Biogenic Amines Vol. 25, issue 4 (2011), pp. 262–282
BIA250411A04

Somatization, but not depression, is characterized by disorders in the tryptophan catabolite (TRYCAT) pathway, indicating increased indoleamine 2,3-dioxygenase and lowered kynurenine aminotransferase activity

Reprinted from: Neuroendocrinology Letters 2011; **32**(3): 264–273.

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Key words: tryptophan; serotonin; inflammation; cytokines; oxidative stress; chronic fatigue

Abstract
Reduced plasma tryptophan occurs in depression and somatization. Induction of indoleamine 2,3-dioxygenase (IDO) with consequent synthesis of tryptophan catabolites (TRYCATs) and lowered tryptophan are associated with the onset of depression in the puerperium and during interferon-alpha treatment. Depression is accompanied by lowered kynurenic acid, a neuroprotectant, or increased kynurenine, a neurotoxic TRYCAT.
To examine plasma tryptophan; kynurenine; kynurenic acid; the kynureni-

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ne / tryptophan (KY/TRP) ratio, indicating IDO activity; and the kynurenine / kynurenic acid (KY/KA) ratio, indicating kynurenine aminotransferase (KAT) activity, in somatization; depression; somatization + depression; and controls. Illness severity is measured by the Somatic Symptom Index (SSI), the Screening for Somatoform Symptoms (SOMS), and the Beck Depression Inventory (BDI).
Tryptophan is significantly lower in patients than in controls and lower in somatization than in depression. KY/TRP is significantly increased in somatization. Kynurenic acid is significantly lower in patients than in controls, and lower in somatization than in depression. KY/KA is significantly higher in somatization and somatization + depression than in depression and controls. There are significant correlations between the severity of somatization, but not depression, and KY/TRP and KY/KA (positive) and tryptophan (negative). Kynurenine and kynurenic acid are significantly correlated in controls, somatization + depression, and depression, but not in somatization.
Somatization is characterized by increased IDO activity and disorders in KAT activity and an increased neurotoxic potential. The TRYCAT pathway may play a role in the pathophysiology of somatizing and "psychosomatic" symptoms through effects on pain, gut motility, the autonomic nervous system, peripheral NMDA receptors, etc. Even more, biological disorders, such as aberrations in the TRYCAT pathway, which are considered to be a hallmark for depression, are in fact attributable to somatization rather than to depression per se. Future research in depression on the TRYCAT pathway should always control for the possible effects of somatization.

INTRODUCTION

There is evidence that depletion of plasma tryptophan, and induction of indoleamine 2,3-dioxygenase (IDO) with consequent synthesis of tryptophan catabolites (TRYCATs) play a role in the onset of depression (Maes *et al.* 2011). The conversion of tryptophan to kynurenine is the first and rate-limiting step in this pathway. Kynurenine is further metabolized into kynurenic acid by kynurenine 2,3-aminotransferase (KAT) or to nicotinamide. IDO is widely expressed in human tissues, e.g. the brain, kidney, lung, spleen, and duodenum, macrophages and dendritic cells (Takikawa *et al.* 1984; Moroni *et al.* 1991). IDO is induced primarily by interferon-γ (IFNγ) and cytokines, like interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) (Maes *et al.* 2011; Oxenkrug 2007). There is

evidence that depression is accompanied by cell-mediated immune activation, as indicated by increased IFNγ and neopterin; and an inflammatory response, with increased production of IL-1β and TNFα (Maes 1995; 2010; Maes *et al.* 2011).

Activation of IDO diverts tryptophan from the 5-HT synthetic route thereby depleting tryptophan and consequently brain 5-HT and leads to the formation of some TRYCATs that are important to depression, e.g. kynurenine, and kynurenic acid (Maes *et al.* 1994; 2011). Indeed, kynurenine may be detrimental as it has depressogenic, anxiogenic, excitotoxic and neurotoxic effects. Kynurenic acid, on the other hand, has neuroprotective effects. Therefore, the kynurenine / kynurenic acid (KY/KA) ratio indicates not only KAT activity, but also the neurotoxic potential generated during IDO activation.

Depletion of plasma tryptophan is frequently observed in depression (DeMyer *et al.* 1981; Joseph *et al.* 1984; Moller 1985; Maes *et al.* 1987a; 1991a; Cowen *et al.* 1989). Low plasma tryptophan is significantly associated to various markers of cell-mediated immune activation in depression (Maes *et al.* 1993; 1994; Song *et al.* 1998). This indicates that cell-mediated immune activation and the inflammatory response in depression have induced IDO thus causing lowered plasma tryptophan. In the early puerperium, IDO activation, as estimated by the kynurenine/tryptophan (KY/TRP) ratio, and TRYCAT production are significantly associated with the development of affective symptoms (Maes *et al.* 2001b; 2002). The onset of affective symptoms during IFNα-based immunotherapy is significantly related to the KY/TRP ratio, plasma kynurenine and the KY/KA ratio (Maes *et al.* 2001a; Bonaccorso *et al.* 2002; Wichers *et al.* 2005b). Raison ***et al.*** (2010) reported that the IFNα-induced synthesis of kynurenine and quinolinic acid, another neurotoxic TRYCAT, are associated with the onset of depression. In depressed patients there are only few data: some authors found increased levels of neurotoxic TRYCATs (Gabbay *et al.* 2010), whereas others found lowered levels of neuroprotective TRYCATs (Myint *et al.* 2007).

Depression shows a high degree of comorbidity with somatization, a multisymptomatic syndrome characterized by unexplained physical symptoms, that has a prevalence rate of around 4–7% in the general population (Escobar *et al.* 1987; Rief *et al.* 2001a). Other terms that are frequently used to describe this prevalent disorder are 'functional somatic symptoms', 'psychosomatic syndrome' or 'somatoform disorders' (Rief *et al.* 2004). There is a considerable overlap between somatization and chronic fatigue syndrome, an illness that shows a strong comorbidity with depression (Rief *et al.* 2010). In primary care, depression is the most common comorbid diagnosis (54.6%) of somatization disorder (Brown *et al.* 1990). In the NIMH Epidemiologic Catchment Area study (Simon & vonKorff

1991), the increasing number of symptoms in subjects with somatization was strongly associated with depression. Recently, we have reported that patients with somatization have a lowered availability of plasma tryptophan and signs of monocytic activation, such as increased soluble IL-1 receptor antagonist (sIL-1RA) levels, and suppressed cell-mediated immunity, as indicated by lowered levels of the sCD8 molecule (Rief *et al.* 2004; 2001b). These results show that monocytic activation may underlie the pathophysiology of somatization. Likewise in related disorders, such as chronic fatigue syndrome and fibromyalgia, signs of inflammation and oxidative stress have been observed (Maes & Twisk, 2010; Maes *et al.* 2011; Brkic *et al.* 2010).

There are, however, no data whether the lowered plasma tryptophan in somatization may be explained by IDO activation or whether other disturbances in the TRYCAT pathway are involved in the pathophysiology of somatization.

This study was carried out to examine: a) whether increased IDO activation may explain lowered tryptophan levels in depression and somatization; b) whether depression is characterized by an increased neurotoxic (kynurenine) potential and/or decreased neuroprotective (kynurenic acid) potential; and c) other disorders in the TRYCAT pathway in somatization disorder.

SUBJECTS AND METHODS

Subjects

One-hundred and forty-six subjects participated in this study: one hundred an eleven patients and 35 normal controls. All patients were admitted to the Roseneck Center for Behavioral Medicine, a German inpatient treatment Center for psychiatric and psychosomatic disorders. Before inclusion in the study, patients were examined by means of self-rating scales, i.e. Beck Depression Inventory (BDI) (Beck *et al.* 1961) and the Screening for Somatoform Symptoms (SOMS) (Rief *et al.* 1997). The SOMS is a self-rating scale that considers the presence of 53 physical symptoms during the last two years. The symptom of the SOMS include all somatoform symptoms mentioned in the classification criteria of DSM-IV somatization disorder, symptoms of the ICD-10 somatization disorder and the ICD-10 somatoform autonomic dysfunction list. Only patients with eight or more unexplained physical symptoms on the SOMS or a BDI-score of 17 or above were allowed to participate in the study. Diagnoses were made using a standardized psychiatric interview carried out by senior psychiatrists or clinical psychologists. We used the German translation of the Structured Clinical Interview for DSM-IV SCID (Wittchen *et al.* 1997) to assess

present and lifetime diagnoses of major depression, somatization disorder, and other psychiatric disorders. In order to facilitate the diagnostic procedures we used the International Diagnostic Checklist – IDCL (Hiller *et al.* 1997).

Depression was diagnosed according to the DSM-IV criteria, using the SCID interview, on preselected patients who had a BDI-score of 17 or above. The diagnosis 'somatization' was based on the DSM-IV criteria for somatization disorder but defined as a multisomatiform disorder with a cut-off score of 8 symptoms or more (SSI-8) on the Somatization Index (SSI). The SSI is thus based on a structured interview based on the DSM-IV criteria for somatization disorder that includes a list of 33 medically unexplained physical symptoms. Our diagnosis of somatizing thus constitutes a subthreshold syndrome of the DSM-IV somatization disorder. In our experience, somatization is a more relevant diagnostic entity than somatization disorder. The criteria for somatization disorder are over-exclusive and do not represent the healthcare relevance of patients with multiple somatoform symptoms (Escobar *et al.* 1989). Previously, we have externally validated the diagnosis "somatization" because we have shown that the SSI-8 criterion constitutes a useful index to differentiate patients with high versus low disabilities (Rief *et al.* 1997). Comparable results have been published using the diagnosis "multisomatiform disorder", defined as 3 or more medically unexplained symptoms plus a long history of somatization (> or = 2 years) (Kroenke *et al.* 1997). Finally, we included 36 patients with somatization (SOM), 36 patients with depression (MDD), 39 patients with 'comorbid' SOM and MDD (SOM+MDD). Nine of the 36 SOM patients fulfilled the criteria for somatization disorder, while 15 of the 38 patients with SOM+MDD were classified as DSM-IV somatization disorder. Patients and controls gave written informed consent after the study protocol was fully explained; the study has been approved by the local ethical committee.

We excluded subjects with any medical illnesses, e.g. inflammatory bowel disorders, diabetes, hypertension, cardiovascular disorders and any condition that could have explained the somatoform symptoms; b) subjects with acute inflammatory or allergic reactions the last 2 months prior to the study; c) patients with life-time diagnoses of other axis-I DSM IV disorders, e.g. psychotic, substance use and organic mental disorders; and d) subjects who had been treated with immunomodulatory drugs, such as glucocorticoids, etc. Only subjects who were free of psychotropic drugs, including antidepressants, were selected to participate in this study. Thirteen women took contraceptive drugs, 8 subjects took thyroxine, and some were taking antioxidants. We have controlled our results by using the following variables as explanatory variables in regression analyses: drug state (drug free versus not drug free), smoking

behavior, alcohol use and use of caffeine, and the body mass index (BMI), as calculated as weight (kg) / body height (in meter).

Severity of depression was measured by means of Beck Depression Inventory (BDI) (Beck *et al.* 1961). The 90-item version of the Symptom Check List-90-Revised (SCL-90) (Derogatis 1994; Rief & Fichter 1992) was employed to measure the general severity of psychopathology according to different symptom dimensions. The SSI was used as a general index for the severity of somatization. The DSM-IV SSI-index is the total score of positively answered symptoms out of 33 bodily complaints. The retest reliability of the SSI is rtt = 0.85 (72 h). Self-rated severity of somatic symptoms was assessed by means of the SOMS (Rief *et al.* 1997).The SSI and the SOMS showed a strong correlation, i.e. r = 0.75.

Methods

After an overnight fast, blood (2 × 7 cc) was sampled at 7:00 a. m. for the assay of the biomarkers. Subjects were instructed to stay in bed until the blood sample was taken. At 8:00 a. m. blood samples were centrifuged at 1 500 g for 10 min at 4 °C and stored at –70 °C until thawed for assay. The initial interview, the self-rating scales and the blood samplings were carried within a time window of 3–4 days. Plasma specimens for the assay of tryptophan, kynurenine, and kynurenic acid were assayed in a single run with a single lot number of reagents and consumables employed by a single operator (who was blind to the diagnosis) in order to minimize variation in the results. Tryptophan was assayed by means of high performance liquid chromatography (HPLC) as previously explained (1996). Serum kynurenine and kunurenic acid were determined by means of HPLC as described by Herve *et al.* (1996). Both TRYCATs were analyzed in deproteinised plasma samples employing reversed phase HPLC with Chromolith performance PR-18e, 4.7 100 mm column (Merck, Darmstadt, Germany). Kynurenine was detected spectrophotometrically at 365 nm and kynurenic acid fluorimetrically at 334 nm and an emission wavelength of 388 nm. The mobile phase was prepared with 250 mM zinc-acetate in distilled water (27.4 g in 500 ml) and pH was brought to 5.8 with acetic acid and made up to a volume of 455 ml with water. A total of 45 ml acetonitrile was added into this mixture. We prepared the kynurenine standards by dissolving kynurenine in water and the kynurenic acid standards by dissolving kynurenic acid in ethanol and hydrochloric acid. For the working standards, stock standards of 5 µM for kynurenine (500 µl) and 100 nM for kynurenic acid (10.0 µl) were diluted in distilled water. Perchloric acid was used for deproteinization. Tryptophan and kynurenine are expressed as µmol/L and kynurenic acid as nmol/L. The intra- and interassay CV values for all analytes

obtained in our laboratory were less than 5% and 7%, respectively. The kynurenine/tryptophan quotient (KY/TRP) was computed, since this ratio allows to estimate IDO activity. The kynurenic acid / kynurenine (KY/KA) quotient was computed as an index for neurotoxic/neuroprotective potential and KAT activity.

Statistics

Group mean differences were examined by means of analysis of variance (ANOVA) and analysis of covariance (ANCOVA). Multiple comparisons between group means were assessed with the Dunn test. Relationships between variables were checked by means of Pearson's product moment correlation coefficients, partial correlation coefficients, multiple regression analyses and canonical correlation analyses. The significance of the difference between two correlation coefficients between the same sets of variables in two independent study groups was calculated employing a Fisher r-to-z transformation. The independence of classification systems was checked by means of analysis of contingence (χ^2-test). Transformations (Box-Cox) were used to normalize the distribution of the data if necessary. The level of significance was set at p=0.05 (two-tailed).

RESULTS

Demographic data

Table 1 shows age, gender ratio, the SSI, SOMS, BDI, SCL-90, and the biomarkers in patients with SOM, SOM+MDD, MDD, and normal controls. There were no significant differences in age (F=1.4, df=3/142, p=0.2) and gender ratio (χ2=4.1, df=3, p=0.2) between the 4 study groups. The SSI was significantly higher in SOM+MDD than in SOM (t=3.76, p=0.0005), MDD (t=14.34, $p<10^{-5}$) and controls (t=19.32, $p<10^{-5}$); higher in SOM than in MDD (t=10.37, $p<10^{-5}$) and controls (t=15.28, $p<10^{-5}$); and higher in MDD than in controls (t=4.99, p=0.00003). The same pattern was observed for the SOMS, BDI and SCL-90, except that the differences in the SOMS between SOM+MDD and SOM were less significant (t=2.40, p=0.016); the BDI (t=1.52, p=0.12) and SCL-90 (t=1.73, p=0.08) were not significantly different between MDD and MDD+SOM.

In the total study group, there were significant correlations between plasma tryptophan and kynurenine (r=0.45, $p<10^{-5}$) and kynurenic acid (r=0.30, p=0.0005). There was a significant and positive correlation between kynurenine and kynurenic acid (r=0.50, $p<10^{-5}$). There was no significant correlation

between the KY/TRP and KY/KA ratios (r=0.09, p=0.3). The effects of age and sex on the biomarkers were examined by means of multiple regression analysis. Age (F=17.6, p=0.0002) and sex (F=14.8, p=0.0004) explained 17.1% of the variance in plasma tryptophan (F=15.7, df=2/141, p=0.00002). Sex (F=9.0, p=0.003), but not age (F=0.7, p=0.6) explained 6.5% of the variance in plasma kynurenine (F=4.9, df=2/141, p=0.008). Sex (F=8.4, p=0.004), but not age (F=3.5, p=0.006) explained 7.9% of the variance in plasma kynurenic acid (F=6.2, df=2/141, p=0.003). Age (F=22.1, p=0.00005) and sex (F=0.3, p=0.6) explained 12.4% of the variance in the KY/TRP ratio (F=11.1, df=2/141, p=0.0001). Age (F=4.4, p=0.036) and sex (F=3.9, p=0.046) explained 5.7% of the variance in the KY/KA ratio (F=4.3, df=2/141, p=0.01). Thus, age had a significant effect on tryptophan (decreasing), the KY/TRP ratio (increasing) and the KY/KA ratio (decreasing). Gender has a significant effect on tryptophan, kynurenine and kynurenic acid (higher in men) and the KY/KA ratio (higher in women). Therefore, we have adjusted the following analyses for possible effects of age and sex by entering these as additional explanatory variables in ANCOVAS or partial correlation coefficients.

Intergroup differences

ANCOVA with age as covariate and sex as second factor showed significant differences in plasma tryptophan between the 4 groups (F=17.7, df=3/137, $p<10^{-5}$). Dunn tests showed that plasma tryptophan was significantly lower in SOM as compared to SOM+MDD (t=2.25, p=0.02), MDD (t=3.68, p=0.0006) and the controls (t=7.61, $p<10^{-5}$). There were no significant differences in plasma tryptophan between SOM+MDD and MDD (t=1.50, p=0.1). SOM+MDD (t=5.52, p=0.00001) and MDD (t=3.95, p=0.0003) patients had lower plasma tryptophan than the controls.

ANCOVA (Table 1) showed significant differences in plasma kynurenine between the groups (F=3.6, df=3/136, p=0.01). Dunn tests showed that patients with SOM (t=3.22, p=0.002) and SOM+MDD (t=2.98, p=0.003) had significantly lower kynurenine than controls. An ANCOVA with plasma tryptophan as additional explanatory variable showed that the intergroup differences in plasma kynurenine disappeared (F=0.2, df=3/135, p=0.9) after adjusting for tryptophan (F=17.5, df=1/135, p=0.0002).

ANCOVA (Table 1) shows significant differences in plasma kynurenic acid between the groups (F=9.3, df=3/137, p=0.00006). Kynurenic acid was significantly lower in SOM and SOM+MDD than in MDD (t=3.22, p=0.002, and t=2.77, p=0.006, respectively) and controls (t=5.21, p=0.00002 and t=4.80,

p=0.00004, respectively). There were no significant differences in kynurenic acid between SOM and SOM+MDD (t=0.52, p=0.61). Kynurenic acid is significantly lower in MDD as compared to controls (t=2.01, p=0.043).

ANCOVA (Table 1) shows significant differences in the KY/TRP ratio between the diagnostic groups (F=2.75, df=3/139, p=0.04). Dunn tests shows a significantly higher KY/TRP ratio in SOM than in SOM+MDD (t=1.96, p=0.048) and controls (t=3.59, p=0.0007), but no significant differences between SOM and MDD (t=1.83, p=0.07) and SOM+MDD (t=1.69, p=0.09) or MDD (t=1.75, p=0.08) versus normal controls.

ANCOVA (Table 1) shows significant differences in the KY/KA ratio between the diagnostic groups (F=8.6, df=3/140, p=0.0001). Dunn tests shows a significantly higher KY/KA ratio in SOM than in MDD (t=3.02, p=0.003) and controls (t=4.52, p=0.00008); a significantly higher KY/KA ratio in SOM+MDD than in MDD (t=2.32, p=0.02) and controls (t=3.86, p=0.0004); and no significant differences between MDD and controls (t=1.54, p=0.1). All above results of ANCOVAs were not affected by entering the drug state, smoking behavior, alcohol use, use of caffeine, or BMI.

Tab. 1. Demographic data, rating scales and measurements of plasma tryptophan, kynurenine, kynurenic acid and the kynurenine/tryptophan (KY/TRP) and kynurenine/kynurenic acid (KY/KA) ratio's in patients with somatization (SOM), SOM and depression (MDD), MDD and in normal controls.

Variables	SOM	SOM+MDD	MDD	controls
Age (years)	43.1 (12.0)	40.2 (10.4)	41.9 (9.9)	38.0 (11.9)
Gender ratio (M/F)	13 / 23	10 / 29	13 / 23	17 / 18
SSI	9.5 (2.3)	11.6 (3.3)	3.7 (2.3)	0.9 (1.0)
SOM	19.0 (5.9)	22.2 (6.5)	12.8 (6.6)	2.4 (2.5)
BDI	13.9 (5.3)	29.2 (7.5)	27.0 (8.2)	2.7 (3.1)
SCL-90	50.1 (16.3)	64.9 (10.8)	59.4 (14.6)	16.4 (12.6)
Tryptophan (μM/L)	58.9 (8.3)	65.8 (10.7)	69.2 (14.6)	81.1 (14.0)
Kynurenine (μM/L)	1.67 (0.30)	1.69 (0.36)	1.80 (0.48)	1.96 (0.42)
Kynurenic acid (nmol/L)	20.95 (11.53)	22.29 (13.59)	28.50 (15.46)	31.80 (11.03)
KY/TRP x 100	2.87 (0.63)	2.59 (0.54)	2.62 (0.64)	2.44 (0.51)
KY/KA	95 (38)	92 (33)	75 (25)	67 (20)

SSI: Somatization Index; SOMS: Screening for Somatoform Symptoms; BDI: Beck Depression Inventory; SCL-90: Symptom Check List-90-Revised; All results are shown as mean (SD). For the results of the statistical analyses: see text

Correlations between tryptophan and the TRYCATs

As described above, there are significant correlations between tryptophan and kynurenine, while ANCOVA showed that the KY/TRP ratio is increased in SOM patients only; there are also correlations between kynurenine and kynurenic acid, while ANCOVA shows that the KY/KA ratio is increased in patients with SOM and SOM+MDD only. This suggests that the rates of both reactions, which are dependent on the levels of IDO and tryptophan, or on KAT and kynurenine may be different between the groups. Therefore, we have computed the correlations between tryptophan and kynunerine, and between kynurenine and kynurenic acid in the 4 study groups separately. Tryptophan and kynurenine were significantly correlated in the controls (r=0.39, p=0.018), SOM+MDD (r=0.37, p=0.02) and MDD (r=0.45, p=0.007), but not in SOM (r=0.14, p=0.6). By means of the Fisher r-to-z transformation no significant differences could be detected between those correlations coefficients. Kynurenine and kynurenic acid were significantly correlated in the controls (r=0.58, p=0.0004), SOM+MDD (r=0.54, p=0.0007) and MDD (r=0.53, p=0.001), but not in SOM (r=0.06, p=0.7). Using the Fisher r-to-z transformation, significant differences were found between the correlation coefficient obtained in SOM and those in normal controls (z=–2.43, p=0.007); SOM+MDD (z=–2.26, p=0.01); and MDD (z=–2.15, p=0.016).

Correlations with severity of illness

In the total study group there were significant correlations between the SSI and the SOMS (r=0.80, $p<10^{-5}$), BDI (r=0.52, $p<10^{-5}$), and SCL-90 (r=0.49, $p<10^{-5}$); the SOMS and the BDI (r=0.62, $p<10^{-5}$) and SCL-90 (r=0.70, $p<10^{-5}$); and between the BDI and the SCL-90 (r=0.74, $p<10^{-5}$). Semipartial correlation analyses (after adjusting for sex and age) showed significant relationships between tryptophan and the SSI (r=–0.36, p=0.00005), the SOMS (r=–0.36, p=0.00007), but not the BDI (r=–0.16, p=0.053) and the SCL-90 (r=–0.17, 0.051); between kynurenine and the SSI (r=–0.16, p=0.047), and the SOMS (r=–0.23, p=0.006), but not the BDI (r=–0.10, p=0.2) and the SCL-90 (r=–0.07, 0.6); between kynurenic acid and the SSI (r=–0.26, p=0.002), the SOMS (r=–0.36, p=0.00008), the BDI (r=–0.18, p=0.03) and the SCL-90 (r=–0.19, 0.03); between the KY/TRP ratio and the SSI (r=0.18, p=0.03), but not the SOMS (r=0.10, p=0.2), the BDI (r=0.04, p=0.6) and the SCL-90 (r=0.08, 0.6); and between the KY/KA ratio and the SSI (r=0.23, p=0.005), the SOMS (r=0.30, p=0.0005), but not the BDI (r=0.15, p=0.06) and the SCL-90 (r=0.17, 0.052).

Table 2 shows the outcome of a canonical correlation analyses whereby the 4 rating scales were entered as dependent variables and plasma tryptophan, the KY/TRP ratio and the KY/KA ratio as independent variables. Two significant eigenvectors were found: the first (r=0.43, $p<0.01$) showed high loadings on the SSI, the SOMS, the SCL-90 (positively loaded), and the KY/KA ratio (positively loaded) and tryptophan (negatively loaded); whereas the second (r=0.21, $p<0.05$) loaded highly on the SSI and the KY/TRP ratio.

DISCUSSION

The first major finding of this study is that, although plasma tryptophan was significantly lower in depressed patients, IDO activity was not increased. These results are in contrast to our primary hypothesis and to previous studies in women in the puerperium and IFNα-treated patients. In these studies, a significant association between increased IDO activity and affective symptoms was detected (Maes *et al.* 2002; 2001b; Bonaccorso *et al.* 2002; Wichers *et al.* 2005b; Raison *et al.* 2010). Also, Gabbay *et al.* (2010) found a significantly higher KY/TRP ratio and lower tryptophan in adolescent depression with melancholic features as compared to non-melancholic depressed adolescents and controls. In patients with coronary artery disease, an elevated KY/TRP ratio was significantly correlated with increased depression scores (Swardfager *et al.* 2009). In malignant diseases, IDO activation and lowered plasma tryptophan parallel the course of the disease and are related to the onset of depressive mood (Brandacher *et al.* 2006). However, also Myint *et al.* (2007) could not find a significant difference in the KY/TRP ratio between depressed patients and controls.

Tab. 2. Results of canonical correlation analysis performed on both the clinical severity ratings, as the first set of variables, and plasma tryptophan, the kynurenine/tryptophan (KY/TRP) and kynurenine/kynurenic acid (KY/KA) ratio, as a second set of variables.

variables	first canonical eigenvector	second canonical eigenvector
SSI	**0.831**	**–0.352**
SOMS	**0.938**	0.175
BDI	0.274	0.211
SCL-90	**0.507**	–0.119
Tryptophan	**–0.856**	0.300
KY/TRP ratio	0.243	**–0.964**
KY/KA ratio	**0.585**	0.079
canonical correlation coefficient	r=0.43 ($p<0.01$)	r=0.21 ($p<0.05$)

The significant loadings (>0.350) are shown in bold.
Tryptophan, the KY/TRP and KY/KA ratios were entered as the residualized values after adjusting for age and gender.

There are other mechanisms in depression that can explain lowered plasma tryptophan even in the absence of IDO activation. A first possible mechanisms is enhanced catabolism of tryptophan along the TRYCAT pathway by activation of liver tryptophan 2,3-dioxygenase (TDO, tryptophan pyrolase EC 1.13.11.11) by glucocorticoids, which are known to be increased in depression (Maes *et al.* 2011). In this respect, it has been shown that dexamethasone or glucocorticoid administration in rats enhances liver TDO activity and consequently lowers tryptophan in the liver, serum and brain, as well as 5-HT in the brain (Morgan & Badawy 1989; Young 1981). TDO knocked-out mice show elevated plasma tryptophan and tryptophan and 5-HT levels in the hippocampus and midbrain (Kanai *et al.* 2009). In humans, administration of 1 mg dexamethasone is sufficient to lower plasma tryptophan in controls and depressed patients (Maes *et al.* 1990a; 1990c). Another inflammatory mechanism that may explain lowered plasma tryptophan in depression is related to serum albumin, one of the negative acute phase proteins, that is reduced in depression (Maes *et al.* 1991b; Song *et al.* 1994). Total plasma tryptophan consists of a major fraction, i.e. 70–90%, that is loosely bound to albumin and a smaller fraction that circulates as free tryptophan (Curzon & Sarna 1984; Fernstrom 1984; Yuwiler *et al.* 1977). The significant and positive correlations that are detected in depression between plasma tryptophan and serum albumin indicate that the reduced serum albumin levels may have depleted plasma total tryptophan (Maes *et al.* 1996; 1997). Finally, the failure to find increased IDO activity in our depressed patients does not mean that the lowered availability of tryptophan in depression is not the consequence of IDO activation. Indeed, previously we have reviewed that activation of IDO may, through reduced plasma tryptophan and increased TRYCAT levels, exert a negative feedback on the primary immune-inflammatory response in depression thereby acting as a counter anti-inflammatory response system (CARS) (Maes *et al.* 2007; 2011; Burdette *et al.* 2010). Tryptophan depletion leads to metabolic shutdown and starvation of immune cells, whereas increased TRYCATs lead to T cell unresponsiveness and anti-inflammatory effects, which in turn attenuate IDO activation (Maes *et al.* 2007; 2011). Thus, subchronic conditions with an activated CARS may be characterized by a normalized KY/TRP ratio and lowered plasma tryptophan levels. Even more, in our study we found that after covarying for tryptophan in an ANCOVA the differences in kynurenine between depressed patients and controls disappeared. This indicates that the lowered plasma levels of kynurenine in depression are a consequence of depletion of its precursor, tryptophan.

The second major finding of this study is that patients with somatization had an increased KY/TRP ratio, indicating increased IDO activity. Therefore, the lowered plasma tryptophan in somatization may be explained, at least in part,

by activation of IDO. We should underscore that the plasma levels of tryptophan are much more reduced in somatization than in depressed patients (this study) and more reduced than for example during treatment with IFNα-based immunotherapy (Bonaccorso *et al.* 2002). In the present study we found that the lowered tryptophan was significantly related to the SSI and the SOMS, but not the BDI and the SCL-90. Also the KY/TRP ratio was significantly associated with the SSI but not the SOMS, BDI or SCL-90. This indicates that lower tryptophan and activated IDO are biomarkers for somatization rather than for depression. Previously, it was found that in depression, lower plasma and CSF tryptophan are associated with anxiety and somatization (Joseph *et al.* 1984), agitation (Curzon 1979), neuromuscular symptoms, anxiety, agitation, depressed mood and catatonia (Lehmann 1972), and psychic anxiety, depersonalization, obsessions, paranoid symptoms and diurnal variation (Maes *et al.* 1990b). IDO activation following IFNα-based immunotherapy is also associated to the onset of somatic symptoms, which develop soon after starting treatment and which predict the outcome of the more cognitive symptoms of depression (Wichers *et al.* 2005a; 2005b). Terre *et al.* (2003) reported that somatic complaints may represent one risk factor for the subsequent development of depression. The activation of IDO in somatization may offer an explanation for the gender-specific rate of somatization and somatization disorder with a 1.6–2/1 and 10/1 female/male prevalence rate, respectively (Ladwig *et al.* 2001; Swartz *et al.* 1990). Thus, women show significantly greater abnormal responses in the TRYCAT pathway than their male counterparts. For example, depressed women excrete significantly more xanthurenic acid, another TRYCAT, following 5 g tryptophan than male patients (Maes *et al.* 1987b). Plasma tryptophan and the KY/TRP ratio following IFNα-based immunotherapy are significantly more depleted and enhanced, respectively, in women than in men (Maes *et al.* 2011; Bonaccorso *et al.* 2002). This indicates that IDO is more vulnerable to immune-inflammatory challenges in women and that these gender-related differences in IDO activation could play a role in the increased prevalence of somatization in females.

The third major finding of this study revolves around the concentrations of the detrimental TRYCAT kynurenine versus that of the neuroprotective TRYCAT kynurenic acid, which were normal versus reduced in depression. First, the negative kynurenine findings are in accordance with those of Myint *et al.* (2007) who found no significant differences in kynurenine concentrations between depressed patients and controls. These negative findings contrast the results that in the pueperium and during IFNα-based immunotherapy the production rate of kynurenine is associated to the onset of depressive symptoms (Maes *et al.* 2001a; Bonaccorso *et al.* 2002; Raison *et al.* 2010). In depressed adolescents, Gabbay *et al.* (2010) found significant increases in kynurenine in adolescents

with melancholic features, while there was a significant correlation between kynurenine levels and severity of depression. Thus, differences in population, i.e. acute (puerperium and IFNα-based treatment) versus subchronic (clinical depression) conditions, and melancholic adolescents versus depressive adults, could explain the differences between these studies. Second, as Myint *et al.* (2007) we found lowered kynurenic acid levels in depression, whereas we were unable, in contrast to Myint *et al.* to find an increased KY/KA ratio in depression (or as they express it a lowered KA/KY index). In Myint's study, however, the results were not adjusted for the significant effects of age on the KY/KA ratio by means of regression analysis. There is another major difference between our study and all above-mentioned studies which may explain differences, i.e. we used a selected group of depressed patients without concomitant 'somatization', whereas all previous researchers did not take somatization into account. As we explained and will explain in the next paragraph, somatization as defined in our study is of critical importance.

So, the fourth major finding of this study is that patients with somatization and comorbid somatization and depression had significantly lowered kynurenic acid levels than depressed patients and normal controls. Likewise, the KY/KA index was significantly increased in both study groups as compared to controls. Correlation analyses revealed significant associations between the KY/KA ratio and the SSI and the SOMS, but not the BDI or the SCL-90. This shows that disorders in the TRYCAT pathway are in fact specific for somatization and not for depression per se and that disorders in the TRYCAT pathway can be found in depressed samples when somatization is present.

The fifth major finding of our study is that there are significant differences in the correlation coefficients between kynurenine and kynurenic acid obtained in somatization (r=0.06, p=0.7) versus those in normal controls (r=0.58, p=0.0004), comorbid somatization and depression (r=0.54, p=0.0007) and depression (r=0.53, p=0.001).This suggests that the rate of product formation (reaction rate of kynurenic acid) from its substrate (kynurenine), which depends on KAT levels, differs considerably between somatization and all other study groups. These findings and the lowered product (kynurenic acid) levels in somatization suggest that the KAT enzyme concentrations are reduced or that the properties of KAT (Km, kcat) are modified in somatization. This may be caused by inhibitory effects by endogenous KAT inhibitors, or through modifications of KAT's properties by post-translational modifications or gene mutations.

The lowered kynurenic acid and thus increased KY/KA ratio, pointing toward a relative kynurenine excess in somatization, and the lower tryptophan availability may play a role in symptom formation in somatization patients by modulating pain, anxiety, the autonomic nervous system, gut motility, peripheral nerve function, ventilation, cardiac functions, etc. Thus, kynurenine contributes to pain and gut motility (Stone & Darlington 2002). Kynurenic acid, on the other hand, has significant antinociceptive and analgesic effects and inhibits intestinal hypermotility (Mecs *et al.* 2009; Kekesi & Horwath 2002; Kaszaki *et al.* 2008). Kynurenine is neurotoxic for the peripheral nerves, whereby an excess of serum kynurenine is associated with the development of peripheral neuropathy (Huengsberg *et al.* 1998). Moreover, peripheral N-methyl-D-aspartate receptors (NMDARs) are detected in deep tissues and are involved in deep tissue pain (Cairns *et al.* 2003).

Injection of glutamate into the masseter muscle provokes afferent discharges in rats and muscle pain in humans through activation of peripheral NMDARs (Cairns *et al.* 2003). The same authors concluded that peripherally acting NMDAR antagonists may constitute effective analgesics for NMDA-related muscle pains. Peripheral NMDARs are now also regarded as novel targets for treatment of neuropathic pain (Wu & Zhuo 2009). NMDARs expressed on spinal afferent neurons are upregulated in the lumbosacral dorsal root ganglia following experimental colitis (Li *et al.* 2006). Peripheral NMDARs play a role in behavioral pain responses to colonic distention in the gut, suggesting that these receptors are important in visceral pain transmission (McRoberts *et al.* 2001). The glutamate-induced activation of the NMDARs is regulated by TRYCATs. Kynurenic acid is the only endogenous antagonist of NMDARs and acts as an inhibitor of glutamate release (Nemeth *et al.* 2005). Thus, a lowered KAT activity or kynurenic acid levels – as detected in somatization – could be involved in the maintenance of persistent pain-related behaviors. Important is that peripheral inflammation – as detected in somatization (Rief *et al.* 2001) – could have increased the expression of those peripheral NMDA receptors that lead to behavioral sensitization during inflammatory pain (Yang *et al.* 2009).

Not only KAT activity but also the depletion of plasma tryptophan may play a role in the onset of somatizing symptoms. It is well established that changes in the availability of plasma tryptophan determine the synthesis of 5-HT in the brain (Moir & Eccleston 1968). There is also evidence that tryptophan depletion techniques [for example through administration of tryptophan-free amino acid drinks coupled with ingestion of large concentrations of amino acids that compete for the same amino acid transporter] induce depressive symptoms in some patients who had suffered from depression (Maes & Meltzer 1995). Tryptophan

depletion also provokes somatization symptoms in humans, e.g. increased visceral perception, and increased pain and higher urge scores during rectal distention in patients with irritable bowel syndrome (Kilkens *et al.* 2004); increased basal ventilation (Struzik *et al.* 2002); increased nausea, headache, light-induced pain and photophobia in migraine patients (Drummond 2006); lowered heart rate in subjects with attention deficit disorder (Zepf *et al.* 2009); increased systolic and diastolic blood pressure and psychological responses to stress (Davies *et al.* 2006), and decreased heart rate variability (Booij *et al.* 2006); increased autonomic stress responses in patients with social anxiety (van Veen *et al.* 2009); increased distress following uncontrollable stressors in humans (Richell *et al.* 2005) and stress-sensitivity in rats (Tanke *et al.* 2008). Behavioral correlates of 5-HT depletion include enhanced pain responses (Harvey *et al.* 1975).

This study and our previous studies (Rief *et al.* 2001) show that the unexplained physical symptoms in somatization may in fact have a biological explanation. Even more, biological disorders, such as aberrations in the TRYCAT pathway, which are considered to be a hallmark for depression, are in fact attributable to somatization rather than to depression per se. These findings have two important consequences. First, future research on depression should always control for the possible intervening effects of somatization. Second, the results of the present study show that the TRYCAT pathway could be a novel target in somatization. Attenuating the effects of IDO activation and in particular enhancing KAT or kynurenic acid levels could prove to be beneficial.

CONFLICTS OF INTEREST

The authors do not report any conflict of interest.

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