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ORIGINAL ARTICLE

N-acetylcysteine and neurodegenerative diseases: Basic and clinical pharmacology

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Abstract

Increasing lines of evidence suggest a key role of oxidative stress in neurodegenerative diseases. Alzheimer's disease, Parkinson's disease, myoclonus epilepsy of the Unverricht-Lundborg type, spinocerebellar degeneration, tardive dyskinesia and Down's syndrome have been associated with several mitochondrial alterations. Oxidative stress can decrease cellular bioenergetic capacity, which will then increase the generation of reactive oxygen species resulting in cellular damage and programmed cell death. First, this review examines the mechanisms of action of N-acetylcysteine (NAC), an antioxidant and a free radical-scavenging agent that increases intracellular GSH, at the cellular level. NAC can act as a precursor for glutathione synthesis as well as a stimulator of the cytosolic enzymes involved in glutathione regeneration. The chemical properties of NAC include redox interactions, particularly with other members of the group XIV elements (selenium, etc.) and ebselen, a lipid-soluble seleno-organic compound. Second, NAC has been shown to protect against oxidative stress-induced neuronal death in cultured granule neurons. Recent findings on the protective effect of NAC against 4-hydroxynonenal (HNE)-induced toxicity in cerebellar granule neurons are summarized. Finally, the protective pharmacokinetics of NAC in humans and the possible usefulness of NAC for the treatment of neurodegenerative diseases are discussed with reference to basic and clinical studies.

Key words: *N-acetylcysteine, ebselen, neuronal death, 4-hydroxynonenal, cerebellar granule neurons*

Introduction

N-Acetylcysteine (NAC) is a thiol-containing compound that has been used in clinical practice since the mid-1950s. NAC was originally introduced for the treatment of congestive and obstructive lung diseases (1), primarily those associated with hypersecretion of mucus, e.g., chronic bronchitis and cystic fibrosis (2,3). NAC has also been used since the mid-1970s as the drug of choice for the treatment of paracetamol intoxication (4). In more recent times, its applications have included attempts to use it for the treatment of pulmonary oxygen toxicity and adult respiratory distress syndrome (ARDS) (5,6), and exploratory studies have been performed to investigate its efficacy in the treatment of a variety of acute and chronic inflammations (7,8), a number of disorders involving the immune system, such as human immunodeficiency virus (HIV)-1 infection (9), and several neurodegenerative diseases (10). In addition to its clinical uses, this compound has, in recent years, been used increasingly as a tool for basic research, particularly in studies of the basic molecular and cellular aspects of the regulation of apoptosis and gene transcription (11,12).

This review covers various pharmacological properties of NAC, focusing particularly on the basic pharmacology of NAC, recent data on the protective effect of NAC against 4-hydroxynonenal (HNE)-induced toxicity in cerebellar granule neurons, human pharmacokinetic data, and the effect of NAC on neurodegenerative diseases.

Basic pharmacology of NAC and its co-factors

NAC has been shown to exert survival-promoting effects in several cell systems (13). Cysteine is transported mainly by the alanine-serine-cysteine (ASC) system, a ubiquitous system of Na^+-dependent neutral amino acid transport, in a variety of cells (14,15); however NAC is a membrane-permeable cysteine precursor that does not require active transport and delivers cysteine to the cell in a unique

way (Figure 1) (16). After free NAC enters a cell, it is rapidly hydrolyzed to release cysteine, a precursor of glutathione (GSH). GSH is synthesized intracellularly by the consecutive actions of γ-glutamylcysteine synthetase (glutamate+cysteine+ATP $\Leftrightarrow$ γ-glutamylcysteine+ADP+P_i,) and GSH synthetase (γ-glutamylcysteine+glycine+ATP $\Leftrightarrow$ GSH+ADP+P_i). The synthesis of GSH is limited by the availability of substrates; cysteine is usually the limiting precursor (17). γ-Glutamylcysteine synthetase is inhibited by feedback from GSH (K_i about 1.5 mM) (18). Thus, under physiological conditions, this enzyme is probably not operating at its maximal rate. In addition, intracellular GSH is maintained in its thiol form by glutathione reductase, which requires NADPH (16). GSH participates nonenzymatically and enzymatically (GSH *S*-transferases (GSTs)) in protection against toxic compounds (19). Perhaps one of its most important functions is protection against oxidative damage caused by reactive oxygen species (ROS), many of which are generated during normal metabolism (20). In addition, GSH can react nonenzymatically with ROS, and GSH peroxidase (and non-selenium (Se) peroxidase) catalyzes the destruction of hydrogen peroxide and hydroperoxides (17). Thus, NAC is an antioxidant and a free radical-scavenging agent that increases intracellular GSH, a major component of the pathways by which cells are protected from oxidative stress. The efficacy of NAC in protecting cells from apoptosis has generally been interpreted within the context of a mechanism involving oxidative stress (21).

The apparent diversity in the pharmacological use of NAC is rather unique, and is due to the multifaceted chemical properties of the thiol of the molecule. These include its nucleophilicity and redox interactions, particularly with other members of the group XIV elements (Se, etc.), providing 'scavenger' and antioxidant properties, as well as the ability to undergo transhydrogenation or thiol-disulfide exchange (TDE) reactions with other thiol redox couples (11,22,23). Ebselen, 2-phenyl-1,2-benzisoselenazol-3[2H]-one, is a lipid-soluble seleno-organic compound that exhibits both glutathione peroxidase-like and antioxidant activity (24). The redox properties of ebselen confer activity as GSH peroxidase to this low-molecular-weight compound. Thus, in the presence of thiols it acts as an enzyme mimic with much lower substrate specificity constraints, as ebselen is more readily accessible than active sites in proteins (25). This explains the relative unspecificity toward the thiol reductant involving NAC, whereas GSH peroxidase is highly specific for GSH. Ebselen has antioxidant activity and reacts rapidly with peroxynitrite ($ONOO^-$/ONOOH) in a bimolecular manner, yielding the selenoxide of the parent molecule, ebselen Se-oxide [2-phenyl-l,2-benzisoselenazol-3(2H)-one 1-oxide], as the sole Se-containing product with 1:1 stoichiometry (26). Ebselen Se-oxide is readily reduced to ebselen by reducing equivalents such as GSH (27), as shown in the GSH peroxidase-like catalytic cycle of ebselen. This reversible reaction allows its utilization for potential sustained defense against peroxynitrite.
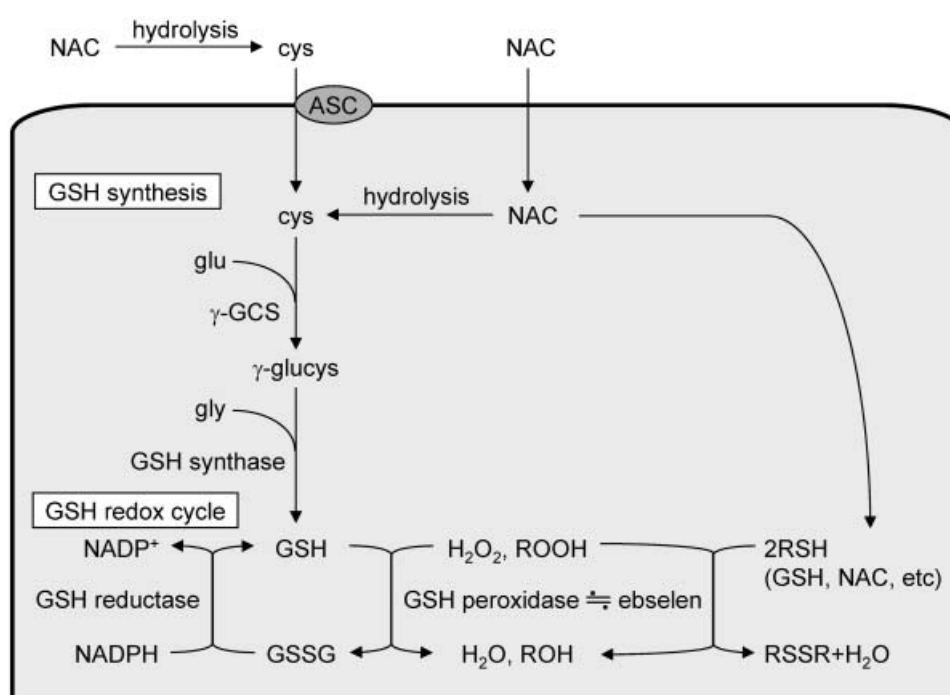

Figure 1. Mechanism of action of N-acetylcysteine. NAC, N-acetylcysteine; ASC, alanine-serine-cysteine (ASC) transport system; γ-GCS, γ-glutamylcysteine synthetase; cys, cysteine; glu, glutamine; gly, glycine; GSH, glutathione.

Effect of NAC on HNE-induced neurotoxicity in cerebellar granule neurons

HNE is an aldehydic product of membrane lipid peroxidation (28), which is reportedly associated with inhibition of the activity of several types of cellular function and signaling, such as membrane transport, microtubule formation mitochondrial respiration and synaptic plasticity, and exhibits cytotoxicity through alkylation (29–35). In addition, elevated levels of HNE have been reported in the brain regions of patients with Alzheimer's disease (AD) in comparison with normal controls (36) and also in plasma from AD patients in comparison with controls (37). An increased HNE level has also been observed in the cerebellum of patients with spino-cerebellar degeneration (SCD) (38). We have shown that HNE-induced neurotoxicity is suppressed by Ac-DEVD-CHO, a caspase-3 inhibitor, in rat cerebellar granule neurons and rat hippocampal neurons, suggesting that HNE-induced neuronal death is attributable to activation of the caspase-3-dependent pathway (39,40).

The mitochondrial detoxification of HNE can occur through phase I or phase II processes (Figure 2). In the phase I process, brain mitochondria oxidize HNE to 4-hydroxynonenoate (HNEAcid) by the ALDHs, ALDH2 and ALDH5A (41,42). The formation of HNEAcid is a major pathway of HNE detoxification, comprising approximately 30–40% of HNE metabolism in multiple systems including the heart, liver, and brain (42–44). ALDH2 and ALDH5A have been shown to be localized in the mitochondrial matrix. Mitochondrial membrane potential is maintained by the respiratory chain, which contains the four electron-transporting complexes I–IV and one H^+-translocating ATP synthetic complex (complex V); electron transporting complex I has NADH-ubiquinone oxidoreductase activity (45). The mitochondrial detoxification phase II process has been shown to involve glutathione conjugation to the electrophilic C3 carbon directly or through the action of GSTs, forming the GSH-HNE adduct S-(4-hydroxy-l-oxononan-3-yl)glutathione (GSHNE) (46). Mitochondria in rat brain possess GST A4-4, an inducible GST isoform with high activity toward HNE (47).

The HNE-modified protein is produced by multiple action of HNE, which affects mitochondrial function, signal transduction, transport function, cytokine production and the cytoskeleton (31). Pretreatment with high concentrations of NAC completely suppresses the formation of HNE-modified protein, mitochondrial injury and neuronal death (48). It is suggested that this protective effect is due to an increase of GSH-HNE conjugation by increased GSH levels after treatment with NAC, whereas low concentrations of NAC are ineffective (48).
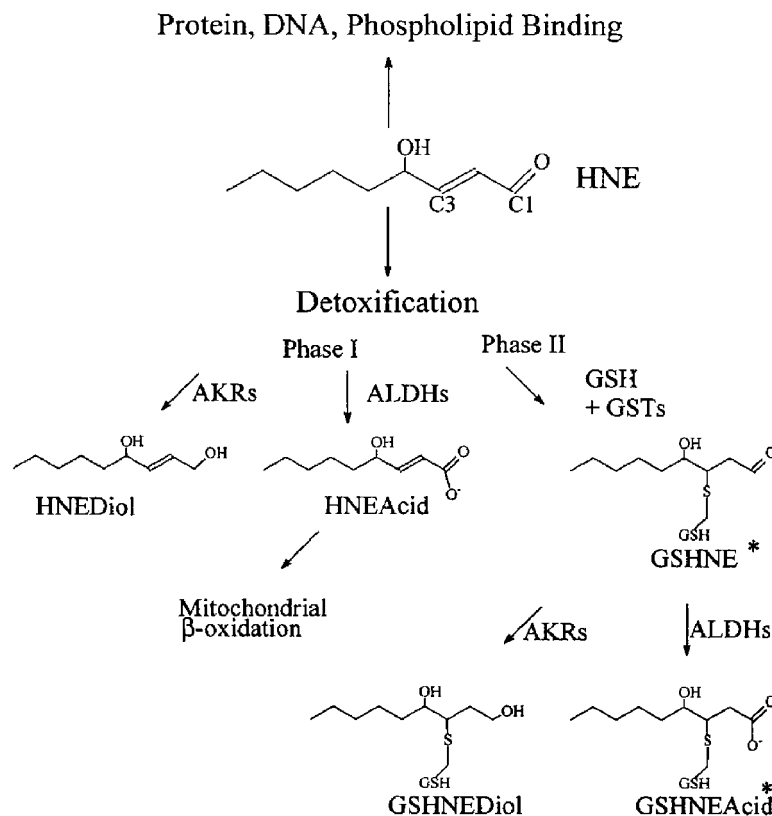

Figure 2. Potential routes of mitochondrial HNE loss and metabolism. HNE is able to alkylate diverse classes of biological molecules. Balancing this toxicity is the metabolism of HNE by multiple phase I and phase II pathways. *GSHNE and GSHNEAcid can dehydrate to form a cyclic hemiacetal and lactone, respectively. Adapted from Meyer MJ et al. 2004 (41).

A previous study in our laboratory has shown that HNE-induced neurotoxicity in cerebellar granule neurons is not suppressed by single administration of ebselen (12). However, ebselen completely protected these neurons from HNE-induced death after pretreatment with low concentrations of NAC that, alone, exhibited no protective effect (48). Pretreatment with high concentrations of NAC, and pretreatment with low concentrations of NAC followed by ebselen treatment, suppressed the HNE-induced decrease in intracellular GSH levels, and the combined treatment partially but significantly restored the level of sulfhydryl base, suggesting that these effects play an important role in the synergistic effect of NAC and ebselen (48). Ebselen has been shown to lack the ability to synthesize GSH, which reacts with HNE (11). In addition, NAC is effective in supplying reducing equivalents for the peroxidase-like activity of ebselen (11). Therefore, it is suggested that ebselen can exert its neuroprotective effect under conditions of increased GSH production, and that combination of NAC pretreatment with subsequent ebselen treatment may be a useful therapeutic approach for neurodegenerative diseases.

Basic pharmacokinetics of NAC in humans

In one typical experiment following a single i.v. infusion of 200 mg NAC, the peak plasma level (*ca.* 200 μM) declined rapidly and biphasically ($\alpha T_{1/2}$ and $\beta T_{1/2} = 6$ and 40 min, respectively) (49). Infused NAC also rapidly forms disulfides in plasma, which prolongs the existence of the drug in plasma for up to 6 h (49). However, following oral ingestion of 200 mg NAC, the free thiol is largely undetectable, and only low levels of oxidized NAC are detectable

for several hours after administration (49). These early data also indicated that the drug was less than 5% bioavailable from the oral formulation. Further pharmacokinetic data were subsequently obtained for different oral formulations of NAC and different dose regimes (50,51), and these generally suggested that the drug itself does not accumulate in the body, but rather its oxidized forms and reduced and oxidized metabolites.

On the other hand, it has been suggested that NAC may cross the blood-brain barrier (BBB) (52). It has been shown that NAC crosses the blood-brain barrier and accumulated in the brain, and that it reverses memory impairment and brain oxidative stress in aged SAMP8 mice (53). In addition to pharmacokinetic studies of NAC at doses indicated for the treatment of various neurodegenerative diseases, attempts have also been made to define the pharmacokinetics of the drug following several doses, as indicated during its use as a chemical adjunct for chemotherapy (54). NAC was found to readily cross the blood-brain barrier when given into the carotid artery, and treatment with NAC can prevent cisplatin-induced ototoxicity in rats (54,55).

Effect of NAC against diseases of the central nervous system

Oxidative stress has been shown to play a pivotal role in neuronal dysfunction and death in various neurodegenerative diseases, including SCD (38), myoclonus epilepsy of the Unverricht-Lundborg type (ULD) (56–58), AD (59), Parkinson's disease (PD) (10,59), tardive dyskinesia (TD) (60,61) and Down's syndrome (DS) (62) (Figure 3).

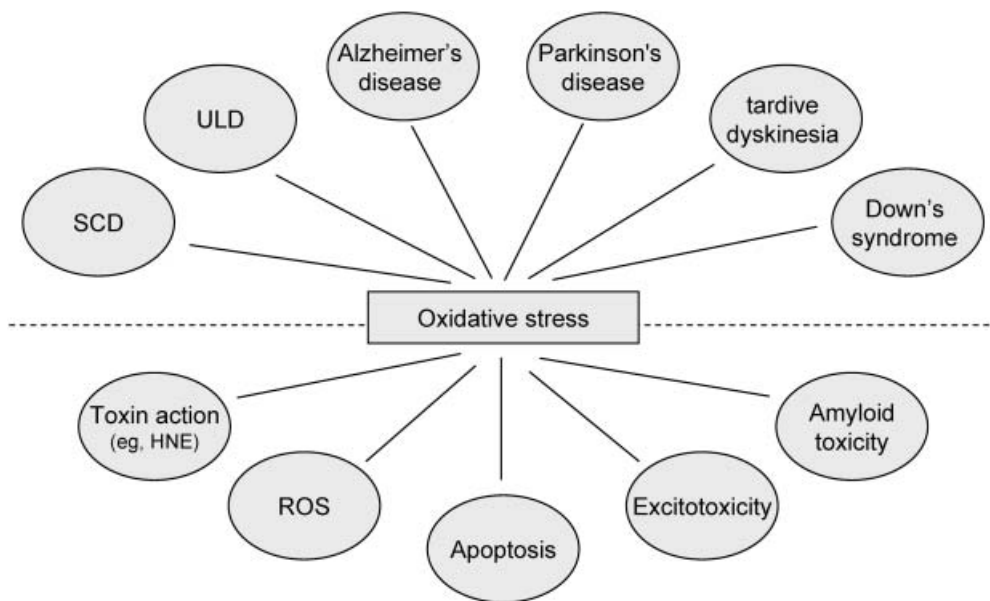SCD is a diverse group of rare, slowly progressive, neurological diseases, often inherited but of

Figure 3. Neurodegenerative diseases and mechanism of cell death thought to be associated with oxidative stress. SCD, spinocerebellar degeneration; ULD, myoclonus epilepsy of the Unverricht-Lundborg type; HNE, 4-hydroxynonenal; ROS, reactive oxygen species.

incompletely understood pathophysiology, which affect the cerebellum and its related pathways. Evidence of oxygen-mediated damage in SCD has been reported (38). If free radical species play an important role during cerebellar degeneration in SCD, then NAC may be therapeutically effective. However, there have been no basic or clinical studies.

ULD is an autosomal recessive disorder that typically develops between the ages of 6 and 15 years with stimulus-sensitive myoclonus and tonic-clonic seizures, followed by the development of a progressive cerebellar syndrome (63). NAC has been tested in patients with ULD, and shown to achieve a dramatic clinical improvement. However, further study is required to confirm anecdotal reports of improved speech, coordination, and gait in patients with ataxia (56–58).

In AD, whose etiology is almost certainly multifactorial, there is both direct and indirect evidence of free radical involvement. Increased levels of lipid peroxides have been reported in the temporal and cerebral cortex of patients with AD as compared with controls (64). Previous studies have demonstrated that GSH is decreased in cortical areas and the hippocampus (65,66). Abnormal GSH metabolism may also be involved in AD. NAC has been tested in some murine models of AD, and these studies provided supportive evidence that administration of NAC buffers oxidative damage in AD (67,68).

PD is due primarily to an abnormality in the substantia nigra, and is to date the most suggestive example of a condition resulting from neural degeneration due to oxidative stress. Increased lipid peroxidation has been reported in PD, although its causality was not established (64); subsequent studies then found that GSH levels were dramatically decreased in PD patients (65,66).

Clinical trials for the treatment of PD have been performed using antioxidants such as vitamin E (69,70) and vitamin C (71). However, no apparent success was reported, possibly due to the poor ability of antioxidants to penetrate the BBB (69,71). On the other hand, it has been suggested that NAC may cross the BBB (52). In addition, it has been reported that NAC exerts a preventive effect in a MPTP-induced mouse model of PD (72), but there have been no clinical trials of NAC for treatment of PD. Further controlled trials involving administration of NAC or GSH precursors, preferably in combination with other antioxidants, are needed.

TD, a movement disorder due to an abnormality in the basal ganglia, has been linked to long-term treatment with neuroleptic drugs. The basal ganglia are exceptionally vulnerable to free-radical overload because they are rich in dopamine as well as other catecholamines. By blocking dopamine receptors, neuroleptics may cause dopamine buildup in the basal ganglia, which then increases free radical production. NAC has been shown to decrease disease severity in both *in vivo* and *in vitro* TD models (60,61), suggesting that further clinical trials may be warranted.

DS, a classic mental deficiency disease resulting from trisomy of chromosome 21, is known to involve increased systemic oxidative stress (62). The 50% overexpression of SOD on chromosome 21 contributes to heightened fluxes of superoxide in all tissues. However, DS is not manifested until after birth, as the mother's antioxidant defenses may protect the fetus until delivery. DS children are also at greatly increased risk of Alzheimer-type dementia as they age (64). Although NAC has been shown to protect neuronal migration in DS models *in vitro* (73), further clinical trials should help to clarify whether supplementation of NAC from birth can delay the onset of dementia in DS patients.

Increasing evidence to date helps in understanding the possible roles of oxidative stress in various neurodegenerative disorders. If oxidative stress does contribute to certain neural degeneration, irrespective of whether it is eventually proven to be a primary or secondary factor in the etiologic progression, the therapeutic rewards are likely to be great. Future clinical trials of NAC, administered in combination with other antioxidants, antioxidant co-factors, and non-antioxidant brain-trophic nutrients, are expected.

Summary

The thiol drug NAC has a diversity of applications in both an experimental setting, as a tool for studies of oxidative stress induced by various agents including HNE, and in a clinical setting, as a therapeutic agent against several neurodegenerative diseases sharing primary or secondary mitochondrial defects that result in ROS overproduction and/or mitochondria-associated apoptosis. As our understanding of such redox processes increases, particularly their roles in specific pathophysiological processes, new avenues will open for the clinical use of NAC. As a drug, NAC represents perhaps the ideal xenobiotic, capable of directly entering endogenous biochemical processes as a result of its own metabolism. In addition, NAC may cross the BBB. Thus, it is hoped that the experience gained with this unique agent will help in future efforts to design antioxidants and chemoprotective principles that are able to more accurately utilize endogenous biochemical processes for therapy of neurodegenerative diseases.

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