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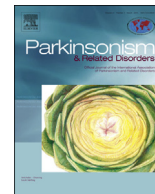
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Cerebrospinal fluid concentrations of N-acetylcysteine after oral administration in Parkinson's disease

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ABSTRACT

Introduction: Depletion of neuronal glutathione may contribute to the pathogenesis of Parkinson's disease (PD). N-acetylcysteine (NAC) can restore neuronal glutathione levels, but it has not been established whether NAC can cross the blood–brain barrier in humans.

Methods: Twelve patients with PD were given oral NAC twice daily for 2 days. Three doses were compared: 7 mg/kg, 35 mg/kg, and 70 mg/kg. NAC, cysteine, and glutathione were measured in the cerebrospinal fluid (CSF) at baseline and 90 min after the last dose. Cognitive and motor functions were assessed pre- and post-NAC administration using the Montreal Cognitive Assessment (MoCA) and the Unified Parkinson's Disease Rating Scale part III motor subscore (UPDRS-III).

Results: Oral NAC produced a dose-dependent increase in CSF NAC concentrations ($p < 0.001$), with the highest dose producing a CSF concentration of $9.26 \pm 1.62 \mu\text{M}$. There were no significant adverse events. NAC had no acute effect on motor or cognitive function.

Conclusion: Orally administered NAC produces biologically relevant CSF NAC concentrations at doses that are well tolerated. The findings support the feasibility of NAC as a potential disease-modifying therapy for PD.

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1. Introduction

Glutathione (GSH) is utilized by cellular enzymes to detoxify reactive oxygen and nitrogen species [1] and to repair oxidatively damaged proteins. GSH levels are selectively decreased in the substantia nigra pars compacta (SNc) of patients with Parkinson's disease (PD) and presumed presymptomatic PD [2–6], and the severity of GSH loss correlates to the degree of neurodegeneration in the SNc [4]. The reduction in GSH in PD cannot be fully explained by the degree of nigral neuronal degeneration observed, since there is significant nigral cell loss in atypical parkinsonian syndromes as well, without concurrent reduction in GSH levels [5]. Moreover, in animal models, reduction in neuronal GSH content leads to

oxidative stress and cell death of SNc neurons out of proportion to other neuronal populations. Several lines of evidence indicate that normalization of GSH levels reduces both oxidative stress and neuronal cell death [3,5,7], and there has been growing interest in supporting neuronal glutathione metabolism as a disease-modifying therapy in PD [6].

GSH itself cannot be taken up by neurons, but N-acetylcysteine (NAC) can restore neuronal GSH levels by functioning as a cell-permeable source of cysteine, the rate-limiting substrate for GSH synthesis [6,8]. In addition, NAC may also have a direct role in neuroprotection as a scavenger of oxygen radicals, and as a modulator of the immune system and mitochondrial processes [9]. Several animal models of PD have shown that NAC ameliorates dopaminergic neuronal loss [10–15]. NAC administered to MPTP-treated mice showed restoration of dopamine, GSH, total tissue thiol levels and glutathione peroxidase activity in the SNc [12,14,15]. NAC has also been shown to increase GSH within the substantia nigra, decrease the loss of dopaminergic terminals, and

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reduce the accumulation of α -synuclein in the striatum in transgenic mice overexpressing wild-type human α -synuclein [13]. Mice with impaired uptake of cysteine into neurons undergo age-related neurodegeneration that is most prominent in the SNC, and again, this is reversed by NAC [10,11].

Recent evidence suggests that NAC can be symptomatically effective in certain psychiatric disorders [16]. Nevertheless, there is uncertainty as to whether NAC can effectively cross the blood–brain barrier (BBB) in human subjects [9]. The objective of this study was to determine whether biologically relevant levels of NAC could be achieved in human cerebrospinal fluid (CSF) after oral administration at doses previously shown to be well tolerated in humans.

2. Methods

2.1. Study design

This was a single-center study evaluating the tolerability and bioavailability of NAC in PD patients. This study received approval by the Institutional Review Board at the University of California, San Francisco (UCSF) and the San Francisco VA Medical Center, and all subjects signed written informed consent prior to their participation.

2.2. Patient population

Eligible patients with PD were enrolled between January 2012 and December 2013. Inclusion criteria were: a diagnosis of PD (using UK Brain Bank criteria) [17]; age > 40 years; and on a stable dopaminergic medication regimen ≥ 2 weeks. Exclusion criteria were: significant cognitive impairment; inability to sign informed consent; mass lesion on brain imaging; INR > 1.4; or ongoing use of antiplatelet or anticoagulant other than aspirin.

2.3. Study intervention

Three different dose trials of NAC were administered, each to a set of three patients receiving either 7 mg/kg, 35 mg/kg, or 70 mg/kg for two days, twice each day. A comparison of CSF NAC concentrations was completed between three patients receiving the solution form at 70 mg/kg, and three patients receiving the capsule form at the same dose. Lumbar punctures were performed prior to the first dose and 90 min after the fourth dose; the CSF was analyzed for cell count, protein, glucose, NAC, cysteine, and GSH levels.

The FDA approved liquid form of NAC (20% solution, USP verified) was purchased from McKesson (CA), a pharmaceutical distributor contracting with Hospira (IL). The capsule form was compounded by UCSF Medical Center's Drug Product Services Laboratory in the Department of Clinical Pharmacy, with NAC active ingredient purchased in powder form (USP verified) from Professional Compounding Centers of America (TX).

2.4. Analytic approach

CSF obtained by lumbar puncture was immediately placed on ice and aliquoted into samples for separate analyses of total thiols and reduced thiols. Samples for total thiol determination were treated for 30 min with 2 mM Tris(2-carboxyethyl) phosphine hydrochloride to convert disulfides to reduced thiols. All samples were then derivitized with 100 μ M N-(9-acridinyl)maleimide (NAM), stabilized with 40 mM formic acid, and stored at -80 C. Standards of cysteine, GSH, and NAC were

prepared in water and treated exactly as the samples. A second set of internal standards was prepared in pooled CSF to control for potential effects of proteins or other CSF constituents on the reduction, derivitization, or analysis procedures. Samples were analyzed by liquid chromatography–tandem mass spectrometry (Integrated Analytical Solutions, Berkeley, CA). Chromatography was done using a 2×10 mm Peek Scientific Duragel G C18 guard cartridge perfused with 0.2% formic acid in water followed by 0.2% formic acid in acetonitrile. Mass spectrometry was done with positive ion polarity, using the following Q1/Q3 ratios for species identification: 396.2/195.2 for NAM-cysteine, 582.5/221.2, for NAM-GSH, and 438.2/309.1 for NAM-NAC. CSF from each group of patients was analyzed separately, for a total of 4 independent analyses.

2.5. Motor and cognitive assessments

Immediate symptomatic improvement from NAC was assessed comparing the Unified Parkinson's Disease Rating Scale part III motor subscores (UPDRS-III) and the Montreal Cognitive Assessment (MoCA) scores, pre- and post-NAC administration (off-medication for 12 h, and off deep brain stimulation for 30–60 min, if applicable).

2.6. Statistics

A paired two-tailed *t* test was used to compare measurements pre- and post-oral NAC administration. A two-tailed *t* test was used to compare CSF concentrations of NAC after the administration of liquid and capsule formulations. A one-way analysis of variance was used to analyze the difference between mean CSF NAC concentrations at different doses. Tests were considered significant at *p* value < 0.05, which was adjusted using the Bonferroni correction for multiple comparisons. All analyses were performed using STATA12 (College Station, TX).

3. Results

Thirteen subjects were enrolled, and twelve completed all study procedures (Table 1). The mean age was 64.1 ± 9.7 years, and the mean duration of disease was 8.5 ± 6.7 years. Oral NAC administration produced a dose-dependent increase in CSF reduced NAC (free thiol form) and total NAC (NAC in disulfide form with NAC or another thiol) ($p < 0.001$), with the highest dose producing a CSF concentration of 9.26 ± 1.62 μ M (Fig. 1). The 70 mg/kg liquid and capsule forms of NAC had comparable effects ($p = 0.46$). NAC did not produce any consistent changes in CSF cysteine or GSH concentrations.

NAC was well tolerated, with only mild transient adverse effects in two subjects, both at the 70 mg/kg dose (one subject with mild nausea, and one with mild diarrhea). The capsule form was preferred over the liquid form, as the latter was malodorous and sour tasting. There was one study dropout due to a post-lumbar puncture headache. There were no dropouts due to adverse effects from NAC. No significant changes in the MoCA ($p = 0.29$) or UPDRS-III ($p = 0.86$) were observed pre- and post-NAC administration. CSF cell count, protein and glucose were within normal limits in all study participants.

Table 1
Baseline characteristics of subjects.

	Age	Gender	Duration of disease	Levodopa equivalent dose (mg)	Hoehn & Yahr stage	MoCA	UPDRS-III (off-medication)
Subject 1	57	M	2.5	573	2	22	39
Subject 2	68	M	8	600	2	27	37
Subject 3	58	M	5	400	2.5	26	43
Subject 4	61	M	5	1640	2	22	40
Subject 5	81	M	7	825	2.5	24	37
Subject 6	57	F	12	300	2	28	37
Subject 7	60	M	8	600	2.5	27	35
Subject 8	79	M	19	3889	2	23	24
Subject 9	71	M	24	675	2	27	29
Subject 10	69	M	3	200	2	30	33
Subject 11	48	M	12	480	2.5	27	62
Subject 12	69	M	3	300	2	28	29
Subject 13 ^a	55	F	2	270	1	28	8

MoCA, Montreal Cognitive Assessment; UPDRS-III, Unified Parkinson's Disease Rating Scale part III motor subscale score.

^a Subject 13 dropped out of the trial due to headache after the first lumbar puncture.

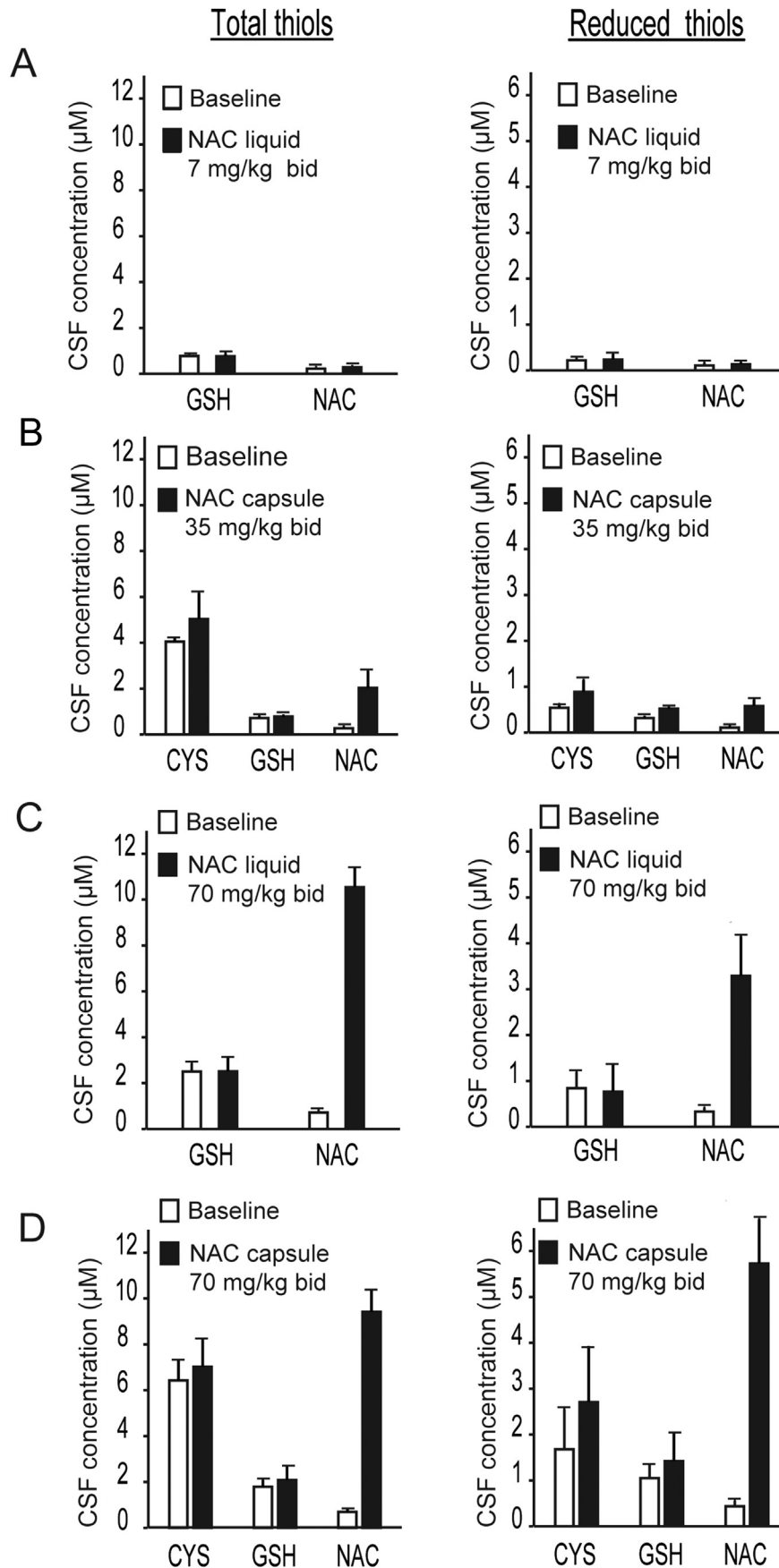


Fig. 1. Cerebrospinal fluid concentrations of N-acetylcysteine, cysteine and glutathione after oral NAC administration. Each panel (A–D) shows mean results obtained in 3 patients given the stated dose. Technical problems prevented cysteine determinations in studies A and C.

4. Discussion

These findings demonstrate that orally administered NAC is well tolerated by PD patients and readily crosses the BBB, producing a dose-dependent increase in CSF NAC. The higher NAC dose produced CSF NAC concentrations that were comparable or higher than CSF cysteine concentrations, suggesting that the levels achieved were biologically significant. Moreover, these NAC concentrations were several-fold higher than the CSF NAC concentrations required to normalize depleted neuronal GSH levels in mice [18].

NAC readily forms disulfide linkages with other thiols, which are reversible. Consequently, NAC present in these disulfide forms might serve as a source of free NAC in the CSF. For this reason, we quantified both reduced and total NAC concentrations, with the total NAC including both reduced and disulfide forms. Results showed that the majority of CSF NAC was in its reduced form, and that reduced and total NAC concentrations increased in parallel with oral NAC administration. The observation that NAC had no consistent effect on CSF GSH levels is not unexpected, because the synthesis of GSH from NAC occurs in the intracellular space. Recent magnetic resonance spectroscopy studies indicate increased brain GSH levels following intravenous administration of NAC, an effect presumably occurring in the intracellular compartment [19].

NAC is already FDA approved for both short term and long-term indications [20], and given the known associations between GSH and PD pathogenesis, several authors have identified NAC as a promising neuroprotective therapy in PD [6,9,21,22]. Prior studies using antioxidants to slow progression of PD have shown no clinical benefit [23]. NAC, however, acts by increasing intracellular levels of GSH, which is used to fuel enzymatic antioxidant functions that are orders of magnitude faster than direct molecular scavengers. Moreover, GSH is also used by enzymes (e.g. glutathione S-transferase) to repair oxidative damage to proteins. Although the patient numbers in this study were small, we detected no evidence of an acute symptomatic benefit of NAC on either cognitive or motor function, suggesting that symptomatic effects are unlikely to complicate the interpretation of NAC as a neuroprotective agent in future studies.

Conflicts of interest

None.

Disclosures

None.

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