Research Paper

The role of anthranilic acid in the increase of depressive symptoms and major depressive disorder during treatment for hepatitis C with pegylated interferon-α2a and oral ribavirin

Tomasz Pawlowski, MD, PhD; Dariusz Pawlak, PhD; Malgorzata Inglot, MD, PhD; Malgorzata Zalewska, PhD; Dominik Marciniak, PhD; Jolanta Bugajska, PhD; Justyna Janocha-Litwin, MD; Krzysztof Malyszczak, MD, PhD

Background: Tryptophan metabolism via the kynurenine pathway is considered the link between the immune and endocrine systems. Dysregulation of serotonergic transmission can stem from the direct influence of interferon-α on the activity of serotonergic receptors 5-HT$_{1A}$ and 5-HT$_{2A}$, and from its indirect effect on tryptophan metabolism. Induction of the kynurenine pathway increases the concentration of neurotoxic kynurenine metabolites, and the activity of kynurenine derivatives is linked to the onset of depression. The aim of our study was to evaluate the relationships between depressive symptoms and kynurenine, tryptophan, anthranilic acid and kynurenic acid concentrations, indolamine 2,3-dioxygenase (IDO) activity and tryptophan availability to the brain. **Methods:** The study followed a prospective longitudinal cohort design. We evaluated 101 patients with chronic hepatitis C who were treated with pegylated interferon-α2a, and 40 controls who were awaiting treatment. We evaluated the relationships between total score on the Montgomery–Åsberg Depression Rating Scale and kynurenine, tryptophan, anthranilic acid and kynurenic acid concentrations, IDO activity and tryptophan availability to the brain. A logistic regression model was adapted for the diagnosis of major depressive disorder at each time point, taking into account changes in parameters of the kynurenine pathway between a given time point and the baseline measurement. **Results:** Of the treated patients, 44% fulfilled the criteria for major depressive disorder at least once during the 24 weeks of treatment. Anthranilic acid concentrations were significantly increased compared to baseline for all time points except week 2. Tryptophan availability showed a significant decrease ($\beta = -0.09$, $p = 0.01$) only in week 12 of treatment. Over time, kynurenine, tryptophan and anthranilic acid concentrations, as well as IDO activity and tryptophan availability to the brain, were significantly associated with total score on the Montgomery–Åsberg Depression Rating Scale. A logistic regression model revealed that participants with decreased tryptophan availability to the brain at 12 weeks of treatment and participants with increased anthranilic acid concentrations at week 24 of treatment were at increased risk for diagnosis of major depressive disorder (odds ratios 2.92 and 3.59, respectively). **Limitations:** This study had an open-label design in a population receiving naturalistic treatment. **Conclusion:** The present study provides the first direct evidence of the role of anthranilic acid in the pathogenesis of inflammation-induced major depressive disorder during treatment for hepatitis C with pegylated interferon-α2a.

Introduction

Tryptophan is an essential amino acid that must be supplied from external sources. Four biosynthetic pathways originate from tryptophan: synthesis of proteins, synthesis of serotonin, synthesis of tryptamine and the kynurenine pathway (Fig. 1). Tryptophan metabolism via the kynurenine pathway is considered the link between the immune and endocrine systems. Lapin and Oxenkrug[1] proposed a mechanism of tryptophan depletion as a result of kynurenine pathway activation, leading to depression. During treatment with interferon, a decrease in serotonin levels has been observed in both plasma[2] and in blood platelets,[3] and this decrease has been correlated with intensified depressive symptoms. Dysregulation of serotonergic transmission can stem from the direct interference of interferon-α in the activity of serotonergic receptors 5-HT$_{1A}$ and 5-HT$_{2A}$, and from its indirect effects on tryptophan metabolism.[4,5]

The induction of the kynurenine pathway increases the concentration of neurotoxic kynurenine metabolites
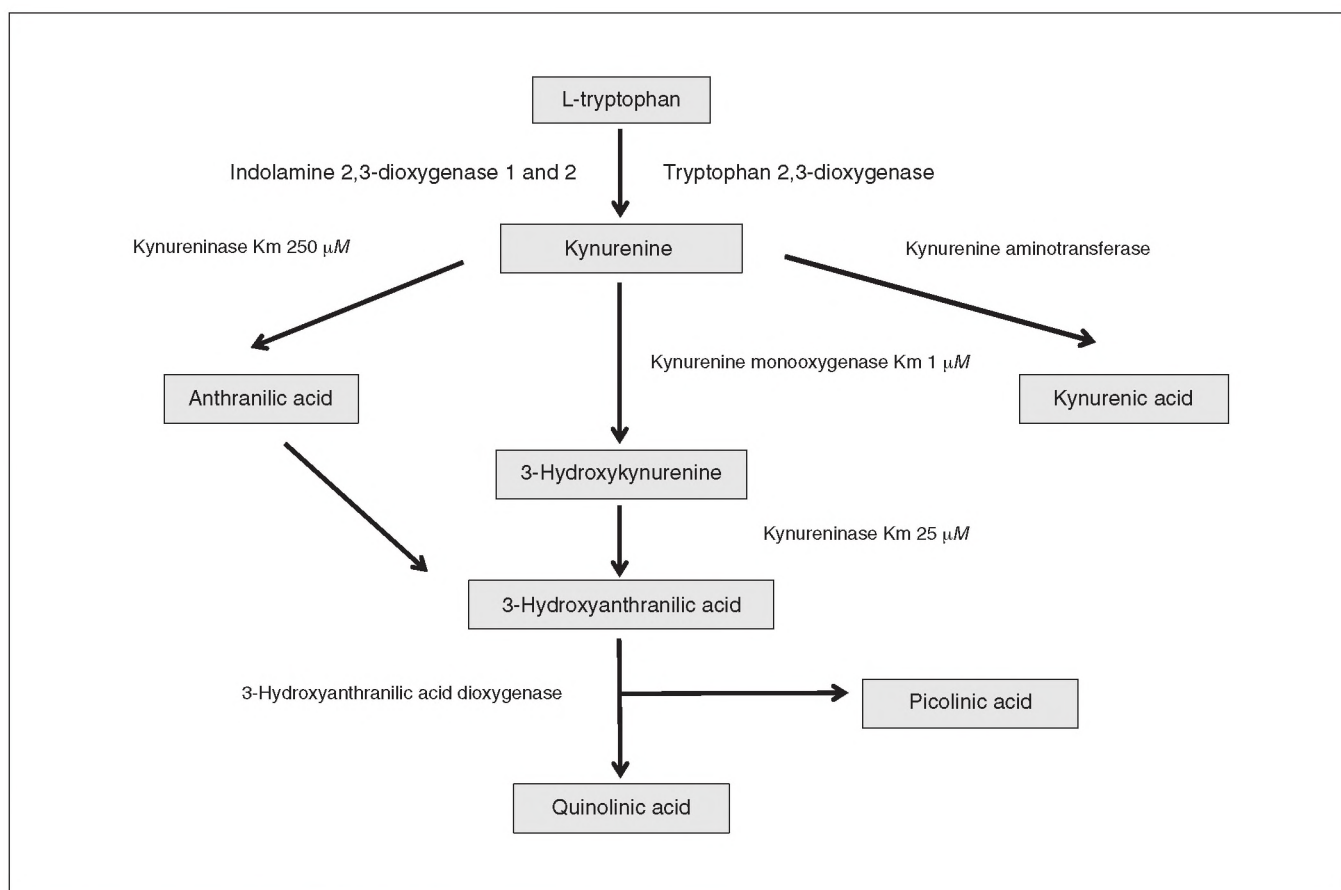
Fig. 1: Diagram of tryptophan metabolism.

3-hydroxykynurenine and quinolinic acid, which have a toxic influence on the central nervous system. The activity of these kynurenine derivatives is linked to the onset of depression.[6] Quinolinic acid is a strong N-methyl-D-aspartate receptor agonist that causes excitotoxicity in the neurons. The imbalance between quinolinic acid and kynurenic acid (an N-methyl-D-aspartate antagonist) might disturb glutamate neurotransmission[7,8] — called the "neurodegeneration hypothesis."[9]

"Sickness behaviour" is a term that describes behavioural (reduced food intake, sleep disturbances, decreased novelty-seeking), emotional (anhedonia) and cognitive (impaired attention) changes induced by inflammation.[10] Similarities between the symptoms of sickness behaviour and depression alerted scholars to the role of inflammation in the pathogenesis of depression.[11–13] The effect of inflammation on the induction of depressive symptoms has been investigated using different stimuli to induce peripheral inflammatory activity. Prospective experimental studies have relied on 1 of 3 paradigms to induce inflammation: vaccination, endotoxin injection and interferon treatment.[14]

Research findings have indicated potential pathomechanisms by which inflammation is connected to depression: altered neuroplasticity (as an effect of decreased expression of brain-derived neuroprotective hormone[15]), altered dopa-

minergic system,[16] sustained activation of the hypothalamic–pituitary–adrenal axis,[17] altered serotonin system[18] and altered glutamate neurotransmission.[19]

Immunotherapy with interferon-α facilitates an opportunity for the prospective observation of the occurrence of depressive symptoms. It induces depression in 20% to 70% of patients.[20] So far, investigations into interferon-α treatment have confirmed a decrease in plasma tryptophan, but not in tryptophan availability to the brain (the ratio of tryptophan to competing amino acids).[2,6,21,22] Decreased tryptophan availability to the brain has been detected in only 1 study, by Capuron and colleagues.[23]

The aim of our study was to evaluate the relationship between kynurenine, tryptophan, kynurenic acid and anthranilic acid concentrations, indolamine 2,3-dioxygenase (IDO) activity, tryptophan availability to the brain and depressive symptoms. We also evaluated the effect of tryptophan catabolites on the diagnosis of depression.

Methods

Sample

Our sample included 141 adults with chronic hepatitis C infection (detectable plasma HCV RNA levels on the Cobas Amplicor HCV Monitor Test version 2.0; Roche Diagnostics)

and compensated liver disease, who qualified for treatment with pegylated interferon-α2a (PEG-IFN-α2a). Patients were at least 18 years old and had previously untreated hepatitis C. All patients were recruited from the same geographic area and belonged to native Polish populations.

Apart from chronic hepatitis C, patients had no other clinical conditions that might have influenced their immune status (e.g., acute or chronic infections, pregnancy, autoimmune and neoplastic diseases), nor were they receiving any immunosuppressive treatment. Patients who were actively using alcohol or taking drugs were excluded from the study. Other exclusion criteria included previous treatment with interferon-α, a history of traumatic brain injury or neurologic or psychiatric disorders at baseline (major depressive disorder [MDD], bipolar disorder, schizophrenia). None of the participants was receiving antidepressants or anxiety medications 6 months before inclusion in the study.

The participants in the current study were also a subsample of participants from previous studies analyzing the effect of interferon treatment on the activation of peripheral blood mononuclear cells ($n = 24$), the outcomes of antiviral treatment ($n = 22$) and alterations in the metabolism of tryptophan after interferon treatment ($n = 76$ and $n = 24$).[24–26]

Informed consent was obtained from each patient in the study, and the study protocol followed the ethical guidelines of the 1975 Helsinki Declaration. Furthermore, the study was approved by the ethical committee at Wroclaw Medical University.

Study design

The study followed a prospective longitudinal cohort design. We examined neurobiological and behavioural variables in study participants receiving treatment with PEG-IFN-α2a and ribavirin (treatment group, $n = 101$) and in a group awaiting treatment (controls, $n = 40$). We conducted a cross-sectional assessment of tryptophan and its metabolites in the peripheral blood of the treatment group at baseline and week 24 to compare them with findings for the control group.

Participants in the treatment group were evaluated 6 times: before they started treatment (week 0) and at weeks 2, 4, 8, 12 and 24. They received PEG-IFN-α2a (Pegasys; Hoffmann-LaRoche) 180 μg once per week. They also received ribavirin (Schering-Plough Corp.) 1000 mg per day if their body weight was less than 75 kg, or 1200 mg per day if their body weight was 75 kg or more. The duration of treatment was 48 weeks, except for patients with genotype 3 hepatitis C, who were treated for 24 weeks.

Participants awaiting treatment with PEG-IFN-α2a and ribavirin (controls) were examined twice: at the beginning of the study and 24 weeks later.

Psychiatric evaluation

Participants underwent a baseline psychiatric evaluation using the Present State Examination from the Schedules of Clinical Assessment in Neuropsychiatry (SCAN 2.0)[27] performed by a senior board-certified investigator. Categorical

depressive episodes during treatment were diagnosed according to the DSM-IV criteria for Axis I disorders. We used the Montgomery–Åsberg Depression Rating Scale (MADRS) to assess the severity of depressive symptoms.[28]

Laboratory assessments

At each assessment point blood samples were collected between 0800 h and 0900 h after overnight fasting. We performed quantitative determination of plasma free amino acids using the Pico-Tag method (Waters). As an internal standard, we used methionine sulfoxide at a concentration of 0.8 mM in 0.1 M HCl (Sigma-Aldrich). The following analytical stages were performed: samples were deproteinized using ultrafiltration (Microcon filter, Millipore); samples were derivatized using phenylisotiocyanate; samples were determined using UV-visible high-performance liquid chromatography; individual amino acids were identified; and concentrations were calculated. Sample separation was performed in reverse-phase mode on the C18 Pico-Tag chromatography column, and absorbance of analytes was measured at 254 nm. We used the Empower program (Waters) to collect and compile data. We determined the following amino acids: tyrosine, valine, isoleucine, leucine and phenylalanine. These amino acids compete with tryptophan for transport across the blood–brain barrier, so they are referred to as competing amino acids (CAAs). We calculated the plasma CAA value by summing the tyrosine, valine, isoleucine, leucine and phenylalanine concentrations.

The method for amino acids analysis used in the present study is controlled by the European Research Network for Inherited Disorders of Metabolism (ERNDIM, MCA Laboratory).

We measured kynurenine concentrations according to Holmes.[29] Using a variable wavelength detector, the column effluent was monitored at 365 nm. The mobile phase consisted of 0.1 M acetic acid and 0.1 M ammonium acetate (pH 4.65) containing 2% acetonitrile, and it was pumped at a flow rate of 0.2 mL/min.

We determined tryptophan, anthranilic acid and kynurenic acid concentrations using high-performance liquid chromatography according to the method of Herve and colleagues.[30] The chromatographic equipment was an Agilent 1200 series liquid chromatography system (Agilent Technologies), composed of a G1322A degasser, a G1311A quaternary pump, a G1329A autosampler and a G1330B thermostat for the autosampler, a HP1050 variable wavelength detector and a HP1046A fluorescence detector. Detectors were connected with a Spherisorb 3 μm ODS2 150 × 2.1 mm column (Waters).

Column effluent was monitored using a programmable fluorescence detector. Optimized conditions were determined by recording fluorescence spectra with a stop-flow technique. Excitation and emission wavelengths were set at 254/404 nm for tryptophan and kynurenic acid, and 320/420 nm for anthranilic acid. The mobile phase consisted of 50 mM acetic acid and 0.25 M zinc acetate (pH 4.9) containing 1.2% acetonitrile, and it was pumped at a flow rate of 0.2 mL/min.

Statistical analysis

We fitted multiple repeated-measures regression models using the generalized estimating equations population-averaged model. In the generalized estimating equations models, we used an exchangeable covariance structure. The effect of explanatory variables on dependent variables was expressed using regression coefficients (β) and 95% confidence intervals (CIs). Analyses were performed on the treatment group only. We used 7 separate models — total MADRS score; kynurenine, tryptophan, kynurenic acid and anthranilic acid concentrations; kynurenine:tryptophan ratio; and tryptophan:CAA ratio — and we used time as an explanatory variable. Then, total MADRS score was regressed using kynurenine, tryptophan, anthranilic acid and kynurenic acid concentrations, tryptophan:CAA ratio and kynurenine:tryptophan ratio as covariates to examine their relationships with depressive symptoms.

We adapted a logistic regression model for the diagnosis of MDD at each time point, taking into account changes in the parameters of the kynurenine pathway between a given point and the baseline measurement. Parameter changes were categorized against the median. We used stepwise elimination of statistically nonsignificant parameters, using $p = 0.1$ as a level to remain in the model. The effect of changes in biochemical parameters of the kynurenine pathway on the diagnosis of depression was expressed using odds ratios (ORs) and 95% CIs.

We estimated IDO enzymatic activity for each time point by calculating the kynurenine:tryptophan ratio $\times 10^3$ (mmol/mol). We calculated the quotient of tryptophan:CAA $\times 10^3$ to estimate the tryptophan availability to the brain.

Results

During the 24 weeks of treatment, 4 patients had to discontinue the treatment early because of somatic side effects. This meant that 97 patients completed the 24 weeks of the treatment; their data were included in the analysis (Table 1).

Depressive symptoms and diagnosis of MDD

Total MADRS score was significantly increased compared to baseline at all time points in the treatment group. As well, 43 patients (44%) fulfilled the criteria for MDD at least once during the 24 weeks of treatment (Table 2).

In the control group, the total MADRS score (mean ± standard deviation [SD]) was 8.0 ± 4.8 at baseline and 9.0 ± 5.2 after 24 weeks (not significant). We observed no major differences in MADRS scores at baseline between the treatment and control groups. We did observe significant differences in MADRS scores between the treatment and control groups at week 24 after the first examination (14.7 ± 7.4 for the treatment group v. 9.0 ± 5.2 for the control group; $p < 0.001$).

None of the participants in the control group fulfilled the criteria for MDD at baseline or at 24 weeks.

Kynurenine, tryptophan, anthranilic acid and kynurenic acid concentrations, IDO activity and tryptophan availability

In the treatment group IDO activity (kynurenine:tryptophan ratio) was significantly increased compared to baseline at all time points. Tryptophan concentrations declined significantly during treatment, but kynurenine concentrations increased markedly compared to baseline for all time points except week 2 (Table 3 and Table 4).

Tryptophan availability (tryptophan:CAA ratio) showed a significant decrease (β = −0.09; $p = 0.01$) only in week 12 of treatment. Anthranilic acid concentration was significantly increased compared to baseline for all time points except week 2. Kynurenic acid concentration showed a statistically significant increase only in week 24 of treatment (β = 0.20; $p = 0.01$) (Fig. 2).

In the control group at baseline, kynurenine levels (mean ± SD) were 2.0 ± 0.75 μmol/L, tryptophan levels were 36.1 ± 8.5 μmol/L, anthranilic acid levels were 42.2 ± 28.6 nmol/L, kynurenic acid levels were 30.2 ± 10.2 nmol/L, IDO activity was 53.3 ± 19.5 and tryptophan availability was 63.8 ± 12.5. We observed no major differences between the treatment and control groups at baseline.

Table 1: Baseline characteristics of study cohort and controls*

Characteristic	Patients treated with PEG-IFN-α-2a and oral RBV (n = 97)	Controls awaiting treatment with PEG-IFN-α-2a and oral RBV (n = 40)
Overall cases, n	97	40
Age, yr	46.2 ± 9.9	44.2 ± 10.0
Sex, M/F	48/49	20/20
Weight, kg	77.8 ± 16.3	77.3 ± 14.6
Alanine aminotransferaze, IU/L	83 ± 52	79 ± 44
Serum HCV RNA × 10⁶ IU/mL	2.0 ± 3.3	1.9 ± 3.0
History of MDD, n	9	3
History of substance abuse, n	31	10
Education, yr	13 ± 3	12 ± 3

HCV = hepatitis C virus; MDD = major depressive disorder; PEG-IFN-α2a = pegylated interferon-α2a; RBV = ribavirin.
*Unless otherwise indicated, values are mean ± standard deviation; all differences between groups were nonsignificant.

Table 2: MADRS score and participants with MDD in the treatment group (*n* = 97)

	Week of treatment					
Characteristic	Baseline	2	4	8	12	24
Total MADRS score, mean ± SD	7.0 ± 4.8	11.3 ± 6.5	13.8 ± 7.5	14.7 ± 7.5	14.1 ± 7.3	14.7 ± 7.4
Patients with MDD, *n* (%)	0	18 (19)	29 (30)	39 (40)	36 (37)	40 (41)

MDD = major depressive disorder; MADRS = Montgomery–Åsberg Depression Rating Scale; SD = standard deviation.

Table 3: Kynurenine, tryptophan, kynurenic acid and anthranilic acid concentrations, IDO activity and tryptophan availability in the treatment group (*n* = 97)

	Week of treatment*					
Characteristic	Baseline	2	4	8	12	24
Kynurenine, µmol/L	2.04 ± 0.66	2.11 ± 0.57	2.13 ± 0.69	2.22 ± 0.64	2.20 ± 0.61	2.41 ± 0.60
Tryptophan, µmol/L	35.1 ± 8.6	34.2 ± 7.1	33.7 ± 6.0	33.1 ± 8.2	32.3 ± 7.8	32.5 ± 7.1
Kynurenic acid, nmol/L	27.6 ± 9.3	26.2 ± 10.5	27.5 ± 12.1	30.5 ± 17.5	31.5 ± 16.5	34.0 ± 18.1
Anthranilic acid, nmol/L	44.6 ± 23.5	52.9 ± 24.4	59.0 ± 29.6	60.7 ± 26.5	49.9 ± 26.4	54.8 ± 18.8
IDO activity (KYN:TRP × 10^3)	57.1 ± 20.0	65.1 ± 20.1	67.1 ± 24.5	76.1 ± 23.8	82.0 ± 29.2	85.4 ± 26.2
Tryptophan availability (TRP:CAA × 10^3)	64.0 ± 13.0	64.2 ± 15.2	64.7 ± 11.9	64.5 ± 12.1	55.6 ± 12.2	59.3 ± 9.4

CAA = competing amino acid; IDO = indolamine 2,3-dioxygenase; KYN = kynurenine; TRP = tryptophan.
*Values are mean ± standard deviation.

Table 4: Multilevel regression analyses indicating differences in kynurenine, tryptophan, kynurenic acid and anthranilic acid concentrations, IDO activity and tryptophan availability for each time point compared with baseline in the treatment group (*n* = 97)*

	Week of treatment									
	2		4		8		12		24	
Characteristic	β	*p* value	β	*p* value	β	*p* value	β	*p* value	β	*p* value
MADRS	4.82	< 0.001	7.32	< 0.001	8.22	< 0.001	7.65	< 0.001	8.25	< 0.001
Kynurenine	0.07	NS	0.09	0.03	0.18	< 0.001	0.16	0.001	0.37	< 0.001
Tryptophan	−0.09	0.007	−0.14	< 0.001	−0.20	< 0.001	−0.28	< 0.001	−0.26	< 0.001
Kynurenic acid	−0.10	NS	−0.01	NS	0.08	NS	0.10	NS	0.20	0.01
Anthranilic acid	0.14	NS	0.37	< 0.001	0.41	< 0.001	0.23	0.004	0.30	< 0.001
IDO activity (KYN:TRP × 10^3)	0.12	< 0.001	0.20	< 0.001	0.30	< 0.001	0.37	< 0.001	0.43	< 0.001
Tryptophan availability (TRP:CAA × 10^3)	0.03	NS	0.06	NS	0.04	NS	−0.09	0.01	−0.06	NS

CAA = competing amino acid; IDO = indolamine 2,3-dioxygenase; KYN = kynurenine; MADRS = Montgomery–Åsberg Depression Rating Scale; NS = not significant; TRP = tryptophan.
*β indicates the regression coefficient.

The second assessment of the control group took place 24 weeks after the first assessment. At this point, kynurenine levels (mean ± SD) were 2.04 ± 0.70 µmol/L, tryptophan levels were 35.2 ± 7.5 µmol/L, kynurenic acid levels were 31.3 ± 12.2 nmol/L, anthranilic acid levels were 47.0 ± 30.2 nmol/L, IDO activity was 59.1 ± 20.2 and tryptophan availability was 62.5 ± 12.1. We detected no major differences between the first and second assessments in the control group.

Laboratory assessment findings and their relationship with MADRS score and diagnosis of MDD

Kynurenine, tryptophan and anthranilic acid concentrations, IDO activity and tryptophan availability were significantly associated over time with total MADRS score (Table 5). A logistic regression model revealed the following (Table 6). At

week 12 of treatment, participants with changes in tryptophan availability that were greater than the median had a higher risk of a diagnosis of MDD (OR 2.92) than participants with changes that were lower than the median. At week 24 of treatment, participants with changes in anthranilic acid concentrations that were greater than the median had a higher risk of a diagnosis of MDD (OR 3.59) than participants with changes lower than the median.

Discussion

Our main finding is connected with the possible role of anthranilic acid in the pathogenesis of depression. We found that anthranilic acid concentrations were significantly increased during treatment with PEG-IFN-α2a and ribavirin; they were significantly associated over time with total
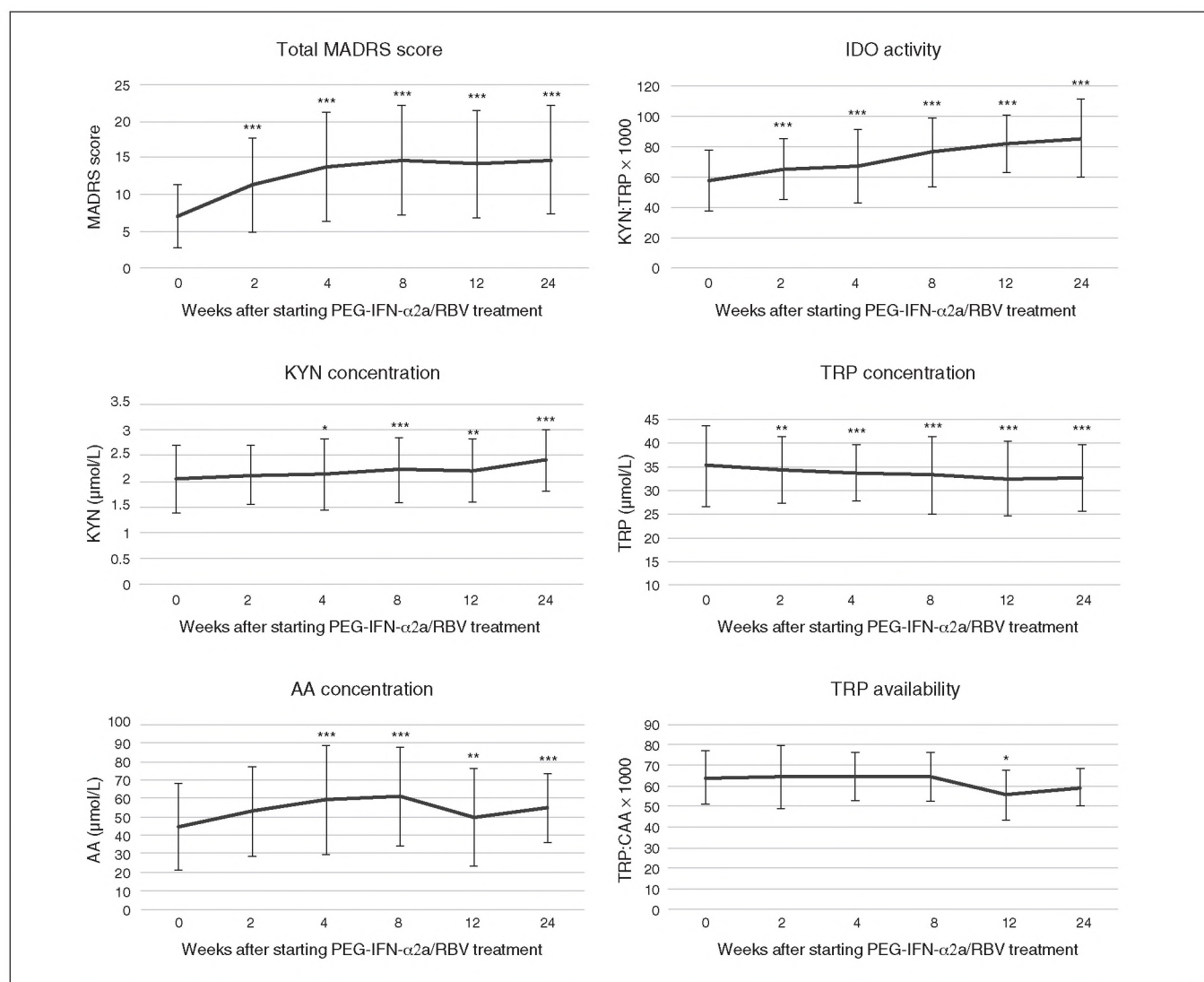
Fig. 2: Changes in MADRS score, IDO activity (KYN:TRP), kynurenine, tryptophan and anthranilic acid concentrations, and tryptophan availability to the brain (TRP:CAA) during 24 weeks of treatment with PEG-IFN-α2a and oral ribavirin. Error bars indicate standard deviation. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Week 0 = baseline. AA = anthranilic acid; CAA = competing amino acid; IDO = indolamine 2,3-dioxygenase; KYN = kynurenine; MADRS = Montgomery–Åsberg Depression Rating Scale; PEG-IFN-α2a = pegylated interferon-α2a; RBV = ribavirin; TRP = tryptophan.

Table 5: Relationship of kynurenine, tryptophan, kynurenic acid and anthranilic acid concentrations, IDO activity and TRP availability with MADRS score in the treatment group ($n = 97$)

Characteristic	MADRS*	
	β (95% CI)	p value
Kynurenine	0.37 (0.12 to 0.62)	0.004
Tryptophan	−1.01 (−1.31 to −0.72)	< 0.001
Kynurenic acid	−0.12 (−0.19 to 0.04)	NS
Anthranilic acid	0.28 (0.04 to 0.47)	0.006
IDO activity (KYN:TRP × 10³)	0.08 (0.02 to 0.12)	0.004
Tryptophan availability (TRP:CAA × 10³)	−0.50 (−0.19 to −0.81)	0.001

CAA = competing amino acid; CI = confidence interval; IDO = indolamine 2,3-dioxygenase; KYN = kynurenine; MADRS = Montgomery–Åsberg Depression Rating Scale; NS = not significant; TRP = tryptophan.
*Linear model for logarythmic values.

MADRS score; and at week 24 of treatment, participants with increased anthranilic acid concentrations (greater than the median) had a higher risk of a diagnosis of MDD (OR 3.59).

Increased anthranilic acid concentrations have been reported in schizophrenia, rheumatoid arthritis and type 1 diabetes.[31–33] To the best of our knowledge, the present study provides the first direct evidence of a role for anthranilic acid in the pathogenesis of inflammation-induced MDD.

Anthranilic acid is one of the products of kynurenine metabolism, which converts kynurenine to anthranilic acid, kynurenic acid and 3-hydroxykynurenine. The conversion of kynurenine to anthranilic acid occurs under the influence of the kynureninase enzyme. Kynureninase is an enzyme present mostly in liver, kidney and spleen cells. Its activity in the brain amounts to only 1% of its activity in the liver.[34] Because

Table 6: Logistic regression model adapted for a diagnosis of MDD at each time point*

Characteristic†	Diagnosis of MDD				
	2	4	8	12	24
Δ Kynurenine	$p > 0.1$	$p > 0.1$	$p > 0.1$	$p > 0.1$	$p > 0.1$
Δ Tryptophan	$p > 0.1$	$p > 0.1$	$p > 0.1$	$p > 0.1$	$p > 0.1$
Δ Kynurenic acid	$p > 0.1$	$p > 0.1$	$p > 0.1$	$p > 0.1$	$p > 0.1$
Δ Anthranilic acid	$p > 0.1$	$p > 0.1$	$p > 0.1$	$p > 0.1$	OR (95% CI) = 3.59 (1.18 to 10.93) $p = 0.024$
Δ IDO activity (KYN:TRP × 10^3)	$p > 0.1$	$p > 0.1$	$p > 0.1$	$p > 0.1$	$p > 0.1$
Δ Tryptophan availability (TRP:CAA × 10^3)	$p > 0.1$	$p > 0.1$	$p > 0.1$	OR (95% CI) = 2.92 (1.01 to 8.42) $p = 0.032$	$p > 0.1$

CAA = competing amino acid; CI = confidence interval; IDO = indolamine 2,3-dioxygenase; KYN = kynurenine; OR = odds ratio; TRP = tryptophan.
*This model takes into account the changes (Δ) in kynurenine, tryptophan, kynurenic acid and anthranilic acid concentrations, IDO activity (KYN:TRP) and TRP availability (TRP:CAA) between a given point and the baseline measurement in treatment group.
†Greater than the median v. less than the median.

of this, anthranilic acid synthesis takes place mostly in the periphery, entering the brain by passive diffusion.[35,36] The next stage in the kynurenine pathway is buildup of 3-hydroxyanthranilic acid (3HAA), which can be synthesized from both anthranilic acid and 3-hydroxykynurenine. Conversion of 3-hydroxykynurenine into 3HAA also occurs under the influence of kynureninase enzymatic activity (i.e., the same enzyme that converts kynurenine into anthranilic acid). The enzyme's propinquity to 3-hydroxykynurenine (Km 25 μM) is 10 times greater than to kynurenine (Km 250 μM),[37] meaning that kynurenine metabolism through 3-hydroxykynurenine is the main source of 3HAA in peripheral tissue (Fig. 1).

Because 3HAA does not cross the blood–brain barrier,[36] nonspecific hydroxylation of anthranilic acid remains the major source of 3HAA in the brain.[38] Thus in the brain, peripheral anthranilic acid is the preferred precursor for 3HAA production, whereas in peripheral tissue, 3HAA is produced from 3-hydroxykynurenine. This ties in with data from a study by Giorgini and colleagues,[39] which showed that genomic depletion of kynurenine 3-monooxygenase (KMO), converting kynurenine to 3-hydroxykynurenine, reduced brain quinolinic acid levels by only about 20%, and anthranilic acid levels were markedly increased.

We know that 3HAA, anthranilic acid and 3-hydroxykynurenine can inhibit the activity of complexes of the respiratory chain,[40] which is connected with impairments in mitochondrial functioning.[20] There is evidence that mitochondria-mediated mechanisms are related to depressive symptoms.[41,42] A correlation has been found between circulating mitochondrial DNA and inflammation.[43]

Mackay and colleagues[44] found strong positive correlations between 3HAA and scores on the MADRS, Hamilton Rating Scale for Depression and Beck Depression Inventory (higher concentrations of 3HAA were associated with greater degrees of depression at 6 weeks in patients with depression treated with fluoxetine).

In their study in mice infected with *Plasmodium berghei* ANKA, Clark and colleagues[45] found that administration of Ro-61–8048 (a high-affinity inhibitor of KMO synthesized

by F. Hoffmann-La Roche Ltd.) generated a 40-fold increase in brain anthranilic acid in controls and a more than 200-fold increase in infected animals. In this case, anthranilic acid appeared to play a protective role, although the theoretically high anthranilic acid levels should have caused a rise in 3HAA in the brain, and, as a result, a rise in quinolinic acid.

Tashiro and colleagues[46] investigated the role of KMO in emotional and cognitive function using KMO knockout mice compared to wild-type mice. Their results suggested that KMO knockout mice may be a novel animal model for MDD. Serum concentrations of kynurenine, kynurenic acid and anthranilic acid in the KMO knockout mice revealed dramatically increased levels of kynurenine (44737% that of wild-type mice), kynurenic acid (9000% that of wild-type mice) and anthranilic acid (1470% that of wild-type mice), as well as a decrease in 3HAA (88% that of wild-type mice) and a lack of 3-hydroxykynurenine in the serum. This finding seems to be consistent with predictions for the kynurenine pathway model (Fig. 1), given that in the case of the KMO enzyme blockade, kynurenine metabolism follows the anthranilic acid and kynurenic acid production pathway.

We are gradually uncovering more and more of the data required to answer the question of how peripheral blood changes in IDO catabolites manifest in the brain. Raison and colleagues[22] found that increased kynurenine in peripheral blood was correlated with increased kynurenine, quinolinic acid and kynurenic acid in cerebrospinal fluid. Kita and colleagues[47] showed that after systemic immune activation, kynurenine in the brain was derived exclusively from blood, transported to the brain by large amino acid transporters,[36] and brain quinolinic acid was derived from blood kynurenine (52%), blood quinolinic acid (40%) and blood 3HAA or 3-hydroxykynurenine (8%). Thus, blood kynurenine is the main source of central nervous system kynurenine, 3-hydroxykynurenine and quinolinic acid. According to our findings, anthranilic acid concentrations were associated with depressive symptoms, and at week 24 of treatment, increased anthranilic acid concentrations (greater than the median) were associated with MDD (OR 3.59). Together with the fact

that anthranilic acid can enter the brain by passive diffusions in significant quantities,[37] anthranilic acid might be regarded as a second (after kynurenine) important accessible "window" into the central nervous system, with respect to the activation of IDO pathways.

So far, the biologic importance of anthranilic acid remains unknown. Originally, it was believed to have been a substrate for 3HAA production, and, consequently, for quinolinic acid. However, increases in anthranilic acid concentration appear to have a protective function and do not lead to a rise in quinolinic acid concentrations,[40,46] which might be connected to the fact that anthranilic acid is a competitive inhibitor of 3HAA dioxygenase and might therefore reduce the conversion of 3HAA to quinolinic acid and picolinic acid.[48] Thus, endogenous levels of anthranilic acid limit the conversion of 3HAA to quinolinic acid. Anthranilic acid might also prevent tryptophan depletion by inhibiting sodium transporters.[49] However, we still know little about the role of anthranilic acid in the brain.

It is hypothesized that the ratio of 3HAA to anthranilic acid is a marker of the assessment of inflammation and its progression,[50] which could act as a compensatory mechanism for the reduction of cell toxicity. According to Darlington and colleagues,[50] a decreased ratio of 3HAA to anthranilic acid is responsible for a reduction in oxidative stress; it is also the antagonist of quinolinic acid and has a "cleanup" effect after brain injury or stroke.

Treatment with PEG-IFN-α2a and oral ribavirin significantly increased concentrations of kynurenine and anthranilic acid, and decreased concentrations of tryptophan, likely due to the induction of IDO activity, which we observed at all time points. Despite a decrease in total tryptophan concentrations, tryptophan availability to the brain was stable up to 8 weeks of treatment. Maintenance of tryptophan availability to the brain — expressed as the ratio of tryptophan to CAAs — was possible thanks to a decrease in CAA levels in the serum. Immunotherapy with interferon-α activates the inflammatory response system, which increases the use of amino acids by leukocytes and the liver. Amino acid oxidation and a higher uptake by the liver of branched-chain amino acids (valine, leucine and isoleucine) are activated.[2] The branched-chain amino acids, along with phenylalanine and tyrosine, compete with tryptophan for transport across the blood–brain barrier. The decrease in CAA levels in the serum during interferon-α immunotherapy has been reported by other researchers.[2,5,6] In light of this, the second important finding of our study was that after 12 weeks of treatment with PEG-IFN-α2a and ribavirin, the homeostatic mechanism of decreasing CAA levels at a rate proportionate to that of the tryptophan decrease broke down, resulting in lower tryptophan availability to the brain (expressed as the tryptophan:CAA ratio). We found that over time, the ratio of tryptophan to CAAs was significantly associated with total MADRS score. These findings corroborate the report of Capuron and colleagues[5] that the development of depressive symptoms during interferon therapy is mediated by reduced tryptophan availability to the brain. As well, from a logistic regression model, our results revealed that at week 12 of treatment, participants with decreased tryptophan availability (greater than the median) were at higher risk (OR 2.92) of a diagnosis of MDD. This result verged on statistical significance because the OR CIs were very wide and the lower bound was very close to 1 (1.02). However, in light of the above findings it also appeared to have clinical significance. Our results confirmed the potential role of tryptophan depletion in MDD. In a study of 58 patients with MDD and 189 controls, Myint and colleagues[51] found that the mean tryptophan index (tryptophan:CAA) in depressed patients was significantly lower than in controls ($p = 0.045$). However, given that mean kynurenic acid concentrations were lower in patients with depression ($p = 0.003$), the authors concluded that a reduction in neuroprotective markers plays an important role in the pathophysiology of MDD.

Our findings also emphasized an interesting aspect of the pathogenesis of depression related to IDO activation. According to Raison and colleagues,[22] immunotherapy with interferon-α significantly decreased tryptophan concentration in the blood but did not change tryptophan concentrations in cerebrospinal fluid. Thus, although the mechanism of peripheral IDO activation causes decreased tryptophan concentrations in the blood, it does not lead to decreased tryptophan availability to the brain, because the levels of CAAs in the serum decrease simultaneously. Independent of the IDO activation mechanism, disturbances in the compensational mechanism for CAA decrease in the peripheral blood are necessary for the decrease in tryptophan:CAA ratio.

Data from our study argue in favour of Dantzer's hypothesis: "It is possible that depression represents a maladaptive version of cytokine-induced sickness, which could occur when activation of the innate immune response is exacerbated in intensity and/or duration."[10]

We showed that the duration of inflammation during treatment for hepatitis C with PEG-IFN-α2a and ribavirin is a very important factor. During different time periods, different mechanisms acquire particular significance. In week 12 of treatment, a "depletion mechanism" in the form of tryptophan availability to the brain was crucial for diagnosis of MDD, whereas in week 24 of treatment it was increased anthranilic acid concentrations that had an impact on the incidence of MDD.

We should remember that MDD is not a unified disease but a heterogeneous syndrome. According to Rantala and colleagues,[52] depression induced by infection is 1 of 12 discrete depression subtypes. The others are induced by long-term stress, loneliness, traumatic experience, hierarchy conflict, grief, romantic rejection, postpartum events, the seasons, chemicals, somatic diseases and starvation. An experimental interferon-α prospective model of MDD could be instrumental in providing causal evidence for an association between inflammation and depression. Dooley and colleagues[14] conclude that inflammation likely plays a role in exaggerated reactivity to negative information, altered reward reactivity and somatic symptoms.

Limitations

Limitations of this naturalistic study stemmed from its open-label design. However, the small number of participants precluded us from conducting further subgroup analyses. It should be noted that each patient was evaluated in detail at 6 time points over 24 weeks. As well, given the lack of concomitant biological measures such as cerebrospinal fluid concentrations of kynurenine, tryptophan, anthranilic acid and kynurenic acid, our findings should be approached with caution.

Conclusion

The main findings of the current work are connected with the role of anthranilic acid and decreased tryptophan availability to the brain in the pathogenesis of inflammation-induced depression during treatment for hepatitis C with PEG-IFN-α2a and ribavirin. Our results reveal that each of these mechanisms acquires clinical importance in the incidence of MDD at a different time point from the beginning of the treatment.

Affiliations: From the Division of Psychotherapy and Psychosomatic Medicine, Department of Psychiatry, Wroclaw Medical University, Wroclaw, Poland (Pawlowski, Malyszczak); the Department of Pharmacodynamics, Medical University of Bialystok, Bialystok, Poland (Pawlak); the Department of Infectious Diseases, Liver Diseases and Acquired Immune Deficiency, Wroclaw Medical University, Wroclaw, Poland (Inglot, Zalewska); the Department of Drugs Form Technology, Wroclaw Medical University, Wroclaw, Poland (Marciniak); the Clinical Biochemistry Department, Jagiellonian University College of Medicine, Krakow, Poland (Bugajska); and the Department of Infectious Diseases and Hepatology, Wroclaw Medical University, Wroclaw, Poland (Janocha-Litwin).

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