The Lancet Psychiatry
Manuscript Draft

Manuscript Number: THELANCETPSYCH-D-17-00232

Title: Treatment of Bipolar Depression with Minocycline and/or Aspirin: An adaptive, 2 X 2 double-blind, randomized, placebo-controlled clinical trial

Article Type: Article (Clinical Trials)

Keywords: Bipolar Disorder; Inflammation; Minocycline; Aspirin

Abstract: Background: Given evidence of chronic inflammation in bipolar disorder (BD), we tested the efficacy of aspirin and minocycline for the treatment of bipolar depression.
Methods: Ninety-nine depressed outpatients with BD were enrolled in a six-week (seven visit), multi-site, double-blind, placebo-controlled trial in which as adjunctive therapy to existing treatment, subjects were randomized (1:1:1:1) to one of four groups: active-minocycline (100 mg b.i.d.) + active-aspirin (81 mg b.i.d.) (M+A); active-minocycline + placebo-aspirin (M+P); placebo-minocycline + active-aspirin (A+P); and placebo-minocycline + placebo-aspirin (P+P). A blinded interim analysis led to the dropping of the M+P and A+P arms from further enrollment giving numbers per group who were included in the analysis of: 30 (M+A), 18 (M+P), 19 (A+P), and 28 (P+P). The main outcome variable was response to treatment. CRP and IL-6 were assayed to measure inflammation, and thromboxane B2 (11-D-TXB2) was assayed to measure compliance to aspirin.
Trial registration: NCT01429272.
Findings: In a two-group analysis, the M+A group showed a greater response rate than the P+P group (p(1-tailed)=0.037, OR=2.89, NNT=4.7). When all four arms were included in the analysis, there was a main effect of aspirin on treatment response that was driven by both the M+A and the A+P groups (p(2-tailed)=0.016, OR=3.75, NNT=4.0). Additionally, there was a significant 3-way interaction between aspirin, minocycline, and IL-6, indicating that response to minocycline was significantly greater in subjects in the M+P group with higher IL-6 concentrations. Further, subjects in the M+P group who responded to treatment had significantly greater decreases in IL-6 levels between baseline and visit 7 compared with non-responders.
Interpretation: The results provide evidence that aspirin and minocycline may be efficacious adjunctive treatments for bipolar depression. Given their potential import, additional studies to confirm and extend these findings are warranted.
Funding: Stanley Medical Research Institute and Laureate Institute for Brain Research.

Treatment of Bipolar Depression with Minocycline and/or Aspirin: An adaptive, 2 X 2 double-blind, randomized, placebo-controlled clinical trial

Jonathan B. Savitz[1,2], Ph.D., T. Kent Teague[3,4,5,6], Ph.D., Masaya Misaki[1], Ph.D., Matt Macaluso[7], D.O., Brent E. Wurfel[1], M.D., Ph.D., Matt Meyer[1], M.D., Douglas Drevets[8], M.D., William Yates[1], M.D., Ondria Gleason[4], M.D., Wayne C. Drevets[1,9] *, M.D., Sheldon H. Preskorn[1,7] *, M.D.

Abstract Count: 298/300
Word Count: 4,085/4,500
References: 30/30

* Joint senior authors.

1. Laureate Institute for Brain Research, Tulsa, OK, USA
2. Faculty of Community Medicine, University of Tulsa, Tulsa, OK, USA
3. Department of Surgery, University of Oklahoma College of Medicine, Tulsa, OK, USA
4. Department of Psychiatry, University of Oklahoma College of Medicine, Tulsa, OK, USA
5. Department of Pharmaceutical Sciences, University of Oklahoma College of Pharmacy, Tulsa, OK, USA
6. Department of Biochemistry and Microbiology, Oklahoma State University Center for Health Sciences, Tulsa, OK, USA
7. Department of Psychiatry and Clinical Trials Unit, University of Kansas School of Medicine, Wichita, Kansas, USA
8. Department of Medicine, Oklahoma University Health Sciences Center, and Oklahoma City VAMC, Oklahoma City, Oklahoma, USA
9. Janssen Research & Development, LLC of Johnson & Johnson, Inc., Titusville, NJ, USA

SUMMARY

Background: Given evidence of chronic inflammation in bipolar disorder (BD), we tested the efficacy of aspirin and minocycline for the treatment of bipolar depression.

Methods: Ninety-nine depressed outpatients with BD were enrolled in a six-week (seven visit), multi-site, double-blind, placebo-controlled trial in which as adjunctive therapy to existing treatment, subjects were randomized (1:1:1:1) to one of four groups: active-minocycline (100 mg b.i.d.) + active-aspirin (81 mg b.i.d.) (M+A); active-minocycline + placebo-aspirin (M+P); placebo-minocycline + active-aspirin (A+P); and placebo-minocycline + placebo-aspirin (P+P). A blinded interim analysis led to the dropping of the M+P and A+P arms from further enrollment giving numbers per group who were included in the analysis of: 30 (M+A), 18 (M+P), 19 (A+P), and 28 (P+P). The main outcome variable was response to treatment. CRP and IL-6 were assayed to measure inflammation, and thromboxane B2 (11-D-TXB_2) was assayed to measure compliance to aspirin. Trial registration: NCT01429272.

Findings: In a two-group analysis, the M+A group showed a greater response rate than the P+P group (p(1-tailed)=0.037, OR=2.89, NNT=4.7). When all four arms were included in the analysis, there was a main effect of aspirin on treatment response that was driven by both the M+A and the A+P groups (p(2-tailed)=0.016, OR=3.75, NNT=4.0). Additionally, there was a significant 3-way interaction between aspirin, minocycline, and IL-6, indicating that response to minocycline was significantly greater in subjects in the M+P group with higher IL-6 concentrations. Further, subjects in the M+P group who responded to treatment had significantly greater decreases in IL-6 levels between baseline and visit 7 compared with non-responders.

Interpretation: The results provide evidence that aspirin and minocycline may be efficacious adjunctive treatments for bipolar depression. Given their potential import, additional studies to confirm and extend these findings are warranted.

Funding: Stanley Medical Research Institute and Laureate Institute for Brain Research.

INTRODUCTION

The treatment of bipolar depression is a major clinical challenge and no conventional antidepressants have been approved by the FDA for the short-term treatment of bipolar depression[1]. Only three pharmacotherapies are FDA-approved for bipolar depression; the combination of olanzapine and fluoxetine (OF), quetiapine monotherapy (QTP), and lurasidone as a monotherapy or adjunctive therapy. These treatments produce numbers needed to treat (NNT) for response of 4 for OF, 6 for QTP, and 5-7 for lurasidone, respectively[2], and all three produce significant side-effects with numbers need to harm (NNH) of 6 for OF (e.g. weight gain), 5 for QTP (e.g. sedation), and 15-16 for lurasidone (e.g. akathisia and nausea), respectively[2]. Thus, new classes of medication are needed.

Given that an inflammatory-like state exists in a *subgroup* of patients with BD[3-5], there is increasing interest in the therapeutic potential of immune-modulating medications[6]. Two particularly promising candidates are minocycline and low-dose aspirin, as reviewed in our published protocol[7] (appendix) and summarized here: both medications are well-tolerated, even with long-term use, well absorbed and brain penetrant, and likely exert anti-inflammatory effects in the brain and the periphery.

Aspirin inhibits cyclo-oxygenase-1 (COX-1) and acetylates COX-2, blocking the conversion of arachidonic acid to prostaglandins and thromboxane A2. At low doses (e.g. “baby” aspirin, 81 mg) aspirin preferentially inhibits COX-1 while at higher doses it additionally reduces COX-2 function. COX-1 is predominantly expressed by microglia and macrophages while COX-2 is predominantly localized to neurons[8]. Importantly, preclinical evidence suggests that inhibition of COX-1 after intracerebroventricular administration of lipopolysaccharide (LPS) is neuroprotective whereas inhibition of COX-2 is detrimental, increasing leukocyte recruitment into the brain and exacerbating tissue damage[9]. Notably, a pharmacoepidemiological study demonstrated that individuals treated with lithium and low dose aspirin (≤ 80 mg/day) were less likely to have a medication event (medication switch or dose change) whereas high-dose aspirin, non-selective NSAIDs, and glucocorticoids were associated with an increase in medication events[10]. While there are no published controlled clinical trials of aspirin as an antidepressant, an open-label study reported aspirin increased the speed of response to SSRIs[11], and an epidemiological study reported that aspirin protected against depression in older men with elevated levels of homocysteine[12].

Minocycline, a tetracycline antibiotic, modulates immune function via multiple mechanisms - for instance, inhibiting the activation, migration, and/or proliferation of T-cells, neutrophils, and microglia, inhibiting the release of pro-inflammatory cytokines, and increasing the release of anti-inflammatory and anti-apoptotic molecules[13]. In preclinical models, minocycline exerts neuroprotective effects, including reversing inflammation-induced inhibition of neural stem cell proliferation[14] and reducing lesion size and/or demyelination in neurological disorders such as Huntington’s disease[15]. Antidepressant-like effects have been reported in

rodents[16] and anecdotally in humans[17, 18]. No randomized controlled trial has been published in patients with primary mood disorders.

Here, we perform the first randomized, placebo-controlled trial of aspirin and minocycline to assess their efficacy as adjunctive treatments for bipolar depression. Given the well-established safety profiles of these drugs, a 2x2 design was chosen for study as it permitted an opportunity to test two mechanistically different anti-inflammatory agents. Second, it allowed an opportunity to test for whether these mechanisms augmented or interfered with each other. If only one of the agents was ineffective and both were well tolerated, then the two cells with the active agent could be combined and compared to the double placebo group plus the group treated with the ineffective drug plus placebo, essentially doubling the statistical power of the study. On the other hand, if the two drugs augment each other or interfere with each other, there would be no advantage of increased power but valuable clinical information could be obtained.

METHODS

Study Design

This was a multi-site, double-blind, placebo-controlled clinical trial in which as adjunctive therapy to existing treatment, subjects were randomized (1:1:1:1) to one of 4 groups: (a) active minocycline (100 mg b.i.d., p.o.) + active aspirin (81 mg b.i.d., p.o.) (M+A); (b) active minocycline (100 mg b.i.d., p.o.) + placebo aspirin (M+P); (c) placebo minocycline + active aspirin (81 mg b.i.d., p.o.) (A+P), and (d) placebo minocycline + placebo aspirin (P+P). The duration of the trial was 6 weeks and comprised 7 visits (baseline and weekly follow-up, figure S1). The study was conducted at 3 sites: the Laureate Institute for Brain Research (LIBR) in Tulsa, the University of Oklahoma College of Medicine (OU), Tulsa, and the University of Kansas School of Medicine (KUSM-W) in Wichita. Ethics approval was obtained through the LIBR site. The study protocol was published prior to the trial [7] (see appendix).

Participants

Based on a pre-study power analysis using data from conventional antidepressant therapy in patients with unipolar depression, the initial goal was to recruit 120 participants (30 per group). However, we underestimated the difficulty of recruiting subjects (see[19]) and had a fixed budget. Several efforts were made to address the recruitment problems but we determined as the study progressed that we could not recruit 120 participants but instead only 100 participants in order to stay within budget. For this reason, a blinded interim analysis was performed at the approximate recruitment midpoint (n=64) by an investigator not involved in the study conduct. The first purpose was to test for futility (i.e., no evidence suggesting any of the cells were separating each other). Given that two cells were appearing to separate, the second purpose was to assess the potential effect

size and perform power calculations to determine whether enrolling the future projected subjects equally into those two cells might achieve sufficient power to test the apparent difference in these two cells. The power calculations predicted that 30 subjects in these two cells would have sufficient power to test for differences in response rates. WCD, who did not assess subjects and was not regularly at any of the sites during this portion of the study, was unblinded and observed that the M+A group was separating from the P+P group. This information was not conveyed to the clinical teams conducting the study. Thus, the design was adapted by eliminating the A+P and M+P groups and randomizing all future participants (projected to be 36 based on the historical rate of participant accrual in the study) to the M+A or P+P groups, resulting in the numbers per group included in the *final statistical analyses* of: 30 (M+A), 18 (M+P), 19 (A+P), and 28 (P+P). See CONSORT diagram (figure 1).

Adaptive trial designs allow for the modification of aspects of a study in a real-time, data-driven manner, and thus can greatly enhance the efficacy of early drug development[20]. The adaptive design is usually employed in early studies to test either many doses of a drug or different combinations or schedules of administration (e.g., oncology), and then is followed by at least one confirmatory conventional study. Nevertheless, the results of an adaptive trial may be accepted by the United States FDA as one of the two pivotal trials needed for drug approval.

Participants were recruited from psychiatric clinics associated with the study sites as well as the general community through radio and print advertisement. Inclusion criteria: (a) meeting DSM-IV-TR criteria for BD I (n=37), BD II (n=57), or BD NOS (n=5) based on the MINI-Plus and an unstructured interview with a psychiatrist; (b) current major depressive episode of ≥4 weeks duration, (c) at least moderately depressed, i.e. a Quick Inventory of Depressive Symptomatology (QID-C16) score >10; (d) stable regimen of medication for ≥4 weeks prior to enrollment (if receiving therapy), (e) 18-65 years of age. Exclusion criteria are detailed in the supplement and a summary of the psychiatric medications taken by the participants during the trial appears in table S1.

The study was approved by the Western IRB and subjects provided written informed consent. The trial was publically registered (www.clinicaltrials.gov), NCT01429272 and minor amendments to the published pre-study protocol are detailed in the supplement.

Study participants were withdrawn if their treating health-care providers made changes in medication to specifically target a depressive symptom. Participants were given an information sheet to take home detailing the procedure to be followed in the case of a missed dose, and requesting that this information be recorded for the investigators. Returned trial medication was audited. Participants were permitted to miss no more than 50% of their study medications in a single week on one occasion. Non-adherent participants received additional counseling about the need for adherence beyond what was routinely given each week. A second

episode of non-adherence resulted in withdrawal from the study. We also obtained a post-study measure of medication adherence for the subjects receiving aspirin, i.e. urinary concentrations of thromboxane B2 (11-D-TXB_2), a downstream metabolite of prostaglandin H2, that is robustly decreased by treatment with aspirin[21].

Randomization and Masking

Participants were randomly assigned to one of the four arms in a double-blind fashion via a central database hosted at LIBR. Randomization was conducted according to CONSORT guidelines using block permutations. Randomization codes were generated by a computer-based random number generator and were held by LIBR staff not involved in the trial until study completion. The investigators and participants were blind to the treatment allocation. Blindness was maintained by ensuring that the packaging, appearance and color of the minocycline, aspirin, and placebo capsules were identical. Commercial minocycline and aspirin tablets were purchased by the manufacturer (Wedgewood Pharmacy, Swedesboro, NJ) and over-encapsulated. Matching placebo capsules were produced.

Biological Samples

Serum samples were obtained at baseline (n=90) and visit 7 (n=80) to measure inflammatory biomarkers. CRP and interleukin 6 (IL-6), were measured using a Meso Scale Discovery (MSD) QuickPlex SQ 120 instrument and MSD V-PLEX assays (CRP: LLOQ=0.05 mg/L; CV=1.9%; IL-6: LLOQ=0.04 pg/mL; CV=3.6%). A spot morning urine was taken and a 11-Dehydro Thromboxane B2 EIA kit (Cayman Chemical Company; Ann Arbor, MI) used to quantify 11-D-TXB_2 (LLOQ=46 pg/mL; CV=4.3%), an indirect marker of COX activity that has been used to measure adherence to aspirin therapy in cardiovascular and cerebrovascular diseases[21]. In addition, for purposes of comparison, we obtained two 11-D-TXB_2 measures from a healthy control sample (n=27), six weeks apart.

Outcome Measures

Based on the interim power calculations, the primary outcome measure was response to treatment defined as a >50% reduction[22] in Montgomery-Asberg Depression Rating Scale (MADRS) for the final two consecutive visits of each participant[23]. Secondary outcome measures were (a) remission, defined as a post-treatment MADRS score of <11[22] for the final two consecutive visits[23] and (b) the change from baseline in the MADRS score.

Adverse events were recorded at each visit and were posed as open-ended questions about any issues with the trial or the study medication in accordance with standard FDA guidance for the execution of such clinical

trials (table S2). Safety was monitored by the study PI, a biweekly consensus meeting of the PI and study staff at each site, and a Data, Safety and Monitoring Board (DSMB) which met biannually.

Hypotheses

Primary hypotheses

Hypothesis 1a: Participants in the M+A group will show a greater response rate than subjects in the P+P group.
Hypothesis 1b: Across all four groups, subjects receiving aspirin and/or minocycline will show a greater response rate than subjects in the P+P group.
Hypothesis 2: The efficacy of aspirin and/or minocycline treatment will be predicted by baseline inflammation such that patients receiving active treatment with higher levels of IL-6 and CRP will show a greater response rate to treatment. Additionally, participants who show a greater decrease in IL-6 and CRP concentrations between visits 1 and 7 will show a greater reduction in MADRS scores.

Secondary hypotheses

(a). Compared to the P+P group, patients receiving aspirin and/or minocycline will show a greater remission rate, and a greater decrease in MADRS scores over time. (b). The remission rate and change in MADRS scores will be predicted by levels of IL-6 and CRP.

Statistical analysis

Our initial published power analysis[7] was based on a meta-analysis of the mean effect size of conventional antidepressant treatment versus placebo in MDD (2.50 – 1.69 = 0.81)[24] which required us to recruit 26 participants per cell to obtain ~80% power. Subsequently we re-performed the power analysis after the blinded interim analysis. This analysis determined that for the response rate, 30 subjects per cell in the two separating groups (M+A versus P+P) would yield ~80% power to detect a difference in this outcome variable (in contrast, the *sample size* needed to provide comparable power to detect a difference in the change in MADRS scores over time was estimated to be considerably larger).

Analyses were performed with R. Analysis of Variance (ANOVA) or the chi-squared test was performed to test for baseline differences in demographic and clinical variables across the four treatment arms. Concurrent medications were coded into four classes, i.e. antidepressants, anticonvulsants, antipsychotics, and anxiolytics (table S1). There was no significant difference between groups in numbers of subjects per medication class, age, sex, and body mass index (BMI). Results also did not differ significantly across sites. Variables were

selected as regressors according to whether their inclusion improved the model measured by Akaike information criterion score. Based on this criterion, age, sex, and body mass index (BMI), but not medication class or study site were included in the analysis models as covariate regressors.

Between group differences in response rate (primary outcome) and remission rate (secondary outcome) were tested with logistic regression with the analysis of deviance test. To evaluate the effects of baseline inflammation on response rate, IL-6 and CRP were entered as additional binary (i.e. low versus high based on a median split) variables. Linear mixed-effect (LME) model analyses were performed to measure of the temporal change in depression ratings (MADRS scores) across visits. Age, sex, and BMI were included as fixed effects. Random effects included subject and autoregressive covariance structure across visits within subject.

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Ninety-nine participants were randomized out of a total of 201 individuals who underwent in-person screening (figure 1). The recruitment period was from March 2012 to August 2015. Initially we believed we had enrolled 100 participants but one person was erroneously counted twice. Seventy-five participants completed all visits. Four participants did not complete any post-baseline visits and were excluded from the analyses. The difference in urine 11-D-TXB_2 concentrations between baseline and visit 7 indicated excellent treatment compliance in the P+A group but at least three participants appeared non-compliant in the M+A group (figure S2). However, rerunning the analyses without these subjects did not substantially alter the results. Baseline characteristics of the sample and the number of participants who completed each visit are shown in tables 1 and 2, respectively. The adverse events per group appear in table S2.

The dropout rate did not differ significantly across groups (χ^2_3=0.83, p=0.842). There was no significant difference across the four arms in the number of subjects who were being treated with antipsychotics (χ^2_3=0.41, p=0.932), mood stabilizers (χ^2_3=0.45, p=0.928), antidepressants (χ^2_3=0.87, p=0.832), or anxiolytics (χ^2_3=2.01, p=0.530) at study entry. Further, there was no difference between groups in the number of individuals with a diagnosis of BD 1, BD II, and BD NOS (χ^2_6=5.54, p=0.476).

Hypothesis 1a: Participants receiving minocycline plus aspirin showed a better response rate compared with participants receiving double placebo (X_1^2=3.21, p(1t)=0.037, odds ratio (OR) = 2.89, NNT=4.7). Given that response rate was selected as the primary outcome variable, we also tested whether by dropping the M+P and A+P groups, we introduced a bias that would further increase the response rate in the M+A versus P+P group in the second half of the clinical trial. When pre-versus post interim analysis status was used as a co-variate in the logistic regression, the M+A group still showed a significantly better response rate than the P+P over the entire trial (X_1^2=3.21, p(1t)=0.037). There was no main effect of pre-versus post interim analysis status (X_1^2=0.002, p=0.961) or interaction between pre-versus post interim analysis status and study arm (X_1^2=1.35, p=0.246).

Hypothesis 1b: There was a significant main effect of aspirin on the clinical response rate (X_1^2=5.76, p(2t)=0.016 with analysis of deviance test, (OR = 3.75, 95% confidence interval (CI) = 1.04-14.68) but no significant effect of minocycline (X_1^2=0.03, p=0.857) or interaction between aspirin and minocycline (X_1^2=0.17, p=0.679) (figure 2). The NNT to obtain a response to aspirin (M+A and A+P versus M+P and P+P) was 4.2. The NNT for the M+A and A+P versus the P+P comparison was 4.0.

Hypothesis 2: There was a significant 3-way interaction between aspirin, minocycline, and IL-6 (X^2_1=4.07, p(2t)=0.044) on response rates. Follow-up analysis showed that subjects in the M+P group with higher baseline IL-6 levels responded better to minocycline than subjects in the M+P group with lower IL-6 levels (X^2_1=7.719, p(2t)=0.005, figure 3). Further, there was a significant interaction between the change in IL-6 and treatment response (X^2_3=11.7, p(2t)=0.001), indicating that subjects in the M+P group who responded to treatment had a significantly greater decrease in IL-6 levels between baseline and visit 7 compared with non-responders in the M+P group. There were no significant interactions between aspirin and CRP or minocycline and CRP, or the 3-way interaction between aspirin, minocycline, and CRP (all p's>0.1).

Secondary Outcomes: (a). There was a significant main effect for aspirin on the remission rate (X_1^2=4.14, p(2t)=0.042, OR=2.52, CI=0.56-12.29, figure 2) but not for minocycline (X_1^2=0.45, p=0.503) or the interaction between aspirin and minocycline (X_1^2=0.35, p=0.554). The NNT for aspirin to obtain remission (M+A and A+P versus M+P and P+P) was 6.5. The NNT for the M+A and A+P versus P+P comparison was 8.0. (b). There were no significant main effects of aspirin or minocycline on the change in MADRS score from baseline, nor was there a significant interaction between aspirin, minocycline, and visit.

DISCUSSION

This is the first randomized controlled trial of adjunctive minocycline and/or aspirin for the treatment of bipolar depression. There were two principal findings.

Firstly, consistent with our findings from the interim analysis, there was a significantly greater sustained clinical response rate in the M+A group (44%) versus the P+P group (21%, NNT=4.7) with participants receiving minocycline plus aspirin approximately twice as likely to respond than those individuals receiving double placebo. For this specific analysis we performed a one-tailed test because of our *a priori* hypothesis (based on the interim analysis) that the M+A group would show a greater response rate than the P+P group. However, irrespective of whether a 1 or 2-tailed test is performed, the large effect size (OR=2.89) raises the possibility that the combination of minocycline and aspirin may be an efficacious adjunctive treatment for bipolar depression. Further studies are needed to test whether the combination of minocycline and aspirin exerts a synergistic effect that is superior to either drug alone.

When all four treatment arms were included in the analysis, there was a significant main effect of aspirin (M+A and M+P combined) on sustained response rates with NNTs of between 4 and 4.2, i.e. equal to or superior to currently approved treatments for bipolar depression. Consistent with these results, the secondary analysis showed that there was a significant main effect of aspirin on sustained *remission* with NNTs between 6.5 and 8.

Our interim analysis suggested we were underpowered to detect a group difference with the LMM analysis because the variance across subjects in the change in MADRS scores was large. This was indeed the case (see figure S3). The other factor contributing to the lack of statistical separation amongst the four groups in the mean reduction in MADRS scores was the presence of four individuals in the P+P group that had a >25-point mean reduction in MADRS scores at visit 7. Thus the *mean* reductions in MADRS scores at visit 7 were approximately -14, -11, -11, and -13 in the A+P, M+P, M+A, and P+P groups, respectively. In contrast, the *median* reductions at visit 7 were -12, -13, -15 and -11 for these groups, respectively (figure S4). The reason for the greater response in the four P+P treated individuals is unknown but could include spontaneous improvement which occurs commonly in a cyclical condition such as BD. The fact that there was a statistically significant doubling of the number of patients who had a sustained response and/or remission in the M+A and A+P groups versus the M+P and/or P+P groups is consistent with the fact that spontaneous response and remission at one time point occurred in four and two subjects in the P+P group, respectively, and demonstrates a clinically meaningful as well as a statistically significant difference between the aspirin and non-aspirin treated groups.

Secondly, participants with higher baseline levels of IL-6 responded better to minocycline administration than patients with lower levels of inflammation. These data resemble findings from a recent clinical trial of the TNF inhibitor, infliximab, for treatment resistant depression in which no overall difference in the change in depression ratings was detected between treatment groups across time[25]. However, infliximab-treated patients with a baseline CRP concentration greater than 5 mg/L had a greater decrease depression ratings compared to the placebo group[25]. In addition, the minocycline-treated participants who showed a greater decrease in IL-6

levels between baseline and visit 7, also showed a larger reduction in depressive symptoms over the trial. This result is consistent with recent studies reporting that depressed patients who respond/remit to treatment with an SSRI or electroconvulsive therapy show a greater decrease in IL-6 levels over time compared with non-responders or remitters[26, 27].

The results from both the infliximab and our current study suggest that caution should be applied in treating depressed patients with anti-inflammatory agents when they do not manifest inflammation. Our results also are consistent with a paper reporting that: (a) anti-inflammatory agents may counteract the antidepressant-like effects of SSRIs in mice and (b) a higher percentage of patients who had versus had not taken an NSAID were treatment resistant to citalopram during the STAR*D trial[28].

The absence of a significant interaction between the efficacy of aspirin treatment and baseline levels of CRP and/or IL-6 may reflect Type II error given the relatively small samples, but also may reflect the clinically non-significant anti-inflammatory effect of low-dose aspirin in autoimmune or other inflammatory disorders. Our results therefore suggest that the therapeutic effect of aspirin may be attributable to a still unknown mechanism, conceivably the effect of COX-1 inhibition on the arachidonic acid cascade[29] or neurotrophic processes[30]. In this regard, this first report of higher baseline 11-D-TXB_2 levels in the BD sample relative to the control sample (see figure S2) is noteworthy as it suggests that the activity of the arachidonic acid pathway is elevated in BD, consistent with previous hypotheses[29].

A limitation of the study is the modest sample size which may have reduced our ability to detect group differences in the change in MADRS score over time. We recommend that future studies test the impact of baseline levels of inflammation on response to minocycline using a more rigorous, formal stratified design. Secondly, in order to ensure the study was not futile and to maximize the efficient use of future recruited participants we performed an interim analysis which could theoretically have biased the results of the study. However, this possibility is mitigated by the fact that a *post-hoc* analysis revealed no statistical difference in response rates, pre- versus post the interim analysis. Thirdly, although we attempted to assess the effects of adjunctive medications by broadly grouping medications by class, we did not control for differences in dose, pharmacokinetics, and drug-drug interactions. Future studies using adjunctive designs should attempt to balance the number and type of co-occurring medications across treatment arms.

Strengths of the study include: (a) the placebo-controlled assessment of two novel therapeutic agents, with the 2x2 design allowing us to evaluate potential additive and antagonistic drug interactions, (b) the use of peripheral blood biomarkers to assess the effect of inflammation on treatment outcome, (c) the utility of the 11-D-TXB_2 measures for assessing treatment compliance in the aspirin groups, and (d) the representative nature of our sample for individuals with bipolar depression, many of whom were inadequately responsive to existing treatments, which remains a serious unmet medical need.

In sum, the study provides preliminary support to the possibility that aspirin and minocycline can be efficacious adjunctive therapies for the treatment of bipolar depression. These findings should encourage further studies in larger samples perhaps using markers of inflammation as inclusion criteria to increase statistical power. Independent confirmation of the therapeutic efficacy of low-dose aspirin has significant potential to advance the treatment of depression given its global availability and affordability, and its relatively benign side-effect profile.

FIGURE LEGENDS

Figure 1: CONSORT flow diagram showing the number of individuals assessed for eligibility in person, the number of participants randomized to each group, the number of individuals lost to follow-up at visit 7 (week 6) and the number of individuals included in the statistical analyses.

Figure 2: (A) Percentage of responders (y-axis) in each of the 4 treatment groups shown individually (reader's left) and the two aspirin groups (M+A and A+P) versus the two non-aspirin groups (M+P and P+P) on the reader's right. (B) Percentage of remitters (y-axis) in each of the 4 treatment groups shown individually (reader's left) and the two aspirin groups (M+A and A+P) versus the two non-aspirin groups (M+P and P+P) on the reader's right.

Figure 3: Bar charts showing the 4 treatment groups divided into responders (green) and non-responders (blue). The natural logarithm of IL-6 is shown on the y-axis. Participants in the M+P group with higher baseline levels of IL-6 were more likely to be classified as treatment responders.

Figure S1: Each session number (total of 7) is encircled, with the timing between sessions indicated in weeks with a 2 business day window on either side of visit target date to complete the visit. Session 1 is the baseline (green star) and session 7 is the study end (purple star). The study duration is 6 weeks.

Figure S2: Plot showing the change (visit 1 versus visit 7) in urine 11-D-TXB_2 concentrations for each individual across the four treatment groups and healthy controls. Points above the x-axis indicate increases in 11-D-TXB_2 concentration over time, while points below the x-axis indicate decreases in 11-D-TXB_2 concentrations over time. The mean change in 11-D-TXB_2 for each group was as follows: -1068±3696 (M+A), 606±2277 (M+P), -3281±3147 (A+P), -473±3918 (P+P), and 468±1516 (HC). There was a significant decrease between visit 1 and visit 7 in 11-D-TXB_2 concentrations in the A+P group (t_{15}=4.2, p=0.001) but not the M+A group (t_{24}=1.4, p=0.162). Interestingly, baseline concentrations of (log-normalized) 11-D-TXB_2 were significantly higher in the entire BD group compared with the healthy control group (t_{43}=2.43, p(2t)=0.019).

Figure S3: Spaghetti plots showing the time course of the change in MADRS score for individual subject (each line is one subject). Responders are shown in green and non-responders in blue.

Figure S4: Illustration of the median change in MADRS scores from baseline (y-axis) at each visit (x-axis) in the M+A (green) versus P+P (blue). The error bars indicate the 68% confidence interval (corresponding to one standard error) estimated with bootstrapping.

Contributors

JS was the lead investigator and wrote the first draft of the paper with input from SHP and WCD; TKT oversaw all of the biomarker analyses; MM (Misaki) performed the statistical analyses; MM (Macaluso), BEW, MM (Meyer), WY, OG, and SHP performed clinical assessments; MM (Macaluso) and SHP developed the TXB_2 component of the study; DD initially had the idea of testing the efficacy of minocycline in depression; WCD and SHP were the senior investigators and PIs on the grant that partly funded the study. All authors were involved in revising the manuscript for publication.

Declaration of Interest

WCD, WY, JS, and SHP are co-inventors on a user patent that has been filed for treating bipolar depression with minocycline and aspirin (“Composition and Method for Treating Bipolar Disorder”, WO2016/090316). WCD is an employee of Janssen Pharmaceutical of Johnson & Johnson, Inc.

Acknowledgments

Funding for the study was received from the Stanley Medical Research Institute (SMRI grant # 10T-1401) and the Laureate Institute for Brain Research (LIBR). Funding for the 11-TXB_2 assays was received from a Wichita Center for Graduate Medical Education and Kansas Biosciences Authority Grant. The authors acknowledge Chibing Tan, Ashlee Rempel, and Brenda Davis for their assistance with the serum and urine processing and biomarker assays.

REFERENCES

1. Correa R, Akiskal H, Gilmer W, Nierenberg AA, Trivedi M, Zisook S. Is unrecognized bipolar disorder a frequent contributor to apparent treatment resistant depression? *J Affect Disord* 2010; **127**(1-3)**:** 10-18.

2. Ketter TA, Miller S, Dell'Osso B, Calabrese JR, Frye MA, Citrome L. Balancing benefits and harms of treatments for acute bipolar depression. *J Affect Disord* 2014; **169 Suppl 1:** S24-33.

3. Padmos RC, Hillegers MH, Knijff EM, Vonk R, Bouvy A, Staal FJ*, et al*. A discriminating messenger RNA signature for bipolar disorder formed by an aberrant expression of inflammatory genes in monocytes. *Arch Gen Psychiatry* 2008; **65**(4)**:** 395-407.

4. Dargel AA, Godin O, Kapczinski F, Kupfer DJ, Leboyer M. C-reactive protein alterations in bipolar disorder: a meta-analysis. *J Clin Psychiatry* 2015; **76**(2)**:** 142-150.

5. Mechawar N, Savitz J. Neuropathology of mood disorders: do we see the stigmata of inflammation? *Transl Psychiatry* 2016; **6**(11)**:** e946.

6. Kohler O, Benros ME, Nordentoft M, Farkouh ME, Iyengar RL, Mors O*, et al*. Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects: a systematic review and meta-analysis of randomized clinical trials. *JAMA Psychiatry* 2014; **71**(12)**:** 1381-1391.

7. Savitz J, Preskorn S, Teague TK, Drevets D, Yates W, Drevets W. Minocycline and aspirin in the treatment of bipolar depression: a protocol for a proof-of-concept, randomised, double-blind, placebo-controlled, 2x2 clinical trial. *BMJ Open* 2012; **2**(1)**:** e000643.

8. Choi SH, Aid S, Bosetti F. The distinct roles of cyclooxygenase-1 and -2 in neuroinflammation: implications for translational research. *Trends Pharmacol Sci* 2009; **30**(4)**:** 174-181.

9. Choi SH, Aid S, Choi U, Bosetti F. Cyclooxygenases-1 and -2 differentially modulate leukocyte recruitment into the inflamed brain. *Pharmacogenomics J* 2010; **10**(5)**:** 448-457.

10. Stolk P, Souverein PC, Wilting I, Leufkens HG, Klein DF, Rapoport SI*, et al*. Is aspirin useful in patients on lithium? A pharmacoepidemiological study related to bipolar disorder. *Prostaglandins Leukot Essent Fatty Acids* 2010; **82**(1)**:** 9-14.

11. Mendlewicz J, Kriwin P, Oswald P, Souery D, Alboni S, Brunello N. Shortened onset of action of antidepressants in major depression using acetylsalicylic acid augmentation: a pilot open-label study. *Int Clin Psychopharmacol* 2006; **21**(4)**:** 227-231.

12. Almeida OP, Flicker L, Yeap BB, Alfonso H, McCaul K, Hankey GJ. Aspirin decreases the risk of depression in older men with high plasma homocysteine. *Transl Psychiatry* 2012; **2:** e151.

13. Moller T, Bard F, Bhattacharya A, Biber K, Campbell B, Dale E*, et al*. Critical data-based re-evaluation of minocycline as a putative specific microglia inhibitor. *Glia* 2016.

14. Zheng LS, Kaneko N, Sawamoto K. Minocycline treatment ameliorates interferon-alpha- induced neurogenic defects and depression-like behaviors in mice. *Frontiers in cellular neuroscience* 2015; **9:** 5.

15. Wang X, Zhu S, Drozda M, Zhang W, Stavrovskaya IG, Cattaneo E*, et al*. Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. *Proc Natl Acad Sci U S A* 2003; **100**(18)**:** 10483-10487.

16. Molina-Hernandez M, Tellez-Alcantara NP, Perez-Garcia J, Olivera-Lopez JI, Jaramillo-Jaimes MT. Desipramine or glutamate antagonists synergized the antidepressant-like actions of intra-nucleus accumbens infusions of minocycline in male Wistar rats. *Prog Neuropsychopharmacol Biol Psychiatry* 2008; **32**(7)**:** 1660-1666.

17. Levine J, Cholestoy A, Zimmerman J. Possible antidepressant effect of minocycline. *Am J Psychiatry* 1996; **153**(4)**:** 582.

18. Miyaoka T, Wake R, Furuya M, Liaury K, Ieda M, Kawakami K*, et al*. Minocycline as adjunctive therapy for patients with unipolar psychotic depression: an open-label study. *Prog Neuropsychopharmacol Biol Psychiatry* 2012; **37**(2)**:** 222-226.

19. Macaluso M, Preskorn S, Savitz J, Wurfel B, Meyer M, Gleason O*, et al*. A Management Strategy for Clinical Trials with a Focus on Study Recruitment: Lessons Learned from an Efficacy Trial in Bipolar Depression 2017; **In Review**.

20. Shen J, Preskorn S, Dragalin V, Slomkowski M, Padmanabhan SK, Fardipour P*, et al*. How Adaptive Trial Designs can Increase Efficiency in Psychiatric Drug Development: A Case Study. *Innov Clin Neurosci* 2011; **8**(7)**:** 26-34.

21. Bruno A, McConnell JP, Mansbach HH, 3rd, Cohen SN, Tietjen GE, Bang NU. Aspirin and urinary 11-dehydrothromboxane B(2) in African American stroke patients. *Stroke* 2002; **33**(1)**:** 57-60.

22. Nierenberg AA, DeCecco LM. Definitions of antidepressant treatment response, remission, nonresponse, partial response, and other relevant outcomes: a focus on treatment-resistant depression. *J Clin Psychiatry* 2001; **62 Suppl 16:** 5-9.

23. Borges S, Chen YF, Laughren TP, Temple R, Patel HD, David PA*, et al*. Review of maintenance trials for major depressive disorder: a 25-year perspective from the US Food and Drug Administration. *J Clin Psychiatry* 2014; **75**(3)**:** 205-214.

24. Rief W, Nestoriuc Y, Weiss S, Welzel E, Barsky AJ, Hofmann SG. Meta-analysis of the placebo response in antidepressant trials. *J Affect Disord* 2009; **118**(1-3)**:** 1-8.

25. Raison CL, Rutherford RE, Woolwine BJ, Shuo C, Schettler P, Drake DF*, et al*. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. *JAMA Psychiatry* 2013; **70**(1)**:** 31-41.

26. Jarventausta K, Sorri A, Kampman O, Bjorkqvist M, Tuohimaa K, Hamalainen M*, et al*. Changes in interleukin-6 levels during electroconvulsive therapy may reflect the therapeutic response in major depression. *Acta Psychiatr Scand* 2017; **135**(1)**:** 87-92.

27. Lindqvist D, Dhabhar FS, James SJ, Hough CM, Jain FA, Bersani FS*, et al*. Oxidative stress, inflammation and treatment response in major depression. *Psychoneuroendocrinology* 2016; **76:** 197-205.

28. Warner-Schmidt JL, Vanover KE, Chen EY, Marshall JJ, Greengard P. Antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) are attenuated by antiinflammatory drugs in mice and humans. *Proc Natl Acad Sci U S A* 2011; **108**(22)**:** 9262-9267.

29. Rapoport SI. Lithium and the other mood stabilizers effective in bipolar disorder target the rat brain arachidonic acid cascade. *ACS Chem Neurosci* 2014; **5**(6)**:** 459-467.

30. Modi KK, Sendtner M, Pahan K. Up-regulation of ciliary neurotrophic factor in astrocytes by aspirin: implications for remyelination in multiple sclerosis. *J Biol Chem* 2013; **288**(25)**:** 18533-18545.

Research in Context

Evidence before this study: Minocycline is a tetracycline antibiotic that has been demonstrated to exert: a) anti-inflammatory and neuroprotective effects in animal models of several inflammatory and neurological disorders, b) antidepressant effects in rodents given lipopolysaccharide or subjected to the forced-swim test, c) antidepressant effects in case-studies of patients with major depressive disorder (MDD) and d) antidepressant effects in an open-label study of patients with unipolar psychosis. Low dose aspirin has been shown to be: a) neuroprotective in preclinical work, b) to have mood-stabilizing effects in people treated with lithium, c) to protect against depression in elderly individuals with high homocysteine levels, and d) to have antidepressant effects in open-label studies of individuals with MDD and men undergoing coronary angiography.

Added value of this study: This is the first randomized, placebo-controlled trial of either minocycline or aspirin for the adjunctive treatment bipolar depression. Further, this is the first study to evaluate the potential therapeutic effects of combination minocycline and aspirin.

Implications of all the available evidence: Here we provide preliminary evidence that low-dose aspirin may be an efficacious augmentation therapy for bipolar depression. Our results also raise the possibility that minocycline may exert modest anti-depressant effects in some patients with elevated concentrations of inflammatory mediators. Additional, well-powered clinical trials that stratify participants according to their baseline inflammatory status are needed to fully evaluate the potential therapeutic efficacy of minocycline and aspirin.

Table 1. Demographic and clinical characteristics of the M+A, M+P, A+P, and P+P groups at baseline.

	Mino+ASA	Mino+Pl.	ASA+Pl.	Pl.+Pl.	Statistic
N (BD)	31	19	19	30	-
N (I/II/NOS)	11/20/0	7/10/2	5/13/1	14/14/2	$\chi^2(6)=5.54$, p=0.476
Age	40.8±9.7	44.8±8.7	40.6±10.2	40.8±10.4	F(3,95)=0.88, p=0.455
Sex (% F)	84	68	68	73	$X^2(3)=2.20$, p=0.531
BMI	31.8±8.6	31.1±5.0	32.1±7.2	30.9±10.1	F(3,95)=0.11, p=0.952
MADRS	28.0±5.7	27.2±5.2	25.9±6.5	29.2±6.2	F(3,95)=1.23, p=0.302
HAM-A	19.9±7.7	19.6±7.3	19.0±7.2	19.9±7.3	F(3,94)=0.08, p=0.972
YMRS	4.7±2.2	3.3±2.4	5.6±3.4	3.9±2.5	F(3,95)=3.13, p=0.029*
CGI Severity	4.1±0.5	4.3±0.5	4.2±0.5	4.3±0.5	F(3,94)=0.93, p=0.428
CRP (mg/L)	8.2±12.9	4.3±5.5	4.5±4.9	4.4±5.3	F(3,86)=1.30, p=0.281
IL-6 (pg/mL)	1.0±0.7	0.9±0.5	1.1±0.7	0.9±0.5	F(3,86)=0.44, p=0.724

Abbreviations: BMI = body mass index; MADRS = Montgomery-Asberg Depression Rating Scale; HAM-A = Hamilton Anxiety Scale; YMRS = Young Mania Rating Scale; CGI = Clinical Global Impressions scale; CRP = c-reactive protein; IL-6 = interleukin 6.

Table 2. Primary and Secondary Outcome Measures at Visits 2-4 (Mean±SD). See table 1 for baseline scores.

Outcome/Visit	V2				V3				V4			
	M+A	M+P	A+P	P+P	M+A	M+P	A+P	P+P	M+A	M+P	A+P	P+P
N	30	18	19	27	27	17	19	27	27	16	18	27
MADRS	21.5±7.1	21.4±8.3	21.6±7.2	25.0±7.4	20.1±6.8	19.8±9.5	17.6±7.3	23.0±9.7	17.8±8.1	16.9±8.5	15.9±6.0	20.3±9.2
HAM-A	15.9±6.3	16.7±7.6	17.1±6.7	18.1±5.8	15.2±7.3	16.2±7.3	16.5±6.9	15.7±7.1	13.5±7.4	14.8±7.1	13.3±5.2	14.0±6.9
CGI-I	3.4±1.0	3.0±1.2	3.7±1.1	3.6±0.9	2.9±1.0	3.2±1.1	2.9±1.0	3.3±1.1	2.6±1.1	2.7±0.9	2.8±1.0	3.1±1.1
CRP (mg/L)	-	-	-	-	-	-	-	-	-	-	-	-
IL-6 (pg/mL)	-	-	-	-	-	-	-	-	-	-	-	-
11-D-TXB_2 (pg/mL)	-	-	-	-	-	-	-	-	-	-	-	-

Table 2 (continued). Primary and Secondary Outcome Measures at Visits 5-7 (Mean±SD)

Outcome/Visit	V5				V6				V7			
	M+A	M+P	A+P	P+P	M+A	M+P	A+P	P+P	M+A	M+P	A+P	P+P
N	27	16	16	26	24	16	14	25	22	15	14	24
MADRS	15.9±9.1	17.7±9.0	15.2±9.3	18.4±8.5	15.5±9.1	16.7±7.6	13.7±9.3	17.6±9.3	14.5±8.9	15.5±8.0	12.3±8.4	16.0±9.6
HAM-A	12.5±7.5	13.1±7.2	12.9±8.6	13.4±6.3	12.5±8.3	12.0±6.6	11.4±7.7	12.4±7.8	11.1±6.6	12.3±7.1	10.1±6.3	12.3±7.4
CGI-I	2.6±1.2	2.8±1.0	2.6±1.0	2.8±1.0	2.5±1.1	2.7±1.0	2.8±1.3	2.7±1.0	2.3±1.2	2.5±1.0	2.4±1.0	2.5±1.1
CRP (mg/L)	-	-	-	-	-	-	-	-	10.3±20.4	2.2±2.2	3.9±4.4	5.0±5.2
IL-6 (pg/mL)	-	-	-	-	-	-	-	-	1.0±0.5	0.8±0.3	0.9±0.5	1.0±0.7
11-D-TXB_2 (pg/mL)	-	-	-	-	-	-	-	-	1,867±2,238	3,323±3,107	963±724	4,565±3,498

Supplementary Tables

Table S1. Psychiatric History and Medication Status of the Participants.

	Mino. + Aspirin	Mino.+ Pl.	Pl.+ Aspirin	Pl.+ Pl.
N	31	19	19	30
Psychiatric History[1]				
Family Psychiatric History (Any) - frequency (%)	21 (91.3)	16 (88.9)	18 (100.0)	27 (90.0)
No information	8	1	1	-
Comorbid Physical History (Current)				
Current smoker - frequency (%)	9 (29.0)	7 (43.8)	3 (18.8)	13 (50.0)
Nervous system disorder - frequency (%)	23 (74.2)	12 (63.2)	8 (42.1)	23 (76.7)
Respiratory system disorder - frequency (%)	15 (48.4)	5 (26.3)	9 (47.4)	11 (36.7)
Cardiovascular disorder - frequency (%)	8 (25.8)	5 (26.3)	4 (21.1)	13 (43.3)
Endocrine - frequency (%)	12 (38.7)	7 (38.9)	8 (42.1)	10 (33.3)
Gastrointestinal disorder - frequency (%)	6 (19.4)	6 (31.6)	6 (31.6)	15 (50.0)
Genitourinary disorder - frequency (%)	3 (9.7)	1 (5.3)	2 (10.5)	6 (20.0)
Musculoskeletal disorder - frequency (%)	18 (58.1)	14 (73.7)	14 (73.7)	3 (10.0)
Comorbid - other - frequency (%)	23 (74.2)	16 (84.2)	15 (78.9)	24 (80.0)
Baseline Medication				
Antidepressant - frequency (%)	13 (41.9)	9 (47.4)	11 (57.9)	15 (50.0)
Anxiolytics - frequency (%)	6 (19.4)	5 (26.3)	7 (36.8)	6 (20.0)
Antipsychotic - frequency (%)	11 (35.5)	8 (42.1)	8 (42.1)	10 (33.3)
Mood stabilizer - frequency (%)	8 (26.7)	7 (36.8)	6 (31.6)	9 (30.0)
Complimentary (including multi-vitamin - frequency (%)	4 (12.9)	5 (26.3)	4 (21.1)	5 (16.7)
Other - frequency (%)	21 (67.7)	14 (73.7)	13 (68.4)	21 (70)
Comorbid Diagnosis (DSM-IV)				

Dysthymic disorder - frequency (%)[2]	1 (3.2)	1 (5.3)	0 (0.0)	0 (0.0)
Anxiety disorders (pooled) - current^ - frequency (%)[3]	5 (16.1)	5 (26.3)	4 (21.1)	10 (33.3)
Post traumatic stress disorder - frequency (%)[4]	7 (22.6)	4 (21.1)	3 (15.8)	3 (10.0)
Eating disorder - frequency (%)[5]	2 (6.5)	1 (5.3)	1 (5.3)	1 (3.3)
Obsessive compulsive disorder - frequency (%)[6]	1 (3.2)	1 (5.3)	1 (5.3)	1 (3.3)

[1]Total frequency, includes multiple medication types for the same participant
[2]Includes 300.04: Dysthymic Disorder
[3]Includes 300.0, 300.02, 300.21: Anxiety Disorder NOS; Generalized Anxiety Disorder; Panic Disorder with Agoraphobia
[4]Includes 309.81: Post-traumatic Stress Disorder
[5]Includes 307.1, 307.5, 307.51: Anorexia Nervosa; Eating Disorder NOS; Bulimia Nervosa
[6]Includes 300.3: Obsessive Compulsive Disorder

Table S2. Summary of Potential Side-Effects Recorded.

Adverse Event	P+P	P+A	M+P	M+A
Nausea	1	0	0	2
Vomiting	2	0	0	0
Suicidal Ideation	0	0	1	0
Transient Ischemic Attack	0	1	0	0
Heart palpitations	0	1	0	0
Dizziness	0	1	0	0
Reduced K+	1	0	0	0
Vaginal itching	0	0	0	1
Vaginal discharge	0	0	0	1
"Food poisoning"	0	0	0	1
Heart burn	0	0	0	1
Burning/itching during urination	0	0	0	1
Headache	0	0	1	0
Bacterial vaginosis	0	0	1	0
Homicidal Ideation	0	1	0	0
Hot/cold sensations	1	0	0	0
Myalgia	1	0	0	0
Diarrhea	0	0	0	1
Rash	1	0	0	0

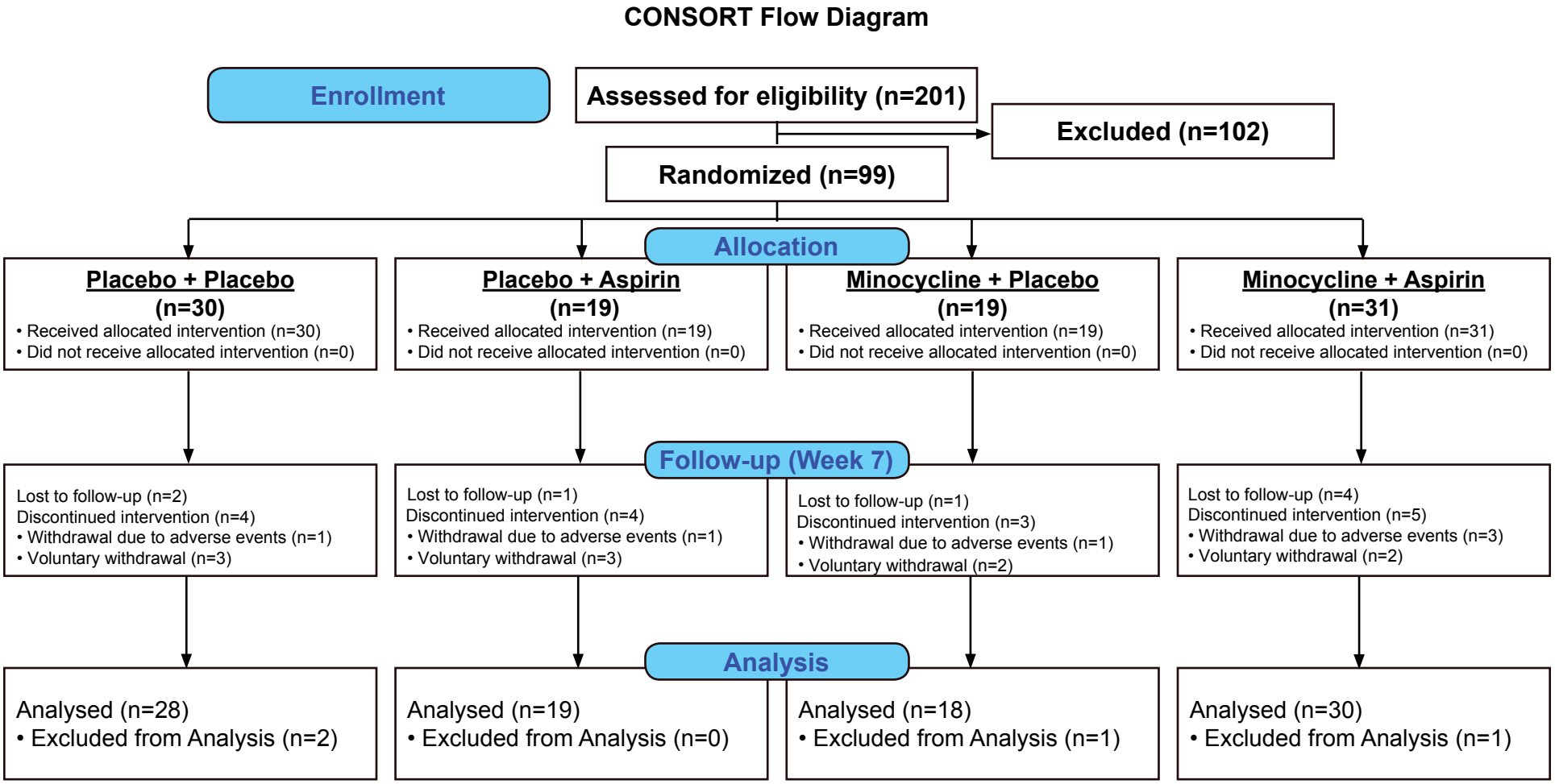

CONSORT Flow Diagram
Enrollment
Assessed for eligibility (n=201)
Excluded (n=102)
Randomized (n=99)
Allocation
Placebo + Placebo
(n=30)
• Received allocated intervention (n=30)
• Did not receive allocated intervention (n=0)
Placebo + Aspirin
(n=19)
• Received allocated intervention (n=19)
• Did not receive allocated intervention (n=0)
Minocycline + Placebo
(n=19)
• Received allocated intervention (n=19)
• Did not receive allocated intervention (n=0)
Minocycline + Aspirin
(n=31)
• Received allocated intervention (n=31)
• Did not receive allocated intervention (n=0)
Follow-up (Week 7)
Lost to follow-up (n=2)
Discontinued intervention (n=4)
• Withdrawal due to adverse events (n=1)
• Voluntary withdrawal (n=3)
Lost to follow-up (n=1)
Discontinued intervention (n=4)
• Withdrawal due to adverse events (n=1)
• Voluntary withdrawal (n=3)
Lost to follow-up (n=1)
Discontinued intervention (n=3)
• Withdrawal due to adverse events (n=1)
• Voluntary withdrawal (n=2)
Lost to follow-up (n=4)
Discontinued intervention (n=5)
• Withdrawal due to adverse events (n=3)
• Voluntary withdrawal (n=2)
Analysis
Analysed (n=28)
• Excluded from Analysis (n=2)
Analysed (n=19)
• Excluded from Analysis (n=0)
Analysed (n=18)
• Excluded from Analysis (n=1)
Analysed (n=30)
• Excluded from Analysis (n=1)

Figure 1

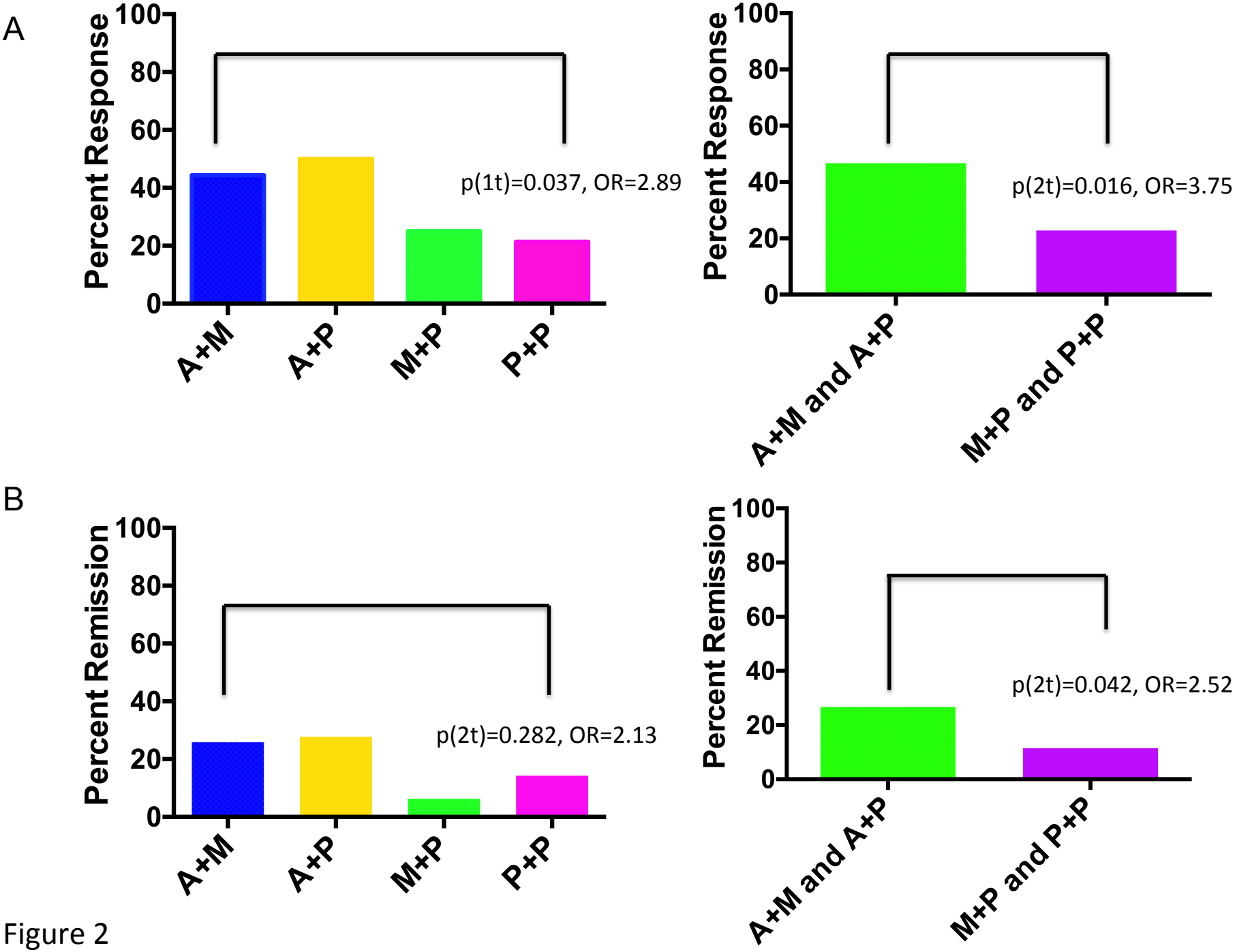

A
Percent Response
100
80
60
40
20
0
A+M
A+P
M+P
P+P
p(1t)=0.037, OR=2.89
Percent Response
100
80
60
40
20
0
A+M and A+P
M+P and P+P
p(2t)=0.016, OR=3.75
B
Percent Remission
100
80
60
40
20
0
A+M
A+P
M+P
P+P
p(2t)=0.282, OR=2.13
Percent Remission
100
80
60
40
20
0
A+M and A+P
M+P and P+P
p(2t)=0.042, OR=2.52

Figure 2

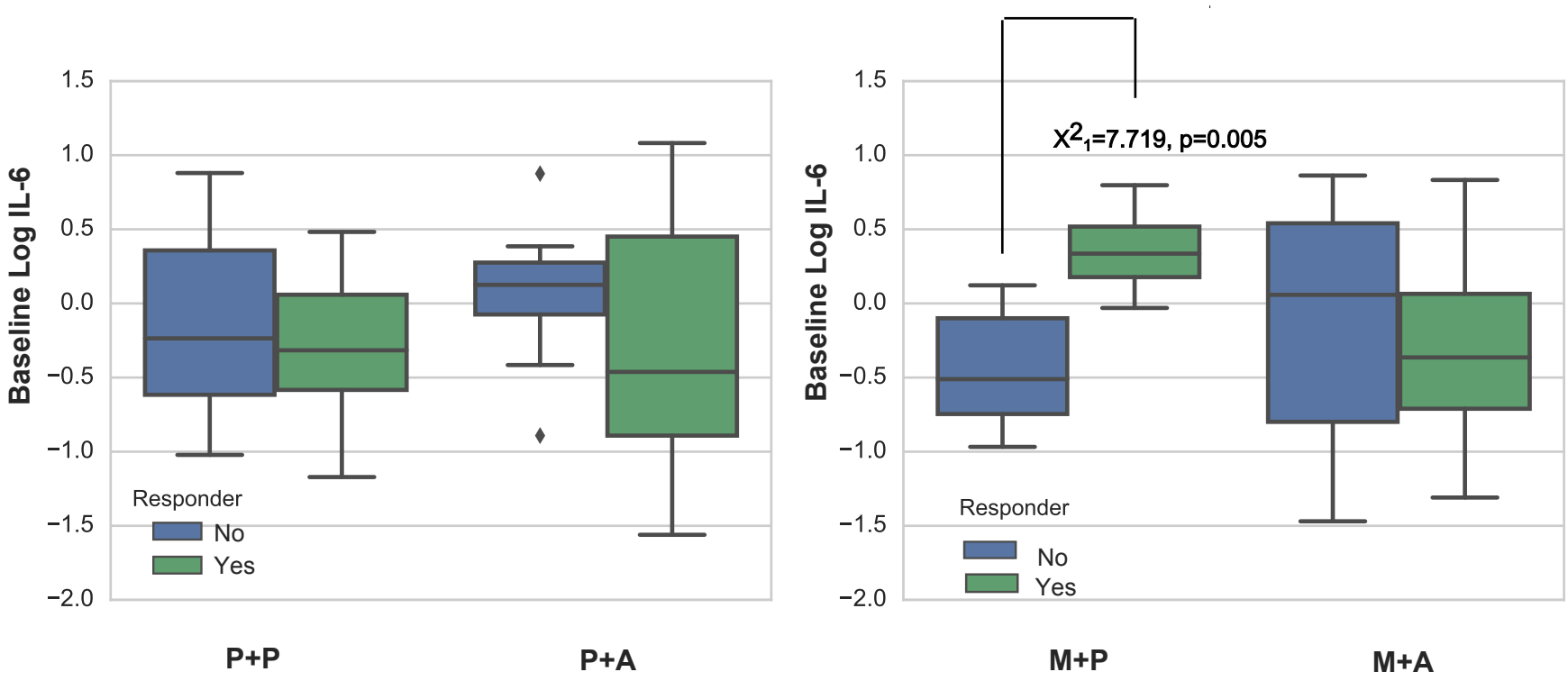
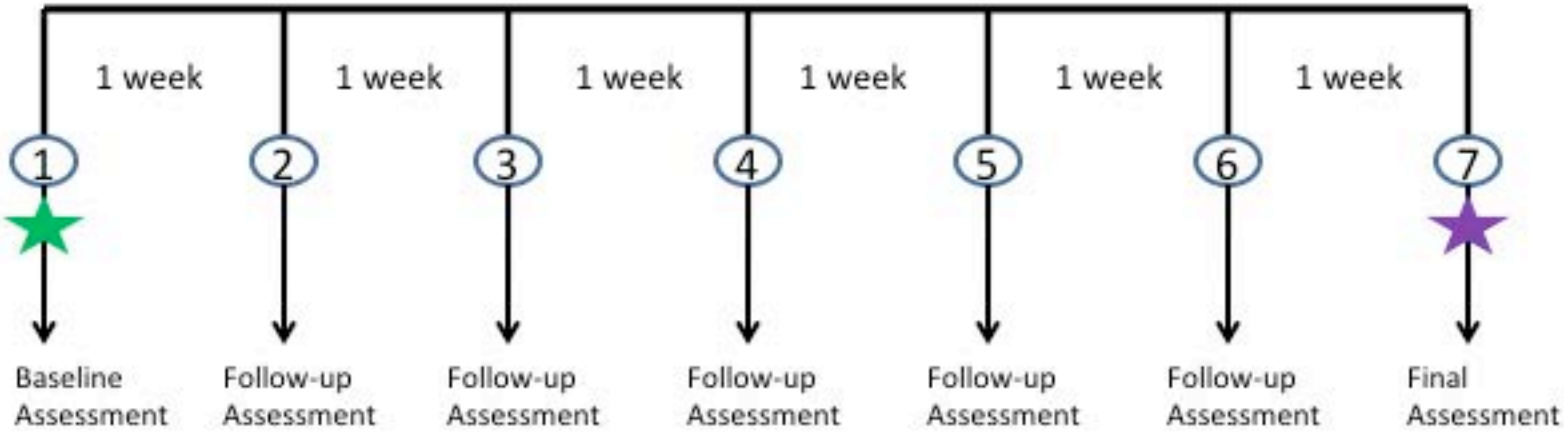

Baseline Log IL-6
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
−2.0
Responder
No
Yes
P+P
P+A
X^2_1=7.719, p=0.005
M+P
M+A

Figure 3



Minocycline dose initiation at 100mg bid

Aspirin dose initiation at 81mg bid

Placebo initiated

Inflammation markers

Inflammation markers

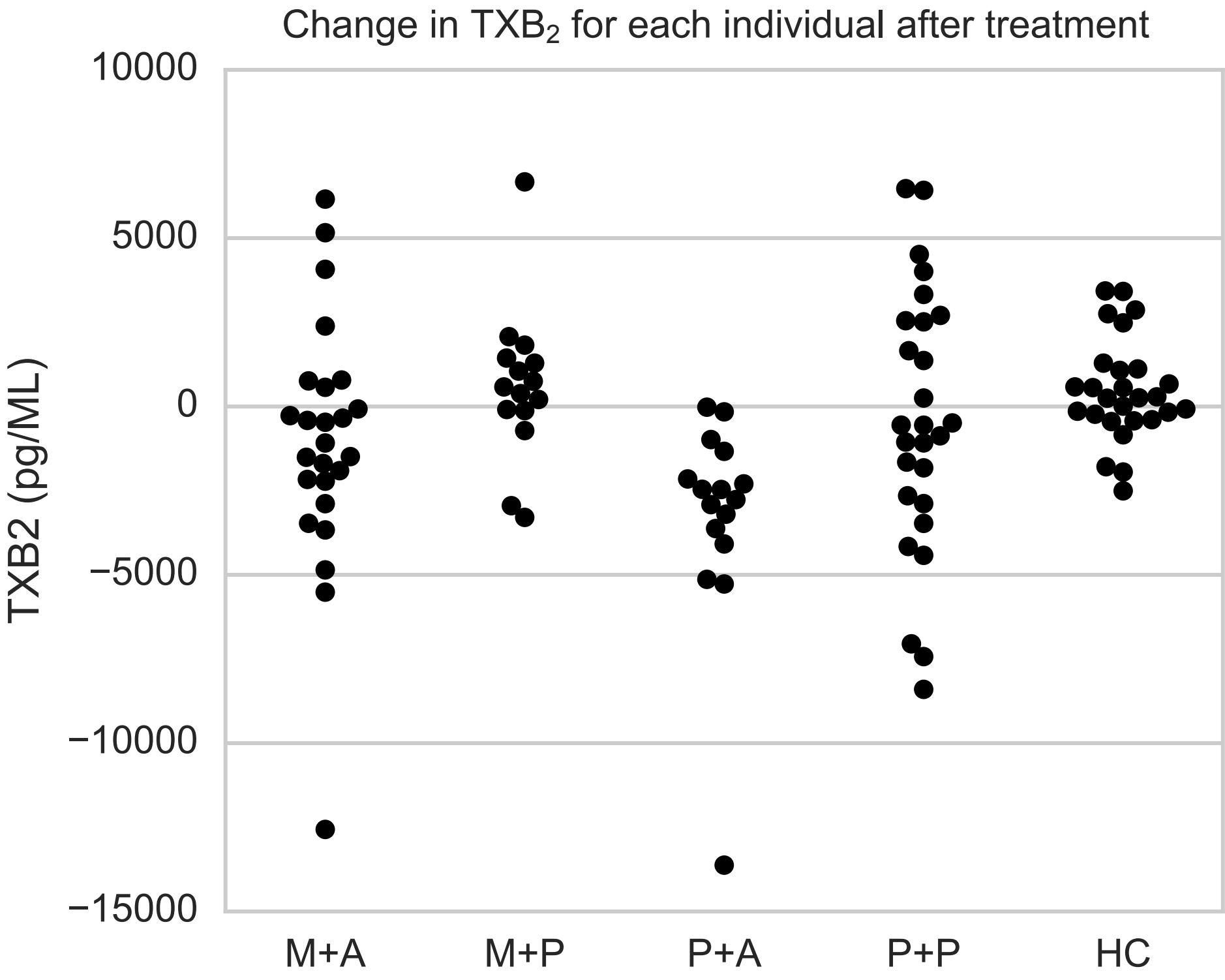

Change in TXB2 for each individual after treatment
10000
5000
0
−5000
−10000
−15000
TXB2 (pg/ML)
M+A
M+P
P+A
P+P
HC

Figure S2

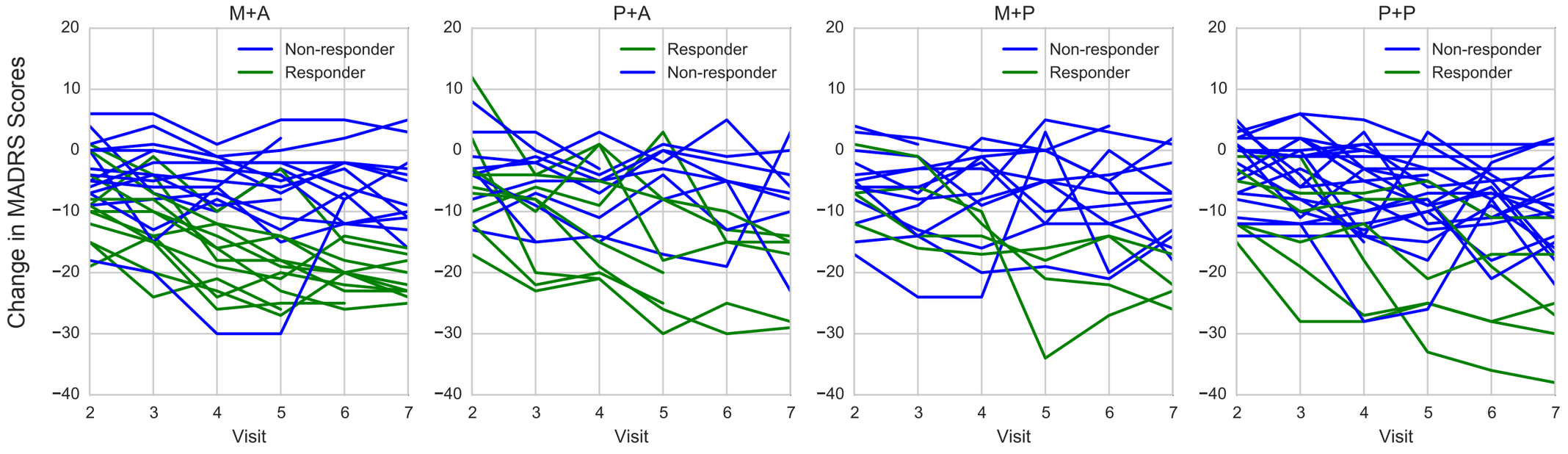

M+A
P+A
M+P
P+P
Non-responder
Responder
Change in MADRS Scores
Visit
20
10
0
−10
−20
−30
−40
2
3
4
5
6
7

Figure S3

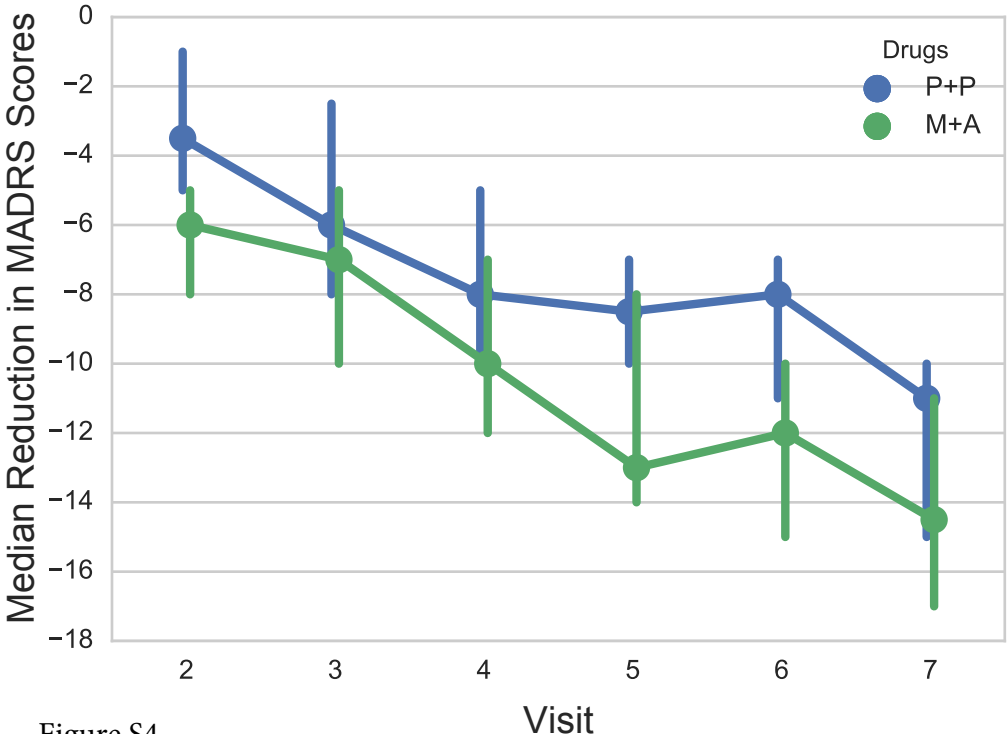

Median Reduction in MADRS Scores
0
−2
−4
−6
−8
−10
−12
−14
−16
−18
2
3
4
5
6
7
Visit
Drugs
P+P
M+A

Figure S4

BMJ open accessible medical research

Minocycline and aspirin in the treatment of bipolar depression: a protocol for a proof-of-concept, randomised, double-blind, placebo-controlled, 2×2 clinical trial

Jonathan Savitz,[1,2] Sheldon Preskorn,[3] T Kent Teague,[4,5,6,7] Douglas Drevets,[8] William Yates,[1] Wayne Drevets[1,5]

To cite: Savitz J, Preskorn S, Teague TK, *et al*. Minocycline and aspirin in the treatment of bipolar depression: a protocol for a proof-of-concept, randomised, double-blind, placebo-controlled, 2×2 clinical trial. *BMJ Open* 2012;**2**:e000643. doi:10.1136/bmjopen-2011-000643

► Prepublication history for this paper is available online. To view these files please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2011-000643).

Received 17 November 2011
Accepted 24 January 2012

For numbered affiliations see end of article.

Correspondence to
Professor Wayne Drevets; wdrevets@laureateinstitute.org

ABSTRACT

Introduction: New medication classes are needed to improve treatment effectiveness in the depressed phase of bipolar disorder (BD). Extant evidence suggests that BD is characterised by neural changes such as dendritic remodelling and glial and neuronal cell loss. These changes have been hypothesised to result from chronic inflammation. The principal aims of the proposed research is to evaluate the antidepressant efficacy in bipolar depression of minocycline, a drug with neuroprotective and immune-modulating properties, and of aspirin, at doses expected to selectively inhibit cyclooxygenase 1 (COX-1).

Methods and analysis: 120 outpatients between 18 and 55 years of age, who meet DSM-IV-TR criteria for BD (type I or II) and for a current major depressive episode will be recruited to take part in a randomised, double-blind, placebo-controlled, parallel-group, proof-of-concept clinical trial following a 2×2 design. As adjuncts to existing treatment, subjects will be randomised to receive one of the four treatment combinations: placebo-minocycline plus placebo-aspirin, active-minocycline plus placebo-aspirin, placebo-minocycline plus active-aspirin or active-minocycline plus active-aspirin. The dose of minocycline and aspirin is 100 mg twice daily and 81 mg twice daily, respectively. Antidepressant response will be evaluated by assessing changes in the Montgomery–Asberg Depression Rating Scale scores between baseline and the end of the 6-week trial. As secondary outcome measures, the anti-inflammatory effects of minocycline and aspirin will be tested by measuring pre-treatment and post-treatment levels of C reactive protein and inflammatory cytokines.

Ethics and dissemination: Minocycline has been widely used as an antibiotic in doses up to 400 mg/day. Low-dose aspirin has been safely used on a worldwide scale for its role as an antithrombotic and thrombolytic. The study progress will be overseen by a Data, Safety and Monitoring Board, which will meet once every 6 months. Results of the study will be published in peer-reviewed publications.

ARTICLE SUMMARY

Article focus

- Clinical trial testing the efficacy of aspirin and/or minocycline in the treatment of bipolar depression.

Key messages

- Extant evidence suggests that mood disorders are associated with inflammation. Aspirin and minocycline exert anti-inflammatory effects and have shown promise in the treatment of major depressive disorder.

Strengths and limitations of this study

- The first study to assess the efficacy of the separate and combined effects of aspirin and minocycline in the treatment of bipolar depression. Aspirin and minocycline will be used to augment conventional treatments in type I bipolar disorder patients, potentially reducing statistical power.

Trial registration number: Clinical Trials.gov: NCT01429272.

INTRODUCTION

The treatment of bipolar depression remains a major challenge for psychiatry. The US Food and Drug Administration has not approved any of the ~25 standard antidepressants for the treatment of bipolar depression, partly because these agents have not been robustly effective in bipolar disorder (BD) patients.[1] Thus, currently approved treatments for bipolar depression include lithium, quetiapine and the combination of olanzapine and fluoxetine.[2] Other treatments used include lamotrigine, conventional antidepressant agents, other

atypical antipsychotics, pramipexole or riluzole (reviewed in Nierenberg *et al*[3]). Unfortunately, the effectiveness of these options also is limited. For example, in a placebo-controlled study in which subjects receiving lithium were randomised to receive either standard antidepressant pharmacotherapy (paroxetine or imipramine) or placebo, those receiving lithium plus an antidepressant did not show a significant improvement over those receiving lithium plus placebo.[4] Similarly, in the STEP-BD trial, 42 of 179 subjects (23.5%) receiving a mood stabiliser plus adjunctive antidepressant drug treatment had a durable recovery, which did not differ significantly from 51 of 187 subjects (27.3%) receiving mood stabiliser plus placebo. Mallinger *et al* reported a similar durable recovery rate in BD depressives treated with mood stabiliser plus paroxetine (27%) but found a higher rate for adjunctive monoamine oxidase inhibitors (MAOIs; 53%),[5] consistent with the findings of previous studies comparing MAOIs versus imipramine.[6 7] Unfortunately, MAOIs are commonly unacceptable to patients.

New classes of antidepressant drugs are needed for bipolar depression. Existing agents exert their primary actions on monoaminergic systems. The efficacy of these agents contributed to the monoamine deficiency hypothesis of depression, which continues to receive empirical support. Nevertheless, the field is in the early stages of a paradigm shift driven by evidence of dendritic remodelling and neuronal atrophy in animal models of depression and of reductions in grey matter volume and glial cell loss at *postmortem* in BD.[8] The neurotrophic effects of lithium, coupled with longitudinal studies demonstrating volumetric changes over time, raise the possibility that mood disorders are underpinned by a neurotoxic process.[8 9] The final common pathway through which neurotoxic agents exert their effect is hypothesised to involve excess glutamatergic signalling.[10]

The glutamatergic model of mood disorders is based on the premise that excessive stimulation of NMDA glutamatergic receptors results in neuronal atrophy and apoptosis of glial and/or neuronal cells and, ipso facto, depression. Evidence for this hypothesis derives from multiple sources. In preclinical models, riluzole, which inhibits neuronal release of glutamate, ceftriaxone, which increases glutamate reuptake, and NMDA receptor antagonists, such as ketamine, ameliorate behavioural analogues of depression.[11] In addition, rats bred to be genetically sensitive to stress show differential expression of NMDA receptors,[12] and behavioural analogues of depression are abrogated in NMDA receptor subunit knockout mice.[13] In humans, increased serum levels of glutamate that resolve with antidepressant treatment were reported in MDD and extended to the cerebrospinal fluid (CSF) *postmortem*.[11] Polymorphisms of the metabotropic glutamate receptor genes, GRM2 and GRM3, and a haplotype of the glutamic acid decarboxylase (GAD2) gene were associated with MDD.[14] Finally, ketamine induced a rapid sustained antidepressant effect in BD[15 16] and riluzole showed promising results in treatment-resistant depression.[15 16]

One potential cause of the disruption in glutamatergic signalling in BD is dysregulation of the immune system. Increased levels of proinflammatory cytokines such as interleukin (IL) 6, IL-1β, interferon α (IFNα), tumour necrosis factor α (TNFα), prostaglandin E2 (PGE2) and chemokine ligand 2 (CCL2) are consistently observed in the blood and CSF of patients with mood disorders, both at baseline and after exposure to stressors.[17 18] Elevated serum levels of (proinflammatory) positive acute-phase proteins (eg, haptoglobin, α1-antitrypsin, ceruloplasmin, C reactive protein (CRP)) but reduced levels of negative acute-phase proteins (eg, albumin and retinal-binding protein) also are reported in mood disorders.[19–21] Furthermore, treatment of hepatitis C with IFNα is known to induce the major depressive syndrome and/or manic symptoms in approximately 40% of patients, and the efficacy of conventional antidepressant drugs is associated with a reduction in inflammation.[18] Moreover, anti-TNF therapy (for psoriasis) can improve mood.[22] Since proinflammatory cytokines can alter brain function, these data are compatible with evidence that an activated inflammatory response system exists in mood disorders which plays a role in their pathophysiology.[23–26]

The overactivity of the hypothalamic–pituitary–adrenal axis in mood disorders may play a role in inflammation since hypersecretion of corticotrophin-releasing hormone (CRH) activates the transcription factor, nuclear factor κB (NF-κB). NF-κB regulates the expression of proinflammatory cytokines in immune cells in the central nervous system (CNS) and periphery and the expression of genes involved in apoptosis.[27] In addition, NF-κB may result in the expression of the class 1 major histocompatibility complex, labelling cells for removal by cytotoxic T cells.[27] Usually, cortisol suppresses this inflammatory response, but chronic stress appears to desensitise the glucocorticoid receptor and by extension, the anti-inflammatory effects of cortisol.[27] Cytokines play a role in desensitising the system to cortisol. For example, IL-1 and TNFα retard dexamethasone-induced translocation of the glucocorticoid receptor from the cytoplasm to the nucleus.[28]

The immunologic and glutamatergic models of BD are complementary because a proinflammatory state is one potential cause of excitotoxicity.[27] Peripheral inflammatory signals activate microglia in the brain, inducing an inflammatory cascade of cytokines and free radicals. Cytokines and reactive oxygen and nitrogen species exert a direct toxic apoptotic effect on oligodendrocytes. Potentially through the loss of oligodendrocytes, oxidative stress can lead to demyelination. Such a process conceivably may account for the reduction in oligodendroglia found *postmortem* in the prefrontal cortex[29] in mood disorders. The inflammatory milieu also compromises astrocyte function, leading to downregulation of glutamate

transporters and impaired glutamate reuptake into astrocytes, further amplifying inflammatory signalling.[27]

In addition, cytokines such as IL-1, IL-6 and TNFα activate indoleamine 2, 3-dioxygenase (IDO). IDO catalyses the breakdown of tryptophan, the amino acid precursor of serotonin and an important regulator of T cell function, into kynurenine (Kyn).[30] Activation of the Kyn pathway shunts tryptophan away from 5-HT synthesis, putatively reducing serotonergic transmission. Kyn is in turn metabolised into quinolinic acid (Quin), a potent NMDA receptor agonist, and neuromodulator involved in lipid peroxidation, which can induce neuronal damage via oxidative stress and over-stimulation of NMDA receptors.[30] Consistent with inflammation-related shunt towards Kyn metabolism, the plasma tryptophan−Kyn ratio was found to correlate inversely with striatal total choline (a putative cell membrane turnover biomarker) in adolescents with melancholic depression.[31]

The messenger RNA (mRNA) transcripts for proinflammatory genes appear particularly sensitive for discriminating BD patients. Microarray gene expression profiles in purified CD14+ monocytes from whole blood of BD subjects, offspring of BD parents and healthy controls (HCs) displayed a distinct mRNA signature representing genes from inflammatory and inflammation-related pathways.[32] The signature showed >80% sensitivity and specificity in BD subjects who were not receiving lithium or antipsychotic drugs (n=11) and in affected offspring of a BD parent (n=13, of whom 10 had only manifested depression). A positive signature also was present in 17 of 38 unaffected offspring (45%) versus 13 of 70 healthy children (19%). Cross-sectional comparisons suggested that lithium and antipsychotic drugs—but not conventional antidepressant drugs—downregulated expression of most inflammatory genes. Thus, when medicated and unmedicated subjects were considered together, only 23 of 42 BD patients (55%) had a positive signature versus seven of 38 HCs (18%). Notably, the IL-6 mRNA level remained elevated in medicated BD subjects and did not differ significantly from unmedicated subjects (table 1), suggesting that this assay identifies a proinflammatory diathesis even in treated cases.

Minocycline is a second-generation tetracycline that may prevent both glutamate-induced excitotoxicity and cytokine-induced inflammation in the CNS and periphery

Minocycline has high lipophilicity enabling efficient transfer across the blood-brain barrier (BBB)[33]—its concentration in CSF reaches 11%−56% of plasma concentrations.[34] Minocycline inhibits the microglia-mediated release of proinflammatory cytokines IL-1β, TNFα, IL-6 and p38,[35] while promoting release of the anti-inflammatory cytokine, IL-10.[34] Moreover, minocycline inhibits matrix metalloproteinases, which process cytokines such as TNFα and IL-1β into their biologically active forms.[35] Minocycline is also an effective scavenger of proapoptotic reactive oxygen species and protects against excitotoxicity by preventing glutamate-induced activation of nitric oxide synthase.[36] Nitric oxide facilitates glutamate release from presynaptic neurons and inhibits glial glutamate transporters, amplifying glutamatergic signalling and contributing to excitotoxic cell death.[10] Minocycline also upregulates a key molecular factor in the apoptosis pathway, B cell CLL/lymphoma 2 (BCL-2),[37] an effect shared by lithium, valproate[38] and certain antidepressant drugs.[39] BCL-2 represses apoptosis induced by cytotoxic insults.[40] Conceivably, minocycline may additionally reduce inflammation indirectly by blocking the translocation of bacteria across the intestinal barrier. In mice exposed to a social stressor, bacteria translocated across the intestinal barrier stimulating the release of circulating cytokines, such as IL-6, and increasing microbicidal activity via inducible nitric oxide synthase.[41] Additionally, stress induced a change in the community structure of the microflora in the cecum with a decrease in the relative abundance of bacteria in the genus Bacteroides and an increase in the relative abundance of bacteria in the genus Clostridium. Notably, these effects were blocked by pre-treatment with a broad-spectrum antibiotic.[41]

Minocycline has neuroprotective and anti-inflammatory properties

Minocycline prevents glutamate-induced apoptosis of neurons in vitro,[42] prevents ischaemia-induced activation of microglia in gerbils,[43] increases hippocampal neuron survival,[44] reduces lesion volume and improves

Table 1 Magnitude of difference in messenger RNA expression between mood disordered and healthy control (HC) samples from Padmos *et al*,[32] showing selected transcripts in unmedicated subjects versus HCs, relative to that of medicated BD subjects

	Unmedicated BD versus HC		Medicated BD versus HC		Affected offspring* versus HC	
Gene symbol	Fold change	p Value	Fold change	p Value	Fold change	p Value
PDE4B	13.73†	<0.001	3.42	<0.001	5.79	<0.001
IL-6	37.92	0.005	9.56	0.006	935.7	<0.001
CCL20	55.49	0.006	6.02	0.10	400.1	<0.001

Sample sizes: unmedicated BD n=11, medicated BD n=31, affected offspring n=13, HCs n=25 for comparisons against BD adults, n=70 for comparisons of offspring.
*Affected with respect to having manifested either a depressive or a manic episode.
†Difference significant between unmedicated versus medicated BD samples.
BD, bipolar disorder; CCL20, chemokine ligand 20IL-6, interleukin 6; PDE4B, phosphodiesterase type 4B.

neurological function in mice with traumatic brain injury[45] and in fragile X syndrome,[46] reduces proinflammatory cytokine expression and improves neurological function and locomotor activity in rats with spinal cord injury,[47] attenuates MDMA-induced neurotoxicity of serotonin and dopamine systems in the cerebral cortex and hippocampus of mice,[48] reduces inflammation in a rat model of rheumatoid arthritis (RA),[49] and delays disease progression and demyelination in rodent models of encephalitis,[50] amyotrophic lateral sclerosis[51] and Huntington's disease (HD).[52] Based on these data, minocycline was employed and has shown promise as a therapeutic agent in human diseases including HD,[53] RA[54] and stroke.[55]

Minocycline has been used to treat psychiatric disorders

Miyaoka *et al*[56] discussed two patients with catatonic schizophrenia who benefited from minocycline. This group then conducted a 4-week trial with minocycline (150 mg/day) in 22 patients with schizophrenia to evaluate its efficacy as an adjunct to antipsychotic drugs.[57] Patients showed a significant improvement in positive and negative symptoms. Levkovitz *et al*[58] recently studied 54 patients with early-stage schizophrenia treated for 6 months with antipsychotic medication and either minocycline (200 mg/day) or placebo in a double-blind trial. Minocycline was associated with a reduction in negative symptoms and improved attention/memory.

The efficacy of minocycline has not been formally tested in mood disorders. In rodents, minocycline reduced immobility during the forced swim test,[59] and co-administration of minocycline synergised the antidepressant-like actions of desipramine (but not fluoxetine).[60] Minocycline also abrogated the depression-like behaviour of rodents exposed to lipopolysaccharide (LPS).[61] Levine *et al*[62] presented the case of a 66-year-old woman with severe BD, who observed that the tetracycline she took for an infection alleviated her depression. When her depression returned post-treatment, minocycline was reinitiated (150 mg/day). After 1 week, her HAM-D score fell from 25 to 8.

Aspirin (acetylsalicylic acid) also holds potential efficacy in BD

The second aim of this study is to assess the antidepressant efficacy of acetylsalicylic acid (ASA) in bipolar depression. Using a 2×2 design, we will obtain data providing estimates of the effect size of ASA relative to placebo, ASA relative to minocycline and ASA in combination with minocycline relative to placebo. These data also will explore the specificity of any effect found for minocycline. The clinical use of low-dose ASA primarily has been driven by its role as an antithrombotic and thrombolytic. Given the exaggerated death rate from cardiovascular (CV) events in BD, this action potentially is advantageous in the management of BD. Nevertheless, the recent literature also supports a role for low-dose ASA in the management of the mood disorder itself, specifically in the amelioration of depressive symptoms.

The mechanism of ASA relates to its capacity to inactivate irreversibly the cyclooxygenase (COX) activity of prostaglandin (PG) H-synthase-1 and PGH-synthase 2 (referred to as COX-1 and COX-2, respectively). Although ASA has a short half-life (15—20 min), ASA's permanent inhibition of COX-1 allows once daily dosing for anucleate platelets. In contrast, because nucleated cells rapidly regenerate this enzyme, a shorter dosing interval is required to persistently impact COX activity in cells that mediate inflammatory processes. Moreover, ASA is 50- to 100-fold more potent in inhibiting platelet COX-1 than monocyte COX-2 activity,[63] so there is nearly a 100-fold variation in the daily dose of aspirin, as higher doses are used to target COX-2 in the management of treating peripheral inflammation (eg, arthritis) or pain. As reviewed below, preliminary evidence obtained in BD suggests beneficial effects are achieved using ASA in low doses, where aspirin would inhibit COX-1 but not COX-2.

Aspirin has neuroprotective and anti-inflammatory properties

In the brain, recent data indicate that genetic manipulation of COX-1 and COX-2 differentially modulate leucocyte recruitment during neuroinflammation and suggest that reduction of COX-1 activity is neuroprotective, whereas reduction in COX-2 activity is detrimental, during a primary neuroinflammatory response (reviewed in Choi *et al*[64]). Choi *et al*[64] propose that these distinct roles reflect the predominant localisation of COX-1 in microglia, which play a major role in mediating neuroinflammation, in contrast to the predominant localisation of COX-2 in pyramidal neurons. For example, Choi *et al*[65] examined the effects of COX-1 or COX-2 deficiency on intracerebroventricular LPS-induced neuroinflammation by comparing COX-1 (−/−) and COX-2 (−/−) knockout mice with wild-type (WT) (+/+) control animals. After LPS, leucocyte infiltration and inflammatory response were attenuated in the COX-1 (−/−) mice but increased in the COX-2 (−/−) mice compared with WT controls. In another study, Choi *et al*[66] examined the effect of COX-1 genetic deletion on the inflammatory response and neurodegeneration induced by β-amyloid and found that in COX-1 (−/−) mice, the Aβ1-42-induced inflammatory response and associated neuronal damage were attenuated compared with WT mice. Compatible with these results, in pharmacoepidemiological studies investigating whether chronic non-steroidal anti-inflammatory drug (NSAID) use reduced the risk of developing Alzheimer's disease (AD), indomethacin, a preferential COX-1 inhibitor, showed beneficial effects, while COX-2 selective inhibitors failed to show any beneficial effect in AD patients with mild-to-severe cognitive impairment. These data suggest the hypothesis that inhibition of COX-1 activity may be a valid therapeutic strategy to reduce the cerebral inflammatory response and neurodegeneration in neuropsychiatric diseases in which neuroinflammatory components play a role in pathophysiology.

Other researchers hypothesised that NSAIDs would be beneficial in BD more specifically because of their ability to downregulate activity in the brain arachidonic acid (AA) cascade by interfering with phospholipase A2 (PLA2) and/or COX function. In rodents, Rapoport and colleagues[67–69] demonstrated that conventional mood stabilisers decrease the AA turnover in phospholipids and the expression of PLA2 and/or COX enzymes. The PLA2 and COX enzymes catalyse, respectively, release of AA from membrane phospholipid and AA conversion to eicosanoids such as prostaglandin E2 and thromboxane B2. The AA cascade is involved in neuroreceptor-initiated signalling and can be pathologically upregulated by neuroinflammation and excitotoxicity.

Nevertheless, aspirin has additional mechanisms that may underlie benefits in neuropsychiatric illness. While low-dose aspirin downregulates AA cascade activity via inhibition of COX-1 activity, in higher doses, it also downregulates COX-2 gene transcription, increases levels of lipoxygenase-derived eicosanoids, such as the anti-inflammatory lipoxin A4, and acetylates COX-2 protein to a modified enzyme that can convert unesterified AA to anti-inflammatory mediators such as 15-epi-lipoxin A4 (reviewed in Stolk *et al*[70]). The acylated enzyme also can convert docosahexaenoic acid (DHA) to 17-(R)-OH-DHA, which, like its metabolites di(R)-OH-DHA (neuroprotectin (R) D1) and tri(R)-OH-DHA (resolvin (R) D1), is highly anti-inflammatory (reviewed in Stolk *et al*[70]). Lithium given chronically to rats with LPS-induced neuroinflammation also increases the brain concentration of 17-OH-DHA. Thus, there may be a synergy between aspirin and lithium in forming anti-inflammatory brain DHA metabolites.

Aspirin appears effective in preliminary studies of mood disorders

Pharmacoepidemiological data in BD supportive of these hypotheses were published by Stolk *et al*.[70] Using the Netherlands-based PHARMO Record Linkage System (which connects pharmacy dispensing records to hospital discharge records of >2 million individuals since 1985), these researchers tested whether NSAIDs or glucocorticoids would ameliorate bipolar symptoms. The target sample consisted of 5145 patients receiving lithium (mean age=48.6±15 years; mean duration of lithium use=847 days), based upon the assumption that lithium treatment is relatively specific to individuals with BD. The main outcome measure was a calculated incidence density of medication events (change in the type or numbers of psychotropic medications prescribed or increase (>30%) in the psychotropic drug dose). Subjects receiving low-dose (≤80 mg/day) aspirin were 17% less likely to have a medication event, a finding that remained significant after adjusting for age, sex, chronic disease score and healthcare utilisation. This effect was selective for low-dose ASA. In contrast, high-dose aspirin or non-selective NSAIDs (ie, regimens expected to inhibit both COX-1 and COX-2), selective COX-2 inhibitors and glucocorticoids did not produce a statistically significant protection. Instead, the co-administration of non-selective NSAIDs and glucocorticoids was associated with statistically significant increases in medication events, suggesting destabilisation of bipolar illness. The finding that low-dose aspirin decreased the number of medication events was particularly noteworthy since aspirin does not significantly augment serum lithium levels in contrast to selective COX-2 inhibitors, which can raise lithium levels.[71] These preliminary observations thus appeared consistent with the hypothesis that COX-1 inhibitors can reduce neuroinflammatory processes and thus benefit BD patients.

Notably, the observation that beneficial effects in BD were conferred by low-dose ASA, but not by non-selective COX inhibitors, COX-2 inhibitors or glucocorticoids, appeared inconsistent with the hypothesis that drugs that downregulate AA cascade activity in general hold therapeutic potential in BD. Thus, the putative neuroprotective effects associated with COX-1 inhibition may contribute specifically to the benefits of low-dose aspirin in BD observed by Stolk *et al* For example, as reviewed above, aspirin and lithium may exert synergistic effects in forming anti-inflammatory brain DHA metabolites (reviewed in Stolk *et al*[70]).

Other data suggest that aspirin exerts antidepressant effects within the context of MDD or CV illness. Mendlewicz *et al*[72] examined the effect of aspirin augmentation of conventional antidepressant pharmacotherapy in 24 patients with MDD who had proven non-responsive after 4 weeks of selective serotonin reuptake inhibitor (SSRI) treatment. Participants were treated openly during the subsequent 4 weeks with aspirin 160 mg/day in addition to their SSRI regimen. The combined administration of SSRI plus aspirin was associated with a response rate of 52.4%. Remission was achieved in 43% of the total sample and 82% of the responder sample. In the responder group, a significant improvement was observed within week 1 and this benefit persisted through day 28. In another study, Ketterer *et al*[73] reported that in 174 men undergoing coronary angiography (of whom 99 were taking low-dose aspirin), aspirin use was associated with less depression and anxiety symptoms.

In contrast, a preliminary study of the selective COX-2 inhibitor, celecoxib, was negative in bipolar depression,[74] potentially compatible with the negative results of COX-2 inhibitors reported by Stolk *et al*.[70] In a double-blind, randomised, add-on clinical trial of celecoxib in patients (n=28) studied during a depressed or mixed episode of BD, no significant difference was observed between the celecoxib and placebo add-on groups at study end point.[74] These results contrasted with those obtained using celecoxib in unipolar depression, however. In MDD, celecoxib augmentation of either reboxetine[75] or fluoxetine[76] was associated with a significant therapeutic effect on depressive symptoms in randomised, double-blind, add-on clinical trials.

METHODS AND ANALYSIS

Participants

One hundred and twenty male or female outpatients between 18 and 55 years of age who meet DSM-IV-TR criteria for BD (type I or II) and for a current major depressive episode will be recruited. The depressive syndrome must have been present for at least 4 weeks and the minimum threshold for depression severity will be set at a 17-item HAM-D score ≥18. Subjects will provide written informed consent as approved by the Western Institutional Review Board (IRB).

Concurrent medications

At study entry, type I BD subjects must have been taking a stable dose of a mood-stabilising medication (lithium, valproate, carbamazepine, lamotrigine, antipsychotic agents) for at least 4 weeks, dosed clinically to target the therapeutic range. Type II BD subjects will be included irrespective of whether they present on a mood stabiliser. To investigate the utility of this augmentation strategy in the population for whom minocycline is most likely to prove therapeutically relevant, volunteers receiving stable doses of mood stabilising, antipsychotic, antidepressant and/or anxiolytic drugs for at least 4 weeks will be included. However, volunteers who currently are receiving more than four psychotropic medications in a daily regimen will be excluded since this condition may signify a more brittle or complex clinical state. Subjects may remain in psychotherapy or have no psychosocial intervention. Volunteers will be excluded if they currently are receiving medications likely to have adverse interactions with minocycline or aspirin, including NSAIDS, warfarin, digoxin, penicillins and isotretinoin products.

For participants who enter the study, the preferred strategy will be for subjects to maintain the same regimen of concurrent medications throughout the 6-week study so that only the study drug regimen will be altered per protocol. Nevertheless, changes to concurrent medications will not affect study status, so long as the medication change does not target a depressive or manic symptom. If changes to concurrent medication regimens are clinically required to address worsening depressive symptoms or the development of manic symptoms, then the subject will be dropped from the study.

Study design

Patients will participate in a randomised, double-blind placebo-controlled trial with a 2×2 design. As adjuncts to existing treatment, subjects will receive placebo-minocycline plus placebo-aspirin, active-minocycline plus placebo-aspirin, placebo-minocycline plus active-aspirin, or active-minocycline plus active-aspirin. The randomisation sequences will be determined by a research staff member who is not obtaining clinical information from the research subject and will be assigned by subject number at consenting. A restricted randomisation (permuted block randomisation) method will be used in which subjects are randomly allocated to each block (n=30) to ensure that equal numbers of participants receive each drug/placebo combination. In order to ensure that experimental group assignment is not skewed across the two trial sites, the study progress will be monitored by individuals who are not involved in the data collection, and in the case of 'drift', adjustments will be made as necessary.

The trial will be conducted over 6 weeks and will comprise seven assessment sessions (figure 1). The subject will be seen at the prescribed time intervals within a window of two business days on either side of visit target date to complete the specified visits.

At each session, a clinical assessment will be conducted using the rating scales listed below, and treatment side effects will be assessed and rated for severity. To preserve the rater blind, the research staff member who conducts the clinical ratings will not be the research staff member who assesses the presence of side effects and will remain blind to the information pertaining to side effects. Subjects who experience severe adverse effects or who develop treatment-associated hypomania or mania will be dropped from the study, instructed to discontinue the study medication and referred for appropriate clinical management of these adverse events.

The primary outcome measure will be the change in the Montgomery–Asberg Depression Rating Scale (MADRS) scores at the seventh assessment session (week 6).

Medication

This pilot proof-of-concept study will adhere to the dosing limits and route of administration for the Food and Drug Administration indications for minocycline's and aspirin's use in other conditions (thus an investigational new drug (IND) is not required). A fixed dose design will be followed, and all medications will be administered via the p.o. route. The pilot data extant for both study drugs support an onset of improvement within 2 weeks, so the 6-week study duration is expected to provide sufficient time to detect an antidepressant effect, to provide information about the persistence of the antidepressant effect over about 1 month from the anticipated onset of effect and to minimise dropouts.

For minocycline, the starting dose will be 100 mg twice daily (total daily dose=200 mg). This dose of 100 mg twice daily has been shown by a substantial literature to produce consistent anti-inflammatory effects in RA and other inflammatory disorders. This also is the dose used in a recent schizophrenia treatment trial.[58] The associated placebo capsules match the appearance of the 100 mg minocycline capsule.

The starting dose of aspirin will be 81 mg p.o. twice daily. This dose is sufficient to inhibit COX-1 and appeared beneficial in stabilising the course of BD in the pharmacoepidemiological study of Stolk *et al*.[70] When aspirin is used as an anti-platelet drug, once daily dosing is sufficient since anucleate platelets do not produce enough COX-1 to overcome the irreversible inhibition of COX-1 within a 24 h period. In contrast, in nucleated

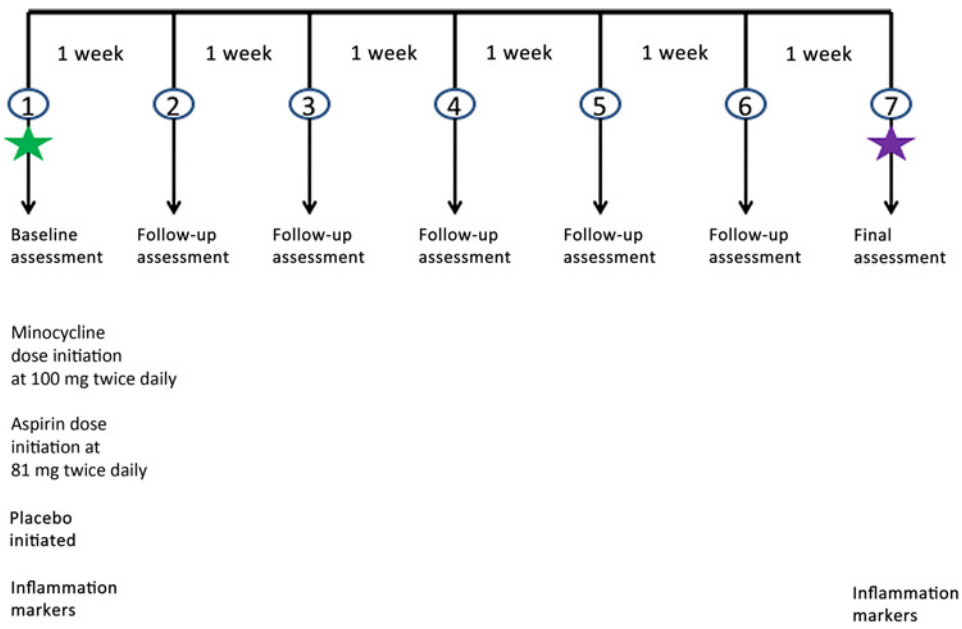


Figure 1 Schematic of study design. Each session number (total of seven) is encircled, with the timing between sessions indicated in weeks with a two business day window on either side of visit target date to complete the visit. Session 1 is the baseline (green star) and session 7 is the study end (purple star). Peripheral blood will be sampled at baseline and study end to assay markers of inflammation. The study duration is 6 weeks.

cells, COX-1 is replenished, so more frequent dosing is required to persistently inhibit COX-1. Thus, we will administer the dose in a twice daily regimen, according to the guidelines described above. A total daily dose of 160 mg was administered in the preliminary study, which reported that aspirin significantly augmented the antidepressant effects of fluoxetine in MDD.[72] The relevant placebo matches the appearance of the aspirin tablet.

Participants will be advised that one of the study drugs may reduce the efficacy of oral contraceptives and to avoid taking the study drugs within 3 h of iron products or of antacids containing calcium, magnesium or aluminium. They also will be advised that one study drug can increase their risk for bleeding during surgical procedure or if combined with other drugs or herbal preparations that reduce haemostasis.

Compensation

Participants will be compensated for participation in the amount of $300.00.

Treatment compliance

To enhance compliance, study participants will be given an information sheet to take home detailing the procedure to be followed in the case of a missed dose and requesting that this information be recorded for the investigators. The number of capsules and tablets remaining in each supply given to the patients will also be counted to evaluate treatment compliance. In cases where treatment compliance is poor, subjects will be excluded from the data analysis, using conventional criteria for defining adequate compliance in a clinical trial.

Psychiatric assessment and clinical ratings

Patients will be evaluated and followed in the outpatient clinics at Laureate Institute for Brain Research (LIBR) or Oklahoma University School of Community Medicine in Tulsa, Oklahoma, or at the University of Kansas Medical Center Research Institute (KUMCRI) in Wichita, KS. The diagnosis of BD will be established using DSM-IV-TR criteria on the basis of an unstructured interview conducted by a psychiatrist and the MINI-Plus administered by trained psychiatric interviewers. The following rating scales will be administered: MADRS, Quick Inventory of Depressive Symptomatology (QUIDS; 16 item), Hamilton Anxiety Rating Scale (HAM-A), Young Mania Rating Scale (YMRS), Universal Fagerstrom (to assess nicotine use), Hollingshead Socioeconomic Scale, Sheehan Disability Scale and the Family Interview for Genetic Studies. Medical assessment will include a physical examination, electrocardiogram, complete blood count, electrolytes and liver function assays (SMA 20), thyroid panel and urinalysis, serum drug and pregnancy tests at study entry and study completion. At each follow-up session, the MADRS, HAM-A, YMRS and Clinical Global Impressions (CGI) scale will be repeated. Physical and psychiatric symptoms will be evaluated and recorded in order to measure the side effect profiles of minocycline and aspirin. Participants will be questioned about adverse reactions, including dizziness, photosensitivity, hyperpigmentation, gastrointestinal (GI) distress or bleeding at each assessment, and will be withdrawn from the study if medically necessary. Vital signs will be measured at entry and at each session.

Immune system measures

The activity of peripheral cytokines correlates with inflammatory processes in the CNS. Peripheral cytokines cross the BBB and can propagate signals across the BBB in the form of small, freely diffusible lipophilic molecules such as prostaglandins, which induce the production of cytokines from glia.[77] The measurement of peripheral markers of inflammation thus serves as a valid, if indirect assessment of CNS inflammation.

To explore predictors and correlates of treatment outcome, blood will be sampled for testing plasma and whole blood peripheral blood monocyte (PBM)-based markers of inflammation at baseline and study end. These markers will include 10 cytokine proteins (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IFNγ and TNFα), high-sensitivity (hs) CRP and RNA expression of candidate genes from peripheral blood monocyte cells (PBMCs). Candidate genes include IL-6, TNF and IRF5 (a factor that mediates monocyte polarisation). The 10 inflammation-related cytokines and the PBMC mRNA will be assayed from plasma at baseline and study end. We selected the markers IL-6, TNF and CRP because they are the most widely implicated in mood disorders. The other cytokines included in the cytokine bead array assays are measured simultaneously with IL-6 and have all been implicated in the general regulation of inflammation. A meta-analysis of >100 studies found that IL-6 and CRP each were significantly elevated in depressed patients with standardised mean difference scores (d) of 0.71 and 0.26, respectively.[78] The associations remained significant after adjustment for body mass index (BMI) and smoking. Moreover, IL-6 has been shown to modulate HPA axis function by inducing CRH release, adrenocorticotropic hormone synthesis and corticosteroid production.[79] CRP production is induced by the proinflammatory cytokines, IL-1, IL-6 and IL-17, and is thus a non-specific marker of systemic inflammation.

Three blood samples will be transported to the immunology laboratory in the Department of Surgery at the University of Oklahoma College of Medicine for each participant at each of the sampling time points (sessions 1 and 7). One sample will be centrifuged to obtain plasma which will be stored at −80°C until analysed. Serum CRP, IL-6, TNF and the other cytokines listed above will be assayed in duplicate with ELISA (CRP high-sensitivity kit; R&D Systems, Oxford, UK) or enhanced cytokine bead array flex kits (Becton Dickinson, Franklin Lakes, NJ, USA) using the manufacturer's reagents and standards. The other two samples will be used to isolate PBMCs and plasma and will be frozen until processed. Monocytes will be isolated from the PBMCs in order to assess mRNA levels similar to the method used by Padmos *et al*.[32] This procedure utilises monoclonal antibodies directed against human CD14 to isolate monocytes in PBM cell suspensions. A magnetic cell sorting system will be used for the separation of the monocytes, and flow cytometry will be used to gauge the purity of the population. Once purity is established, total RNA will be isolated from the monocytes using an RNeasy kits (Qiagen, Valencia, CA, USA) according to the manufacturer's directions. RNA will then be reverse transcribed to complementary DNA using standard commercial kits. Reverse transcription−PCRs will be performed using the Dynamo Sybr Green HS Master Mix (New England Biolabs, Ipswich, MA, USA) and custom primers will be synthesised by a commercial laboratory. Real-time reverse transcription−PCRs will be run using a Cepheid Smart Cycler II or similar instrument. Additional aliquots of serum and plasma will be stored so that other inflammatory markers can be tested in the future using Luminex bead arrays and/or additional available technologies.

Source of compounds tested

Minocycline and aspirin are available on a generic basis and are manufactured within the USA by several companies. The identity of the active medicines and placebos will be blinded using placebos that match the appearance of the active drugs. The medications and placebos have been formulated by Wedgewood Pharmacy (Swedesboro, New Jersey, USA). The study minocycline capsule and chewable aspirin tablet are identical in appearance to their corresponding placebos.

Outcome measures and data analysis

Antidepressant response will be evaluated by assessing changes in MADRS scores at assessment session 7 (ie, 6 weeks). Our a priori hypothesis is that minocycline and/or aspirin plus existing medication will exert greater antidepressant effects than placebo plus existing medication by study completion. Assuming that there are equal numbers of subjects in each treatment group, this hypothesis will be statistically assessed using a group (for the four treatment cells)-by-session (1 vs 7) repeated measures analysis of variance (ANOVA). If the ANOVA statistic is significant, between- and within-group t tests will be used in planned comparisons to identify the nature of the effect leading to the significant overall ANOVA statistic. We expect to find a significant group-by-session interaction, attributable to a greater reduction in MADRS scores in the minocycline and aspirin groups compared with the placebo group between session 1 and session 7. If there is an imbalance in the number of subjects across groups, (eg, due to differential dropout rates during the first treatment week), the data analysis will be conducted with a mixed-effects model.

A Mixed-Effect Model Repeated Measure (MMRM)[80] will be used to derive missing data points as this method has been shown to be superior to last observation carried forward which can inflate the type I error rates.[81] The last observation carried forward and observed cases approaches to data imputation will be used post hoc to provide further confirmation of the results obtained under the MMRM analysis.

In order to test whether the putative antidepressant effects of minocycline or aspirin have a rapid onset, as a post hoc analysis, the ANOVA will be repeated using MADRS ratings from the assessment that follows the first week of exposure to active drug versus the corresponding change under placebo; that is, session 2. Post hoc tests will be performed to assess the significance of changes in the secondary clinical outcome measures (QUIDS 16, HAM-A, YMRS, CGI-I).

The rate of completion in the four cells also will be considered an outcome measure. The completion rate

in the minocycline and/or aspirin arms may be influenced more by dropouts due to side effects, while the completion rate in the placebo group may be influenced more by dropouts due to non-response. Two different measures of completion rate will be obtained: completion of week 1 of the study (baseline to week 1) and completion of the study (baseline to week 6). Differences between the groups in completion rates will be assessed with a χ^2 test or a logistic regression.

We will test the hypothesis that minocycline and aspirin reduce inflammation (eg, CRP, IL-6, IL-6 mRNA) more than placebo using statistical analyses similar to those described above. If the assay results are normally distributed, then a group-by-session repeated measures ANOVA with CRP, IL-6 and nine other cytokine levels as dependent variables and BMI, smoking status and time of blood draw as covariates will be used to assess anti-inflammatory effects of minocycline and aspirin. Mixed-effect models will be used if necessary. If the CRP or inflammatory cytokine data are not normally distributed (Kolmogorov–Smirnov test) or if the equality of statistical variance assumption across assessments is violated (Levene's test), then Friedman's ANOVA will be used to test for CRP or inflammatory cytokine differences between groups. If the Friedman's ANOVA statistic is significant, Wilcoxon sign-ranked tests will be used for post hoc analysis of group differences. Non-specific factors that influence CRP and inflammatory cytokine levels include time of day, presence of infection, treatment with anti-inflammatory medications, smoking, obesity and alcohol abuse. We will attempt to control for these potential confounds by measuring BMI and recording NSAID and nicotine use (Universal Fagerstrom Scale) and by excluding individuals who have recently abused substances or who have intercurrent infections. The serum CRP concentration shows minimal diurnal variability in adults[82] but IL-6 and other cytokine levels vary across time of day.[83] To minimise cytokine measurement variability due to circadian fluctuations, we will schedule patient assessment sessions at the same time each day. Since this may not always be possible, we will record the time of day that each blood draw is made, divide the day into quartiles: 07:00–10:00, 10:00–12:00; 12:00–15:00 and 15:00–18:00 and use these data as a covariate in the statistical analyses.

To test whether baseline levels of CRP and inflammatory cytokines predict response to minocycline or aspirin, we will subclassify the participants using conventional criteria[84] as achieving full response (≥50% reduction in MADRS score from baseline), partial response (<50% but ≥25% reduction) or non-response (<25% reduction). Patients achieving remission (post-treatment MADRS score ≤10) will also be identified. A non-parametric alternative to the ANOVA statistic, the Mann–Whitney test, will be used to compare remitted and non-remitted groups in baseline levels of inflammatory cytokines and CRP if the data are not normally distributed. Ideally, the impact of baseline levels of inflammation on treatment response would be tested more rigorously using a formal stratified design. However, in order to conduct a stratified trial with, for example, eight experimental groups (4 × high versus low inflammation), the sample size of the study would have to be doubled, which would significantly increase costs and decrease feasibility. Nevertheless, this stratification approach would be important to consider for future studies if promising results are obtained in this clinical trial.

Statistical power

A recent meta-analysis of 96 antidepressant treatment studies found that the average effect size of a placebo treatment is 1.69 compared with 2.50 for an antidepressant treatment.[85] We calculated that in order to detect an effect size of 0.81 (ie, the difference between 2.50 and 1.69) with an 80% probability (two-sided test, α=0.05), we will require a sample size of 26 subjects per group (http://hedwig.mgh.harvard.edu/sample_size/size.html). This effect size may correspond to approximately three points on the MADRS. Thus, given our sample size of 30 per group, we should have sufficient power to test specific aim 1, allowing for a 13% dropout rate during week 1 of the study (dropouts after completion of study week 1 will be included in the analysis under the MMRM approach described above).

As discussed above, a recent meta-analysis[78] of cross-sectional studies of serum-derived IL-6 and CRP in depression calculated effect sizes of 0.71 for IL-6 and 0.26 for CRP. Based on these effect sizes, a sample size of 26 would yield >80% probability of detecting significant depression-related changes in IL-6 but only a 60% probability of detecting a depression-related change in CRP. There are three reasons why we believe that these CRP power estimations are not applicable to this study. First, the effect sizes derived from the meta-analysis are based on cross-sectional studies. Given the effect of variables such as smoking, diet, exercise and BMI on proinflammatory cytokines, a within-subjects design is likely to reduce non-depression-related sources of variance and substantially increase statistical power. Second, we are examining the effect of mood on IL-6 and CRP levels and are treating patients with minocycline and aspirin, drugs known to possess anti-inflammatory properties. We therefore suggest that our proposed study is likely adequately powered to detect any true changes in plasma IL-6, CRP and the other inflammatory cytokines across treatment blocks.

Regarding IL-6 mRNA gene expression in PBMs, Padmos *et al*[82] reported a 38-fold increase in IL-6 mRNA levels in unmedicated patients with BD compared with HC. Since minocycline reduces IL-6 levels (see above), we expect our study to have very high power to detect differences between groups, as well as changes in response to minocycline. The simultaneous detection of nine other inflammation-related cytokines, in addition to IL-6 (using newer more sensitive technology),

will provide much finer resolution of the effects on inflammatory cascades than that measured in previous studies.

ETHICS AND DISSEMINATION

Gender/minoritypaediatricc inclusion for research

Women and minorities will be included in the study without prejudice according to their representation in the study population. Participants will be recruited from the greater metropolitan areas of Tulsa, Oklahoma, and Wichita, Kansas, and efforts will be made to ensure that our subject population resembles the gender, ethnic and racial composition of these areas.

Exclusion criteria

The following exclusion criteria apply: (1) inability to provide informed consent; (2) age of onset of BD>40 years; (3) serious risk of suicide; (4) current delusions or hallucinations sufficient to interfere with the capacity to provide informed consent; (5) current manic symptoms (depressed BD patients with concurrent manic symptoms have been found to be more likely to experience adverse reactions in antidepressant treatment trials[86]); (6) medical illness including as hepatic impairment, renal dysfunction, bleeding diatheses (eg, hemophilia), cerebrovascular disease or heart disease, hypertension that is inadequately controlled by medication, diabetes mellitus or known peptic ulcer disease; (7) abuse of drugs or alcohol within the preceding 6 months or substance dependence within the last 5 years; (8) daily alcoholic beverage consumption equivalent to ≥3 oz. of alcohol; (9) asthma or known allergies or hypersensitivities to tetracycline antibiotics, aspirin or other NSAIDs; (10) current use of drugs that could increase the risks associated with aspirin or minocycline administration, namely other antibiotic medications, other NSAIDs or anticoagulants (eg, warfarin), acetazolamide or methotrexate; (11) known HIV or other chronic infection including but not limited to viral hepatitis and (12) pregnant or nursing women and women who are attempting to conceive during the 6-week study period will also be excluded.

Specimens, records and data collection

A physician, registered nurse or trained phlebotomist will utilise a sterile technique to draw 60 ml of blood by venipuncture. Participants will also be asked to submit a urine sample. A physician, registered nurse or trained technician will collect electrocardiogram data from the subject in a private exam room.

Recruitment and consent procedure

Volunteers will be recruited from the community as well as from the clinical services at the Laureate Psychiatric Clinic and Hospital and the Oklahoma University School of Community Medicine in Tulsa, Oklahoma, USA, and from the clinical services affiliated with the KUMCRI. Volunteers may be referred from sources that include physicians, newspaper advertising, self-help organisations, self-referral and WIRB-approved flyers posted at local universities, schools, churches and grocery stores. Participants may be prescreened through screening protocols based at LIBR or KUCRI. We plan to recruit a total of 120 participants.

All participant interactions including consenting will be conducted in private interview/exam rooms. These rooms are secured from public areas via combination locked doors that are only accessible to authorised personnel. Prospective participants will receive an explanation of the objectives, procedures and hazards of this protocol that is appropriate to their level of understanding. The right of the subject to decline to participate or to withdraw from the study at any time will be made clear.

Non-English speaking participants will not be recruited.

After the consent form is verbally explained to the participant and any questions have been answered, the researcher will leave the room to allow the participant to read the consent form thoroughly. Family members will be allowed to be present and to discuss the consenting process with the participant. After the consent is read, the researcher will return and answer any additional questions the participant may have. The researcher will remind the subject that participation is strictly voluntary and that they have the right to withdraw at any time. Participants will be asked to arrive 30 min early in order to have sufficient time for the consenting process.

Subject risks

The risks of behavioural testing are minimal. The risks of blood drawing are also minimal. Possible mild side effects of the blood draw include mild pain or bruising at the site of the venipuncture.

Minocycline has been used a broad-spectrum antibiotic for many years in doses up to 400 mg/day.[34] It has been used on a chronic basis to treat acne and RA, often for many years, in hundreds of thousands of patients. The most commonly encountered side effects are upset stomach, diarrhoea, dizziness, drowsiness, ataxia, vertigo, headache and vomiting. Prolonged use can be associated with pigmentation of the skin, gums or teeth. Between 1975 and 2006, the WHO Collaborating Center for International Drug Monitoring listed 122 cases of adverse drug reactions to intravenous minocycline; most commonly, abnormal hepatic function and thrombocytopaenia.[34] These included cases of serious liver injury, including irreversible drug-induced hepatitis and fulminant hepatic failure that was fatal in two cases, thought to be due to triggering or unmasking autoimmune hepatitis. One case of autoimmune-related glomerulonephritis has been reported. The role of oral minocycline in precipitating these conditions has not been clearly established. Minocycline also has been associated with idiopathic intracranial hypertension (pseudotumor cerebri). Long-term trials have shown that minocycline is well tolerated. In a 2-year trial of minocycline (200 mg/

day) for RA, three of 30 patients withdrew due to fingernail discolouration, dizziness or erythematous rash.[54] Of 11 patients with HD treated with minocycline (100 mg/day) for 2 years, one complained of nausea in the first 3 weeks and two of sedation,[53] while in a 6-month trial of minocycline for amyotrophic lateral sclerosis, the mean tolerated dose was 387 mg/day and the most common adverse effects were GI.[87] Five of 36 patients with schizophrenia withdrew from a 6-month trial of minocycline (200 mg/day) due to indigestion (n=2), pigmentation (n=2) or a suicide attempt (n=1).[58]

Low-dose aspirin has been safely used in many millions of patients on a worldwide scale for its role as an antithrombotic and thrombolytic. A meta-analysis of >100 randomised trials in high-risk patients indicated that low-dose ASA reduced CV death by 15% and prevented non-fatal vascular events by about 30%.[88] These data stand in striking contrast to the data obtained in COX-2 inhibitors, which can increase CV risk. In clinical trials of several COX-2 selective and non-selective NSAIDs of up to 3 years duration have shown an increased risk of serious CV thrombotic events, myocardial infarction and stroke, which have in many cases been fatal.[89] Patients with known CV disease or risk factors for CV disease are at greater risk for such events during chronic treatment with COX-2 inhibitors. Evidence from human pharmacology and genetics, genetically manipulated rodents and other animal models and randomised trials indicates that this is consequent to suppression of COX-2-dependent cardioprotective prostaglandins, particularly prostacyclin.[90]

Aspirin does not cause a generalised bleeding abnormality unless given to patients with an underlying haemostatic defect (eg, haemophilia, uraemia or that induced by anticoagulant therapy). Aspirin-induced impairment of primary haemostasis cannot be separated from its antithrombotic effect and is similar at all doses ≥75 mg/day.[91] The risk of intracranial bleeding is exceedingly rare (<0.1% in high-risk populations) but is higher in individuals with cerebrovascular disease.[88] Hypertension that is inadequately controlled by medication often is considered a contraindication to aspirin because of the concern that possible benefits in the prevention of CV events may be counterbalanced by an increased risk of cerebral bleeding. However, hypertensive patients whose blood pressure is well controlled appear protected from myocardial infarction by aspirin therapy without an increase in the number of cerebral haemorrhages or strokes.[92] Moreover, aspirin therapy does not affect blood pressure or the response of hypertension to antihypertensive agents.[91 93]

NSAIDs as a class can cause serious GI adverse events including inflammation, bleeding, ulceration and perforation of the stomach, small intestine or large intestine, which rarely have proven fatal. In controlled clinical trials, the percentage of patients reporting one or more GI complaints has ranged from 4% to 16%.[91] The mechanism underlying this adverse effect appears attributable to the inhibition of COX-1. Thus, the incidence of GI side effects has been higher for NSAIDs with more potent effects at COX-1, such as aspirin and indomethicin. For example, in controlled trials, the incidence of GI side effects for aspirin and indomethacin has been about twice as high as that for ibuprofen, a non-selective COX inhibitor, in equally effective doses for arthritis. Nevertheless, the incidence of GI side effects associated with aspirin is dose dependent and thus is markedly lower when using aspirin in the low-dose range planned for the current study. Notably, the risk of GI bleeding is not reduced by using the enterically coated aspirin formulations but is thought to be lower during concomitant use of omeprazole.[91] The effects of warfarin and NSAIDs on GI bleeding are synergistic such that the users of both drugs together have a risk of serious GI bleeding higher than users of either drug alone. Fortunately, the risk of GI bleeding, which reflects the inhibition of prostaglandins in the stomach (from systemic rather than local exposure), is much smaller when using low-dose as opposed to high-dose aspirin.

Low-dose aspirin has not been reported to alter renal function and does not reduce effectiveness of ACE inhibitors for HTN (in contrast to other NSAIDs).[93 94] However, aspirin can inhibit the renal clearance of acetazolamide and methotrexate potentially leading to increased blood concentrations of and toxicity from these agents. Salicylate can displace other drugs which are protein bound, especially phenytoin and valproic acid, increasing their free drug concentrations in plasma. This may increase side effects, toxicity and/or efficacy for displaced drugs. If the BD subjects are currently receiving valproic acid preparations (eg, divalproex), then the plasma levels of these agents will be monitored for potential changes.

Aspirin may cause a severe allergic reaction that may include hives, asthma (wheezing), facial swelling and shock. Aspirin overdose can be fatal at 30 g or higher.

In sum, we believe that our 2×2 design is appropriate for trials involving experimental drugs that already have been well studied with respect to toxicity, as is the case with aspirin and minocycline. A parallel arm design, as opposed to a 2×2 factorial design, would be more clearly informative in the case of an experimental drug for which the toxicity and drug interaction potential have not been thoroughly studied in human subjects.

Protected Health Information protection

Paper copies of consents, screening forms, the Research Privacy Form and any other forms, testing results or papers containing Protected Health Information (PHI), will be stored in a secured medical records room with access granted only to authorised personnel.

Electronic data that contain PHI will be managed in accordance with ISO 27000 series information security standards with policies developed from current NIST guidelines (SP 800-66) for HIPAA and HITECH

compliance. Specific controls implemented to protect PHI are derived from NIST 800-122 and include (but not limited to) the following:

1. Access Enforcement (AC-3)—Individual user accounts, role based access control, access control lists;
2. Separation of Duties (AC-5)—de-identification of data as appropriate, acquire/analyze/manage firewall;
3. Least Privilege (AC-6)—to ensure PHI data is only available to persons with established need for access;
4. Remote Access (AC-17)—Secure VPN, encrypted end devices;
5. Access Control for Mobile Devices (AC-19)—Password login, remote destruction capabilities;
6. Auditable Events (AU-2) + Monitoring: Log detailed server and network information, alert for problems;
7. Analysis, and Reporting (AU-6)—Procedures to audit system records for inappropriate activity.
8. User Identification and Authentication (IA-2)—username/secure password and two factor authentication will be required when appropriate.
9. Media Access, Marking, Storage, and Transport (MP-2,3,4,5)—Records will be asset tagged and marked to their PHI status, PHI data will be secured and managed by professional system administrators and will be transported via encryption (VPN, USB, File);
10. Media Sanitization (MP-6)—Data will be destroyed by SFHS in accordance with their policies and procedures;
11. Transmission Confidentiality (SC-9)—Encryption will be used when needed for all avenues of data transmission (wireless, network, etc).

To protect subject confidentiality, blood samples will be anonymized as follows:

1. Last name: All participants will be assigned the last name 'LIBR.'
2. First name: The first name will be a secure alpha cryptographic hash based on LIBR user ID. This technique is the gold standard in computer security for one-way correlation of data.

Benefits versus risks

The participant may benefit from participation if either study drug produces an antidepressant effect. Participants will also receive a free clinical evaluation; more frequent treatment visits than are typical in practice, diligent follow-up in terms of symptoms and side effects and physical and psychiatric monitoring during the study. The risks of delaying alternative treatments are minimal in relation to the potential long-term benefits to the subjects and the importance of knowledge that may reasonably result. The importance of the knowledge that will likely be gained from this study clearly exceeds the associated potential risks.

Alternative treatment

It is possible that some patients may feel better with talk therapy. Participating in any type of talk therapy with their psychiatrist or psychologist does not require dropping out of this study. Subjects will be encouraged to contact the study investigators, particularly the physician in the study, with any questions they may have regarding alternatives to treatment through this research study. The study investigators will assist in referring the subject to another physician for treatment after their participation in the study has ended.

Physical and psychological testing, blood draws, urine samples and electrocardiogram data provide no known risks to persons other than those listed in the exclusion criteria, whereas the combinatory power of these measures may provide information relevant to understanding the pathophysiology of BD.

Data and safety monitoring plan

This study involves more than minimal risk. The study progress will be overseen by a Data, Safety and Monitoring Board. The Data, Safety and Monitoring Board is composed of three members who will meet in person or per telephone at least once every 6 months to review relevant study data including adverse events and dropout rates.

Any unanticipated adverse events will be reported immediately to the IRB of record and to the LIBR Human Protection Administrator. Any adverse events will be included in the annual IRB report.

Dissemination of results

The study results will be presented at national and/or international biomedical scientific meetings and published in peer-reviewed journals.

REGISTRATION

In accordance with the recommendations of the International Committee of Medical Journal Editors,[95] the proposed trial is registered in a public registry (http://www.clinicaltrials.gov/, Identifier: NCT01429272).

Author affiliations
[1]Laureate Institute for Brain Research (LIBR), Tulsa, Oklahoma, USA
[2]Department of Medicine, Tulsa School of Community Medicine, University of Tulsa, Tulsa, Oklahoma, USA
[3]University of Kansas Clinical Research Institute, Wichita, Kansas, USA
[4]Department of Surgery, University of Oklahoma College of Medicine, Tulsa, Oklahoma, USA
[5]Department of Psychiatry, University of Oklahoma College of Medicine, Tulsa, Oklahoma, USA
[6]Department of Pharmaceutical Sciences, University of Oklahoma College of Pharmacy, Tulsa, Oklahoma, USA
[7]Department of Microbiology and Biochemistry, Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma, USA
[8]Department of Medicine, Oklahoma University Health Sciences Center, and the Oklahoma City VAMC, Oklahoma City, Oklahoma, USA

Contributors All authors made a significant contribution to the conception and design of the study protocol. The protocol was written by JS and WD and was critically reviewed by SP, KT, DD and WY. All authors gave approval for the publication.

Funding This work was funded by the Stanley Medical Research Institute, grant number 10T-1401. The SMRI assisted with the study design but plays no role in the collection, management, analysis and interpretation of data; writing of the report or the decision to submit the report for publication.

Competing interests None.

Patient consent Obtained.

Ethics approval Ethics approval was provided by Western Institutional Review Board.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. Correa R, Akiskal H, Gilmer W, *et al*. Is unrecognized bipolar disorder a frequent contributor to apparent treatment resistant depression? *J Affect Disord* 2010;127:10–18.
2. Tohen M, Vieta E, Calabrese J, *et al*. Efficacy of olanzapine and olanzapine-fluoxetine combination in the treatment of bipolar I depression. *Arch Gen Psychiatry* 2003;60:1079–88.
3. Nierenberg AA, Ostacher MJ, Calabrese JR, *et al*. Treatment-resistant bipolar depression: a STEP-BD equipoise randomized effectiveness trial of antidepressant augmentation with lamotrigine, inositol, or risperidone. *Am J Psychiatry* 2006;163:210–16.
4. Nemeroff CB, Evans DL, Gyulai L, *et al*. Double-blind, placebo-controlled comparison of imipramine and paroxetine in the treatment of bipolar depression. *Am J Psychiatry* 2001;158:906–12.
5. Mallinger AG, Frank E, Thase ME, *et al*. Revisiting the effectiveness of standard antidepressants in bipolar disorder: are monoamine oxidase inhibitors superior? *Psychopharmacol Bull* 2009;42:64–74.
6. Himmelhoch JM, Thase ME, Mallinger AG, *et al*. Tranylcypromine versus imipramine in anergic bipolar depression. *Am J Psychiatry* 1991;148:910–16.
7. Thase ME, Mallinger AG, McKnight D, *et al*. Treatment of imipramine-resistant recurrent depression, IV: a double-blind crossover study of tranylcypromine for anergic bipolar depression. *Am J Psychiatry* 1992;149:195–8.
8. Savitz J, Drevets WC. Bipolar and major depressive disorder: neuroimaging the developmental-degenerative divide. *Neurosci Biobehav Rev* 2009;33:699–771.
9. Savitz JB, Drevets WC. Imaging phenotypes of major depressive disorder: genetic correlates. *Neuroscience* 2009;164:300–30.
10. Wang Y, Qin ZH. Molecular and cellular mechanisms of excitotoxic neuronal death. *Apoptosis* 2010;15:1382–402.
11. Mitchell ND, Baker GB. An update on the role of glutamate in the pathophysiology of depression. *Acta Psychiatr Scand* 2010;122:192–210.
12. Ryan B, Musazzi L, Mallei A, *et al*. Remodelling by early-life stress of NMDA receptor-dependent synaptic plasticity in a gene-environment rat model of depression. *Int J Neuropsychopharmacol* 2009;12:553–9.
13. Boyce-Rustay JM, Holmes A. Genetic inactivation of the NMDA receptor NR2A subunit has anxiolytic- and antidepressant-like effects in mice. *Neuropsychopharmacology* 2006;31:2405–14.
14. Tsunoka T, Kishi T, Ikeda M, *et al*. Association analysis of group II metabotropic glutamate receptor genes (GRM2 and GRM3) with mood disorders and fluvoxamine response in a Japanese population. *Prog Neuropsychopharmacol Biol Psychiatry* 2009;33:875–9.
15. Diazgranados N, Ibrahim L, Brutsche NE, *et al*. A randomized add-on trial of an N-methyl-D-aspartate antagonist in treatment-resistant bipolar depression. *Arch Gen Psychiatry* 2010;67:793–802.
16. Zarate CA Jr, Payne JL, Quiroz J, *et al*. An open-label trial of riluzole in patients with treatment-resistant major depression. *Am J Psychiatry* 2004;161:171–4.
17. Dowlati Y, Herrmann N, Swardfager W, *et al*. A meta-analysis of cytokines in major depression. *Biol Psychiatry* 2010;67:446–57.
18. Pace TW, Miller AH. Cytokines and glucocorticoid receptor signaling. Relevance to major depression. *Ann N Y Acad Sci* 2009;1179:86–105.
19. Maes M, Scharpe S, Van Grootel L, *et al*. Higher alpha 1-antitrypsin, haptoglobin, ceruloplasmin and lower retinol binding protein plasma levels during depression: further evidence for the existence of an inflammatory response during that illness. *J Affect Disord* 1992;24:183–92.
20. Motivala SJ, Sarfatti A, Olmos L, *et al*. Inflammatory markers and sleep disturbance in major depression. *Psychosom Med* 2005;67:187–94.
21. Song C, Dinan T, Leonard BE. Changes in immunoglobulin, complement and acute phase protein levels in the depressed patients and normal controls. *J Affect Disord* 1994;30:283–8.
22. Tyring S, Gottlieb A, Papp K, *et al*. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. *Lancet* 2006;367:29–35.
23. Drexhage RC, Knijff EM, Padmos RC, *et al*. The mononuclear phagocyte system and its cytokine inflammatory networks in schizophrenia and bipolar disorder. *Expert Rev Neurother* 2010;10:59–76.
24. Leonard BE. The immune system, depression and the action of antidepressants. *Prog Neuropsychopharmacol Biol Psychiatry* 2001;25:767–80.
25. Dantzer R, O'Connor JC, Freund GG, *et al*. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* 2008;9:46–56.
26. Maes M. Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2011;35:664–75.
27. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* 2009;65:732–41.
28. Pariante CM, Pearce BD, Pisell TL, *et al*. The proinflammatory cytokine, interleukin-1alpha, reduces glucocorticoid receptor translocation and function. *Endocrinology* 1999;140:4359–66.
29. Ongur D, Drevets WC, Price JL. Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc Natl Acad Sci U S A* 1998;95:13290–5.
30. Raison CL, Dantzer R, Kelley KW, *et al*. CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. *Mol Psychiatry* 2010;15:393–403.
31. Gabbay V, Klein RG, Katz Y, *et al*. The possible role of the kynurenine pathway in adolescent depression with melancholic features. *J Child Psychol Psychiatry* 2010;51:935–43.
32. Padmos RC, Hillegers MH, Knijff EM, *et al*. A discriminating messenger RNA signature for bipolar disorder formed by an aberrant expression of inflammatory genes in monocytes. *Arch Gen Psychiatry* 2008;65:395–407.
33. Zemke D, Majid A. The potential of minocycline for neuroprotection in human neurologic disease. *Clin Neuropharmacol* 2004;27:293–8.
34. Elewa HF, Hilali H, Hess DC, *et al*. Minocycline for short-term neuroprotection. *Pharmacotherapy* 2006;26:515–21.
35. Hailer NP. Immunosuppression after traumatic or ischemic CNS damage: it is neuroprotective and illuminates the role of microglial cells. *Prog Neurobiol* 2008;84:211–33.
36. Pae CU, Marks DM, Han C, *et al*. Does minocycline have antidepressant effect? *Biomed Pharmacother* 2008;62:308–11.
37. Wang J, Wei Q, Wang CY, *et al*. Minocycline up-regulates Bcl-2 and protects against cell death in mitochondria. *J Biol Chem* 2004;279:19948–54.
38. Chen G, Zeng WZ, Yuan PX, *et al*. The mood-stabilizing agents lithium and valproate robustly increase the levels of the neuroprotective protein bcl-2 in the CNS. *J Neurochem* 1999;72:879–82.
39. Kosten TA, Galloway MP, Duman RS, *et al*. Repeated unpredictable stress and antidepressants differentially regulate expression of the bcl-2 family of apoptotic genes in rat cortical, hippocampal, and limbic brain structures. *Neuropsychopharmacology* 2008;33:1545–58.
40. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol* 2008;9:47–59.
41. Bailey MT, Dowd SE, Galley JD, *et al*. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain Behav Immun* 2011;25:397–407.
42. Tikka T, Fiebich BL, Goldsteins G, *et al*. Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. *J Neurosci* 2001;21:2580–8.
43. Cornet S, Spinnewyn B, Delaflotte S, *et al*. Lack of evidence of direct mitochondrial involvement in the neuroprotective effect of minocycline. *Eur J Pharmacol* 2004;505:111–19.
44. Yrjanheikki J, Keinanen R, Pellikka M, *et al*. Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc Natl Acad Sci U S A* 1998;95:15769–74.
45. Sanchez Mejia RO, Ona VO, Li M, *et al*. Minocycline reduces traumatic brain injury-mediated caspase-1 activation, tissue damage, and neurological dysfunction. *Neurosurgery* 2001;48:1393–9; discussion 99–401.
46. Bilousova TV, Dansie L, Ngo M, *et al*. Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. *J Med Genet* 2009;46:94–102.
47. Stirling DP, Khodarahmi K, Liu J, *et al*. Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury. *J Neurosci* 2004;24:2182–90.
48. Zhang L, Shirayama Y, Shimizu E, *et al*. Protective effects of minocycline on 3,4-methylenedioxymethamphetamine-induced neurotoxicity in serotonergic and dopaminergic neurons of mouse brain. *Eur J Pharmacol* 2006;544:1–9.

49. Greenwald RA, Moak SA, Ramamurthy NS, *et al*. Tetracyclines suppress matrix metalloproteinase activity in adjuvant arthritis and in combination with flurbiprofen, ameliorate bone damage. *J Rheumatol* 1992;19:927–38.
50. Brundula V, Rewcastle NB, Metz LM, *et al*. Targeting leukocyte MMPs and transmigration: minocycline as a potential therapy for multiple sclerosis. *Brain* 2002;125:1297–308.
51. Zhu S, Stavrovskaya IG, Drozda M, *et al*. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature* 2002;417:74–8.
52. Wang X, Zhu S, Drozda M, *et al*. Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. *Proc Natl Acad Sci U S A* 2003;100:10483–7.
53. Bonelli RM, Hodl AK, Hofmann P, *et al*. Neuroprotection in Huntington's disease: a 2-year study on minocycline. *Int Clin Psychopharmacol* 2004;19:337–42.
54. O'Dell JR, Blakely KW, Mallek JA, *et al*. Treatment of early seropositive rheumatoid arthritis: a two-year, double-blind comparison of minocycline and hydroxychloroquine. *Arthritis Rheum* 2001;44:2235–41.
55. Lampl Y, Boaz M, Gilad R, *et al*. Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. *Neurology* 2007;69:1404–10.
56. Miyaoka T, Yasukawa R, Yasuda H, *et al*. Possible antipsychotic effects of minocycline in patients with schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 2007;31:304–7.
57. Miyaoka T, Yasukawa R, Yasuda H, *et al*. Minocycline as adjunctive therapy for schizophrenia: an open-label study. *Clin Neuropharmacol* 2008;31:287–92.
58. Levkovitz Y, Mendlovich S, Riwkes S, *et al*. A double-blind, randomized study of minocycline for the treatment of negative and cognitive symptoms in early-phase schizophrenia. *J Clin Psychiatry* 2010;71:138–49.
59. Molina-Hernandez M, Tellez-Alcantara NP, Perez-Garcia J, *et al*. Antidepressant-like actions of minocycline combined with several glutamate antagonists. *Prog Neuropsychopharmacol Biol Psychiatry* 2008;32:380–6.
60. Molina-Hernandez M, Tellez-Alcantara NP, Perez-Garcia J, *et al*. Desipramine or glutamate antagonists synergized the antidepressant-like actions of intra-nucleus accumbens infusions of minocycline in male Wistar rats. *Prog Neuropsychopharmacol Biol Psychiatry* 2008;32:1660–6.
61. O'Connor JC, Lawson MA, Andre C, *et al*. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol Psychiatry* 2009;14:511–22.
62. Levine J, Cholestoy A, Zimmerman J. Possible antidepressant effect of minocycline. *Am J Psychiatry* 1996;153:582.
63. Cipollone F, Patrignani P, Greco A, *et al*. Differential suppression of thromboxane biosynthesis by indobufen and aspirin in patients with unstable angina. *Circulation* 1997;96:1109–16.
64. Choi SH, Aid S, Bosetti F. The distinct roles of cyclooxygenase-1 and -2 in neuroinflammation: implications for translational research. *Trends Pharmacol Sci* 2009;30:174–81.
65. Choi SH, Aid S, Choi U, *et al*. Cyclooxygenases-1 and -2 differentially modulate leukocyte recruitment into the inflamed brain. *Pharmacogenomics J* 2010;10:448–57.
66. Choi SH, Bosetti F. Cyclooxygenase-1 null mice show reduced neuroinflammation in response to beta-amyloid. *Aging (Albany NY)* 2009;1:234–44.
67. Bosetti F, Weerasinghe GR, Rosenberger TA, *et al*. Valproic acid down-regulates the conversion of arachidonic acid to eicosanoids via cyclooxygenase-1 and -2 in rat brain. *J Neurochem* 2003;85:690–6.
68. Weerasinghe GR, Rapoport SI, Bosetti F. The effect of chronic lithium on arachidonic acid release and metabolism in rat brain does not involve secretory phospholipase A2 or lipoxygenase/cytochrome P450 pathways. *Brain Res Bull* 2004;63:485–9.
69. Ramadan E, Basselin M, Rao JS, *et al*. Lamotrigine blocks NMDA receptor-initiated arachidonic acid signalling in rat brain: implications for its efficacy in bipolar disorder. *Int J Neuropsychopharmacol* 2011:1–13.
70. Stolk P, Souverein PC, Wilting I, *et al*. Is aspirin useful in patients on lithium? A pharmacoepidemiological study related to bipolar disorder. *Prostaglandins Leukot Essent Fatty Acids* 2010;82:9–14.
71. Phelan KM, Mosholder AD, Lu S. Lithium interaction with the cyclooxygenase 2 inhibitors rofecoxib and celecoxib and other nonsteroidal anti-inflammatory drugs. *J Clin Psychiatry* 2003;64:1328–34.
72. Mendlewicz J, Kriwin P, Oswald P, *et al*. Shortened onset of action of antidepressants in major depression using acetylsalicylic acid augmentation: a pilot open-label study. *Int Clin Psychopharmacol* 2006;21:227–31.
73. Ketterer MW, Brymer J, Rhoads K, *et al*. Is aspirin, as used for antithrombosis, an emotion-modulating agent? *J Psychosom Res* 1996;40:53–8.
74. Nery FG, Monkul ES, Hatch JP, *et al*. Celecoxib as an adjunct in the treatment of depressive or mixed episodes of bipolar disorder: a double-blind, randomized, placebo-controlled study. *Hum Psychopharmacol* 2008;23:87–94.
75. Muller N, Schwarz MJ, Dehning S, *et al*. The cyclooxygenase-2 inhibitor celecoxib has therapeutic effects in major depression: results of a double-blind, randomized, placebo controlled, add-on pilot study to reboxetine. *Mol Psychiatry* 2006;11:680–4.
76. Akhondzadeh S, Jafari S, Raisi F, *et al*. Clinical trial of adjunctive celecoxib treatment in patients with major depression: a double blind and placebo controlled trial. *Depress Anxiety* 2009;26:607–11.
77. Maier SF. Bi-directional immune-brain communication: implications for understanding stress, pain, and cognition. *Brain Behav Immun* 2003;17:69–85.
78. Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med* 2009;71:171–86.
79. Renner U, De Santana EC, Gerez J, *et al*. Intrapituitary expression and regulation of the gp130 cytokine interleukin-6 and its implication in pituitary physiology and pathophysiology. *Ann N Y Acad Sci* 2009;1153:89–97.
80. Mallinckrodt CH, Clark WS, David SR. Accounting for dropout bias using mixed-effects models. *J Biopharm Stat* 2001;11:9–21.
81. Siddiqui O, Hung HM, O'Neill R. MMRM vs. LOCF: a comprehensive comparison based on simulation study and 25 NDA datasets. *J Biopharm Stat* 2009;19:227–46.
82. Meier-Ewert HK, Ridker PM, Rifai N, *et al*. Absence of diurnal variation of C-reactive protein concentrations in healthy human subjects. *Clin Chem* 2001;47:426–30.
83. Vgontzas AN, Bixler EO, Lin HM, *et al*. IL-6 and its circadian secretion in humans. *Neuroimmunomodulation* 2005;12:131–40.
84. Nierenberg AA, DeCecco LM. Definitions of antidepressant treatment response, remission, nonresponse, partial response, and other relevant outcomes: a focus on treatment-resistant depression. *J Clin Psychiatry* 2001;62(Suppl 16):5–9.
85. Rief W, Nestoriuc Y, Weiss S, *et al*. Meta-analysis of the placebo response in antidepressant trials. *J Affect Disord* 2009;118:1–8.
86. Goldberg JF, Perlis RH, Ghaemi SN, *et al*. Adjunctive antidepressant use and symptomatic recovery among bipolar depressed patients with concomitant manic symptoms: findings from the STEP-BD. *Am J Psychiatry* 2007;164:1348–55.
87. Gordon PH, Moore DH, Gelinas DF, *et al*. Placebo-controlled phase I/II studies of minocycline in amyotrophic lateral sclerosis. *Neurology* 2004;62:1845–7.
88. Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ* 2002;324:71–86.
89. Fries S, Grosser T. The cardiovascular pharmacology of COX-2 inhibition. *Hematology Am Soc Hematol Educ Program* 2005:445–51.
90. Grosser T, Yu Y, Fitzgerald GA. Emotion recollected in tranquility: lessons learned from the COX-2 saga. *Annu Rev Med* 2010;61:17–33.
91. Patrono C, Coller B, FitzGerald GA, *et al*. Platelet-active drugs: the relationships among dose, effectiveness, and side effects: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 2004;126(3 Suppl):234S–64S.
92. Hansson L, Zanchetti A, Carruthers SG, *et al*. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT Study Group. *Lancet* 1998;351:1755–62.
93. Zanchetti A, Hansson L, Leonetti G, *et al*. Low-dose aspirin does not interfere with the blood pressure-lowering effects of antihypertensive therapy. *J Hypertens* 2002;20:1015–22.
94. Teo KK, Yusuf S, Pfeffer M, *et al*. Effects of long-term treatment with angiotensin-converting-enzyme inhibitors in the presence or absence of aspirin: a systematic review. *Lancet* 2002;360:1037–43.
95. De Angelis C, Drazen JM, Frizelle FA, *et al*. Clinical trial registration: a statement from the international Committee of medical journal Editors. *N Engl J Med* 2004;351:1250–1.

Minocycline and aspirin in the treatment of bipolar depression: a protocol for a proof-of-concept, randomised, double-blind, placebo-controlled, 2×2 clinical trial

Jonathan Savitz, Sheldon Preskorn, T Kent Teague, et al.

BMJ Open 2012 2:
doi: 10.1136/bmjopen-2011-000643

Notes