Brain, Behavior, and Immunity 46 (2015) 55–59

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Short Communication

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

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ARTICLE INFO

Article history:
Received 3 September 2014
Received in revised form 5 February 2015
Accepted 5 February 2015
Available online 14 February 2015

Keywords:
Major depressive disorder
Inflammation
Kynurenine
Kynurenic acid
Quinolinic acid
Remission
Anhedonia

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 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.

1. Introduction

Circulating biomarkers of inflammation such as C-reactive protein (CRP) and pro-inflammatory cytokines such as IL-1β 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 (Fig. 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

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http://dx.doi.org/10.1016/j.bbi.2015.02.007
0889-1591/

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.

2. 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 8 am and 11 am. Serum samples were collected with BD Vacutainer serum tubes, processed according to the standard BD Vacutainer protocol, and stored at −80 °C.

Table 1
Demographic, clinical and biomarker data for the dMDD, the rMDD, and the HC groups (Mean ± SD).

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

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 3000 rpm. 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).