A Double-Blind, Randomized, Placebo-Controlled Study of Aspirin and *N*-Acetylcysteine as Adjunctive Treatments for Bipolar Depression

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ABSTRACT

Objective: Neuroinflammation has been implicated in the pathophysiology of bipolar disorder. Some evidence shows that nonsteroidal anti-inflammatory drugs (NSAIDs) have promising antidepressant effects. The antioxidant *N*-acetylcysteine (NAC) may enhance the effects of NSAIDs. No study has, however, tested the adjunctive therapeutic benefits of an NSAID and NAC in bipolar disorder.

Methods: The sample included 24 medicated patients diagnosed with *DSM-IV-TR* bipolar disorder who were aged 18–65 years and had a Montgomery-Asberg Depression Rating Scale (MADRS) score ≥ 20. Participants were randomly assigned to receive either aspirin (1,000 mg), NAC (1,000 mg), combined aspirin and NAC (1,000 mg each), or placebo. Data were collected between 2013 and 2017. The primary outcome was a ≥ 50% reduction in MADRS scores. Participants completed mood and global functioning questionnaires. They also underwent blood tests prior to and following 8 and 16 weeks of treatment. A Bayesian analytic method was adopted, and posterior probability distributions were calculated to determine the probability of treatment response.

Results: Following the first 8-week treatment phase, individuals on treatment with placebo and NAC + aspirin had a similar probability for successful treatment response (about 70%). Following a 16-week treatment period, NAC + aspirin was associated with higher probability of treatment response (67%) compared to placebo (55%), NAC (57%), and aspirin (33%). There was no treatment effect on interleukin-6 and C-reactive protein levels at either 8 or 16 weeks.

Conclusions: The coadministration of NAC and aspirin during a period of 16 weeks was associated with a reduction in depressive symptoms. The adverse effects were minimal. These preliminary findings may serve as a starting point for future studies assessing the efficacy, tolerability, and safety of anti-inflammatory and antioxidant agents in the treatment of bipolar depression.

Trial Registration: ClinicalTrials.gov identifier: NCT01797575

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Bipolar disorder is a chronic, severe psychiatric disorder that constitutes the sixth leading cause of disability worldwide and affects 2.6% of the US population.[1–3] The treatment of bipolar disorder poses great challenges due to the unpredictable clinical course of the disease, duration of the mood episodes, and heterogeneous nature of the clinical symptoms. For instance, approximately 37% of patients relapse within a year and 60% relapse within 2 years after recovering from an episode.[2] Patients with bipolar depression are at particularly elevated risk for relapse, recurrence, self-harm, and suicide.[3] Few treatments have proven efficacy, and there are no consensus guidelines for the treatment of bipolar depression.

The pathophysiology of bipolar depression is controversial, but there is evidence for contributions of neuroinflammation.[4] A meta-analysis of human studies measuring blood cytokine levels in major depression[5] found that levels of interleukin-6 (IL-6)—signaling molecules known to mediate central and peripheral inflammation—are elevated, thus indicating potential immune system dysregulation. Levels of C-reactive protein (CRP)—a common measure of systemic inflammation—are elevated (> 3 mg/L) in patients with mood disorders.[6–10] High CRP levels have been linked to psychological distress, increased risk for hospitalization,[11] and treatment response.[12–15] Notably, individuals with pretreatment CRP levels greater than 5 mg/L displayed reduced depressive symptoms following a 12-week trial with infliximab.[16] Inflammation also has been linked to the structural and functional brain abnormalities observed in bipolar disorder, specifically in brain regions involved in mood regulation.[17–19]

There is limited but promising evidence for the antidepressant benefits of nonsteroidal anti-inflammatory drugs (NSAIDs; eg, acetylsalicylic acid [aspirin], celecoxib) and antioxidative (eg, *N*-acetylcysteine [NAC]) adjunct treatments in mood disorders.[20–22] The anti-inflammatory effect of NSAIDs has been associated with the reduction in levels of cyclooxygenase (COX), a rate-limiting enzyme responsible for the synthesis of proinflammatory prostaglandins.[23] A large meta-analysis of 6,262 medicated individuals with depression taking cytokine inhibitors or NSAIDs[24] showed a moderate adjunct antidepressant effect for these treatments. An open-label study in which patients with major depressive disorder were administered 160 mg/d of aspirin[25] yielded a 52.4% response rate, and remission was achieved in 82% in the responder sample. This improvement was maintained across the 28-day treatment period. Furthermore, a 3-month double-blind trial in which aspirin 1,000 mg/d was given to patients

Clinical Points

- Patients with bipolar depression have an increased risk of relapse and often do not respond to traditional treatments.
- Inflammatory and oxidative stress processes may contribute to the development and maintenance of bipolar disorder, but few clinical trials have tested anti-inflammatory and antioxidant treatments in bipolar depression.
- This study shows that 16-week treatment with aspirin 1,000 mg/d and *N*-acetylcysteine 1,000 mg/d reduces depressive symptoms.

with symptomatic schizophrenia found promising results for both positive and negative symptoms.[26] However, the therapeutic effects of aspirin in bipolar disorder have yet to be confirmed.

N-acetylcysteine has well-established anti-inflammatory, antioxidant, and glutamatergic modulating effects through the cysteine-glutamate antiporter.[27,28] NAC inhibits the expression and release of proinflammatory cytokines, including IL-6.[29] Further, it can be converted to cysteine, a substrate for the cysteine-glutamate antiporter. This antiporter promotes the uptake of cysteine and inhibits the transport of glutamate into the extracellular space and the synaptic release of glutamate.[30] The cysteine component of NAC combines with glutamate and glycine to produce glutathione, a major endogenous antioxidant.[31] Abnormalities in the glutamatergic system have been associated with increased impulsivity, a core feature of bipolar, substance abuse, and gambling disorders.[32] As a cysteine precursor, NAC influences the glutamatergic system and may, therefore, hold potential for treating these psychiatric conditions.[33] Meta-analytic evidence supports the use of NAC for its antidepressant benefits in bipolar disorder.[34] In a 6-month randomized clinical trial (RCT) that included 2,000 mg/d of NAC,[20] bipolar disorder patients showed improvement in depressive symptoms per scores on the Montgomery-Asberg Depression Rating Scale (MADRS).[35] In another RCT,[36] depressive symptoms decreased after a 10-week treatment period with NAC 1,200 mg/d adjunct with valproate and paroxetine.[37] Further, in patients with early psychosis, 6-month treatment with NAC 2,700 mg/d as an add-on to standard medication was associated with improved cognitive performance and increased brain levels of glutathione (measured via magnetic resonance spectroscopy).[38] This finding is important because it suggests that NAC may be able to cross the blood-brain barrier and improve the oxidative state of the brain.

The temporal pattern of the therapeutic benefits of NAC effects is, however, still poorly understood. In individuals with bipolar disorder, an 8-week open-label treatment phase with 1,000 mg/d of NAC adjunct to conventional treatment led to decreased depressive symptoms compared to placebo treatment.[20] Nevertheless, during the subsequent 16-week double-blind phase, NAC was comparable to placebo.[39] Methodological differences in terms of study design and sample may be the primary explanation for such divergent findings.

Findings of studies examining the interaction between inflammatory processes and oxidative stress support the hypothesis that the therapeutic benefits of NSAIDs may be enhanced by the simultaneous administration of NAC.[40] The anti-inflammatory action of NSAIDs is typically associated with reduced synthesis of the COX enzyme and decreased levels of prostaglandins, which are primary oxidative stress biomarkers.[23,41] In cell studies, NAC has been found to counteract oxidative stress even further by enhancing the inhibitory effects of the NSAIDs rofecoxib and diclofenac on the production of prostaglandin E2.[40] However, the therapeutic effects of NAC adjunct to NSAIDs in humans are still underexplored and require further investigation.[42]

In summary, there is some evidence for the antidepressant benefits of aspirin (a COX-1 and COX-2 inhibitor) and NAC (an antioxidant agent). It is unclear whether aspirin and NAC have similar therapeutic efficacy and whether administering both aspirin and NAC would have additional benefits relative to NAC or aspirin alone. To address these questions, we conducted a 16-week placebo-controlled, double-blind, randomized study to test the antidepressant effects of NAC and aspirin separately and in combination. We applied Bayesian statistical analyses to the existing data to yield probabilistic estimates of treatment effect size. Further, on the basis of recent findings suggesting that patients with relatively high inflammatory marker levels may be more responsive to anti-inflammatory treatments, we measured CRP and IL-6 levels prior to and following treatment.

METHODS

Participant Recruitment

Participants were recruited from the outpatient clinic of the University of Texas Health Science Center at Houston (UT) (N = 36). To be included in this study, participants had to be aged between 18 and 65 years; have a diagnosis of bipolar disorder type I or II according to the *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition, Text Revision (*DSM-IV-TR*); and currently be in a depressive or mixed episode. Ascertainment of bipolar disorder diagnosis was done through the Structured Clinical Interview for *DSM-IV-TR* Axis I Disorders, Patient Edition (SCID-I/P).[43] An independent psychiatrist or trained research assistant administered the SCID-I/P to all participants. All participants had a MADRS[35] score ≥ 20 at study entry. Participants had to be on therapeutic doses of a mood-stabilizing drug or combinations of such agents for at least 1 month. Changes in the doses of the psychotropic medications during the trial were not allowed.

Exclusion criteria included the current presence or past history of severe chronic diseases. For safety reasons, concurrent intake of NSAIDs (other than those administered in this study) or anticoagulants was not allowed, and pregnant women were excluded. Comorbid psychiatric

conditions were allowed. However, we excluded patients who met SCID-I/P criteria for substance abuse or dependence in the past 2 months. The study protocol was approved by the UT institutional review board, and informed consent was obtained from all the participants. This study is registered at ClinicalTrials.gov (identifier NCT01797575).

Study Design

This was a double-blind, randomized, placebo-controlled trial conducted during 16 weeks to compare aspirin and NAC (individually and in combination) with placebo, adjunctive to treatment as usual in patients with BD with MADRS scores ≥ 20. Patients were randomly assigned to receive 1 of the following 4 treatments: aspirin (1,000 mg/d [500 mg twice daily]), NAC (1,000 mg/d [500 mg twice daily]), combined aspirin and NAC at same doses as when administered separately, or placebo (sugar pill). The choice of treatment doses was based on previous trials of adjunctive aspirin[26] and NAC[44] treatments whereby these doses were efficacious and well tolerated. Treatment with aspirin and/or NAC was adjunctive to patients' ongoing treatment regimen for a 16-week period. Adherence was assessed by pill counts of returned packs. Responders were defined as showing a ≥ 50% reduction in baseline MADRS scores. A researcher not otherwise involved in the trial and analysis carried out participant randomization and group allocation based on a computer-generated allocation sequence. Trial personnel and investigators conducting clinical assessments and data analysis were blinded to the participants' treatment. Treatments were administered in identical numbers and capsule formulations in sealed containers.

Procedures

After providing written informed consent, bipolar disorder patients underwent a SCID-I/P interview[43] to confirm their psychiatric diagnosis, followed by a physical examination and routine laboratory tests to rule out relevant medical problems. Prior to being assigned to a treatment group, eligible participants filled out demographic and mood questionnaires and underwent blood tests to evaluate levels of inflammatory markers (IL-6 and CRP). The same tests were administered at weeks 8 and 16 of the clinical trial. Individuals were withdrawn from the trial if they failed to take treatment for 7 consecutive days, ceased use of effective contraception or had a confirmed pregnancy, or withdrew consent or if serious adverse effects associated with the trial compounds emerged. Participant report of adverse effects was recorded, appropriately managed according to medical assessment, and reported to the UT Institutional Review board and funding agency. The primary outcome measure of the current study was the MADRS score. As part of this study, we considered 2 end points: (1) end of the first 8 weeks of treatment (week 8) and (2) end of the second 8 weeks of treatment (week 16). Figure 1 provides a CONSORT diagram that illustrates the flow of participants through different stages of the study. Data were collected between 2013 and 2017.

Measures

The clinical interview included the MADRS[35] (primary outcome measure) and the Young Mania Rating Scale (YMRS).[45] Participants were also administered the Global Assessment of Functioning (GAF) of the *DSM-IV-TR*[46] to evaluate changes in social, occupational, and psychological functioning. Plasma IL-6 (Quantikine, R&D Systems, Minneapolis, Minnesota) and CRP (MyBiosource, San Diego, California) levels were determined by highly sensitive enzyme-linked immunosorbent assay kits. All samples were assayed in duplicate by a technician blinded to the clinical status of the subjects.

Statistical Analysis

Exploratory analyses. Statistical analyses were conducted in R version 3.4.3[47] and SPSS Statistics Version 24.[48] Descriptive statistics summarized baseline characteristics of the sample. Analyses of variance (ANOVAs) and χ^2 analyses were used to test for demographic and clinical differences across groups at baseline. Repeated-measures ANOVAs were conducted to compare changes in MADRS, YMRS, and GAF scores across treatments over time. The relationship between inflammation and MADRS scores was explored using Pearson coefficients of correlation on the intention-to-treat sample (n = 30).

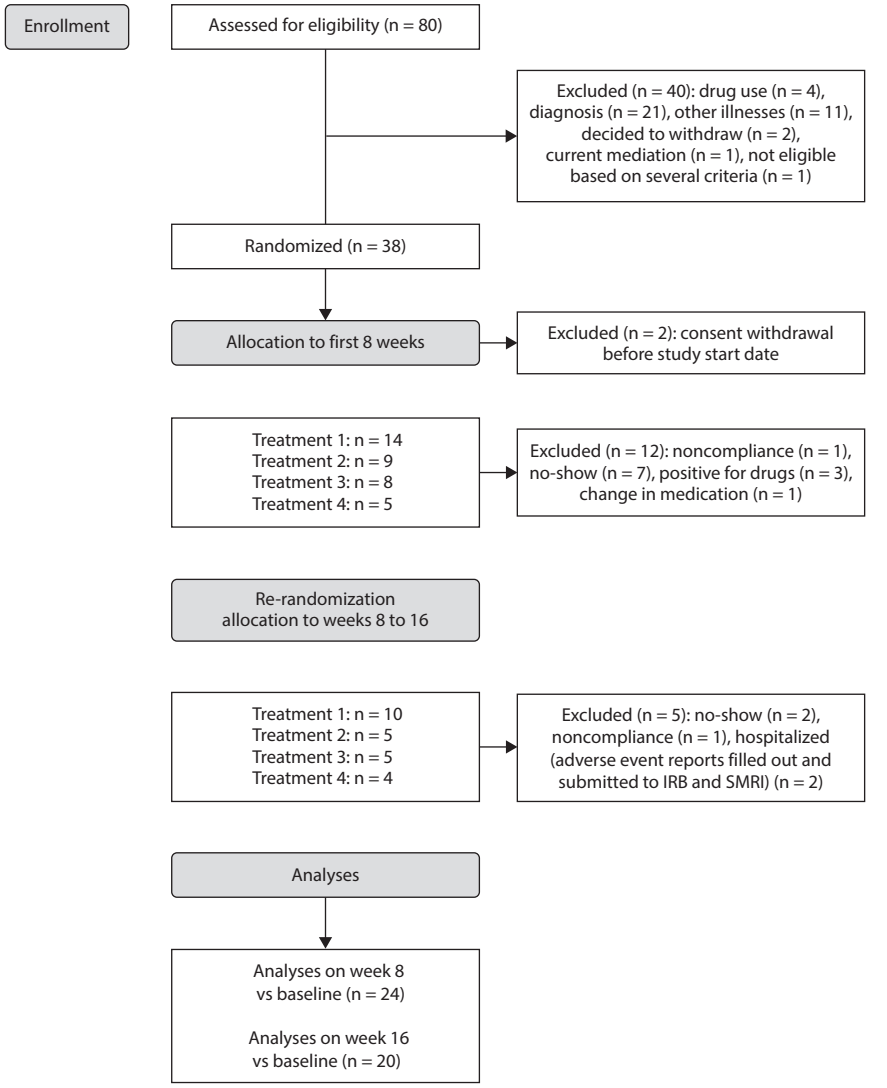
Treatment-related changes in CRP and IL-6 levels were examined using 2 repeated-measures ANOVAs. In these analyses, treatment group was the within-subject factor and the independent variables were CRP and IL-6 data collected at baseline and week 8 (for the first set of analyses) and at baseline and week 16 (for the second set of analyses). CRP and IL-6 values below the limit of detection were substituted by a constant value equal to half of the limit of detection (IL-6 = 0.039 pg/mL divided by 2 = 0.02; hs-CRP [ie, high-sensitivity CRP]: 0.156 ng/mL divided by 2 = 0.08).

On the basis of previous evidence that individuals with high pretreatment inflammatory levels (CRP > 5 mg/L) are more likely to respond to anti-inflammatory treatments, we also compared MADRS difference scores between low (≤ 5 mg/L) and high (> 5 mg/L) CRP individuals using 2 sets of univariate analyses. The first set used the difference between week 8 and baseline MADRS scores, and the second set used the difference between week 16 and baseline MADRS scores.

All analyses were conducted on the sample of randomized participants who adhered to the clinical trial instructions as specified in the study protocol (week 8: n = 24; week 16: n = 20). Results were corrected for multiple comparisons, and the significance threshold was set at $P \leq .05$.

Bayesian analyses. Bayesian statistical reasoning was used to quantify the likelihood of change in MADRS scores across treatments. The rationale for using Bayesian statistics was based on evidence that conventional medication-development trials in psychiatry are exceedingly long and expensive, require a large number of participants, and risk being uninformative if initial drug or dose selection prove to be incorrect. The application of Bayesian statistical methods is a relatively new approach that addresses these

Figure 1. CONSORT Diagram Showing the Flow of Participants Through Each Stage of the Randomized Trial



Abbreviations: IRB = institutional review board, SMRI = Stanley Medical Research Institute.

issues well.[49,50] Bayesian methods are better equipped than non-Bayesian (frequentist) regression models to investigate research questions involving small sample sizes.[51,52] Further, the primary outcome of Bayesian methods—the posterior probability of an effect—is the actual quantity researchers are typically interested in when they use *P* values from frequentist inference. In other words, Bayesian methods provide estimates of the probability that effect sizes are greater (or less) than some clinically meaningful value. A Bayesian analytic approach involves the estimation of the so-called conditional expected power and integrates prior information on treatment effects using conditional prior distribution. This approach recognizes the importance of uncertainty in the study parameters and exploits past performance of and expert information on a treatment when considering the power of a study.[53,54] Given the small sample size of our study, this approach allowed a better assessment of treatment efficacy and discarded heterogeneity due to a mixture of noncompliance and dropout. Specifically, we evaluated the proportion of patients reporting a ≥ 50% decrease in baseline MADRS scores as a function of treatment based on a β-binomial model. The β-binomial model used a ~β (1,1) prior distribution. Distributional assumptions were evaluated via inspection of residual plots and formal statistical tests. Priors were specified as vague and diffuse. No adjustment for multiple testing was necessary for Bayesian analyses due to their conformity to the likelihood principle.[49]

RESULTS

Demographic and Baseline Clinical Findings

Eighty patients with a bipolar disorder diagnosis were initially screened to obtain a sample of 38 subjects who were randomized to treatment (see the CONSORT flowchart in Figure 1). Twenty-four participants completed the first 8-week treatment phase, and 20 participants completed the

Table 1. Demographic, Clinical, and Inflammatory Characteristics of Participants With Bipolar Disorder Who Completed the 8- and 16-Week Treatment Phases

Variable	Placebo	NAC + Placebo	NAC + Aspirin	Aspirin + Placebo	χ^2/F	*P*	Repeated ANOVAs
Responders, n/total n							
Week 8	6/8	5/8	3/4	2/4			
Week 16	4/7	3/5	3/4	1/4			
Age, mean ± SD, y	39.13 ± 9.99	36.38 ± 7.05	40 ± 17.64	49 ± 15.21	1.09	.38	
Female, n/total n	6/8	5/8	1/4	3/4	0.66	.88	
Race, n	4 white 2 Hispanic 1 African American 1 multiracial	5 white 1 African American 1 American Indian 1 unreported	2 white 2 Hispanic	2 white 1 African American 1 Asian	17.15	.84	
Education, mean ± SD, y	14.5 ± 2.51	14.13 ± 1.81	13.5 ± 2.39	13.75 ± 2.06	0.22	.88	
Comorbidities, individual or combination, n	5 agoraphobia 3 GAD 3 panic disorder 1 PTSD 5 phobias 2 OCD 2 BED 4 alcohol use 8 substance use	1 agoraphobia 4 GAD 4 panic disorder 2 phobia 1 BED 2 alcohol use 4 substance use	2 GAD 3 panic disorder 1 PTSD 1 phobia 2 eating disorder 1 OCD 1 alcohol abuse 1 substance use	1 GAD 1 panic disorder 1 PTSD 2 phobia 1 bulimia 2 alcohol abuse/ dependence 6 substance use		> .05	
Medication, monotherapy or combination	2 lithium 4 anticonvulsants 5 antidepressants 4 antipsychotics	1 lithium 5 anticonvulsants 7 antidepressants 3 antipsychotics 4 benzodiazepines	2 lithium 3 anticonvulsants 2 antidepressants 1 antipsychotics	4 anticonvulsants 2 antidepressants 2 antipsychotics 1 benzodiazepines		> .05	
MADRS score, mean ± SD							
Baseline	22.88 ± 4.09	19.38 ± 4.87	19.5 ± 1	22.5 ± 10.47	0.73	.55	
Week 8	7.13 ± 8.29	10.13 ± 9.78	7.5 ± 10.47	12 ± 11.34	0.3	.83	$F_{3,20} = 0.52$, $P = .67$, $\eta^2 = 0.07$[a]
Week 16	13.6 ± 13.83	11.38 ± 10.36	5 ± 6.25	7.25 ± 5.91	0.58	.64	$F_{3,16} = 0.52$, $P = .68$, $\eta^2 = 0.09$[b]
YMRS score, mean ± SD							
Baseline	4.38 ± 4.63	5.5 ± 3.46	5.75 ± 4.27	4 ± 5.42	0.01	.9	
Week 8	1.88 ± 2.3	2.5 ± 1.92	2 ± 1.41	0.75 ± 1.5	0.72	.55	$F_{3,20} = 0.07$, $P = .97$, $\eta^2 = 0.011$[a]
Week 16	3.83 ± 3.54	2 ± 2.33	1.66 ± 2.08	4.25 ± 7.22	0.5	.69	$F_{3,17} = 0.77$, $P = .53$, $\eta^2 = 0.12$[b]
GAF score, mean ± SD							
Baseline	58.38 ± 6.89	58 ± 6.83	59.3 ± 3.69	58 ± 10.61	0.14	.93	
Week 8	72.37 ± 9.47	65.29 ± 10.06	69.5 ± 9.95	67.5 ± 10.1	0.58	.64	$F_{3,19} = 0.57$, $P = .64$, $\eta^2 = 0.08$[a]
Week 16	68.5 ± 16.49	70.28 ± 10.85	69 ± 7.21	67 ± 12.46	0.03	.99	$F_{3,16} = 0.06$, $P = .98$, $\eta^2 = 0.01$[b]
Plasma IL-6 level, mean ± SD, pg/mL							
Baseline	2.3 ± 1.47 (n = 6)	3.22 ± 2.02 (n = 7)	0.85 ± 0.79 (n = 3)	1.27 ± 0.83 (n = 3)	2.04	.15	
Week 8	1.87 ± 0.55 (n = 5)	3.53 ± 0 (n = 1)	1.76 ± 0.64 (n = 2)	0.78 ± 0.43 (n = 3)	7.32	.02[c]	
Week 16	1.71 ± 1.11 (n = 6)	2.6 ± 1.84 (n = 6)	1.72 ± 1.15 (n = 2)	0.90 ± 0.57 (n = 3)	1.07	.4	
Plasma CRP level, mean ± SD, ng/L							
Baseline	17.30 ± 20.74 (n = 6)	17.65 ± 13.51 (n = 7)	5.1 ± 2.86 (n = 3)	6.85 ± 4.72 (n = 3)	0.83	.5	
Week 8	7.4 ± 6.1 (n = 5)	41.09 ± 0 (n = 1)	17.02 ± 9.21 (n = 2)	6.82 ± 8.4 (n = 3)	5.69	.04[c]	
Week 16	13.10 ± 17.98 (n = 6)	17.69 ± 10.98 (n = 6)	9.39 ± 7.05 (n = 2)	10.76 ± 11.29 (n = 3)	0.28	.84	
Adverse effects, n	1 hospitalized	0	0	1 skin rash 1 hospitalized	12.86	.32	

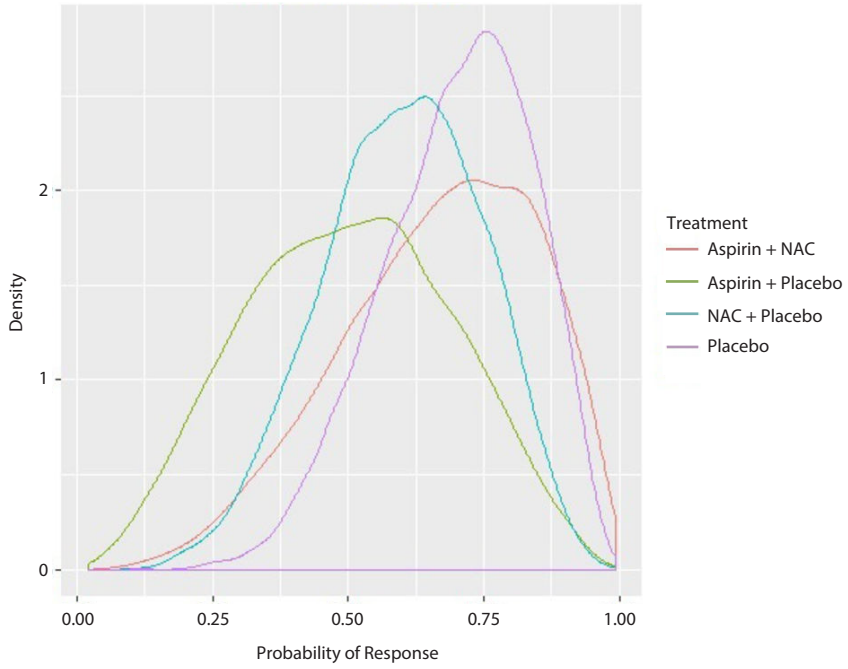
[a]Including baseline and week 8 data.
[b]Including baseline and week 16 data.
[c]Significant for NAC + placebo versus placebo but based on only 1 data point.
Abbreviations: ANOVA = analysis of variance, BED = binge-eating disorder, CRP = C-reactive protein, GAD = generalized anxiety disorder, IL-6 = interleukin-6, GAF = Global Assessment of Functioning, MADRS = Montgomery-Asberg Depression Rating Scale, NAC = *N*-acetylcysteine, OCD = obsessive-compulsive disorder, PTSD = posttraumatic stress disorder, YMRS = Young Mania Rating Scale.

entire 16-week treatment period. As illustrated in Table 1, at baseline, the 4 treatment groups were comparable overall in terms of age; sex; MADRS, YMRS, and GAF scores; IL-6 and CRP levels; medication; and comorbidities ($P > .05$). Participants in the aspirin + placebo group were, however, marginally older than those assigned to other treatment groups. Repeated-measures ANOVAs did not yield statistically significant differences in MADRS, YMRS, and GAF scores across treatments over time (Table 1). In terms of safety, 2 participants were discontinued from the study after being hospitalized for suicidal behaviors and manic relapse. Adverse event reports were filled out and submitted to UT Institutional Review Board and the Stanley Medical Research Institute. Table 1 provides demographic and clinical information of each treatment group at baseline, week 8, and week 16.

Bayesian Analyses

Week 8 vs baseline. Bayesian analyses were used to quantify the evidence for the existence of an effect of

Figure 2. Posterior Distribution of Response Rates in Each Treatment at the End of Week 8



Abbreviation: NAC = *N*-acetylcysteine.

treatment. This approach tests the probability of an effect of treatment given the data. As previously mentioned, a 50% decrease in baseline MADRS scores was the primary outcome measure of this study. At week 8, the mean response probability rates and credibility intervals (in descending order) were 0.7 (95% CI, 0.39 to 0.93) for placebo, 0.67 (95% CI, 0.28 to 0.95) for aspirin + NAC, 0.6 (95% CI, 0.3 to 0.86) for NAC + placebo, and 0.5 (95% CI, 0.15 to 0.86) for aspirin + placebo. Comparison of probability distributions across treatments showed that placebo had a 19.42% greater probability of success compared to aspirin + placebo (95% CI, −0.26.35 to 62.54), 9.91% compared to NAC + placebo (95% CI, −30.56 to 48.53), and 3.01% compared to aspirin + NAC (95% CI, −39.98 to 49.04) (Figure 2).

Week 16 vs baseline. At week 16, 11 of 20 participants displayed a 50% decrease in MADRS scores from baseline (Table 1). Preliminary analyses showed the posterior probability of treatment response (in descending order) was 0.67 (95% CI, 0.54 to 0.81) for aspirin + NAC, 0.57 (95% CI, 0.45 to 0.7) for NAC + placebo, 0.55 (95% CI, 0.44 to 0.67) for placebo, and 0.33 (95% CI, 0.2 to 0.45) for aspirin + placebo. Aspirin + NAC had a 33.26% greater chance of success compared to aspirin + placebo (95% CI, 16.2 to 52.01), 11.1% compared to placebo (95% CI, −4.95 to 27.87), and 9.53% compared to NAC + placebo (95% CI, 7.72 to 27.33) (Figure 3).

Exploratory Analyses: IL-6 and CRP Levels

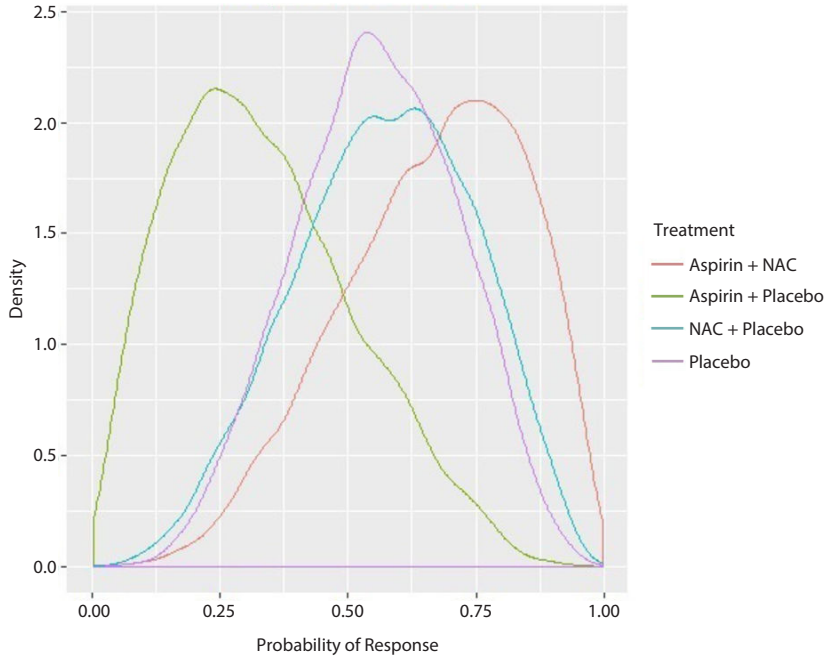
At baseline, plasma levels of IL-6 ranged from 0.85 ± 0.79 to 3.22 ± 2.02 mg/L, and plasma CRP levels ranged from 5.10 ± 2.86 to 17.65 ± 13.51 mg/L (Table 1). Correlations between baseline MADRS scores and inflammatory markers were not statistically significant: $r = 0.12$ ($P = .51$) for CRP and $r = 0.14$ ($P = .46$) for IL-6. Repeated-measures ANOVAs showed an overall effect of treatment on IL-6 levels ($F_{3,6} = 5.33$, $P = .04$, partial $\eta^2 = 0.73$) after the first 8-week treatment phase. There was no treatment effect on CRP values ($F_{3,6} = 0.42$, $P = .74$, partial $\eta^2 = 0.18$). Post hoc tests showed that NAC + placebo was associated with a significant decrease in IL-6 levels ($P = .003$). However, this finding was based on only 1 data point for this treatment group (Table 1). When values collected following the full 16-week trial are considered, there was no effect of treatment on levels of either IL-6 ($F_{3,11} = 2.12$, $P = .16$, $\eta^2 = 0.37$) or CRP ($F_{3,11} = 0.27$, $P = .85$, $\eta^2 = 0.07$).

To explore the relationship between CRP values and MADRS further, we separated individuals into low (≤ 5 mg/L) and high (> 5 mg/L) CRP groups. At baseline, MADRS scores were comparable ($F_{1,28} = 0.02$, $P = .88$) between the low (mean ± SD = 21.21 ± 4.77) and high (mean ± SD = 21.5 ± 5.42) CRP groups. Similarly, changes in MADRS scores from baseline after the 8- and 16-week trials did not differ between low and high CRP individuals (week 8: $F_{1,17} = 2.85$, $P = .11$; week 16: $F_{1,15} = 0.29$, $P = .6$).

DISCUSSION

This study is the first to test the efficacy of adjunct aspirin and NAC—individually and in combination—on depressive symptoms in a sample of adult bipolar disorder

Figure 3. Posterior Distribution of Response Rates in Each Treatment at the End of Week 16



Abbreviation: NAC = *N*-acetylcysteine.

patients. Our findings show that after the first 8-week treatment phase, placebo and NAC + aspirin have a similar probability of treatment response (based on a 50% reduction in baseline MADRS scores). After a 16-week treatment period, NAC + aspirin was associated with a higher probability of treatment response compared to placebo and the other treatments. Considering the preliminary nature of this study and previous probability threshold selected in previous medication trials, a posterior probability above 0.6 was considered sufficient evidence for the existence of an effect.[55,56]

These preliminary findings suggest that the coadministration of NAC and aspirin may be beneficial in decreasing the severity of depressive symptoms in bipolar disorder patients in the long term. Notably, symptomatic improvement emerged only after 16 weeks of treatment. This result is in line with previous studies[57,58] whereby NAC 1,000 mg/d as an add-on treatment was tested in 69 antipsychotic-medicated individuals with schizophrenia and improvement occurred in both the positive and negative symptom domains after a 24-week treatment period. In bipolar disorder, oral NAC 2,000 mg/d led to a reduction in depressive symptoms (per MADRS scores) over a period of 6 months compared to a placebo treatment.[20] As in our study, the beneficial effects of NAC emerged toward the end of the 20-week NAC treatment phase.[20] These "delayed" beneficial effects appear to support previous evidence that NAC and aspirin trigger a cascade of complex, "slow-moving" biological processes related to mitochondrial function and glutathione production.[33,59–61,62] This hypothesis warrants further exploration.

It is noteworthy that although NAC can cross the blood-brain-barrier,[63] its bioavailability is limited (6%–10%).[64] In light of our findings, this may mean that high doses (1,000 mg/d and above) and/or long-term administration of NAC (≥ 16 weeks) may be necessary to observe therapeutic benefits. There is also some evidence showing that the NAC-derived compound *N*-acetylcysteine amide (NACA) has greater cellular permeability and increased bioavailability than NAC within the central nervous system.[64] NACA may therefore be a more effective and fast-acting treatment than NAC.

Less is known about the effects of aspirin in bipolar disorder, as the majority of the studies have focused on individuals with schizophrenia, depression, and cardiovascular diseases. A large study[21] on the effects of NSAIDs on lithium-treated individuals with bipolar disorder showed that bipolar disorder patients receiving low-dose (< 80 mg/d) aspirin were less likely to change lithium doses or change medication compared to those receiving other NSAID and glucocorticoid medications. This finding is intriguing as, while low doses of aspirin are likely to down-regulate only COX-1, higher doses target both COX-1 and COX-2. A higher dose of aspirin would therefore lead to greater conversion of arachidonic and docosahexaenoic acid into highly anti-inflammatory mediators.[21] Another potential explanation of this finding is the presence of a synergistic effect of lithium and a low dose of aspirin generating anti-inflammatory substances. This hypothesis has, however, not been tested so far.

Unlike previous findings,[12,13] in our study the correlation between MADRS scores and CRP levels was not significant.

One could argue that these findings could have been confounded by factors such as age and gender.[65] In our sample, the correlation between IL-6 and age was statistically significant ($r = -0.44$, $P = .01$) but not the one between CRP and age ($r = -0.303$, $P = .103$). When we corrected our repeated and univariate ANOVAs focusing on IL-6 for age, these results were still not significant. The small sample size and the low number of data points for some treatment groups (eg, at week 8, the NAC + placebo group had only 1 data point for CRP and IL-6 levels) do, however, restrict the interpretation of these findings.

It is important to address the limitations of this study. Despite great effort, randomization rates were lower than expected, and the small sample size limited the generalization of our findings. Further, although previous RCTs included 2 or 3 treatment groups, ours was a 4-arm study. This study design was ideal to compare the effects of placebo to those of NAC, aspirin, and NAC + aspirin. However, the comparison of 4 treatment groups of such small sample size presents a methodological challenge even when using robust Bayesian methods. Further, the small sample size limited the statistical power of our frequentist analyses (as shown by the findings of our repeated-measures ANOVAs) and would have allowed us to detect differences of large effect size only. Future dose-finding studies should also explore whether the dose of NAC and aspirin used in this study was optimal or whether lower or greater doses would have been more efficacious. Further, given the potential synergistic effect of aspirin and lithium, a future study should consider covarying or stratifying analyses for medication type. Although we did not observe severe side effects, the tolerance profile of NAC and aspirin in bipolar disorder should be explored further.

In conclusion, the coadministration of NAC and aspirin during a period of 16 weeks is associated with a reduction in depressive symptoms. These preliminary findings may serve as a starting point for future studies assessing the efficacy, tolerability, and safety of anti-inflammatory agents in the treatment of bipolar depression.

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