N-Acetyl Cysteine as a Glutathione Precursor for Schizophrenia—A Double-Blind, Randomized, Placebo-Controlled Trial

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Background: Brain glutathione levels are decreased in schizophrenia, a disorder that often is chronic and refractory to treatment. N-acetyl cysteine (NAC) increases brain glutathione in rodents. This study was conducted to evaluate the safety and effectiveness of oral NAC (1 g orally twice daily [b.i.d.]) as an add-on to maintenance medication for the treatment of chronic schizophrenia over a 24-week period.

Methods: A randomized, multicenter, double-blind, placebo-controlled study. The primary readout was change from baseline on the Positive and Negative Symptoms Scale (PANSS) and its components. Secondary readouts included the Clinical Global Impression (CGI) Severity and Improvement scales, as well as general functioning and extrapyramidal rating scales. Changes following a 4-week treatment discontinuation were evaluated. One hundred forty people with chronic schizophrenia on maintenance antipsychotic medication were randomized; 84 completed treatment.

Results: Intent-to-treat analysis revealed that subjects treated with NAC improved more than placebo-treated subjects over the study period in PANSS total [−5.97 (−10.44, −1.51), $p = .009$], PANSS negative [mean difference −1.83 (95% confidence interval: −3.33, −.32), $p = .018$], and PANSS general [−2.79 (−5.38, −.20), $p = .035$], CGI-Severity (CGI-S) [−.26 (−.44, −.08), $p = .004$], and CGI-Improvement (CGI-I) [−.22 (−.41, −.03), $p = .025$] scores. No significant change on the PANSS positive subscale was seen. N-acetyl cysteine treatment also was associated with an improvement in akathisia ($p = .022$). Effect sizes at end point were consistent with moderate benefits.

Conclusions: These data suggest that adjunctive NAC has potential as a safe and moderately effective augmentation strategy for chronic schizophrenia.

Key Words: Adjunct therapy, clinical trials, glutathione, n-acetyl cysteine, schizophrenia

Abnormalities of brain glutathione (GSH) metabolism in schizophrenia may offer a new target for pharmacological intervention. Glutathione, responsible for the detoxification of reactive oxygen and other radical species (1), is decreased (−27%) in the cerebrospinal fluid of drug-naïve patients with schizophrenia, reflecting a decrease in medial prefrontal cortex GSH (−52%) as detected by in vivo magnetic resonance spectroscopy (MRS) (2). Postmortem assay of the caudate region also showed a decrease of GSH (−41%) in patients with schizophrenia compared with normal control subjects (3). Polymorphisms in the genes for glutamate cysteine ligase modifier subunit (*gclm*) (4) and the catalytic subunit for glutamate cysteine ligase (*gclc*) (5), which both participate in GSH synthesis, suppress both protein expression and GSH levels and are linked to the risk for schizophrenia. Abnormal metabolism of neurotransmitters dopamine and glutamate, characteristic of schizophrenia, induce neuronal oxidative stress that is exaggerated by GSH deficiency (6–9).

We hypothesized that by augmenting production of GSH, N-acetyl cysteine (NAC) treatment may be of clinical benefit in the treatment of schizophrenia. Cysteine is the rate-limiting precursor for GSH synthesis, but oral supplementation with pure cysteine is not efficiently bioavailable (10,11). However, oral NAC rapidly increases plasma cysteine levels, replenishing depleted GSH pools systemically (12). Systemic administration of NAC prevents brain GSH depletion (13–18), with neuroprotective benefits in a variety of neurodegenerative disease models (19–23).

Our current aim was to study the efficacy and tolerability of 2 g daily (1 g twice daily [b.i.d.]) of NAC compared with placebo in patients with chronic schizophrenia who were being maintained on antipsychotics.

Methods and Materials

Study Design

The study was conducted from November 2002 until July 2005. Individuals were assigned using simple randomization (24) to treatment with NAC or placebo in a double-blind fashion. An independent coordinator generated the allocation sequence. The participants were enrolled by trial clinicians, who were blinded to treatment allocation. The randomization sequence was concealed until the end of the trial. All participants remained on their usual antipsychotic medication for the duration of the trial.

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Participants were recruited through advertisements, referral by clinicians, and database screening. Study sites were four private and public general psychiatry inpatient and outpatient facilities in Victoria, Australia, and one public clinic in Lausanne, Switzerland. The participants were residents of the regional vicinities. After complete description of the study to the subjects, written informed consent was obtained at baseline as per protocol.

N-acetyl cysteine was purchased from Zambon, Italy. Purity was 99.8% by high-performance liquid chromatography (HPLC). DFC Thompson, Sydney, Australia, performed encapsulation of the active compound and the inert placebo. The bottles were sealed, dispensed by pharmacy, and returned to pharmacy for pill counts.

Dose Rationale

All randomized participants received two NAC (500 mg) capsules twice daily (2 g daily) or matching placebo capsules. We selected a daily dose that was at the upper dosing range for published clinical trials of 12 weeks to 12 months duration, studying oral NAC treatment for systemic medical conditions, and reporting evidence of tolerability and some efficacy (25–28).

Inclusion and Exclusion Criteria

To be included, participants were required to meet *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) (29) criteria for schizophrenia and have a Positive and Negative Symptoms Scale (PANSS) total score of ≥55, or at least two of the positive and/or negative items being >3, or have a Clinical Global Impression-Severity (CGI-S) ≥3. They needed to have the capacity to consent to the study and be aged between 18 and 65 years. Both inpatients and outpatients were eligible. Participants needed to be currently taking an antipsychotic agent and to be utilizing effective contraception if female and of childbearing age. Exclusion criteria included abnormal renal, hepatic, thyroid, or hematological findings; a systemic medical disorder (30); and positive pregnancy screening at baseline. Individuals who were taking a mood stabilizer (e.g., lithium, valproate, carbamazepine) were excluded, as were those currently taking drugs known to prevent GSH depletion (500+ mg of NAC per day, 200+ μg of selenium per day, or 500+ IU of Vitamin E per day). Individuals on psychoactive medications for other indications (including antidepressants) needed to be on those agents for ≥1 month prior to randomization. Individuals with a prior adverse reaction to NAC or any component of the preparation or who were unable to comply with the treatment protocol were also excluded.

Participant Evaluation

Assessors were all either clinical psychologists or medical practitioners, who were trained on the measures used. Withdrawal from the trial occurred if participants ceased taking their trial medication for 7 consecutive days, ceased effective contraception, or became pregnant. A change in primary antipsychotic or the addition of a mood stabilizer required withdrawal. Dose changes to existing medications were not an exclusion criterion but were monitored. Participants were withdrawn from the study if they withdrew consent or developed serious adverse events associated with the study drug.

End Points

Participants were assessed at baseline using a structured clinical interview (Mini International Neuropsychiatric Interview [MINI], DSM-IV). The primary efficacy outcome measures were the PANSS Total scale, as well as the positive, negative, and general subscales as co-primary outcomes. Secondary readouts included Clinical Global Impression (CGI) Improvement (CGI-I) and Severity (CGI-S) scales, which were chosen to index treatment effects not necessarily solely attributable to changes in psychotic illness. Additionally, functioning was measured using the Global Assessment of Functioning (GAF) scale and the Social and Occupational Functioning Assessment Scale (SOFAS). Extrapyramidal adverse effects were appraised using the Abnormal Involuntary Movements Scale (AIMS), the Simpson-Angus Scale (SAS), and the Barnes Akathisia Scale (BAS). Cognitive tests including digit span (forwards and backwards), word learning, trail making (A and B), and verbal fluency were done at baseline and end point in a small subset of the subjects (n = 32 at baseline; n = 20 at end point). Tolerability was assessed by endorsement scores on a checklist of 44 somatic items. Blinded investigators, who were all experienced clinicians, performed the assessments. Formal training on the PANSS and other rating scales was conducted to optimize reliability prior to the study.

Efficacy measures were repeated every 2 weeks for the first 8 weeks or on the day of study termination if the participant withdrew prior to 8 weeks. After 8 weeks, evaluations were every 4 weeks until 24 weeks, whereupon the treatment was stopped. Postdiscontinuation follow-up was held 4 (±2) weeks after completion to determine any change in participant status. An improvers analysis was performed on subjects with a CGI-I score of ≤3 at any four or more visits. While plasma glutathione levels were not assayed in every subject, a substudy determined that NAC at this dose significantly increased plasma glutathione (31).

Physical and neurological examinations were performed at baseline, as were tests of renal, thyroid, hematological, and hepatic function. Adverse events were tabulated. Blood pressure, pulse, and weight were monitored at each visit.

Randomization occurred at visit 1. End point was defined as the last postbaseline value obtained for a participant for a given measure during the treatment phase. For those participants who completed the 24-week study period, end point corresponded to the week 24 (visit 9) observation.

Statistical Analysis

See Supplement 1.

Results

Study Population

Of 665 people screened, 140 were enrolled, of which 71 were randomized into the placebo group and 69 into the treatment (NAC) group. One hundred eleven participants completed up to week 8; 84 completed up to week 24; and 61 completed the week 28 postdiscontinuation visit (Figure 1 in Supplement 2). Five individuals were inpatients at randomization. There were no significant differences between the two groups for any baseline measures (Table 1). Clozapine (45% of participants) and olanzapine (20% of participants) were the two most commonly used primary antipsychotics, with no significant difference in their use between the treatment groups. Other atypical antipsychotics (risperidone, quetiapine, and aripiprazole) and typical depot antipsychotics accounted for the remainder. The mean doses of chlorpromazine equivalents (32,33) in the placebo group (598.2 mg [SE 56.1]) and the NAC group (716.4 mg [SE 57.0]) were not significantly different. There was a nonsignificant mean dose increase of 20.6 mg chlorpromazine equivalents in the NAC group and 73.1 mg in the placebo group between visits 1 (baseline) and 9 (week 24). Treatment adherence data were

Table 1. Baseline Characteristics of Participants

Characteristic[a]	Placebo Group (n = 71)	NAC Group (n = 69)	All Participants (n = 140)
Age[b] –Years ± SD	36.1 ± 11.7	37.2 ± 10.1	36.6 ± 10.9
Male Sex[c] – Number (%)	50 (70)	48 (70)	98 (70)
Duration of Illness[b] – Years ± SD	12.1 ± 9.6[e]	12.4 ± 8.2[f]	12.2 ± 8.9[g]
Admission Frequency Score[d] – Median (range)[k]	1 (0–7)	1 (0–7)	1 (0–7)
Smoking[c] –Number of Participants (%)	49 (69)	46 (66)	95 (68)
Alcohol Use[c]– Number of Participants (%)	41 (58)	33 (48)	74 (53)
Substance Use[c]– Number of Participants (%)	13 (18)	9 (13)	22 (16)
Prior Suicide Attempt[c] – Number of Participants	4[h]	5[i]	9[j]

NAC, N-acetyl cysteine.

[a]Differences between the NAC and placebo groups were not statistically significant ($p \leq .05$) based on two sample t test (equal variance), Fisher's exact test, or Kruskall-Wallis analysis.

[b]Two sample t test.

[c]Fisher's exact test.

[d]Kruskall-Wallis analysis.

[e]The data were obtained from 67 participants.

[f]The data were obtained from 64 participants.

[g]The data were obtained from 131 participants.

[h]The data were obtained from 70 participants.

[i]The data were obtained from 68 participants.

[j]The data were obtained from 138 participants.

[k]Admissions data were scored on the basis of 1 = 1 admission, 2 = 2 admissions, 3 = 3 admissions, 4 = 4 admissions, 5 = 5 admissions, 6 = 6–10 admissions, 7 = more than 10 admissions.

determined by an audit of returned medication packs, which found a nonsignificant 5.9% and 2.2% discrepancy in the placebo and NAC groups, respectively, over the 24-week treatment period.

Kaplan-Meier survival analysis showed that the dropout rate over the 28-week trial period for all reasons, for patient-initiated reasons (withdrew consent, lost to follow-up, nonadherent, noncompliant, or nonreliable), or for clinician-initiated reasons (adverse event, added mood stabilizer, primary antipsychotic changed or stopped, withdrawal by investigator) was not different between the NAC and placebo groups ($p > .1$ for all comparisons).

Outcome Measures

There were significantly greater improvements observed in the NAC treatment group compared with the placebo group for PANSS negative (least squares [LS] mean difference ± SE, 1.8 ± .8, $p = .018$), PANSS general (LS mean difference ± SE, 2.8 ± 1.3, $p = .035$), and PANSS total (LS mean difference ± SE, 6.0 ± 2.3, $p = .009$) scores at week 24 when compared with baseline using last observation carried forward (LOCF) analysis of covariance (ANCOVA) (Table 2). However, there were no differences observed in PANSS measures when comparing changes from baseline to week 8 (Table 2), suggesting that the clinical benefit was dependent on longer duration of exposure to NAC. We also performed mixed model repeated measures (MMRM) analysis on the PANSS scales but did not detect a significant difference between NAC and placebo over all visits. However, an ANCOVA analysis of the completer dataset confirmed the pattern seen with the primary analysis, with a somewhat greater magnitude of effect. Significantly greater improvements were observed in the NAC treatment group compared with the placebo group for PANSS negative (LS mean difference ± SE, 2.1 ± .9, $p = .028$), PANSS general (LS mean difference ± SE, 4.4 ± 1.6, $p = .005$), and PANSS total (LS mean difference ± SE, 8.6 ± 2.7, $p = .002$) scores at week 24, while the PANSS positive subscale showed a trend toward significance with this analysis (LS mean difference ± SE, 1.7 ± .9, $p = .064$).

Clinical Global Impression-Severity scores, on average, reduced significantly over all visits for the NAC treatment group compared with the placebo group [mean difference (95% confidence interval [CI]): −.26 (−.44, −.08), $p = .004$; Table 2, Figure 1A). Similarly, for CGI-I scores, NAC-treated subjects exhibited a greater clinical improvement than placebo-treated control subjects over all visits [mean difference (95% CI): −.22 (−.41, −.03), $p = .025$; Table 2, Figure 1B].

The onset of clinical benefit was rapid on the CGI scales, with scores significantly improved (using MMRM analysis for CGI-S and Fisher's categorical analysis for CGI-I) in the NAC group compared with the placebo group within 2 weeks (CGI-I, Figure 1B) and 4 weeks (CGI-S, Figure 1A) of commencing treatment. While the placebo group improved between weeks 4 to 8 so that significance of the difference between groups was lost on CGI-I in that interval and on CGI-S at week 8, overall the benefit of NAC treatment compared with placebo was sustained over the treatment interval (24 weeks) with significant improvement at weeks 4, 6, 12, 16, and 24 on CGI-S (Figure 1A). At weeks 12, 16, and 24, significantly more subjects (≈25%) in the NAC treatment group showed improvement on CGI-I compared with placebo (Figure 1B). We also performed ANCOVA for CGI-S scores at two predefined intervals. Table 2 shows illustrative data from the end of week 8 (a customary treatment interval for antipsychotic trials) and from the end of treatment (week 24). N-acetyl cysteine-treated subjects improved compared with placebo at both intervals (week 8, LS mean difference ± SE, .24 ± .11, $p = .027$; week 24, LS mean difference ± SE, .32 ± .13, $p = .022$; Table 2). To clarify the magnitude of the differential clinical improvement between NAC and placebo groups in mean CGI-S scores, we also analyzed the shifts in CGI-S scores from baseline. Mixed model repeated measures analysis revealed that the maximum difference between placebo and NAC groups was at 16 weeks of treatment (Figure 1A). At that visit, 9 of 44 remaining placebo subjects had improved by 1 or more CGI-S points (range 1–2) from their baseline scores. By comparison, NAC treatment was associated with 21 of 44 remaining subjects improving from baseline ($p = .007$) by a range of 1 to 3 points. Therefore, the

Table 2. Efficacy and Functioning Outcome Measures: Changes at Week 8 and Week 24 Compared with Baseline in All Randomized Patients, Using LOCF Analysis[e]

	Within Placebo Group			Within NAC Group			Between Placebo-NAC Differences	
Outcome Measure	Mean Baseline (SD)	Mean Overall[b] Change at Week 8 (95% CI)	Mean Overall[b] Change at Week 24 (95% CI)	Mean Baseline (SD)	Mean Overall[b] Change at Week 8 (95% CI)	Mean Overall[b] Change at Week 24 (95% CI)	LS Mean Difference at Week 8 (95% CI)[a]	LS Mean Difference at Week 24 (95% CI)[a]
CGI-S	4.00 (.83)	−.08 (−.24, .08)	−.03 (−.23, .17)	3.90 (.89)	−.32 (−.48, −.15)[g]	−.35 (−.56, −.14)[f]	.24 (.03, .45)[e]	.32 (.05, .59)[e]
CGI-I[c]	—[c]	3.20 (2.95, 3.45)	3.45 (3.18, 3.73)	—[c]	3.14 (2.90, 3.37)	2.88 (2.64, 3.12)	—[c]	—[c]
PANSS Positive	15.9 (5.3)	−1.9 (−2.8, −0.9)[g]	−1.8 (−2.9, −.7)[f]	16.4 (5.5)	−1.6 (−2.6, −.6)[f]	−2.3 (−3.5, −1.1)[g]	−.3 (−1.5, .1)	.5 (−1.1, 2.1)
PANSS Negative	16.9 (6.2)	−.7 (−1.6, .3)	.24 (−.8, 1.2)[d]	15.1 (6.1)	−.2 (−1.2, .8)	−1.6 (−2.7, −.5)[d]	−.5 (−1.8, .8)	1.8 (.3, 3.3)[d,e]
PANSS General	31.6 (8.5)	−3.3 (−4.7, −1.9)[g]	−1.6 (−3.4, .1)[d]	32.5 (8.0)	−1.7 (−3.2, −.3)[e]	−4.4 (−6.4, −2.5)[d,g]	−1.6 (−3.4, .3)	2.8 (.2, 5.4)[d,e]
PANSS Total	64.4 (16.3)	−6.2 (−8.8, −3.7)[g]	−2.9 (−5.8, .9)[d]	64.0 (15.4)	−3.6 (−6.3, −.9)[f]	−8.8 (−12.2, −5.5)[d,g]	−2.6 (−6.1, .8)	5.9 (1.5, 10.4)[d,f]
GAF	49.3 (12.8)	2.2 (−.2, 4.6)	1.9 (−1.0, 4.7)	50.6 (15.1)	2.7 (.2, 5.3)	4.5 (1.5, 7.5)[f]	−.5 (−3.7, 2.7)	−2.6 (−6.6, 1.3)
SOFAS	50.9 (9.9)	−.4 (−3.2, 2.3)	−1.6 (−5.2, 2.0)	56.6 (12.4)	−.2 (−3.5, 3.0)	−.7 (−5.0, 3.6)	−.2 (−3.9, 3.5)	−.9 (−5.7, 3.8)
BAS	.86 (1.47)	−.03 (−.37, .30)	.12 (−.21, .46)	.96 (1.83)	−.23 (−.58, .11)	−.42 (−.77, −.06)	.20 (−.24, .64)	.54 (.08, 1.00)
SAS	1.37 (1.68)	−.11 (−.35, .13)	−.05 (−.33, .22)	1.87 (1.63)	−.05 (−.30, .21)	−.17 (−.46, .13)	−.06 (−.39, .26)	.12 (−.27, .50)
AIMS	1.66 (2.98)	−.23 (−.77, .31)	−.32 (−.87, .24)	2.71 (4.58)	.08 (−.48, .65)	−.44 (−1.03, .16)	−.31 (−1.03, .41)	.12 (−.65, .89)

AIMS, Abnormal Involuntary Movements Scale; ANCOVA, analysis of covariance; BAS, Barnes Akathisia Scale; CGI-I, Clinical Global Impression-Improvement; CGI-S, Clinical Global Impression-Severity; CI, confidence interval; GAF, Global Assessment of Functioning; LOCF, last observation carried forward; LS mean, least squares mean; NAC, N-acetyl cysteine; PANSS, Positive and Negative Symptoms Scale; SAS, Simpson-Angus Scale; SOFAS, Social and Occupational Functioning Assessment Scale.

[a]Between treatment group LS means (placebo minus NAC), CI and *p* values are from LOCF ANCOVA model with terms baseline score, treatment, and investigator.

[b]Within treatment group LS means, CI and *p* values are from LOCF ANCOVA model with terms baseline score, treatment, and investigator.

[c]CGI-I does not measure baseline score. All subsequent measures refer to baseline status. Mean (CI) refers to score at that time point.

[d]Within and between treatment group LS means, CI and *p* values are from LOCF ANCOVA model with terms baseline score, treatment, investigator, and treatment by investigator (interaction).

[e]Mean difference significant at $p < .05$.

[f]Mean difference significant at $p < .01$.

[g]Mean difference significant at $p < .001$.

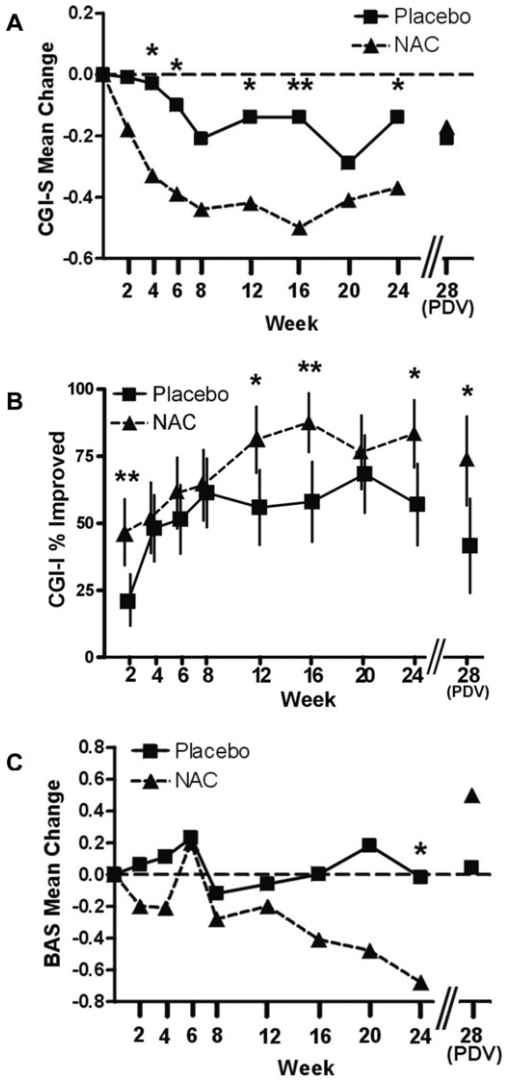

Figure 1. Score for symptoms during the double-blind phase of the trial. **(A)** Mean change in CGI-S from baseline over the study period. Severity is rated on a 7-point scale (1 = normal to 7 = extremely ill). *$p < .05$ versus placebo, **$p < .01$ versus placebo. p values are from MMRM adjusted for baseline score and investigator. **(B)** CGI-I: proportion of participants with a score of 3 or less (improvement) over the study period. Proportions with 95% confidence intervals are indicated. *$p < .05$, **$p < .01$. p values are from Fisher's exact test. **(C)** Mean change in BAS from baseline showing the trend toward improvement over the study period. *$p = .022$ (not significant post–Simes-Hochberg correction for multiple testing) versus placebo. p values are from MMRM adjusted for baseline score and investigator. BAS, Barnes Akathisia Scale; CGI-I, Clinical Global Impression-Improvement; CGI-S, Clinical Global Impression-Severity; MMRM, mixed model repeated measures; NAC, N-acetyl cysteine; PDV, postdiscontinuation visit.

number of patients who exhibited a clinician-observed improvement by CGI-S was more than twofold greater in the NAC group than in the placebo group.

To characterize the quality of the clinical improvement detected by the CGI-I, a MMRM analysis on improvers for all outcomes was performed. The improvement on CGI-I was accompanied by significant improvement on PANSS positive (LS mean difference −1.33, 95% CI: −2.41, −.25, $p = .0162$), negative (LS mean difference −1.87, 95% CI: −2.96, −.78, $p = .0009$), general (LS mean difference −2.99, 95% CI: −4.53, −1.45, $p = .0002$), and total (LS mean difference −5.87, −8.78, −2.96, $p = .0001$) subscales, as well as on CGI-S (LS mean difference −.44, 95% CI: −.63, −.25, $p < .0001$), GAF (LS mean difference +4.61, 95% CI: 1.95–7.27, $p = .0008$), and SOFAS (LS mean difference +5.50, 95% CI: 2.63–8.37, $p = .0003$) but not on the SAS, BAS, or AIMS. Therefore, the treatment effect observed on CGI-I probably reflects improvement of schizophrenia symptoms and not merely general health.

There were no between-group differences on functioning, as measured by the GAF scale or the SOFAS (Figure 2). However, ANCOVA revealed a significant within-group improvement from baseline to end point on the GAF scale (mean overall change ± SE of +4.5 ± 1.5 points) for the NAC treatment group but not for the placebo group (mean overall change ± SE of +1.9 ± 1.4 points; Table 2). This was also confirmed by MMRM analysis where the average improvement over all visits of the NAC group was significant (+3.1 ± 1.0 points, $p = .0026$), but the overall average change from baseline (+1.5 ± 1.0 points) for the placebo group was not significant. No effects on cognition were seen in the subset of subjects that received cognitive assessment.

Post hoc analyses revealed no differences between NAC and placebo groups for baseline predictors of outcome: treatment (clozapine compared with other antipsychotics), gender, age, duration of illness, comorbidity, and number of hospitalizations.

A calculation of the effect sizes (Cohen's *d*) by ANCOVA of the benefits after 24 weeks of NAC treatment on CGI-S, PANSS negative, PANSS general, and PANSS total rating scales, revealed moderate improvements ranging from .43 to .57 (Figure 2).

Postdiscontinuation Measures

The treatment benefit of NAC on CGI-S at the treatment end point (week 24) was lost upon washout (week 28, the postdiscontinuation visit) (LS mean difference ± SE: .10 ± .17, $p = .54$; Table 3, Figure 1A). However, the proportion of participants who were clinically improved when referred to baseline, on the CGI-I scale, remained significantly greater in the NAC group at week 28 (Figure 1B). Similarly, the significant improvement for the NAC group compared with the placebo group observed at week 24 on scores for PANSS positive, PANSS general, PANSS total, and the near-significant improvement on the BAS were not evident after treatment discontinuation (Table 3). In addition, the significant NAC within-group improvement on GAF scores at week 24 was lost postdiscontinuation (Table 3).

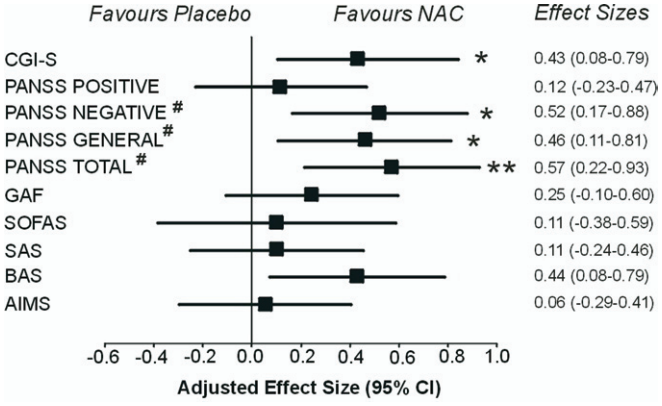

Figure 2. Adjusted effect size at week 24 compared with baseline for outcome measures. Data are mean effect size (Cohen's *d* statistic) ± 95% confidence intervals. All analyses were adjusted for baseline and investigator using ANCOVA. Significant effects are asterisked: *$p < .05$ versus placebo, **$p < .01$ versus placebo. Abbreviations as in Table 2.

Table 3. Efficacy and Functioning Outcome Measures: Change at Posttreatment Discontinuation (Washout, Week 28) in All Randomized Patients Compared with Week 24

	Within Placebo Group		Within NAC Group		Between Placebo-NAC Differences
Outcome Measure	Mean Week 24 (SD)	Mean Overall[b] Change Between Week 24 and Week 28 (95% CI)	Mean Week 24 (SD)	Mean Overall[b] Change Between Week 24 and Week 28 (95% CI)	LS Mean Difference (95% CI)[a]
CGI-S	3.97 (1.05)	−.06 (−.30, .18)	3.50 (1.02)	−.17 (−.41, .08)	.11 (−.23, .44)[e]
CGI-I[c]	3.45 (1.11)	3.45 (3.00, 3.90)	2.88 (.93)	2.93 (2.47, 3.39)	N/A
PANSS Positive	14.2 (5.9)	−.2 (−1.3, .8)	14.5 (5.6)	−.9 (−2.0, .1)	.7 (−.7, 2.2)
PANSS Negative[d]	15.9 (6.5)	−1.0 (−2.1, .1)	13.7 (4.9)	.6 (−.5, 1.7)	−1.6 (−3.2, .0)[e]
PANSS General[d]	29.1 (10.0)	−.7 (−2.5, 1.2)	28.8 (8.5)	.2 (−1.7, 2.1)	−.9 (−3.4, 1.7)[e]
PANSS Total[d]	59.2 (19.3)	−2.1 (−5.0, .7)	57.0 (16.1)	.6 (−2.4, 3.6)	−2.7 (−6.8, 1.4)[e]
GAF	49.8 (14.8)	1.0 (−1.9, 3.86)	54.4 (15.4)	.4 (−2.7, 3.5)[e]	.6 (−3.5, 4.7)
SOFAS	51.2 (12.8)	−2.0 (−4.8, .9)	58.8 (12.7)	−.8 (−3.8, 2.2)	−1.2 (−4.9, 2.5)
BAS	.86 (1.67)	.11 (−.31, .53)	.42 (.93)	.42 (−.02, .86)	−.31 (−.91, .29)
SAS	1.20 (1.60)	−.22 (−.56, .11)	1.61 (1.25)	.30 (−.06, .65)	−.52 (−.99, −.05)
AIMS	1.23 (2.93)	−.67 (−1.32, −.02)	2.24 (3.46)	−.27 (−1.04, .50)	−.40 (−1.41, .61)

Abbreviations as in Table 2.

[a]Between treatment group LS means (placebo minus NAC), CI and *p* values are from LOCF ANCOVA model with terms baseline score, treatment, and investigator.

[b]Within treatment group LS means, CI and *p* values are from LOCF ANCOVA model with terms baseline score, treatment, and investigator.

[c]CGI-I does not measure baseline score. All subsequent measures refer to baseline status. Mean (CI) refers to score at that time point.

[d]Within and between treatment group LS means, CI and *p* values are from LOCF ANCOVA model with terms baseline score, treatment, investigator, and treatment by investigator (interaction).

[e]Significant improvement at week 24 that was not evident after posttreatment discontinuation (washout, week 28).

Effects on Abnormal Movements

Over all visits, there were no significant differences detected between the placebo and NAC groups on the SAS or AIMS scores. Baseline to week 24 LOCF end point changes indicated that the NAC group had reduced akathisia on the BAS scale compared with the placebo group as a product of time on treatment (Figure 1C), almost reaching significance at week 24 after correction for multiple testing ($p = .022$). The effect size (Cohen's *d* statistic) of the benefits after 24 weeks of NAC treatment on the BAS revealed a moderate improvement of .44 (Figure 2).

Adverse Effects and Safety

There were no significant effects of NAC on safety parameters or reported adverse events. See Supplement 1 for details.

Discussion

The results of this study support the possibility that adjunctive treatment of chronic schizophrenia with 2 g/day oral NAC reduces clinical severity as measured by CGI-S (Figure 1A) and PANSS scores (Table 2) and improves global measures of symptomatology as measured by the CGI-I scores (Figure 1B). The response on the PANSS, with improvement on total, general, and negative components, but little improvement on positive symptoms is noteworthy. This may imply that the benefit of NAC could be confined to stable chronic patients, such as the current cohort. However, the deficit of brain glutathione in schizophrenia is not known to be confined to a particular form or stage of the illness, and there is growing evidence of genetic predisposition via polymorphisms in the synthetic pathway that lowers levels of glutathione (5). Therefore, NAC may yet benefit acute schizophrenia or schizophreniform illness, and it is premature to predict what aspects of the illness may respond.

Both trial groups were treated with antipsychotic medication but ~25% more participants taking adjunctive NAC demonstrated clinical improvement on the CGI-I than participants on placebo at weeks 12, 16, and 24 (Figure 1B). At the maximum point of differentiation between NAC and placebo groups, the raters detected clinical improvement from baseline (using CGI-S) in more than twice as many NAC-treated subjects compared with placebo-treated subjects ($p = .007$). Further supporting the likelihood of a NAC treatment effect, the significant benefits that were detected were lost after a 4-week washout (Figure 1A, Table 3), with the exception of the CGI-I.

While the improvement on CGI scales was detected by the more robust MMRM analysis, improvements on the PANSS negative, total, and general scales were observed using ANCOVA LOCF, which does not fully account for treatment effects on the dropouts. The dropouts may have potentially led to bias in favor of the NAC therapy, but a completer analysis validated findings of the ANCOVA LOCF approach. Alternatively, the apparent improvement in CGI scales may reflect changes in clinical features outside parameters measured by the PANSS, such as mood, a clinical observation of the study. However, survival analysis found no significant difference in the dropout rates between NAC and placebo groups for either clinician- or patient-initiated reasons. In addition, the majority of withdrawals from the study could be explained by the data observed, only three patients were lost to follow up, and discontinuation rates were similar between the groups, supporting the likelihood that clinical data on the dropouts are missing at random and therefore the MMRM analysis is valid. The MMRM analysis also found that improvement on the CGI scale was accompanied by significant improvement on the PANSS subscales, suggesting that the observed clinical improvement was likely driven by resolution of psychotic illness.

A moderate benefit of NAC at end point for akathisia was also evident (Figures 1C and 2) on the BAS, which approached significance ($p = .022$). The lack of effect on the AIMS and SAS may reflect the low basal scores in these measures because of atypical antipsychotics being the predominant maintenance medication. These results support further examination of NAC as a neuroprotective treatment for extrapyramidal symptoms (EPS).

This trial had several methodological limitations. Firstly, we had no prior data to base power calculations. As a result, while the PANSS outcomes reached significance on the ANCOVA, they did not reach significance on the MMRM analysis, which probably is due to underpowering. Secondly, we tested only one dose. Thirdly, we lacked a biomarker of glutathione status to help gauge the biological effects of the NAC dose. Fourthly, it was not possible to predict an adequate duration of treatment for a clinical effect. Fifthly, 45% of participants were on clozapine, indicating that the cohort was enriched for treatment resistance, and this may have contributed to the lack of clear efficacy on positive symptoms and to the moderate effect sizes. Finally, as there are high levels of comorbidity in schizophrenia, it is possible that our results may have been influenced by unidentified clinical variables, e.g., mood. Indeed, the potential benefits of NAC for other major psychiatric conditions such as mood disorders merit investigation. Despite these limitations, we found evidence of significant benefit. These data may now be utilized for future studies. The studies will need to increase power; the treatment duration may need to be increased; the design may need to follow more of those subjects who were excluded by the current protocol (e.g., include subjects where medication was changed); the Calgary Depression Scale for Schizophrenia could be included; and cognitive testing could be performed. N-acetyl cysteine treatment of less chronically ill individuals might feasibly be associated with greater effect sizes. Future trials could include dose-finding studies, the examination of NAC augmentation of specific antipsychotic agents, the examination of benefits in acute psychotic illness, and NAC treatment potential for prodromal or first-episode illness.

There is increasing evidence implicating deficits in oxidative defenses in major psychiatric illness (34). Glutathione is the most fundamental antioxidant substrate. The deficiencies of glutathione and related enzymes imply that the brain is vulnerable to oxidative stress in schizophrenia. For instance, there is increased lipid peroxidation, which may be inhibited by atypical antipsychotics (35,36). The typical antipsychotic haloperidol induces oxidative stress, which NAC is able to reverse (37). Recent evidence indicates that the mechanism of ketamine-induced psychosis involves elevated brain superoxide that is rescued by a catalytic scavenger (38). While we theorized that NAC would augment brain glutathione levels, the mechanism of benefit is still uncorroborated. It is possible, for example, that by providing more substrate for the formation of cystine, NAC may favorably impact on the glutamate system via the cystine-glutamate antiporter where cystine is exchanged for glutamate by glia (39,40). Supporting this possibility, NAC recently was reported to improve mismatch negativity in schizophrenia, an auditory evoked potential that reflects *N*-methyl-D-aspartate (NMDA) receptor activity (31).

Antioxidants have various biochemical target specificities and varied penetration across the blood-brain barrier (BBB). Whereas all antioxidant scavengers can, to some extent, spare glutathione, we reasoned that the drop in brain glutathione in schizophrenia is best remedied by direct supplementation of precursor. Other antioxidants that promote glutathione recycling have been studied in schizophrenia. Benefit was reported for a combination of vitamins C, E, and omega 3 fatty acids (41), as well as for monotherapy with vitamin C (42). These strategies help convert oxidized glutathione into reduced glutathione, whereas NAC acts to increase the production of new glutathione (43). In future studies, it will be valuable to obtain plasma GSH and cysteine levels, as well as *gclm* and *gclc* genotype, to correlate with treatment response. Similarly, to show that the depletion of brain GSH in schizophrenia observed by in vivo MRS (44) is corrected by NAC treatment and corresponds with clinical improvement would strongly support the proposed mechanism of replenishing brain GSH.

Improvement was seen on the CGI-I at 2 weeks (Figure 1B) and the CGI-S at 4 weeks (Figure 1A), while improvement on the PANSS and a trend to improvement on the BAS emerged only toward 24 weeks of treatment (Table 2, Figures 1C and 2). While the mechanism of action of NAC here is unproven, it is unlikely that NAC acts at a receptor level, and the delayed benefits, as well as the possible effects on akathisia, support a potentially neuroprotective mechanism. The loss of treatment benefits shortly after discontinuation may be important for clinical use and for understanding the mechanisms underlying efficacy. Glutathione undergoes rapid biochemical turnover. Given that a genetic diathesis cannot be altered by NAC treatment, the benefits of glutathione supplementation would be expected to wear off rapidly once the precursor is stopped. The relatively slow onset of some aspects of benefit may be related to the need to reverse years of accumulated oxidative and neurochemical changes.

There is a need for additional pharmacological approaches to schizophrenia. While the benefits of NAC treatment in our current study are moderate at best, the patient group being treated is notoriously refractory to treatment, so that even modest benefits are of interest. It is possible that NAC therapy may elicit more robust responses in other categories of psychosis, such as acute schizophrenia. Therefore, our results represent a promising first step. The multicenter, outpatient setting for our study of chronically ill patients encourages consideration of NAC supplementation in this setting as a potential augmentation strategy, especially as it is safe, relatively inexpensive, and available over-the-counter. The results of this trial indicate that modulation of cysteine-glutathione biology in schizophrenia via NAC supplementation represents a promising new approach that warrants further investigation.

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Supplementary material cited in this article is available online.

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