Neurology, Psychiatry and Brain Research 32 (2019) 22–29

Contents lists available at ScienceDirect

Neurology, Psychiatry and Brain Research

journal homepage: www.elsevier.com/locate/npbr

Celecoxib augmentation of escitalopram in treatment-resistant bipolar depression and the effects on Quinolinic Acid

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ARTICLE INFO

Keywords:
Bipolar depression
Treatment-resistance
Inflammation
Celecoxib
Kynurenines
Quinolinic acid

ABSTRACT

Objectives: Treatment-resistance is high in bipolar disorder and is associated with a pro-inflammatory state and diversion of tryptophan toward the kynurenine pathway. This study as part of a large clinical trial, sought to determine, if modulation of the inflammatory response by inhibiting cyclooxygenase-2 (COX-2) with celecoxib combined with escitalopram, would convert treatment-resistant bipolar depression to response or remission and whether blood levels of quinolinic acid (QA) differ from healthy controls and change with treatment response.
Methods: This was a randomized, double-blind, two-arm, placebo-controlled study. Subjects who met study criteria were randomized to receive escitalopram + celecoxib, or escitalopram + placebo. Inflammation biomarkers and kynurenine pathway intermediates were determined at baseline and weeks 4 and 8.
Results: Patients receiving the celecoxib combination showed improved response and higher remission rate. All patients had significantly lower QA levels at baseline compared to healthy controls. QA values did not change significantly over time, but a downtrend was noted through treatment. Responders had marginally lower QA values than non-responders. Factors that might have led to low QA levels may include prior exposure to a variety of psychoactive agents.
Conclusions: Although QA did not significantly change, symptom reduction and remission occurred more frequently in the celecoxib group, demonstrating the beneficial effect of inflammation modulation.

1. Introduction

Bipolar disorder (BD) is a serious mental illness characterized by periods of depression and manic or hypomanic episodes, with a life prevalence of 4.4%, according to the National Institute of Mental Health based on the National Comorbidity Survey. It is a complex disorder with a range of presentations causing unusual shifts in cognition, mood, behavior, energy, activity levels, and diminished ability to carry out day-to-day tasks. The depressive phase of BD can impair the individual's quality of life to a significant degree. In bipolar disorder type I (BD I), depressive symptoms occur in 31.9% of the weeks the patient experiences symptoms, while manic/hypomanic symptoms occur in 8.9% of the weeks the patient is symptomatic (Judd et al., 2002). In bipolar disorder type II (BD II), depressive symptoms occur in 50.3% of the weeks the patient experiences symptoms, and hypomanic/manic symptoms in 1.3% of the weeks the patient is symptomatic (Judd et al., 2003). Amongst 258 bipolar patients admitted from 1996 to 1999 to the Stanley Foundation Bipolar Network, 25% were ill with depressive symptoms averaging 214 days/year while 40% were ill with depressive symptoms averaging 120 days/year (Post, Denicoff et al., 2003). Treatment for the depressive phase of BD poses a greater challenge than for the manic phase (Post, Leverich et al., 2003). The suicide rate is highest in patients with BD, occurring most frequently after severe and recurrent depressive episodes referred to as mixed affective states (Tondo, Isacsson, & Baldessarini, 2003). Patients with bipolar depression generally respond more poorly than patients with major depression, and the non-response rate to anti-depressant therapy is 1.6 times higher in bipolar depressed patients. Failure to respond during antidepressant therapy is 3.4 times more common in bipolar compared to unipolar depressed patients (Ghaemi et al., 2004).

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https://doi.org/10.1016/j.npbr.2019.03.005
Received 15 January 2019; Received in revised form 13 March 2019; Accepted 18 March 2019
Available online 23 March 2019
0941-9500/

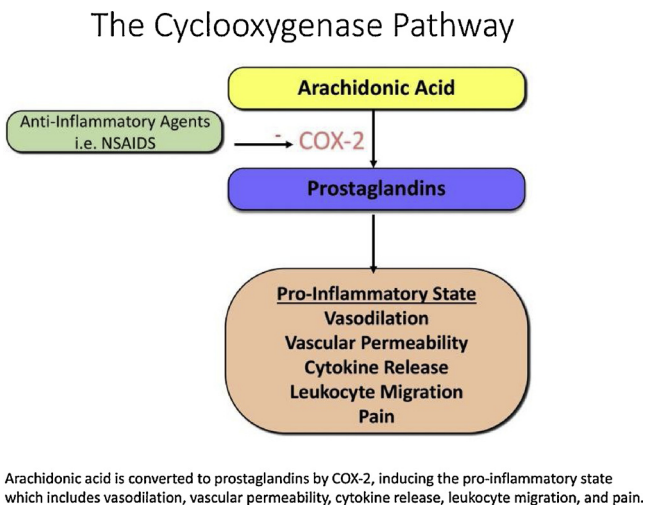

Fig. 1. The Cyclooxygenase Pathway.

Both unipolar and bipolar depression are known for the high number of non-responders, partial responders and non-remitters indicating an urgent need to seek innovative treatment strategies with pharmacologic interventions beyond the monoamine theory. The sustained pro-inflammatory status found in a high percentage of subjects suffering from unipolar or bipolar depression may delay or diminish the antidepressant response. The chronic subthreshold pro-inflammatory status in bipolar patients, especially in those whose treatment-resistance is associated with dysregulation of tryptophan metabolism favoring kynurenine pathway intermediates, may interfere with antidepressant drug effectiveness. In the cyclooxygenase (COX) pathway, arachidonic acid is converted to prostaglandins by COX-2, inducing the pro-inflammatory state which includes vasodilation, vascular permeability, cytokine release, leukocyte migration, and pain (Fig. 1). Therefore, we hypothesized that a non-steroidal anti-inflammatory (NSAID) agent, such as celecoxib (CBX), that selectively inhibits COX-2 should attenuate or even reverse the pro-inflammatory status and thereby normalize kynurenine pathway intermediates and improve treatment outcome.

Escitalopram (ESC) has been shown to be an effective treatment in bipolar depression (Fonseca, Soares, Hatch, Santin, & Kapczinski, 2006). It was associated with significant improvement as measured by the HAM-D total score, which showed a mean reduction from baseline to endpoint of 12 points, suggesting that ESC in association with mood stabilizers can be an effective and reasonably well-tolerated treatment for patients with moderate-to-severe bipolar depression. The switch to mania rate was similar to what is described in the literature for SSRI's (Fonseca et al., 2006).

Tryptophan metabolism and the kynurenine pathway have been implicated in the pathophysiology of depression (Anderson, Jacob, Bellivier, & Geoffroy, 2016; Myint & Kim, 2003) (Fig. 2). The pro-inflammatory state in mood disorders is believed to lead to an imbalance in this pathway and contribute to the etiopathology of bipolar disorders (Kim, Jung, Myint, Kim, & Park, 2007). Quinolinic acid (QA) is a metabolite of the kynurenine pathway and a NMDA-R agonist (Bender & McCreanor, 1985). Accumulation of QA likely results in excitotoxic neurodegenerative changes (Guillemin, 2012), and Hoekstra et al. (2006) have demonstrated NMDA-R involvement in manic relapse in bipolar I disorder. We measured kynurenine pathway intermediates as part of a comprehensive project on treatment resistant bipolar depression. In this publication we present the findings pertaining to QA, given the emphasis this compound has received in the world literature. The complete presentation of the clinical data from this clinical trial will be published elsewhere.

2. Materials and methods

2.1. Study population

The study was approved by the Institutional Review Board of Loyola University Medical Center and the Review Board of the Medical Faculty of the LMU and was conducted according to the principles of the Declaration of Helsinki. Potential candidates were screened to determine eligibility for the study by meeting inclusion criteria and their capability of understanding the study and giving informed consent. Males and females between the ages of 21 and 65 years who met DSM-IV criteria for bipolar disorder (BD I or II) depressed phase, without comorbid medical or psychiatric diagnoses, or substance abuse/dependence during the preceding 12 months, were considered for this study.

The diagnosis of Bipolar Depression was established by a comprehensive psychiatric examination and a structured interview using the MINI to determine the DSM-IV based diagnosis and any history of substance abuse. Mania was ruled out by the clinical interview. A trained and experienced interviewer other than the study psychiatrist conducted the structured interview. Cardiovascular and/or inflammatory disease was ascertained by history, physical and laboratory exam and record review. If the subject met study criteria and signed informed consent, s/he was randomized to receive ESC + celecoxib (CBX) or ESC + Placebo (PBO), at doses of 10 mg of ESC orally twice daily, 200 mg of CBX orally twice daily, and PBO matched to CBX orally twice daily.

Eligible bipolar depressed patients had to have previously failed to respond to two or more adequate trials with an antidepressant, or experienced a breakthrough depressive episode in spite of being maintained on a mood stabilizer and/or an atypical antipsychotic agent. To quantify the degree to which study patients were treatment resistant we used the Maudsley Staging Method to obtain a resistance score. The scale utilizes a variety of factors to quantify treatment resistance in depression, including duration of depressive symptoms, symptom severity, number of treatment failures, and whether or not the patient had received psychopharmacological augmentation or electroconvulsive therapy (Fekadu, Wooderson, Markopoulou, & Cleare, 2009; Fekadu, Wooderson, Donaldson et al., 2009). Each patient was assigned a score with a range of 3 (minimal resistance) to 15 (maximal resistance). Seventy percent of our subjects had scores between 5 and 8, while 30% had scores between 9 and 13. A minimum score of 18 on the first 17 items of the 21-item Hamilton Depression Scale (HAM-D17) was required for admission into the study.

Patients had to agree to undergo a washout from: Vitamin E, fish oils > 600 IU/day, non-aspirin NSAIDs, aspirin > 81 mg/day, H2 receptor antagonists, and Ginko biloba for at least two weeks, to refrain from caffeine on morning of blood drawing, and to institute lights-out at 23:00 h on nights before blood drawings.

Exclusion criteria included hypertension, anemia, liver disease, kidney disease, arthritis, diabetes, recurrent migraines, epilepsy, stroke, gum disease, and autoimmune disease. Additional exclusion criteria were: any abnormal findings on physical exam, ECG, or blood/urine tests, any infections; any physical pain including fibromyalgia; history of peptic ulcer complicated by perforation, hemorrhage, or obstruction; and symptoms of peptic ulcer within 4 weeks of enrollment date. Other exclusion criteria included current use of lithium, a stimulant, hormonal birth control and any corticosteroids (with the exception of hormone replacement therapy), and anticoagulant agents. Subjects had to be smoke and nicotine-free for greater than 3 months prior to the assessment to be considered for the study. Sensitivity or allergy to study medications or a need to receive agents contraindicated in combination with celecoxib or escitalopram were additional exclusion criteria as was sleep disorder, with the exception of insomnia or hypersomnia associated with BD.

No adverse effects resulting from the combination treatment were reported. In a systemic review and meta-analysis for celecoxib, 6 studies

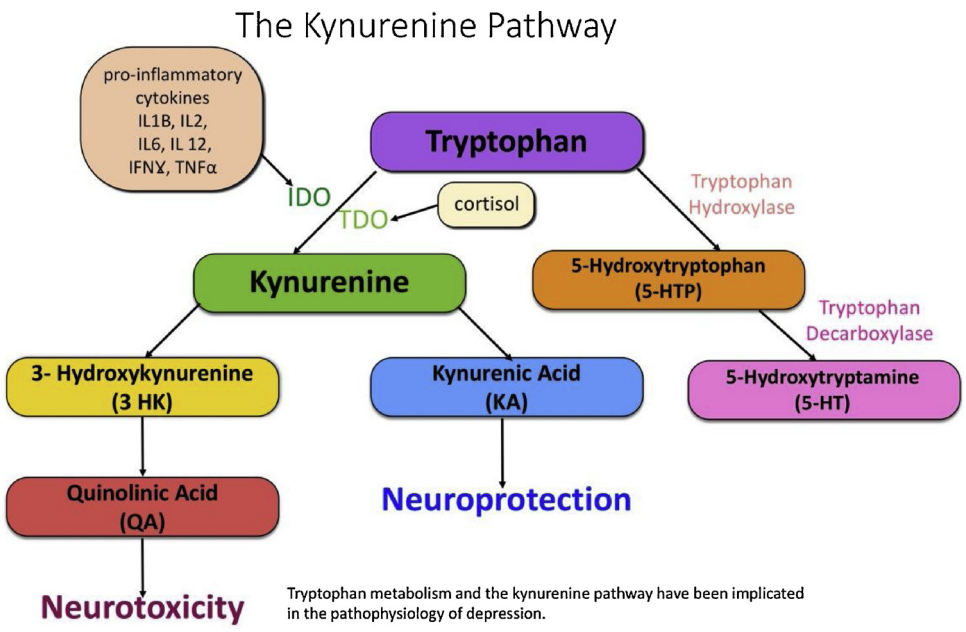

Fig. 2. The Kynurenine Pathway.

reporting on adverse effects found no evidence of an increased number of gastrointestinal or cardiovascular events after 6 weeks or infections after 12 weeks of anti-inflammatory treatment compared with placebo. All trials were associated with a high risk of bias owing to potentially compromised internal validity (Köhler et al., 2014). Another study which hypothesized that celecoxib may have a therapeutic role in acute bipolar mania reported no serious adverse events (Mousavi et al., 2017).

For Escitalopram, in Fonseca et al.'s 2006 open trial of adjunctive escitalopram in bipolar depression, adverse events emerged in 75% of the patients usually of mild-to-moderate severity, including headache, somnolence, insomnia, cloudy vision, dizziness, and anxiety; GI side effects including nausea, dry mouth, iron taste, vomiting; and, sexual dysfunction, joint pain, dry eyes, tachycardia. Four dropouts took place due to manic switch, hypomanic symptoms, and hospitalization due to the emergence of suicidal ideation and psychosis (Fonseca et al., 2006). Also, a case report also showed Restless Leg Syndrome Induced by Escitalopram and Lithium Combined with Quetiapine Treatment in Bipolar II Disorder: A Case Report (Chen, 2016). There are no studies with the combination of celecoxib and escitalopram, therefore any potential adverse effects can only be estimated based on each therapeutic agent used as monotherapy.

2.2. Study design

The study consisted of a screening visit, a minimum 2-week washout, a 1-week placebo run-in period, and 8 weeks of treatment. At the screening visit, subjects underwent a brief interview and were given an explanation of the study. If they passed the screening evaluation and agreed to participate, they were asked to sign the IRB-approved consent form. They then underwent comprehensive assessments that included quantifying severity of depression and anxiety (HAM-D, HAM-A). At the end of this screening visit, they were instructed to taper the antidepressant, if they had been taking any, for at least two weeks. All prospective study participants had to be stable on mood stabilizing medication which at times delayed the start of active study participation. They were then placed on a 1-week blinded placebo run-in phase until the next visit, which was the baseline visit. Throughout the study, patients were maintained on a mood stabilizer (with the exception of lithium) and/or an atypical antipsychotic, as selective COX-2 inhibitors can increase serum lithium concentrations leading to toxicity. Increased serum lithium concentration reports exist for aspirin, sulindac, and 14 other NSAIDs, including celecoxib and rofecoxib (Phelan, Mosholder, & Lu, 2003).

At the baseline visit they were rated in a blinded manner, as if they had received active medication. If they continued to score at least 18 on the 17-item HAM-D scale, they were randomized to receive escitalopram (ESC) + celecoxib (CBX), or escitalopram (ESC) + placebo (PBO). The study pharmacist generated the randomization code, kept it in sealed envelopes, and dispensed the medication. At every visit thereafter, subjects were handed a medication installment to last until the following visit. Subjects were asked to return the used medication vials at the following visit so that a pill count could be made to ensure compliance. If at the baseline visit the subject scored HAM-D ≤ 17, they were designated placebo responders and were offered conventional care. The overall study was powered for 70 patients (35 in each arm of the study) for the clinical outcomes of response and remission, to complete 8 weeks of active medication to be considered study completers. The primary outcome of this study was treatment effectiveness. We expected an effect size of 0.60 with a two-group analysis of covariance using baseline measurements of mood and pain perception scores as covariates. Eighty patients were enrolled, to allow for an anticipated 10% drop-out rate. Fifty-five patients qualified as completers, 24 in the placebo arm and 31 in the celecoxib arm. Of these 55 patients, only 47 subject had complete biological data for statistical analysis. Age and sex distribution of the study participants are shown in Table 1.

The primary endpoints pertain to the main hypothesized outcome, namely, a significantly better-than-placebo mood response for

Table 1
Demographics of BDD subjects vs healthy controls - age and sex distribution.

Healthy controls (from our database):	
# females	22
# males	16
Avg age	40
Age Range:	21–65
BDD Subjects:	
#females	28
#males	27
Avg Age	55
Age Range:	20–65

ESC + CBX that is not related to altered pain perception. The HAM-D rating scale (17 items from the 21-item scale) was the main measure of clinical improvement. We defined onset of response as the day of treatment when a 30% decline in the initial (day 0) HAM-D score is recorded and is maintained for two consecutive weeks. We defined treatment response time as the day of treatment when a 50% reduction in the initial HAMD score occurs. We defined symptom remission as a HAM-D score of ≤7 at the end of treatment. We established whether the combination of ESC + CBX showed improvement augmentation over the antidepressant efficacy of monotherapy based on a) a significantly earlier group response (earlier decline in HAM-D scores), and/or, b) a more robust group improvement in HAM-D scores at 8 weeks of treatment, and/or, c) more patients with symptom remission than in the ESC + PBO group at 8 weeks.

The second end point was a reduction in pro-inflammatory biomarkers as well as neurotoxic metabolites including Quinolinic Acid, at end of treatment in patients randomized to receive one of the two treatment options. The baseline values were compared to age and sex matched controls from our existing normative dataset of healthy subjects.

Second tier end points were exploratory in nature, not powered for in our study since existing data in the literature is insufficient to calculate an accurate sample size for them. This paper's focus on Quinolinic Acid is merely one part of a larger clinical paper that is in preparation.

2.3. Healthy control subjects

Healthy control subjects (HC) were obtained from our database (Halaris et al., 2015). The healthy control subjects had been recruited previously, and blood samples had been stored and frozen at -80 C. Serum samples were analyzed for Quinolinic Acid in the Kynurenine Pathway, at the same time that patient samples were processed. Blood samples for biomarker analyses were obtained from HC subjects only once because based on our experience, measured values are fairly stable barring intercurrent illnesses or stressful life events. All eligible HC subjects underwent the same screening assessments after providing written informed consent, as approved by the Institutional Review Board of the institution. Subjects were admitted into the study only if the screening test results fell within normal range. Once deemed eligible, a baseline visit was scheduled. The exclusion criteria for HC subjects were presence of any kind of medical or mental illness, an inflammatory process, gum disease, substance use, mental illness or substance use amongst first degree relatives, pregnant or lactating females, and females taking oral contraceptives. The HAM-D and BDI scores had to be less than 5. A total of 27 HC subjects were included.

2.4. Biochemical analyses

After an overnight fast, 20 ml of antecubital venous blood was drawn between 08:00 and 10:00 AM at baseline, week-4, and week-8 follow-up visits. For the HC subjects, blood was taken only upon completion of all assessments to confirm physical and mental health status. Both plasma and serum samples were obtained and immediately stored at -80^{O} C until analyzed. Serum samples were sent to the Institute of Laboratory Medicine of the University Hospital, LMU Munich for analyses. The metabolite QA was measured by Ultra Performance Liquid Chromatography/Mass Spectrometry (UPLC-MS), using a Waters Acquity UPLC connected to a Xevo TQ MS triple-quadrupole mass spectrometer, equipped with a Z-spray ESI ion source (Waters, Milford, MA, USA). Separation was carried out using a Kinetex XB-C18, 2.6 µm, 2.1 × 150 mm column (Phenomenex, Torrance, CA, USA). The blood CBX and ESC levels were also measured by UPLC-MS/MS using a Waters Acquity UPLC connected to a Xevo TQD MS triple-quadrupole mass spectrometer, equipped with a Z-spray ESI ion source (Waters, Milford, MA, USA). Separation was carried out using a PerfectSil Target ODS-3 HD, 5 µm, 2.1 x 100 mm column (MZ Analysentechnik GmbH, Mainz, Germany). The analyses were performed under GLP regulations, and the method was standardized regularly by participating in the EQAS scheme of German TDM laboratories.

2.5. Statistical analyses

Non-parametric Wilcoxon Rank Sum tests were used to assess for differences in baseline QA levels first between BD depressed patients and HC subjects, then by treatment response. Univariable exact binary logistic regression models were then used to examine the odds of treatment response and remission, respectively, as a function of drug therapy. A binomial distribution was specified for each response variable, while a logit link was used to estimate the odds ratio (OR) associated with treatment assignment. Supplementary linear mixed effects models compared QA levels over time by drug therapy and response, adjusting for sex, age, and BMI. In these models, random intercepts were allowed for each patient to account for their multiple observations over the eight-week study period.

Finally, an estimate of total number needed to treat (NNT) was derived by taking the inverse of the absolute risk reduction associated with patients at each level of drug therapy. First, the percentage of patients who did not respond in the CBX and PBO arms was recorded. The proportion of patients in the CBX cohort was then divided by the proportion in the PBO group and the inverse of that result is the NNT, assuming the rates observed in this sample of patients is representative of the population. This process was then repeated to derive similar estimates examining non-remission rates. All statistical analyses were conducted using SAS 9.4 (Cary, NC).

While adjusting for important covariates in a multivariable model does not set the groups equivalent on those criteria, it does account for the effect these imbalances might have on the relationship of interest. In other words, by adjusting for age in our multivariable model we appropriately accounted for the variability this observed imbalance introduces when trying to examine the association between the variable of interest (treatment group) and outcome. In doing so, we are then able to state the relationship that exists between treatment group and the outcome is not due to age differences. To further validate the reported findings, a supplemental univariable analysis examining the influence of only a patient's age on the outcome was run. Not surprisingly (based on our prior reported results), a significant association was not observed ($p = 0.59$). Therefore, the imbalance in age, while an initial limitation in our setting, was appropriately accounted for in our analysis via statistical adjustment and found to have no impact.

3. Results

Patients receiving ESC + CBX were 4.13 (95 CI: 1.03–18.48) times more likely to respond to treatment compared to those randomized to ESC + PBO (exact $p = 0.04$) (Table 2) (Table 3). Further, the proportion of non-responders in the CBX and PBO groups was 22% and 55%,

Table 2
HAM-D Scores of BDD Patients.

Group	n	HAM-D BL	HAM-D Wk8
All Patients	47	22.3	10.3
ESC + PBO Responders	7	24.9	9.1
ESC + PBO Remitters	2	18.0	7.0
ESC + PBO Non-Responders	11	20.4	17.0
ESC + CBX Responders	4	26.5	11.0
ESC + CBX Remitters	17	22.6	4.5
ESC + CBX Non-Responders	6	20.7	16.2
All Responders at Wk 8	11		9.8
All Remitters at Wk 8	19		4.8
All Non-Responders at Wk 8	17		16.7

Table 3
Univariable exact logistic regression model results assessing treatment response.

Drug Therapy	Valid N	Odds Ratio (95 CI)	Exact p
Celecoxib	47	4.13 (1.03-18.48)	0.04
Placebo (Ref)		–	

Patients receiving Escitalopram + Celecoxib were 4.13 times more likely to respond to treatment compared to those randomized to Escitalopram + Placebo.

Table 4
Univariable exact logistic regression model results assessing remission.

Drug Therapy	Valid N	Odds Ratio (95 CI)	Exact p
Celecoxib	47	14.34 (2.59-153.17)	< 0.001
Placebo (Ref)		–	

Patients receiving Escitalopram + Celecoxib were 14.34 times more likely to experience remission compared to those on Escitalopram + Placebo.

respectively. Thus, the Number Needed to Treat (NNT) is 3, meaning 3 patients would have to be treated with ESC + CBX to prevent one treatment failure.

Patients receiving CBX were also 14.34 (95 CI: 2.59–153.17) times more likely to experience remission compared to those on PBO (exact $p < 0.001$) (Table 4). The observed proportion of patients receiving CBX who did not experience remission was 37%, while the proportion of individuals receiving the PBO combination who did not experience remission was 90%. Assuming these rates are representative of the population, then the Number Needed to Treat (NNT) is 2, meaning 2 patients would have to be treated with ESC + CBX to prevent one remission failure.

Patients in both treatment groups (ESC + CBX/ESC + PBO) had significantly lower QA levels at baseline compared to HC subjects ($p < 0.001$) (Table 5, Fig. 3). Adjusting for sex, age, and BMI, bipolar patients receiving CBX (Mean = 55.69, SE = 6.27) had comparable QA values to those of patients receiving PBO (Mean = 64.90, SE = 7.20, p = 0.34). The interaction between drug therapy and time was not statistically significant ($p = 0.28$), indicating patients' QA levels did not change significantly over time in response to either drug treatment (Table 6, Fig. 4).

Overall, patients who responded to treatment (Mean = 50.73, SE = 6.33) had marginally lower QA values to patients who did not respond to treatment (Mean = 70.98, SE = 7.88, $p = 0.06$) (Table 7). The interaction between treatment response and time was not significant ($p = 0.82$), indicating patients' QA values did not change significantly over time based on their response to therapy (Fig. 5).

4. Discussion

We have demonstrated that bipolar depressed patients (BDD) receiving CBX add-on to ESC therapy were far more likely to respond to treatment and remit, as compared to those patients who received the ESC + PBO combination. These findings are consistent with a growing body of evidence that COX-2 inhibitor-mediated modulation of inflammation enhances clinical response and promotes remission in bipolar depression. Müller et al. (2006) found that unipolar depressed patients receiving reboxetine + CBX showed a significantly greater antidepressant response by week-5 than the group on reboxetine + PBO. Nery et al. (2008) studied non-responsive bipolar patients during depressive or mixed episodes and noted a statistically significant improvement in the first week of treatment with add-on CBX. Akhondzadeh et al. (2009) reported that MDD patients receiving the combination of fluoxetine and CBX showed greater response compared to the PBO group. Krause, et al. (2017) added CBX or PBO to reboxetine in unipolar depressed patients, and demonstrated that CBX add-on also had beneficial effects. Arabzadeh et al. (2015) also found that celecoxib is effective adjuvant therapy to valproic acid in treating manic episodes of bipolar mood disorder.

The links between QA, neurotoxicity, and neuroprogressive structural changes in neuropsychiatric diseases are well documented. QA is an NMDA-R agonist (Bender & McCreanor, 1985), and an accumulation of QA promotes excitotoxicity associated with neurodegenerative changes (Guillemin, 2012). Steiner et al. (2011) reported high QA in microglia of post-mortem prefrontal cortex and hippocampus of unipolar and bipolar depressed patients. While QA is a known excitotoxin at the NMDA receptor, its role as the metabolic precursor to nicotinamide adenine dinucleotide (NAD) may contribute to the mechanistic basis of immune involvement in bipolar neuroprogression. NAD is a critical coenzyme and oxidating agent important for redox metabolism, purine synthesis, cell signaling (Billington et al., 2006), and synaptic transmission (Durnin et al., 2012). Emerging reports indicate that the NAD/tryptophan signaling pathway is involved in the activation of innate and adaptive immune activation independent of exposure to antigen (Biefer, Vasudevan, & Elkhal, 2017). The antigen-independent nature of this type of immune activation points to the involvement of the NAD/tryptophan in distinct pathways, separate from canonical dendritic cell-mediated antigen presentation to major

Table 5
Baseline Quinolinic Acid levels by group.

Group	Valid N	Quinolinic Acid (ng/ml)	p
Bipolar	43	53.80 (40.80 – 71.40)	< 0.001
Healthy Control	24	90.53 (75.04 – 102.13)	

Note: Valid sample N = 67 (In the bipolar group four blood samples were inadequate for analysis).
Estimates reported as medians with interquartile range in parentheses.
Patients in both treatment groups (Escitalopram + Celecoxib / Escitalopram + Placebo) had significantly lower Quinolinic Acid levels at baseline compared to healthy control subjects.

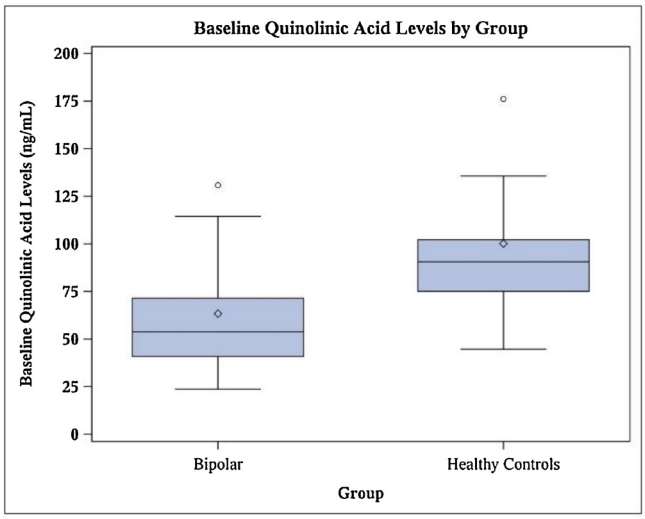

Fig. 3. Baseline Quinolinic Acid levels by treatment group.
Note: A Wilcoxon Rank Sum test was used to assess for differences in patient QA levels at baseline.
-Estimates are expressed as medians with interquartile range due to the small sample size at baseline (N = 67).
-Patients in both treatment groups had significantly lower QA levels at baseline compared to HC subjects ($p < 0.001$).

Table 6
Quinolinic Acid levels by treatment group.

Quinolinic Acid (ng/ml)	Valid N	Celecoxib	Placebo	Total	p
Baseline	42	57.15 (6.58)	70.66 (7.55)	63.90 (5.01)	0.18
Week 4	40	54.34 (6.58)	63.98 (7.68)	59.16 (5.05)	0.34
Week 8	40	55.60 (6.58)	60.07 (7.68)	57.84 (5.05)	0.66

Note: Estimates reported as means with standard errors in parentheses.
Overall, patients receiving Celecoxib had comparable QA levels to those of patients receiving Placebo.
The interaction between drug therapy and time was not statistically significant, indicating patients' Quinolinic Acid levels did not change significantly over time in response to either drug treatment.

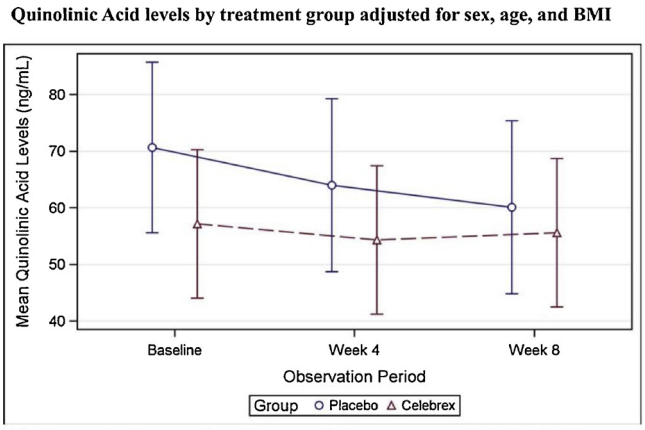

Fig. 4. Patients' Quinolinic Acid levels by drug therapy adjusted for sex, age, and BMI.
Note: A linear mixed effects model was used to estimate Quinolinic Acid levels over time by treatment group after adjusting for Sex, Age, and BMI.
-Estimates are expressed as means with 95% confidence intervals, and random interceptors accounted for multiple observations over the 8-week study period.

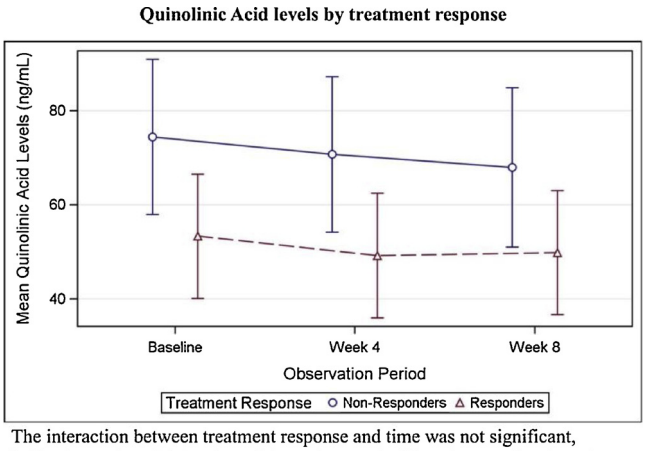

Fig. 5. Quinolinic Acid levels by treatment response.
Note: A linear mixed effects model was used to estimate Quinolinic Acid levels over time by treatment group after adjusting for Sex, Age, and BMI.
-Estimates are expressed as means with 95% confidence intervals, and random interceptors accounted for multiple observations over the 8-week study period.

histocompatibility complex type II (MHCII), that may play a role in the low-grade chronic inflammation linked to bipolarity. While the etiology of the underlying state of inflammation is a matter of ongoing exploration, it is likely multifactorial, and ultimately contributory to kynurenine pathway activation via induction of indoleamine-2,3-dioxygenase (IDO) which regulates the first, rate-limiting step of tryptophan into the kynurenine pathway. The role of prostaglandins in this process cannot be understated. Begemann et al. (2008) uncovered a link between rapid cycling bipolarity and peripheral expression of prostaglandin D synthase (PTGDS) and prostaglandin D2 11-ketoreductase (AKR1C3) in circulating mononuclear cells. PTGDS is preferentially expressed in the CNS and mediates synthesis of prostaglandin D2 from prostaglandin H2. AKR1C3 mediates synthesis of prostaglandin F2 from prostaglandin D2. Celecoxib treatment for over 5 months was associated with a reduction in severity rating of both depressed and manic episodes, suggesting that prostaglandins play a role in rapid cycling, and that major depression and bipolar disorder should be viewed as systemic diseases.

We hypothesized that QA would be elevated in our treatment-resistant BDD patients, and that co-administration of CBX with ESC would reverse treatment resistance in a manner consistent with predicted QA changes. We further hypothesized that modulation of the inflammatory response via inhibition of COX-2 would exert a "normalizing" effect on specific kynurenine pathway intermediates (particularly QA) consistent with a return to baseline or HC levels. Paradoxically, we found significantly *lower* baseline QA levels in our BD patients compared to HC subjects. This unanticipated finding requires explanation, since the chronic proinflammatory state in bipolarity is typically associated with QA-mediated neurotoxicity (Birner et al., 2017), linked to the chronic and relapsing nature of bipolar disorder. An increase in neurotoxicity may be paralleled by reduced neuroprotection, as demonstrated in the study by Savitz et al. (2015), who determined that the neuroprotective index of KynA/QA was significantly lower in the BD subjects relative to the healthy controls, but found no difference in any individual kynurenine metabolites in the BD group vs. healthy controls. In the present study, our non-suicidal treatment-resistant BDD patients showed normal PIC/QUIN ratios compared to HC, both at baseline and week 8 (manuscript in preparation). These inconsistencies are counterbalanced

Table 7
Quinolinic Acid levels by treatment response.

Quinolinic Acid (ng/ml)	Valid N	Responders	Non-Responders	Total	*p*
Baseline	43	53.28 (6.62)	74.40 (8.27)	63.84 (5.14)	0.06
Week 4	41	49.12 (6.65)	70.66 (8.30)	59.89 (5.15)	0.05
Week 8	41	49.80 (6.61)	67.89 (8.51)	58.85 (5.22)	0.11

Note: Estimates reported as means with standard errors in parentheses.
Overall, patients who responded to treatment had marginally lower QA values compared to patients who did not respond to treatment.

by select studies which indicated an inverse relationship between QA and treatment outcome in unipolar depression (Krause et al., 2017). This inverse relationship is compatible with the low baseline QA associated with treatment-resistant status in our study. Provided that peripheral QA levels are reflective of central QA levels, it is plausible that pretreatment blood QA is a candidate predictive biomarker for response to inflammatory modulation in treatment-resistant BD.

An important contextual consideration is the possible influence of past pharmacologic treatments on the observed QA levels. The low baseline QA levels could be a molecular artifact of multiple prior trials of pharmacologic interventions, including failed trials. To minimize the risk of switching into mania/hypomania, our BDD patients were stabilized on a mood stabilizer (valproic acid, lamotrigine, oxcarbazepine, carbamazepine) and/or an antipsychotic agent for at least two weeks prior to exposure to the antidepressant/celecoxib combination. As such, the biochemical changes we have observed may be, at least to some degree, conflated with metabolic effects of prior mood stabilizers which are associated with neuroprotective properties in their own right. In vitro work by Kocki, Wielosz, Turski, and Urbanska (2006) demonstrated that phenobarbital, felbamate, phenytoin and lamotrigine enhanced the production of the neuroprotective metabolite kynurenic acid in a Kynurenine Aminotransferase I (KAT I) dependent manner. Likewise, central kynurenic acid and kynurenine levels were boosted in response to valproic acid (Maciejak et al., 2013) as well as lithium and mianserin (Wood, Harwood, & Coppen, 1978). These metabolic changes are not consistent across the array of pharmacologic therapies, as amitriptyline did not significantly increase plasma kynurenine concentration in depressed patients (Wood et al., 1978), and preclinical studies of ifenprodil, carbamazepine, and nifedipine did not attenuate quinolinate-induced injury (Trescher, McDonald, & Johnston, 1994). Importantly, the metabolic consequences of pharmacologic intervention are also nuanced by pathway specific considerations. For instance, Mary, Wahl, and Stutzmann (1995) reported that lamotrigine was insufficient to rescue quinolinate-induced neuronal damage in rats from the standpoint of glutamatergic transmission, whereas McGeer and Zhu (1990) reported lamotrigine-mediated neuroprotection on the basis of choline acetyltransferase and glutamate decarboxylase enzymatic activities.

A final consideration is that the molecular expression of adaptive changes in response to treatment may not parallel symptom changes, and may follow a different time course. Variations in this regard may be a function of distinct neuroprogressive trajectories based on etiopathology. Significant reductions in HAM-D and related scores appeared by treatment week 8, which correlated with a notable but insignificant downtrend in QA levels by week 8. While QA levels did not significantly differ according to treatment, responders had significantly lower QA at week 4 compared to non-responders, although the significance of this downtrend was not sustained by week 8. Admittedly, this reduction in QA after inflammatory modulation in treatment-resistant bipolar depression was not as robust as the reduction in QA we observed in response to escitalopram in unipolar depression (Halaris et al., 2015), but this variation may be due to differences in disease complexity and treatment resistance. Nonetheless, the reduction in neurotoxic metabolites 3-hydroxykynurenine and QA in response to ESC alone, suggests that the antidepressant effect of ESC may in part be mediated in a kynurenine pathway-dependent manner (Halaris et al., 2015). If the chronic pro-inflammatory state is associated with a pathological shunt toward the kynurenine pathway, interruption of this shunt via inflammatory modulation could contribute to the reversal of treatment resistance in bipolar depression. In this context, the downtrend in QA may be part of a molecular signature associated, causally or correlatively, with an overall reduction in neurotoxic burden underlying the remarkable reversal of treatment resistance in bipolar depression after inflammatory modulation.

5. Conclusion

In a prior study involving unipolar depression, we demonstrated that clinical response to ESC may occur in part by reduction of neurotoxic kynurenine pathway intermediates, including the excitotoxin QA. In the current study involving treatment-resistant bipolar depression, we described the robust clinical response to inflammatory modulation in terms of baseline or reactive metabolic changes in the kynurenine pathway, with particular attention to QA. We found that lower baseline QA levels correlated with treatment-resistant bipolar status. While QA levels did not significantly differ according to treatment, responders had significantly lower QA at week 4 compared to non-responders, although the significance of this downtrend was not sustained by week 8. Altogether, these results reinforce the involvement of immune system activation in neuropsychiatric disease, and more specifically, the growing link between kynurenine pathway activation and treatment-resistance in bipolar depression.

Ethical statement

The current study was approved by the Institutional Review Board of Loyola University Medical Center and the Review Board of the Medical Faculty of the LMU and was conducted according to the principles of the Declaration of Helsinki.

Declaration of interest statement

The authors have no conflict of interest to report.

Acknowledgments

This work was supported by a grant from the Stanley Medical Research Institute (SMRI). Grant No.10T-1401 awarded to Dr. Angelos Halaris.

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