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Reduction of kynurenic acid to quinolinic acid ratio in both the depressed and remitted phases of major depressive disorder

Jonathan Savitz, Ph.D.^{*,1,2}, Wayne C. Drevets, M.D.³, Brent E. Wurfel, M.D., Ph.D.^{1,4}, Bart N. Ford, B.S.¹, Patrick S.F. Bellgowan, Ph.D.^{1,2}, Teresa A. Victor, Ph.D.¹, Jerzy Bodurka, Ph.D.^{1,5}, T. Kent Teague, Ph.D.^{6,7,8}, and Robert Dantzer, DVM, Ph.D.⁹

¹ Laureate Institute for Brain Research, Tulsa, OK, USA

² Faculty of Community Medicine, University of Tulsa, Tulsa, OK, USA

³ Janssen Pharmaceuticals of Johnson & Johnson, Inc., Titusville, NJ, USA

⁴ Department of Psychiatry, University of Oklahoma College of Medicine, Tulsa, OK, USA

⁵ College of Engineering, University of Oklahoma, Tulsa, OK, USA

⁶ Departments of Surgery and Psychiatry, University of Oklahoma College of Medicine, Tulsa, OK, USA

⁷ Department of Pharmaceutical Sciences, University of Oklahoma College of Pharmacy, Tulsa, OK, USA

⁸ Department of Biochemistry and Microbiology, Oklahoma State University Center for Health Sciences, Tulsa, OK, USA

⁹ Division of Internal Medicine, Department of Symptom Research, MD Anderson Cancer Center, Houston, TX, USA

Abstract

Low-grade inflammation is characteristic of a subgroup of currently depressed patients with major depressive disorder (dMDD). It may lead to the activation of the kynurenine-metabolic pathway and the increased synthesis of potentially neurotoxic metabolites such as 3-hydroxykynurenine (3HK) and quinolinic acid (QA), relative to kynurenic acid (KynA). Nevertheless, few studies have examined whether abnormalities in this pathway are present in remitted patients with MDD (rMDD). Here we compared the serum concentrations of kynurenine metabolites, measured using high performance liquid chromatography with tandem mass spectrometry, across 49 unmedicated subjects meeting DSM-IV-TR criteria for MDD, 21 unmedicated subjects meeting DSM-IV-TR

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* Jonathan Savitz Laureate Institute for Brain Research 6655 S. Yale Ave Tulsa, OK 74136 Phone: 918 502 5104 Fax: 918 502 5135 jsavitz@laureateinstitute.org.

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criteria for rMDD, and 58 healthy controls (HCs). There was no significant group difference in the concentrations of the individual kynurenine metabolites, however both the dMDD group and the rMDD group showed a reduction in KynA/QA, compared with the HCs. Further, there was an inverse correlation between KynA/QA and anhedonia in the dMDD group, while in the rMDD group, there was a negative correlation between lifetime number of depressive episodes and KynA/QA as well as a positive correlation between the number of months in remission and KynA/QA. Our results raise the possibility that a persistent abnormality exists within the kynurenine metabolic pathway in MDD that conceivably may worsen with additional depressive episodes. The question of whether persistent abnormalities in kynurenine metabolism predispose to depression and/or relapse in remitted individuals remains unresolved.

INTRODUCTION

Circulating biomarkers of inflammation such as C-reactive protein (CRP) and pro-inflammatory cytokines such as IL-1 β and IL-6 have been reported to be elevated in individuals with major depressive disorder (MDD) (Howren et al., 2009). Cytokines and other inflammatory molecules may directly affect neurophysiological function, mood, and emotion (Miller et al., 2013). However, inflammation may also affect mood and behavior indirectly by activating the tryptophan degrading enzyme, indoleamine 2,3 dioxygenase (*IDO*), increasing the formation of neuroactive kynurenine-pathway metabolites, including kynurenic acid (KynA), 3-hydroxykynurenine (3HK), and quinolinic acid (QA) (Dantzer et al., 2011).

Kynurenine is metabolized along two main branches to form either KynA or alternatively, 3HK, 3-hydroxyanthrallic acid (3-HAA), and QA (figure S1). Both the preclinical literature and human studies of known inflammatory and/or neurodegenerative disorders have led to the hypothesis that microglial-derived 3HK and QA are neurotoxic while astrocyte-derived KynA is neuroprotective (Amaral et al., 2013; Myint and Kim, 2003; Stone et al., 2012). While this model is likely overly simplistic, our previous results showing reductions in KynA/3HK and/or KynA/QA in depressed patients with MDD (Savitz et al., 2015) and bipolar disorder (BD) (Savitz et al., 2014a) along with positive correlations between KynA/3HK and/or KynA/QA and hippocampal volume in the MDD and BD groups, are arguably consistent with this model.

Few studies have measured both KynA and QA-pathway metabolites in the *same* depressed subjects with *primary* MDD. Our previous reports of mood disorder-associated reductions in KynA/3HK and/or KynA/QA are partially consistent with two studies that found reductions in KynA in groups of depressed patients compared with healthy controls (Maes et al., 2011; Myint et al., 2007), and an *ex vivo* study of skin fibroblasts derived from BD subjects that reported disproportionate elevations in 3HK relative to KynA after stimulation with pro-inflammatory cytokines (Johansson et al., 2013). Moreover, (Bay-Richter et al., 2015) recently reported persistent decreases in KynA and increases in QA in the cerebrospinal fluid of predominantly depressed subjects up to 2 years after a suicide attempt.

A question that has to our knowledge, not been addressed in the literature is whether the putative depression-associated changes in kynurenine metabolism are temporally restricted

to the depressive episode or whether these abnormalities are present both within *and* between episodes, constituting a trait-like abnormality. Here we present preliminary data suggesting the existence of a sustained abnormality in the kynurenine metabolic pathway in MDD.

METHODS

Subjects provided written informed consent after receiving a full explanation of the study procedures and risks, as approved by the IRB overseeing the study.

All dMDD (n=49), rMDD (n=21), and healthy control (HC, n=58) participants were interviewed with the Structured Clinical Interview for the DSM-IV-TR. In addition, unstructured psychiatric interviews with board-certified psychiatrists were obtained on all dMDD and rMDD participants. We previously published results from 29 of the dMDD subjects and 20 of the HCs making up the current sample in the context of a study examining associations between kynurenine metabolites and gray matter volumes of the hippocampus and amygdala (Savitz et al., 2015).

The majority of the dMDD subjects had Hamilton Depression Rating Scale (HAM-D, 24-item) and Montgomery-Asberg Depression Rating Scale scores (MADRS) in the moderately-to-severely depressed range (table 1). Anhedonia was assessed with the Snaith-Hamilton Pleasure Scale (SHAPS; higher scores being indicative of greater anhedonia). The rMDD subjects were not only required to meet DSM-IV-TR criteria for full remission but were also asymptomatic at the time of the study with a MADRS score of <10 (corresponding to the non-depressed range). The unmedicated dMDD and rMDD groups had not received any psychotropic medication for at least 4 weeks (8 for fluoxetine) prior to the blood-draw. Exclusion criteria were as follows: serious suicidal ideation or behavior; medical conditions or concomitant medications likely to influence CNS or immunological function including cardiovascular, respiratory, endocrine and neurological diseases, and a history of drug or alcohol abuse within 6 months or a history of drug or alcohol dependence within 1 year.

The HCs met the same exclusion criteria except that they had no personal or family (first-degree relatives) history of psychiatric illness assessed using the Structured Clinical Interview for the DSM-IV-TR and the Family Interview for Genetic Studies.

Subjects fasted overnight and blood was sampled between 8am and 11am. Serum samples were collected with BD Vacutainer serum tubes, processed according to the standard BD Vacutainer protocol, and stored at -80° C.

Concentrations of tryptophan (TRP), kynurenine (KYN), kynurenic acid (KynA), 3-hydroxykynurenine (3HK), and quinolinic acid (QA) were measured blind to diagnosis by Brains Online, LLC in 2 separate batches. The metabolite concentrations were determined by high performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) detection using their standard protocols. The intra-assay and inter-assay coefficients of variation are provided in tables S1 and S2.

High-sensitivity C-reactive protein (hs-CRP) was measured in a clinical laboratory using the Kamiya Biomedical K-Assay. A commercially available colorimetric sandwich ELISA was used to quantify serum levels of IL-1RA (R&D Systems) blind to diagnosis. Serum samples were stored at -80°C until use and thawed on ice the day of the assays. To remove any precipitate, samples were centrifuged for 15 min at 3000rpm. Each sample was run in duplicate according to the manufacturer's instructions using the provided reagents. Two control serum samples were run on each plate to determine inter-assay variation and were used to normalize the data across plates. The mean inter-assay coefficient of variation and lower detection limit were 2% and 31.2 pg/mL, respectively.

We selected IL1-RA for the following reasons: (1) We wanted to use a marker of immune activation that differs somewhat from CRP, and IL-6, which is arguably most often measured in the depression literature, is partly responsible for CRP production. (2) IL-1RA is sufficiently elevated in the plasma to avoid too many undetectable values in our limited population sample. (3) There are existing reports of elevated IL-1RA in depressed patients (Dahl et al., 2014; Howren et al., 2009).

Deviations from normality were tested using the Kolmogorov-Smirnov test and non-normally distributed variables were $\log_{10}$ normalized. Differences in kynurenine pathway metabolites between the diagnostic groups were evaluated with ANOVA (two-tailed). Sex, which trended towards differing among the subject groups (table 1), and analysis batch (first versus second) were used as covariates. For the primary variables of interest (KynA/3HK and KynA/QA) a statistical threshold of $p < 0.05$ was used for determining statistical significance whereas for the individual metabolites and KYN/TRP we used a Bonferroni correction for multiple testing ($p < 0.008$).

RESULTS

The KynA/3HK and KynA/QA ratios appeared to be normally distributed. In contrast, IL-1RA and all the individual kynurenine metabolites were non-normally distributed and were therefore $\log_{10}$ normalized prior to the use of parametric statistics.

After controlling for sex and batch effects, there was no significant effect of diagnosis on CRP, IL1-RA, and any of the individual kynurenine metabolites although IL-1RA concentrations trended higher in the dMDD group versus the HCs ($p = 0.059$). Consistent with the hypothesis that inflammation activates the kynurenine metabolic pathway, IL-1RA was significantly correlated with a proxy measure of *IDO*, Kyn/TRP ($r_s = 0.22$, $p = 0.045$).

There was however, a significant effect of diagnosis on the KynA/QA values ($F_{2,123} = 3.8$, $p = 0.025$), but not on the KynA/3HK values ($F_{2,123} = 2.3$, $p = 0.109$). Protected *post-hoc* pairwise comparisons showed that both the dMDD and the rMDD groups had significantly reduced KynA/QA concentration ratios compared with the HC group (figure 1B, dMDD versus HC: $p = 0.029$; rMDD versus HC: $p = 0.047$; dMDD versus rMDD: $p = 0.781$). KynA/QA was significantly correlated with both IL-1RA ($r = -0.22$, $p = 0.046$) and CRP ($r = -0.27$, $p = 0.012$) in the entire sample.

We then tested whether KynA/QA was significantly associated with various clinical variables. In the dMDD group there was a significant inverse correlation between KynA/QA and SHAPS anhedonia scores ($r=-0.31$, $p=0.041$, figure 1C) but not with the severity of depression as measured with the MADRS ($r=-0.08$, $p=0.610$) or HAM-D ($r=-0.21$, $p=0.132$). In addition, there was no significant association between KynA/QA and scores on the motor agitation item of the HAM-D ($r=-0.17$, $p=0.257$). Within the rMDD group there was a significant negative correlation between lifetime number of depressive episodes and KynA/QA ($r=-0.66$, $p=0.002$). However, the correlation between lifetime number of depressive episodes and KynA/QA was not statistically significant in the dMDD group ($r=-0.31$, $p=0.121$). Further, in the rMDD group there was a significant positive correlation between the number of months in remission (defined using DSM-IVTR criteria) and the KynA/QA ratio ($r=0.49$, $p=0.028$, figure 1D).

DISCUSSION

Here we replicate our previous reports of reductions in KynA/QA in currently depressed subjects with MDD (Savitz et al., 2015) or BD (Savitz et al., 2014a) and extend the finding to rMDD subjects in long-term remission, suggesting the existence of a persistent abnormality in the kynurenine metabolic pathway in MDD. The significant group difference in the KynA to QA ratio rather than in the individual metabolites suggests that it is the relative levels of KynA to QA rather than the absolute concentrations of KynA and QA that is the most salient abnormality in depression – most likely because KynA and QA-pathway metabolites are simultaneously present but exert distinct, and under some conditions competing, physiological effects. The inverse association between KynA/QA and the degree of anhedonia in the subjects with MDD is interesting given the well-characterized link between inflammation-induced sickness behavior and anhedonia (Dantzer et al., 2008; Maes et al., 2012; Yirmiya et al., 2000). Since KynA/QA was *not* significantly correlated with the severity of depression as rated using a broad symptom-based scale, our finding additionally raises the possibility that the relative levels of KynA to QA might influence the development of anhedonic symptoms rather than the severity of the major depressive syndrome *per se*.

At first glance there appears to be a disjunction between the absence of a significant correlation between KynA/QA and anhedonia in the rMDD group and our suggestion that a decrease in KynA/QA is trait-related marker of depression. However, because anhedonia is a core symptom of MDD, individuals with overt anhedonic symptoms appear unlikely to meet DSM criteria for remission, reducing the power to detect a significant relationship between KynA/QA and anhedonia *when anhedonia is measured with the SHAPS*. This possibility is reflected by the lack of significant difference in the mean SHAPS scores between the rMDD and HC groups (table 1). Conceivably more sensitive markers of anhedonia such behavioral or neurophysiological responses to reward and/or positively-valenced stimuli, which have been shown to be abnormal in rMDD subjects (McCabe et al., 2009; Pechtel et al., 2013; Victor et al., 2010), might have shown associations with kynurenine metabolites in the rMDD group.

Few studies have examined the immunological profiles of MDD patients *during remission*. Those studies that have been published indicate a normalization of peripheral cytokine

levels in patients who remit with treatment (Frommberger et al., 1997; Narita et al., 2006). However, the majority of studies are confounded by pharmacological treatment since some classes of antidepressants (e.g. selective serotonin reuptake inhibitors), appear to exert anti-inflammatory effects while other classes of antidepressants may be pro-inflammatory (e.g. serotonin-norepinephrine reuptake inhibitors and tricyclic antidepressants) (Hamer et al., 2011; Hannestad et al., 2011; Vogelzangs et al., 2012). In contrast, studies of *unmedicated*, clinically-remitted MDD patients have reported elevations in pro-inflammatory cytokines (Maes et al., 1995), CRP and serum amyloid A (Kling et al., 2007), but decreases in immunoglobulin A (Gold et al., 2012), compared with HCs. Further, a reduction in the 5-hydroxyindoleacetic acid to kynurenine ratio was found in the cerebrospinal fluid of unmedicated rMDD patients compared with both dMDD patients and HCs, suggesting greater shunting of TRP away from serotonin synthesis towards the kynurenine metabolic pathway (Kaddurah-Daouk et al., 2012). In aggregate these data support the hypothesis that a proportion of MDD patients suffer from persistent low-level, state-independent inflammation. Our data suggest a slightly different picture since we did not observe significant differences between the rMDD and HC group in CRP and IL-1RA levels or KYN/TRP, a proxy measure of *IDO* activity. Rather our data raise the possibility of a persistent abnormality further “downstream” in the kynurenine metabolic pathway, i.e. an imbalance between the synthesis/breakdown of KynA and QA in the periphery.

Persistent abnormalities in kynurenine metabolism may have implications for pathophysiology. Mood disorders are increasingly conceptualized as neuropathological conditions characterized by reductions in gray matter volume, dendritic atrophy and/or glial cell loss (Manji et al., 2001; Savitz et al., 2014b). Elevations in the potentially neurotoxic metabolites, 3HK and QA have been reported in neurodegenerative and/or inflammatory disorders such as Alzheimer's disease, Huntington's disease, Parkinson's disease, and systemic lupus erythematosus with neuropsychiatric manifestations (Guidetti and Schwarcz, 2003; Ogawa et al., 1992; Rahman et al., 2009; Schwarcz et al., 2012; Vogelgesang et al., 1996). Consistent with the possible neurotoxic effects of kynurenine metabolites in depression, we previously reported a positive correlation between KynA/QA and hippocampal volume in depressed MDD patients (Savitz et al., 2015).

If our finding that an inflammation-related imbalance in the kynurenine metabolic pathway is present in a subset of fully-remitted, non-symptomatic patients proves reproducible, then this raises questions concerning the risk-benefit ratio of discontinuing certain classes of antidepressant medications in these patients and whether treatment with anti-inflammatory medications would be beneficial in a subset of long-term remitters. Nevertheless, the results reported here should be treated as preliminary given the modest sample size, the fact that historical information such as number of episodes is subject to recall errors, and the cross-sectional nature of the study design which does not allow us to draw conclusions about whether the putative kynurenine-pathway abnormality is a consequence of prior depressive episodes, or whether it is a vulnerability factor for MDD.

In sum, we present preliminary evidence to support the existence of a persistent trait-like abnormality in kynurenine metabolism in MDD which may worsen with additional episodes

of depression. Our results highlight the importance of early intervention and raise questions concerning the appropriate clinical management of long-term remitters.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- There was no significant difference in CRP and IL-1RA across groups
- There was no significant difference in activation of the kynurenine pathway (KYN/TRP)
- Depressed and remitted subjects show reduced kynurenic acid : quinolinic acid ratio
- An abnormality in kynurenine metabolism may persist in fully-remitted patients
- Our data raise questions concerning the optimal treatment of remitted individuals

Figure 1.

A. Scatterplot showing the difference in KynA/3HK across the dMDD, rMDD, and healthy control (HC) groups. The dMDD subjects are represented by blue circles, the rMDD subjects are represented by green squares, and the healthy controls by pink triangles. The mean and standard error of the mean are displayed for each group in black. After adjusting for sex and batch effects, the omnibus ANOVA showed no significant group differences ($F_{2,123}=1.8$, $p=0.109$).

B. Scatterplot showing the difference in KynA/QA across the dMDD, rMDD, and HC groups. The dMDD subjects are represented by blue circles, the rMDD subjects are represented by green squares, and the healthy controls by pink triangles. The mean and standard error of the mean are displayed for each group in black. After adjusting for sex and batch effects, the dMDD and the rMDD groups had significantly reduced KynA/QA concentrations compared with the HC group ($F_{2,123}=3.8$, $p=0.025$; dMDD versus HC: $p=0.029$; rMDD versus HC: $p=0.047$; dMDD versus rMDD: $p=0.781$).

C. Correlation between the degree of anhedonia (SHAPS score, x-axis) and KynA/QA (y-axis). The dMDD subjects are represented by blue circles and the rMDD subjects are represented by green squares. The dashed lines represent the lines of best fit. In the dMDD group, KynA/QA was inversely correlated with anhedonia (Pearson's $r=-0.31$, $p=0.041$; Spearman's $r_s=-0.34$, $p=0.023$). There was no significant association between KynA/QA and anhedonia in the rMDD group (Pearson's $r=-0.11$, $p=0.626$; Spearman's $r_s=0.04$, $p=0.875$).

D. Correlation between the number of months in remission (x-axis) and KynA/QA (y-axis) in the rMDD group. The solid black line represents the line of best fit. KynA/QA was

positively correlated with length of time in remission (Pearson's $r=0.49$, $p=0.028$; Spearman's $r_s=0.52$, $p=0.018$).

Table 1

Demographic, clinical and biomarker data for the dMDD, the rMDD, and the HC groups (Mean $\pm$ SD).

	dMDD	rMDD	HC
N	49	21	58
Sex (% F)	78	57	57
Age	35.4 $\pm$ 9.8	30.8 $\pm$ 12.2	32.8 $\pm$ 10.7
Age of onset	15.6 $\pm$ 6.7	20.0 $\pm$ 9.7	-
Number of episodes	8.6 $\pm$ 15.1	3.3 $\pm$ 3.1	-
Treatment Naïve (Y/N)	23/26	8/13	
Months off medication	45.7 $\pm$ 49.6	79.0 $\pm$ 81.0	-
Months in remission	-	44.4 $\pm$ 39.6	-
Number Hospitalizations	0.2 $\pm$ 0.6	0 $\pm$ 0	-
Suicide attempts	0.4 $\pm$ 0.6	0.2 $\pm$ 0.7	-
BMI	28.4 $\pm$ 5.3	25.8 $\pm$ 5.1	27.8 $\pm$ 5.6
HAM-D (24-item)	26.1 $\pm$ 5.7	2.8 $\pm$ 2.3	0.8 $\pm$ 1.4
MADRS	30.0 $\pm$ 6.1	2.3 $\pm$ 2.0	0.8 $\pm$ 1.8
SHAPS	31.0 $\pm$ 5.5	20.4 $\pm$ 4.4	18.7 $\pm$ 5.1
hs-CRP (pg/mL)	3.5 $\pm$ 3.9	2.1 $\pm$ 2.5	2.9 $\pm$ 4.5
IL-1RA	564.3 $\pm$ 468.4	347.0 $\pm$ 202.0	388.8 $\pm$ 228.9
TRP (μM)	53.8 $\pm$ 10.0	61.6 $\pm$ 14.0	60.0 $\pm$ 19.3
KYN (nM)	1.94 $\pm$ 0.47	1.96 $\pm$ 0.46	1.93 $\pm$ 0.45
KYN/TRP	0.037 $\pm$ 0.013	0.033 $\pm$ 0.009	0.033 $\pm$ 0.008
KynA (nM)	39.9 $\pm$ 9.4	37.6 $\pm$ 15.4	43.5 $\pm$ 17.1
3HK (nM)	37.4 $\pm$ 12.3	31.0 $\pm$ 7.6	34.8 $\pm$ 16.4
QA (nM)	400.0 $\pm$ 179.0	338.9 $\pm$ 123.5	339.5 $\pm$ 111.8
KynA/3HK	1.15 $\pm$ 0.37	1.21 $\pm$ 0.39	1.32 $\pm$ 0.40
KynA/QA	0.11 $\pm$ 0.03	0.11 $\pm$ 0.04	0.13 $\pm$ 0.05

Note: There was a trend towards sex differences across the groups ($X^2=5.6$, $p=0.060$) but no significant differences in age ($F_{2,125}=1.6$, $p=0.198$) or BMI ($F_{2,125}=1.6$, $p=0.201$) across the groups. Accurate data for number of depressive episodes were not available for 19 individuals with dMDD and 1 person with rMDD. CRP data were available for 36 subjects with dMDD, 13 rMDD subjects, and 37 healthy controls. IL-1RA data were available for 37 individuals with dMDD, 18 rMDD subjects, and 31 healthy controls. SHAPS data were available for 44 subjects with dMDD, 21 rMDD subjects, and 44 healthy controls.

Abbreviations: dMDD= major depressive disorder in current depressive episode; rMDD=major depressive disorder in remission; HC=Healthy Control; BMI=Body Mass Index; HAM-D=Hamilton Depression Rating Scale; SHAPS=Snaith-Hamilton Pleasure Scale; hs-CRP= high sensitivity C-reactive protein; TRP=Tryptophan; KYN=Kynurenine; 3HK=3-hydroxykynurenine; KynA=Kynurenic Acid; QA=Quinolinic Acid.