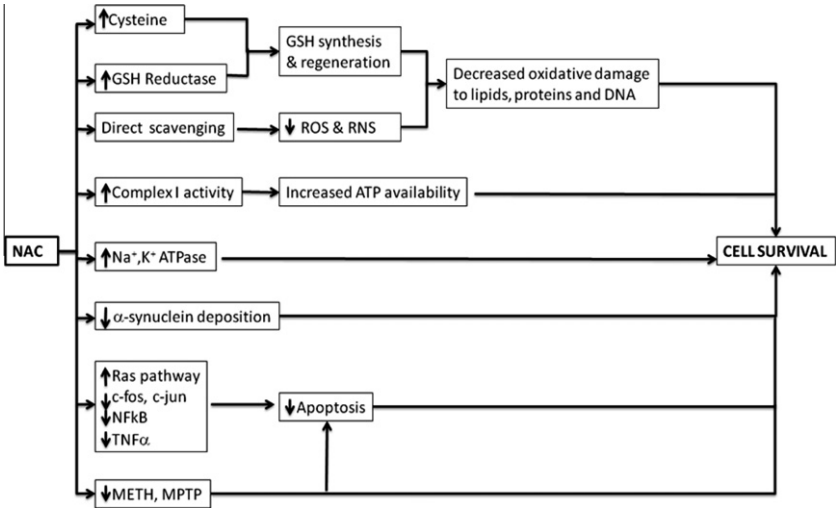

Fig. 1. Potential therapeutic actions of *N*-acetyl-cysteine (NAC) against dopaminergic neuro-degeneration.

advanced cases and seems to affect also non-nigral brain areas, muscle and fibroblasts of PD subjects [15,16].

Swerdlow's group carried out an elegant experiment using cybrids cells to confirm that the mitochondria in PD were at least 20% less efficient in Complex I activity, produced higher levels of ROS, and rendered their host cells more susceptible to MPTP-induced cell death [15]. They suggested that Complex I defect was in the mitochondrial DNA of PD patients caused by parental inheritance or by oxidative damage on the mitochondrial DNA. This constitutive defect in Complex I help to explain why some individuals develop PD following toxin exposure, while others do not. MPTP is not toxic by itself but into the brain it is transformed to the toxic product MPP+(1-methyl-4-phenylpyridinium) by MAO-B. MPP+ is selectively taken up by the dopaminergic neurons of the substantia nigra and selectively taken up by their mitochondria where it inhibits Complex I activity [13]. Then, a vicious cycle develops, which causes oxidative damage and bio-energetic deficiency into the neuron.

Six genes identified as α-synuclein (SNCA), ubiquitin C-terminal hydrolase like 1 (UCH-L1), parkin (PRKN), LRRK 2, PINK 1 and DJ-1 have been reliably linked to PD and/or to neurodegeneration of the parkinsonian type. These single gene mutations with the notable exception of LRRK 2 are responsible for only a small number of patients with PD. The LRRK 2 gene (PARK8) is the most common cause (5–7%) of familial PD to date [17].

Oxidative stress and glutathione deficiency

The earliest reported biochemical change identified in the substantia nigra of early PD patients is a significant depletion of reduced glutathione (GSH), which may promote morphological mitochondrial damage by ROS [18,19]. GSH (γ-L-glutamyl-L-cysteinyl-glycine) is the most abundant intracellular non-protein and water-soluble thiol antioxidant and it is synthesized by two-step reaction [20]. The mitochondrial GSH is dependent on the uptake from the cytosol since they lack the enzymes for the GSH synthesis [20]. GSH plays an important role in scavenging ROS and reactive nitrogen species (RNS) and in recycling other antioxidants and is kept in its thiol-reduced form (>98%) by glutathione disulfide (GSSG) reductase that maintains optimal GSH/GSSG ratios into the cell [20].

The magnitude of GSH depletion seems to parallel the severity of the disease and is the earliest known indicator of the substantia nigra degeneration preceding detectable losses in both mitochondrial Complex I activity and striatal dopamine content [21]. Since Complex I impairment results in the generation of ROS [22] in agreement with reports of elevated markers of oxidative damage to lipids, proteins and DNA in the substantia nigra of patients with PD [23] and it has been demonstrated that exposure of mitochondrial membranes to nitric oxide resulted in selective and persistent inhibition of Complex I activity via S-nitrosation of critical thiol groups in the enzymatic complex, the inhibition of Complex I activity may be reversible by restoring mitochondrial GSH levels [24–26]. Besides, it has been shown that Complex I inhibition following prolonged dopaminergic GSH depletion in vitro was reversible with dithiothreitol, suggesting that it involved a reversible cysteine thiol modification. Then, it seems that GSH depletion in the substantia nigra of PD patients result in increased ROS and RNS generation leading to Complex I inhibition with subsequent mitochondrial dysfunction that significantly affects glutathione synthesis closing the vicious circle that ultimately leads to dopaminergic cell death [26].

In addition, oxidative stress may increase the accumulation of toxic forms of α-synuclein through oxidative ligation to dopamine playing a central role in PD [27,28], suggesting that increased oxidative stress due to early GSH deficiency in the substantia nigra may lead to enhanced toxicity of α-synuclein in dopaminergic neurons in PD.

NAC and Parkinson's disease

Having in account the accumulative oxidative damage in PD patients, some clinical studies have been performed using antioxidants in the treatment of PD with controversial results [29–31].

The central implication of GSH deficiency in PD has stimulated many investigations to find new potential approaches for maintain or restore GSH levels in these patients. Moreover, the use of GSH as a therapeutic agent is limited by its very short half-life in human plasma (<3 min) and difficulty to cross cell membranes, being necessary high doses to reach therapeutic levels [20,32]. Under physiological conditions, the cellular availability of cysteine is considered to be the rate-limiting factor in the synthesis of GSH. However, cysteine is toxic at high concentrations as the result of free radicals generation during cysteine autoxidation [33]. As a consequence, compounds that can be metabolized to cysteine could be used as pro-drugs to increase neuronal GSH levels.

NAC is the simplest cysteine pro-drug that can be systemically administered to deliver cysteine to the brain [8,34–37], acting as a

precursor for glutathione synthesis as well as a stimulator of the cytosolic enzymes involved in glutathione regeneration. Increase in Complex I activities in vivo and in vitro in mitochondria isolated from pre-synaptic terminals of aged mice was proposed 10 years ago as evidence that NAC was able to cross the blood brain barrier having reparative effects on brain mitochondria and against age-associated memory decline [9,34,35]. Since GSH levels become more depleted in the substantia nigra as the disease progresses, NAC may contribute to GSH repletion, which in addition to its potent antioxidant effects by direct scavenging of ROS can make this antioxidant ideal for counteract mitochondrial impairment in the substantia nigra of PD patients [38]. Furthermore, we have shown that NAC can prevent dopamine induced programmed cell death in cultured human cortical neurons [39] and also it can increases mitochondrial complex IV specific activity both in vitro and in vivo in synaptic mitochondrial preparations from aged mice [9,34].

Systemic administration of NAC increases brain levels of glutathione in mice [34,37,40,41], reduces markers of oxidative damage [38], increases brain synaptic [34,36] and non-synaptic brain [42] mitochondrial Complex I activities and protects against MPTP toxicity [43,44] and dopamine-induced cell death [39,45]. Besides, dietary supplementation of NAC during 1 year was able to counteract age-related decrease in rat brain expression of subunit 39 kDa and ND-1 of the mitochondrial respiratory Complex I and other subunits of the mitochondrial oxidative phosphorylation [46].

In view of the above, there are sufficient scientific evidence that Complex I inhibition by prolonged GSH depletion may be due, at least partially, to a reversible age-related event involving cysteine residues with impact on its enzymatic activity. This may be reversible by restoring GSH to normal levels and suggests that therapeutics toward the maintenance of cellular GSH concentration within dopaminergic neurons would be beneficial in PD. Moreover, in vitro studies have shown that NAC is able to restore Complex I age-related activity decline suggesting a direct role of NAC on cysteine residues [34,42].

On the other hand, recent studies using positron emission tomography suggested that chronic use of methamphetamine (METH) causes the reduction of dopamine transporter in the human brain, suggesting that this is the mechanism of neurotoxicity in humans [47]. These findings are supported by a report that demonstrates that the densities of dopamine transporter are significantly decreased in the postmortem striatum of chronic METH users [48]. Although the precise mechanisms of METH-induced neurotoxicity in dopaminergic nerve terminals are not fully known a recent positron emission tomography study demonstrated that NAC administration significantly attenuated the reduction of dopamine transporter in the monkey striatum 3 weeks after the administration of METH [49], possibly rescuing GSH levels in the striatum [50]. Therefore, it is likely that NAC would be a suitable substance for the treatment of neurotoxicity in dopaminergic nerve terminals related to the chronic use of METH in humans.

Oral NAC administration protected against loss of dopaminergic terminals associated with over-expression of α-synuclein in a mouse model [51]. The results of this study showed that striatal tyrosine hydroxylase positive terminal density was increased in NAC-treated α-synuclein over-expressing mice compared to α-synuclein over-expressing mice with a control diet. This also correlated with a decrease in α-synuclein immuno-labeling in the brains of over-expressing mice treated with NAC. Moreover, NAC supplementation significantly increased GSH concentrations in the substantia nigra of transgenic mice over-expressing α-synuclein [51].

There is growing evidence that NAC may play a role against programmed cell death (PCD) in postmitotic cells and oligodendrocites in vitro [39,45,52,53]. Moreover, since alterations in mitochondrial structure and function are early events in apoptosis [54] and NAC can prevent ROS accumulation, telomere shortening and cell death in an in vitro model that disrupt mitochondrial electron transport function it is conceivable that this thiolic antioxidant could act in vivo against PCD in PD [55]. NAC also inhibited the expression of c-fos and c-jun genes and TGFβ-1 mediated apoptosis in human ovarian carcinoma cells [56]. In addition, long-term treatment with NAC affected NF-kappaB signaling in the brain of mice by increasing cytoplasmic retention of NF-kappaB thus preventing its action as a transcription factor in the nucleus [51]. Since increased activation of NF-kappaB may contribute to the pathology in models of Parkinson's disease, it is possible that NAC actions against modification of sulfhydryl groups in the proteins involved in regulating cell survival and NF-kappaB pathway were linked to reduced NF-kappaB activity in these models [57,58], being another beneficial action of NAC.

NAC can also inhibit TNFα-induced PCD in human neuronal and U937 cells by the preservation of mitochondrial integrity and function since NAC was able to partially prevent the mitochondrial membrane depolarization induced by this cytokine [39,59,60].

One study showed that NAC is a potent scavenger of both H_2O_2 and toxic quinones derived from dopamine and it prevented also dopamine-mediated inhibition of Na^+, K^+-ATPase activity suggesting another mechanism for the use of NAC in the treatment of PD [61]. Then, NAC may act against Na^+, K^+-ATPase inhibition, counteracting intracellular damage pathways that lead to death of dopaminergic neurons.

Recommendations for future research

They are necessary double blind randomized, placebo and well-designed and controlled clinical studies for test the probable benefice of oral NAC administration in ameliorate the symptoms and slow down the progression of PD. The age of onset of the symptoms can be important in order to classify the patients in two or even three sub-groups that will receive NAC. Standardization of patients' variables, doses of NAC used and reporting results will facilitate inter-study comparisons.

The reliability and validity of the Unified Parkinson Disease Rating Scale (UPDRS) has been widely documented, and it is currently the most common instrument used to measure the progression of PD. Investigators should report baseline, endpoint, and change in UPDRS scores, along with their respective standard deviations, since some researchers only report the motor sub-score in their investigations. It will be important that the activities of daily living (ADL) score be reported as well. Investigators should make sure to power their efficacy studies appropriately in order to make conclusive findings.

Given that most patients may be under actual treatments with L-dopa into, and given that an important treatment outcome is whether an additional drug allows for a decrease in L-dopa dose, data regarding actual L-dopa doses are quite important in the evaluation of NAC benefice.

While PD is mainly a disease of the elderly, it does occur in young patients as well, and it would be inappropriate to assume that patients with early onset of PD should necessarily be treated the same as patients with older onset of PD. For these reasons and for the possibility of variable and even contradictory results, it would be necessary to classify patients in various groups. We recommend NAC supplements based on scientific rationale for three population sub-groups: (a) persons at high risk for PD, (b) patients with early stage PD and (c) patients on established L-dopa therapy in combination with NAC. These recommendations should be assessed for efficacy in a well-designed clinical trial.

In regard to the oral NAC administration, a minimal dose of 600 mg/day and a maximum of 1800 mg/day would be into the

range of use. It would be important to record whatever adverse effect or intolerance during the treatment and also what is the cause of the treatment interruption.

Conclusions

The present paper shows that many experimental data in the last 10 years support the concept that NAC may be a singular substance for the treatment and prevention of PD and that a well-designed clinical trial is justified.

Conflict of interest statement

No conflict of interest is declared.

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