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Serotonin-immune interactions in major depression: lower serum tryptophan as a marker of an immune-inflammatory response

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Abstract Serum total tryptophan and the five competing amino acids (CAA), i.e., valine, leucine, tyrosine, phenylalanine, and isoleucine were determined in 35 major depressed subjects of whom 27 with treatment resistant depression (TRD), and 15 normal controls. Twenty-five of the depressed subjects had repeated measurements of the amino acids both before and after antidepressive treatment. The following immune-inflammatory variables were assayed in the above subjects: serum zinc (Zn), total serum protein (TSP), albumin (Alb), transferrin (Tf), iron (Fe), high-density lipoprotein cholesterol (HDL-C), number of peripheral blood leukocytes, and the $CD4^+/CD8^+$ T cell (T-helper/T-suppressor) ratio. Serum tryptophan and the tryptophan/CAA ratio were significantly lower in major depressed subjects than in normal controls. The tryptophan/CAA ratio was significantly lower in patients with TRD than in patients without TRD and normal controls. There were no significant alterations in any of the amino acids upon successful therapy. There were significant correlations between serum tryptophan and serum Zn, TSP, Alb, Tf, Fe, and HDL-C (all positive), and number of leukocytes and the $CD4^+/CD8^+$ T-cell ratio (all negative). The tryptophan/CAA ratio was significantly and negatively related to the number of leukocytes and the $CD4^+/CD8^+$ T-cell ratio. The results suggest that (a) TRD is characterized by lower availability of serum tryptophan; (b) the availability of tryptophan may remain decreased despite clinical recovery; and (c) the lower availability of tryptophan is probably a marker of the immune-inflammatory response during major depression.

Key words Cytokines · Acute phase response · Immunology · Serotonin · Tryptophan

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Introduction

There is converging evidence that disorders in the central and peripheral neurotransmission of serotonin (5-HT) and in immune functions are implicated in the pathogenesis or pathophysiology of major depression. A frequently reported abnormality in the metabolism of 5-HT in major depression is a lowered availability of serum/plasma tryptophan to the brain (for review see Maes and Meltzer 1995). Total or free tryptophan and the ratio of tryptophan to the sum of amino acids, known to compete for the same cerebral uptake mechanism, i.e., the competing amino acids (CAA) tyrosine, valine, leucine, isoleucine, and phenylalanine, are indicators of the availability of tryptophan to the brain (Curzon and Sarna 1984; Fernstrom 1984) and, hence, for the synthesis of 5-HT in the brain (Moir and Eccleston 1968). Under normal conditions, tryptophan circulates in the blood with a major fraction, i.e., 70–90%, loosely bound to serum albumin (Alb; McMenamy and Oncley 1958; Yuwiler et al. 1977). It is thought that the blood-brain barrier (BBB) transport site of tryptophan strips off Alb as it passes through the brain capillaries (Pardridge 1979; Smith et al. 1990). Thus, the influx of tryptophan into the brain depends on the plasma concentrations of all tryptophan, i.e., free and/or total tryptophan, as well as on the serum/plasma concentrations of Alb and the CAA (Yuwiler et al. 1977; Sainio et al. 1996).

There are many reports that serum/plasma tryptophan as well as the ratio of tryptophan to the sum of the CAA are significantly reduced in patients with major depres-

sion (for review see Maes and Meltzer 1995). Plasma tryptophan levels following ingestion of a large oral dose of tryptophan have been reported to be lower in depressed patients than in normal controls (Russ et al. 1990; Deakin et al. 1990). Recent studies suggest that an acute reduction of serum tryptophan can induce depressive symptomatology. In normal humans lowering of plasma tryptophan by dietary means has been reported to cause an acute lowering of mood, which was inversely related to postingestion plasma tryptophan levels (Young et al. 1985; Smith et al. 1987). In recently remitted depressed patients receiving selective 5-HT reuptake inhibitors (SSRIs), acute tryptophan depletion coupled with ingestion of large concentrations of CAA led to a rapid clinically significant return of depressive symptoms (Delgado et al. 1990; Heninger et al. 1992). In addition, it is probable that lower plasma tryptophan availability is causally related to lower central presynaptic 5-HT activity, since diets causing a decrease in plasma tryptophan availability also reduce CSF levels of 5-HIAA in humans (Perez-Cruet et al. 1974). It has been reported that the tryptophan/CAA ratio was inversely related to the response to a variety of antidepressive agents such as tryptophan, imipramine, amitriptyline and selective 5-HT reuptake inhibitors (Møller et al. 1986; Møller 1985).

There is now some evidence that major depression is accompanied by an immune and an acute phase (AP) response. Firstly, major depression is accompanied by an increased number of peripheral blood mononuclear cells (PBMCs) such as leukocytes, monocytes, $CD4^+$ T (T-helper cells), activated T-cells (e.g., $HLD\text{-}DR^+$ and $CD25^+$ T cells) and an increased $CD4^+/CD8^+$ T-cell ratio; increased serum concentrations of soluble interleukin-2-receptor (sIL-2R) and neopterin; and increased secretion of prostaglandins (e.g., PGE2) in serum or CSF (reviews in Maes 1995; Maes et al. 1995b; Muller 1995; Seidel et al. 1996a). Secondly, major depression is also accompanied by an AP response, as demonstrated by higher serum concentrations of positive AP proteins (APPs), such as haptoglobin (Hp), and elastase, lower serum concentrations of negative APPs, such as albumin (Alb) and transferrin (Tf), low concentrations of serum zinc (Zn), and lowered levels of high-density lipoprotein cholesterol (HDL-C) and serum iron (Fe; reviews in Maes et al. 1994a, 1994c, 1996a; Deger et al. 1996; Song et al. 1994; Sluzewska et al. 1996). It should be added that an AP response has also been observed in the olfactory bulbectomized rat model of depression and the chronic mild stress model of depression in the rodent (Song and Leonard 1994; Sluzewska et al. 1994). We have hypothesized that an increased secretion of pro-inflammatory cytokines, such as IL-1β, IL-6, and interferon γ (IFNγ), which repeatedly occurs in major depression (Maes et al. 1993a, b, 1995a; Sluzewska et al. 1995; Seidel et al. 1996b), may underlie the AP and immune response. Indeed, IL-1β and IL-6 synergize strongly in T-cell differentiation or proliferation and are the major mediators of the AP response, whereas IFNγ activates the production of monocytic cytokines (reviews in Dinarello 1991; Hirano 1991).

Recently, we have hypothesized that multiple and complex interactions between disturbances in the turnover of 5-HT and the immune of AP response participate in the pathophysiology of major depression (Maes et al. 1993b, 1994d, 1996b). Indeed, highly significant relationships were found between serum tryptophan availability and markers of an immune or AP response, such as plasma Hp (negative), serum Tf (positive), IL-6 production of peripheral blood mononuclear cells (negative), serum neopterin (negative), and serum Alb (positive correlation; Maes et al. 1993b; 1994d). Reduced availability of tryptophan during an immune or AP response may be caused by the following: (a) Peripheral amino acids are used for leukocyte activity and synthesis of APPs in the liver (Moldawer et al. 1987); (b) Indoleamine 2,3 dioxygenase (IDO), a major tryptophan catabolizing enzyme, is induced during an AP response by cytokines such as IFN-γ and IL-2 (Takikawa et al. 1984; Fuchs et al. 1990); (c) The decrease in serum Alb observed in major depression may be accompanied by lower total tryptophan concentrations and, consequently, by a lowered extraction of tryptophan by the brain (Maes et al. 1996b).

The aims of the present study were to examine whether (a) treatment-resistant major depression (TRD) is characterized by lowered availability of tryptophan to the brain; (b) there are significant alterations in the availability of tryptophan upon successful antidepressant therapy, and (c) there are significant relationships between a lowered availability of tryptophan and markers of the immune or AP response, such as serum Zn, Alb, Fe, number of leukocytes, and the $CD4^+/CD8^+$ T-cell ratio.

Subjects and methods

Fifty subjects participated in this study, i.e., 35 major depressed patients and 15 normal controls. The major depressed patients were admitted to the TRD ward of the University Department of Psychiatry, AZ, Stuivenberg, Antwerp, Belgium. Patients between the ages of 25 and 75 years were included if they met the diagnostic criteria of the DSM-III-R for major depression. The semistructured interview for DSM-III-R (Spitzer et al. 1990) was used to assess the clinical diagnosis. All subjects gave informed consent to participate in these studies. Severity of depression was measured with the 17-item Hamilton Depression Rating Scale (HDRS; Hamilton 1960). Staging of depression based on prior treatment response was made according to the criteria of Thase and Rush (1995), i.e., 0: no single adequate trial with antidepressants; 1: nonresponse to one adequate trial with selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), or heterocyclic antidepressants (HCAs); 2: nonresponse to two adequate trials with antidepressive agents from different classes, e.g. TCAs, HCAs, or SSRIs; 3: stage 2 plus failure to respond to one augmentation therapy; 4: stage 3 plus failure to respond to two augmentation strategies; and 5: stage 4 plus failure to respond to electroconvulsive therapy. Treatment resistance was defined as a failure to respond to at least two adequate trials with antidepressants from different classes (Thase and Rush 1995); thus, stages 2–5 are regarded as TRD.

Inclusion criteria for the normal volunteers and depressed patients included: normal blood tests, such as serum electrolytes, urea, creatinine, liver function tests, such as γGT, SGPT, SGOT, and thyroid function tests, such as FT4 and basal thyroid secreting hormone; subjects who were free of medical illnesses, such as endocrine, metabolic or (auto)immune disorders, such as lupus ery-

thematosus, inflammatory bowel disease, rheumatoid arthritis, diabetes; subjects who were free of any infections, inflammatory or allergic reactions for at least 2 weeks prior to the blood samplings; and subjects who were free of drugs known to affect immune or endocrine functions and of hormonal preparations, including glucocorticoids and oral contraceptives. Exclusion criteria for normal volunteers were: use of any medication for at least 1 month prior to blood sampling; use of psychotropic drugs; substance use or abuse; and past, present, or family history of psychiatric disorder. The authors have excluded the following patients: patients with other axis-I diagnoses, such as schizophrenia or schizoaffective disorders, primary anxiety disorders (e.g., obsessive-compulsive disorder, panic, and posttraumatic stress disorder), psychoactive substance use disorders within the past 6 months; anorexia nervosa and bulimia, and organic mental disorders. Also, patients who were treated with fluoxetine or trazodone during the index episode were omitted from these studies.

In normal volunteers and major depressed subjects blood was always drawn at 7:45 a.m. (± 15 min) for the assay of serum amino acids and the other immune-inflammatory markers. After admission to hospital, patients accepted into the study underwent a wash-out period of 10 days. All antidepressants and any drugs used to augment their activity were withdrawn. Low doses of benzodiazepines were allowed during the study span in cases of severe sleep disorders and anxiety. Prior to admission, patients had been treated with sertraline (100–150 mg/day), paroxetine (20–40 mg/day), mianserine (30–60 mg/day), lofepramine (210 mg/day), imipramine (150–200 mg/day), moclobemide (300–450 mg/day), citalopram (20–40 mg/day), maprotiline (150–200 mg/day), dothiepin (150 mg/day), iproclozide (10 mg/day), clomipramine (150–200 mg/day), amitriptyline (150–250 mg/day), nortriptyline (200 mg/day), or doxepine (125 mg/day). The agents used to augment the efficacy of antidepressants in patients belonging to stage 3 included lithium, buspirone, triiodothyronine, ketoconazole (n = 1; this trial was discontinued some months prior to entering the study), sulpiride, and flupentixol. The pretreatment or baseline HDRS was completed at the end of this wash-out period. Twenty-five major depressed patients had blood samplings for assays of serum amino acids both at baseline and after treatment with antidepressants during a 5-week period. They were treated with trazodone 100 mg/day (n = 7); trazodone 100 mg/day + pindolol 7.5 mg/day (n = 7); or trazodone 100 mg/day + fluoxetine 20 mg/day (n = 11). The 17-item HDRS score was again completed 5 weeks after starting treatment with antidepressants. A good clinical response was defined as a 50% or greater decrease in the 17-item HDRS score from baseline to 5 weeks later (Thase and Rush 1995).

Serum amino acids were determined by means of a high-performance liquid chromatography (HPLC) method as previously described by us (Maes et al. 1990). All samples from normal controls and depressed subjects (both before and after treatment) were analyzed at the same time and in one and the same run. The intra-assay coefficients of variation (CV values expressed as a percentage) computed on the present sample were as follows: tryptophan 3.3%, tyrosine 3.8%, valine 3.0%, phenylalanine 3.2%, isoleucine 3.4%, and leucine 3.7%. The tryptophan/valine + leucine + isoleucine + tyrosine + phenylalanine (CAA) ratio was computed and multiplied by 100. Total serum protein (TSP) was determined by means of the Kodak Ektachem Analyzer (Kodak, Vilvoorde, Belgium). The interassay CV obtained in our laboratory was 2.16%. The percentage of Alb was determined electrophoretically by means of the Beckman Paragon SPE agarose system (Beckman Paragon, Analis, Belgium) with densitometric quantitation of the protein fractions. The interassay CV value is 3.05% (n = 26). Serum Zn was determined by means of an atomic absorption spectrometric method using the Perkin Elmer 2380. In our laboratory the interassay CV was 8.5% (n = 25). Serum Tf was determined by means of a turbidimetric method. The interassay CV is 4.75% (mean 310 µg/dL, n = 52). Serum Fe was assayed on the Ektachem analyzer (single slide method). The interassay CV values were 2.76% at 240 µg/dL (n = 30) and 7.21% at 75 µg/dL (n = 30). White blood cell and lymphocyte counts were determined by means of a Coulter STKS fully automated total blood cell counter. Three parameters of the sample were simultaneously measured in the flow cell, i.e., volume, radiofrequency conduction, and light scatter. The interassay CV values were 1.5% for leukocytes and 3.1% for lymphocytes. Flow cytometry of peripheral blood leukocytes was used to enumerate the $CD4^+$ and $CD8^+$ subset populations by surface markers (Cytostat Coulter Clone). After incubation for 20 min at 20° C with fluorescein labelled monoclonal antibody, red blood cells were lysed, leukocytes were fixed with the Q-Prep method (Coulter), and fluorescence was determined with EPICS Profile 2. The results were expressed as the percentage of positive lymphocytes bearing the surface markers. The $CD4^+/CD8^+$ ratio was computed.

Statistics

The independence of classification systems was ascertained by means of analysis of contingence (χ^2 test). Relationships between variables were assessed by means of Pearson's product moment or Spearman's rank order correlation coefficients or by means of (multiple) regression analysis. An automatic step-up method was used with a p-to-enter of $p = 0.05$. Group mean differences were examined by means of analysis of variance (ANOVA), analysis of covariance (ANCOVA), multivariate ANOVA (MANOVA), or linear discriminant analysis (LDA). Repeated-measures ANOVAs were employed to examine differences in the pre- and posttreatment serum amino acids. The diagnostic performance of the biological variables for major depression was checked by means of receiver operating characteristics (ROC) analysis with computation of the area under the ROC curve, sensitivity, specificity, and predictive value of a positive (PV^+) and a negative test result (PV^-). The κ statistic was computed (Cohen 1960). The significance was set at $\alpha = 0.05$ (two tailed).

Results

Demographic data

Table 1 shows the demographic data of the subjects in this study. There were no significant differences either in age ($F = 0.5$, $df = 1/48$, $p = 0.5$) or male/female ratio ($\chi^2 = 1.4$, $df = 1$, $p = 0.2$) between normal volunteers and major depressed patients. The mean (± SD) HDRS score in the 35 major depressed subjects was 24.5 (± 3.8). In these patients the number of previous depressive episodes was 3.9 (± 4.4), the length of the index depressive episode was 17.3 (± 16.8) months, and the mean duration of illness was 11.7 (± 9.4) years.

There was a significant negative correlation between age and tryptophan ($r = -0.34$. $p = 0.01$; regression pooled over the study groups of normal volunteers and depressed subjects). There were no significant correlations between

Table 1 Demographic data of the 15 normal volunteers (NV) and 35 major depressed (MD), subdivided into these with and without treatment-resistant depression (TRD)

Variables	NV	MD	Non-TRD	TRD
Age (years)[a]	47.5 (15.0)	50.7 (14.4)	47.7 (15.3)	11.7 (9.4)
Men/women ratio	10/5	17/18	3/5	14/13

[a] Shown as mean (SD)

age and any of the other amino acids or the tryptophan/CAA ratio. By means of ANOVA, we found significantly higher serum valine (272 ± 43 vs 247 ± 47 μmol/L; $F = 4.0$, $df = 1/48$, $p = 0.048$) and leucine (163 ± 30 vs 147 ± 25 μmol/L; $F = 4.2$, $df = 1/48$, $p = 0.04$). in men than in women. There were no significant gender differences in any of the other serum amino acids or in the tryptophan/CAA ratio. In any case, we adjusted for possible age and gender (and menopausal state) effects by using age, gender, and menopausal state as additional covariates in regression analyses.

Serum amino acids and depression

Table 2 shows the mean values of the amino acids in normal volunteers and major depressed subjects. ANCOVAs showed significantly lower serum tryptophan and tyrosine values and a significantly lower tryptophan/CAA ratio in major depressed subjects than in normal volunteers. Using tryptophan and tryptophan/CAA values as discriminatory variables, a highly significant separation was found between patients with major depression and normal controls (MANOVA: Wilks $\lambda = 0.73$; $F = 8.0$, $df = 2/94$, $p = 0.0009$; LDA: $F = 17.7$, $df = 1/48$, $p = 0.0002$). The LDA score performed well in separating both groups: The area under the ROC curve was 80.3% and the optimal threshold value showed the following diagnostic performance: sensitivity 68.6%; specificity 86.7%; PV^+ 92.3% ($\kappa = 0.47$, $t = 3.7$, $p = 0.0008$). In major depressed subjects, no significant correlations were found between serum amino acids or the tryptophan/CAA ratio and the HDRS score. There were no significant relationships between number of depressive episodes, duration of illness, length of the index depressive episode, or staging of depression based on treatment nonresponsivity and any of the serum amino acids or the tryptophan/CAA ratio.

There were no significant differences in any of the pretreatment values of the serum amino acids between patients with and without TRD. For example, serum tryptophan was not significantly different ($F = 0.00$, $df = 1/33$, $p = 0.98$) between subjects with (mean 58 ± 8 μmol/L) and without TRD (mean 58 ± 9 μmol/L). The tryptophan/CAA ratio was significantly lower in patients with TRD (mean 8.8 ± 1.6) than in those without TRD (mean 10.2 ± 1.2) and normal controls (ANCOVA with age and gender as covariates: $F = 6.0$, $df = 2/44$, $p = 0.005$).

Table 2 shows the pre- and posttreatment values of the serum amino acids in the 25 major depressed subjects (8 without TRD and 17 with TRD) who had repeated measurements both before and after treatment with antidepressants. The posttreatment HDRS score (mean 12.3 ± 7.2) was significantly lower ($F = 113$, $df = 1/24$, $p < 10^{-4}$) than the baseline values (mean 25.2 ± 3.4). There were 9 nonresponders and 16 responders to antidepressant therapy. Of the 8 patients without TRD, 7 responded to treatment, suggesting that these subjects did indeed not suffer from TRD. There were no significant effects of time or time × clinical response status on any of the amino acids, except for phenylalanine. Posttreatment phenylalanine was significantly lower than pretreatment phenylalanine ($F = 9.9$, $df = 1/21$, $p = 0.005$). There were no significant correlations between the Δ HDRS values (i.e., pre- minus posttreatment values) and the Δ changes in serum amino acids or the tryptophan/CAA ratio. Serum tryptophan ($F = 21.4$, $df = 1/38$, $p = 0.001$) and the tryptophan/CAA ratio ($F = 5.1$, $df = 1/38$, $p = 0.03$) were significantly lower in major depressed patients after repeated treatment with antidepressants than in normal volunteers. The pre- and posttreatment serum concentrations of all amino acids did not significantly differ between therapy responders and therapy nonresponders.

Significant relationships

Patients with major depression had significantly lower serum Zn (93 ± 12 vs 115 ± 12 μg/dL; $F = 28.3$, $df = 1/36$, $p = 0.00004$); TSP (66 ± 6 vs 74 ± 5 mg/dL; $F = 25.4$ $df = 1/43$, $p = 0.00006$); serum Alb (41 ± 4 vs 44 ± 4 mg/dL; $F = 4.5$, $df = 1/43$, $p = 0.04$); Tf (251 ± 36 vs 290 ± 27 μg/dL; $F = 12.3$, $df = 1/42$, $p = 0.001$); Fe (76 ± 31 vs 106 ± 45 μg/dl; $F = 6.7$ $df = 1/43$, $p = 0.01$); HDL-C (42 ± 11 vs 54 ± 13 mg/dL; $F = 6.1$, $df = 1/41$, $p = 0.02$); and a higher $CD4^+/CD8^+$ T-cell ratio (2.6 ± 1.3 vs 1.8 ± 0.8; $F = 7.4$, $df = 1/35$, $p = 0.009$) than normal controls (all results of

Table 2 Measurements of serum amino acids and the tryptophan (TRP)/competing amino acids (CAA) ratio in normal volunteers (NV) and major-depressed subjects (MD), and in major-depressed patients both before (pre-TR) and after (post-TR) treatment with antidepressants

Categories	TRP	Tyrosine	Valine	Phenyl-alanine	Isoleucine	Leucine	TRP/CAA ratio (× 100)
NV	69 (11)	77 (12)	262 (45)	83 (9)	83 (26)	164 (34)	10.5 (1.8)
MD	58 (8)[a]	69 (11)[b]	260 (48)	83 (12)	78 (15)	153 (27)	9.1 (1.7)[d]
Pre-TR	56 (8)	68 (9)	253 (41)	82 (10)	76 (14)	152 (36)	9.1 (1.7)
Post-TR	56 (7)	69 (17)	252 (67)	73 (14)[c]	78 (19)	147 (36)	9.3 (1.6)

All results are shown as mean (± SD); amino acids are expressed as μmol/L

[a] Significantly lower than NV ($F = 16.3$, $df = 1/45$, $p = 0.0004$)

[b] Significantly lower than NV ($F = 6.3$, $df = 1/45$, $p = 0.01$)

[c] Significantly lower than pre-TR ($F = 9.9$, $df = 1/22$, $p = 0.005$; repeated-measures ANOVA)

[d] Significantly lower than NV ($F = 7.7$, $df = 1/45$, $p = 0.008$); all results of ANCOVAs with age, gender, and menopausal state as covariates

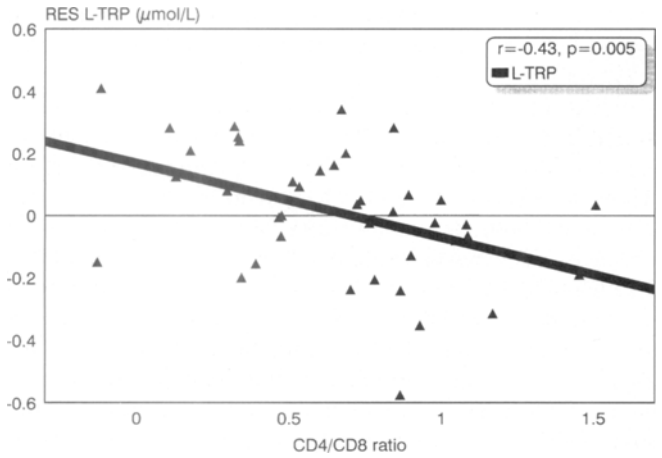

Fig. 1 Correlation between the residual tryptophan values (*RES L-TRP*, adjusted for age) and the $CD4^+/CD8^+$ ratio (in Box-Cox transformation)

ANCOVAs with age, gender, and menopausal state as covariates). Using tryptophan, the tryptophan/CAA ratio, Zn, TSP, Alb, Tf, Fe, and HDL-C as discriminatory variables we found a highly significant separation of major depression from normal controls (LDA: $F = 78.3$, $df = 1/35$, $p < 10^{-4}$). The LDA showed that the distance between the centroids of both groups was 2.96 S.D. The LDA score performed well in separating major depressed patients from normal controls: The area under the ROC curve was 98.5%; at the optimal threshold value, we found a sensitivity of 86.4%, a specificity of 100%, a PV^+ of 100%, and a PV^- of 83.3% ($\kappa = 0.84$, $t = 9.3$, $p < 10^{-4}$).

In the study group as a whole, we detected significant correlations between baseline serum tryptophan and Zn ($r = 0.40$, $p = 0.009$), TSP ($r = 0.48$, $p = 0.0009$), Alb ($r = 0.37$, $p = 0.008$), Tf ($r = 0.38$, $p = 0.007$), Fe ($r = 0.44$, $p = 0.002$), HDL-C ($r = 0.32$, $p = 0.03$), the $CD4^+/CD8^+$ ratio ($r = -0.43$, $p = 0.005$), and number of leukocytes ($r = -0.30$, $p = 0.05$; all results of semi-partial correlation analyses after adjusting serum tryptophan for age). Figure 1 shows the inverse correlation between tryptophan and the $CD4^+/CD8^+$ ratio. There were significant relationships between the tryptophan/CAA ratio and Zn ($r = 0.33$, $p = 0.03$), the $CD4^+/CD8^+$ ratio ($r = -0.48$, $p = 0.002$), and HDL-C ($r = 0.45$, $p = 0.002$). Using all immune-inflammatory variables, age, and diagnostic classification (major depressed subjects vs normal volunteers; entered as a dummy variable) as explanatory variables, automatic step-up multiple regression analyses showed that 46% ($F = 7.1$, $df = 2/25$, $p = 0.001$) of the variance in tryptophan was explained by TSP ($f = 5.9$, $p = 0.02$), the $CD4^+/CD8^+$ ratio ($F = 7.5$, $p = 0.01$), and number of leukocytes ($F = 4.1$, $p = 0.05$). Diagnostic classification was not significant in this regression analysis ($F = 0.2$, $p = 0.7$). Automatic step-up multiple regression analyses showed that 41% ($F = 8.9$, df 2/26, $p = 0.001$) of the variance in the tryptophan/CAA ratio was explained by the regression on the $CD4^+/CD8^+$ ratio ($F = 13.0$, $p = 0.002$) and number of leukocytes ($F = 7.1$, $p = 0.01$). Diagnostic classification was not significant in this regression analysis ($F = 0.09$, $p = 0.8$). There were no significant relationships between any of the other amino acids and the immune-inflammatory markers measured in this study.

There were no significant differences in the tryptophan or tryptophan/CAA values of patients and normal volunteers between the seasons, excluding possible effects of the seasons on our results. There were no significant relationships between serum tryptophan and the HDRS items anorexia ($r = 0.09$, $p = 0.6$) or weight loss ($r = 0.16$, $p = 0.6$). There were no significant correlations between the tryptophan/CAA ratio and either anorexia ($r = -0.15$, $p = 0.6$) or weight loss ($r = 0.2$, $p = 0.2$).

Discussion

The results of the present study confirm previous reports that major depression is accompanied by lower serum tryptophan concentrations and a lowered tryptophan/CAA ratio (review in Maes and Meltzer 1995). To the best of our knowledge, this is a first report that the availability of tryptophan is lower in patients with TRD than in non-TRD patients and normal controls. However, one of the flaws in this type of study is the assessment of TRD. Indeed, the diagnosis of TRD is made retrospectively and is made by patients' self-report (Thase and Rush 1995). Therefore, the accuracy of staging of depression based on prior treatment nonresponse may be questionable (Thase and Rush 1995). Nevertheless, the results suggest that the lowered tryptophan/CAA ratio in depression is probably a marker for treatment resistance. As in one of our previous reports (Maes et al. 1994b), lower serum tyrosine values were found in major depression.

In the present study there were no significant alterations in any of the amino acids upon successful therapy. These findings are in agreement with those of Lucca et al. (1994) who found no significant effects of the responder-nonresponder status on the changes in tryptophan availability during treatment. Healy et al. (1983) found no change in the concentration of either free or total tryptophan during drug treatment. Ashcroft et al. (1973) did not detect any alterations in the CSF levels of tryptophan upon recovery. DeMyer et al. (1981), on the other hand, found that as the HDRS score improved, the plasma tryptophan/CAA ratio increased significantly, suggesting an association between increased tryptophan availability to the brain and a good clinical response.

The major findings of this study are the significant relationships between the availability of tryptophan and established markers of the AP and immune response in major depression:

1. As in one of our previous reports (Maes et al. 1996b) this study found significant positive correlations between serum tryptophan concentrations, and TSP and serum Alb. This is also a first report that the availability of tryptophan to the brain is significantly related to lower serum Zn, Fe, and HDL-C. Thus, the results of this study are in agree-

ment with previous findings that the availability of tryptophan is significantly related to APPs, such as Hp and Tf, and to IL-6 production by PBMCs (Maes et al. 1993b). A relationship between lowered total serum tryptophan and hypoalbuminemia was also observed in untreated patients with chronic renal failure (Walser and Hill 1993).

2. An important part of the variance in the availability of tryptophan is explained by the number of leukocytes and the $CD4^+/CD8^+$ ratio, suggesting that the lower availability of tryptophan is related to the immune response in depression. These findings concur with those of a previous paper, reporting a significant inverse relationship between serum tryptophan or the tryptophan/CAA ratio and serum neopterin, a sensitive marker of activation of the lymphocytic/monocytic arms of cell-mediated immunity (Maes et al. 1994d). In patients with HIV infection, high serum, or CSF neopterin, low-serum tryptophan concentrations and an inverse relationship between both factors is observed (Fuchs et al. 1990). It has been shown that the increased catabolism of tryptophan (peripheral or in the CNS) in patients with HIV infection is secondary to immune activation with induction of IDO (Heyes et al. 1992). In another study, therapy with OKT3 was associated with low-serum tryptophan and an increased catabolism of tryptophan into one its metabolites, i.e., kynurenine (Holmes et al. 1992). Therefore, it may be suggested that the lower availability of tryptophan to the brain in patients with major depression reflects, at least in part, the immune and AP response in that illness. It should be stressed that there were no significant relationships between any of the other serum amino acid concentrations, including tyrosine, and any of the immune-inflammatory variables.

There is now ample evidence that the lower availability of tryptophan in depression is not related to the malnutrition that could accompany the anorexia or weight loss of major depression (Maes et al. 1993b). In the present study we were unable to find significant relationships between the availability of tryptophan to the brain and anorexia or weight loss. In addition, in a previous study, we did not detect nutritional disorders in major depression, indicating protein loss due to malnutrition (Maes et al. 1993b). There are many reports that physiologically relevant differences in food intake do not induce significant alterations in the tryptophan/CAA ratio (review in Curzon 1990). Moreover, a low $CD4^+/CD8^+$ ratio is widely accepted as fundamental to wasting protein-energy malnutrition (Lee and Woodward 1996), whereas this ratio is significantly increased in the major-depressed patients. Another factor that may lower serum tryptophan availability and suppress cell-mediated immunity is hypercortisolemia, which frequently occurs in major depression (for reviews see Maes 1995; Maes and Meltzer 1995). Previously, we have shown that there is no inverse relationship between the endogenous hypersecretion of cortisol (i.e., 24 h urinary free cortisol excretion) in depression and lower serum tryptophan, although acute administration of dexamethasone may lower serum tryptophan (Maes et al. 1990; Maes and Meltzer 1995). Thus, these results suggest that diminished availability of tryptophan in depression is probably not related to hypercortisolemia (Maes et al. 1993b).

There is now evidence that lowered serum tryptophan concentrations could affect protein metabolism and, in particular, that of albumin. Thus, tryptophan limitation causes a decrease in Alb nuclear transcripts and this decrease precedes the decrease in Alb mRNA, suggesting that the latter is caused by a decrease in Alb gene transcription (Marten et al. 1994). In addition, in tryptophan-limited cells a destabilization of Alb mRNA is observed (Marten et al. 1994). Thus, tryptophan restriction may affect the synthesis of proteins such as Alb. Therefore, the positive relationships between serum total tryptophan and Alb or TSP may in part be caused by lower serum Alb inducing lower total tryptophan, but perhaps also by the inhibiting effect of tryptophan restriction on protein synthesis.

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References

American Psychiatric Association (1987) Diagnostic and statistical manual of mental disorders (DSM-III-R) 3rd edn, revised. American Psychiatric Press, Washington, DC

Ashcroft GW, Blackburn IM, Eccelston D, Glen AIM, Hartley W, Kinloch NE, Lonergan M, Murray LG, Pullar IA (1973) Changes on recovery in the concentrations of tryptophan and the biogenic amine metabolites in the cerebrospinal fluid of patients with affective illness. Psychol Med 3:319–325

Cohen J (1960) A coefficient of agreement for nominal scales. Educ Psychol Meas 20:37–46

Curzon G (1990) Availability of amino acids to the brain and implication for transmitter amine function. In: Richardson MA (ed) Amino acids in psychiatric disease. American Psychiatric Press, Washington DC, pp 33–48

Curzon G, Sarna GS (1984) Tryptophan transport to the brain: newer findings and older ones reconsidered. In: Schlossberger HG, Kochen W, Linzen B, Steinhart H (eds) Progress in tryptophan and serotonin research. Walter de Gruyter, Berlin, pp 145–157

Deakin JF, Pennell I, Upadhyaya AJ, Lofthouse R (1990) A neuroendocrine study of 5HT function in depression: evidence for biological mechanisms of endogenous and psychosocial causation. Psychopharmacology 101:85–92

Deger O, Bekaroglu M, Orem A, Orem S, Uluutku N, Soylu C (1996) Polymorphonuclear (PMN) elastase levels in depressive disorders. Biol Psychiatry 39:357–363

Delgado PL, Charney DS, Price LH, Landis H, Heninger GR (1990) Neuroendocrine and behavioral effects of dietary tryptophan restriction in healthy subjects. Life Sci 45:2323–2332

DeMyer MK, Shea PA, Hendric HG, Yoshimura NN (1981) Plasma tryptophan and five other amino acids in depressed and normal subjects. Arch Gen Psychiatry 38:642–646

Dinarello CA (1991) Interleukin-1. In: Thompson AW (ed) The cytokine handbook. Academic Press, London, pp 47–82

Fernstrom JD (1984) Tryptophan availability and serotonin synthesis in rat brain: effects of experimental diabetes. In: Schlossberger HG, Kochen W, Linzen B, Steinhart H (eds) Progress in tryptophan and serotonin research. Walter de Gruyter, Berlin, pp 161–172

Fuchs D, Moller AA, Reibnegger G, Stockle E, Werner ER, Wachter H (1990) Decreased serum tryptophan in patients with HIV-1 infection correlates with increased serum neopterin and with neurologic/psychiatric symptoms. J AIDS 3:873–876

Hamilton M (1960) A rating scale for depression. J Neurol Neurosurg Psychiatry 23:56–61

Healy D, Carney PA, Leonard BE (1983) Monoamine-related markers of depression: changes following treatment. J Psychiatry Res 17:251–260

Heninger GR, Delgado PL, Charney DS, Price LH, Aghajanian GK (1992) Tryptophan-deficient diet and amino acid drink deplete plasma tryptophan and induce a relapse of depression in susceptible patients. J Chem Neuroanat 5:347–348

Heyes MP, Brew BJ, Saito K, Quearry BJ, Price RW, Lee K, Bhalla RB, Der M, Markey SP (1992) Inter-relationships between quinolinic acid, neuroactive kynurenines, neopterin and beta 2-microglobulin in cerebrospinal fluid and serum of HIV-infected patients. J Neuroimmunol 40:71–80

Hirano T (1991) Interleukin-6. In: Thomson AW (ed) The cytokine handbook. Academic Press, London, pp 169–190

Holmes EW, Russell PM, Kinzler GJ, Reckard CR, Flanigan RC, Thompson KD, Bermes EW (1992) Oxidative tryptophan metabolism in renal allograft recipients: increased kynurenine synthesis is associated with inflammation and OKT3 therapy. Cytokine 4:205–213

Lee WH, Woodward BD (1996) The CD4/CD8 ratio in the blood does not reflect the response of this index in secondary lymphoid organs of wealing mice in models of protein-energy malnutrition known to depress thymus-dependent immunity. J Nutr 126:849–859

Lucca A, Lucini V, Catalano M, Alfano M, Smeraldi E (1994) Plasma tryptophan to large neutral amino acids ratio and therapeutic response to a selective serotonin uptake inhibitor. Neuropsychobiology 29:108–111

McMenamy RH, Oncley JL (1958) The specific binding of L-tryptophan to serum albumin. J Biol Chem 233:1436–1440

Maes M (1995) Evidence for an immune response in major depression: a review and hypothesis. Progr Psychopharmacol Biol Psychiatry 19:11–38

Maes M, Meltzer HYM (1995) The serotonin hypothesis of major depression. In: Bloom F, Kupher D (eds) Pschopharmacology: the fourth generation of progress. Raven Press, New York, pp 933–944

Maes M, Schotte C, Scharpé S, Martin M, Blockx P (1990) The effects of glucocorticoids on the availability of L-trytophan and tyrosine in the plasma of depressed patients. J Affect Disord 18:121–127

Maes M, Bosmans E, Meltzer HY, Scharpé S, Suy E (1993a) Interleukin-1β: a putative mediator of HPA-axis hyperactivity in major depression? Am J Psychiatry 150:1189–1193

Maes M, Meltzer HY, Scharpe S, Bosmans E, Suy E, Minner B, Meester I de, Calabrese J, Vandervorst C, Raus J, Cosyns P (1993b) Relationships between lower plasma L-tryptophan levels and immune variables in depression. Psychiatr Res 49:11–27

Maes M, Langhe J de, Scharpé S, Meltzer HY, Cosyns P, Suy E, Bosmans E (1994a) Haptoglobin phenotypes and gene frequencies in unipolar major depression. Am J Psychiatry 151:112–116

Maes M, Meltzer HY, Cosyns P, Schotte C (1994b) Evidence for the existence of major depression with and without anxiety features. Psychopathology 27:1–13

Maes M, Scharpé S, D'Haese W, Broe M de, Cosyns P (1994c) Hypozincaemia in depression. J Affect Disord 31:135–140

Maes M, Scharpé S, Meltzer HY, Okayli G, Bosmans E, D'Hondt P, Vanden Bossche B, Cosyns P (1994d) Increased neopterin and interferon γ secretion and lower availability of L-tryptophan in major depression: further evidence for activation of cell-mediated immunity. Psychiatr Res 54:143–160

Maes M, Meltzer HY, Bosmans E, Bergmans R, Vandoolaeghe E, Ranjan R, Desnyder R (1995a) Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. J Affect Disord 34:301–309

Maes M, Smith R, Scharpé S (1995b) The monocyte-T lymphocyte hypothesis of major depression. Invited editorial. Psychoneuroendocrinology 20:111–116

Maes M, Vandevyvere J, Vandoolaeghe E, Bril T, Vandoolaeghe E, Neels H (1996a) Disorders in iron metabolism and the erythron in major depression: further evidence for an inflammatory response. J Affect Disord 40:23–33

Maes M, Wauters A, Verkerk R, Neels H, Van Gastel A, Cosyns P, Scharpé S, Desnyder R (1996b) Lower L-tryptophan availability in depression: a marker of a more generalized disorder in protein metabolism. Neuropsychopharmacol 15:243–251

Marten NW, Burke EJ, Hayden JM, Straus DS (1994) Effect of amino acid limitation on the expression of 19 genes in rat hepatoma cells. FASEB J 8:538–544

Moir ATB, Eccleston D (1968) The effect of precursor loading in the cerebral metabolism of 5-hydroxyindoles. J Neurochem 15:1093–1108

Moldawer LL, Georgieff M, Lundholm K (1987) Interleukin-1, tumour necrosis factor-alpha (cachectin) and the pathogenesis of cancer cachexia. Clin Physiol 7:263–274

Møller SE (1985) Tryptophan to competing amino acids ratio in depressive disorder: relation to efficacy of antidepressive treatments. Acta Psychiatr Scand (Suppl) 72:1–30

Møller SE, Beurs P de, Timmerman L, Tan BK, Leynse-Ybema HJ, Cohen Stuart MH, Hopfner Petersen HE (1986) Plasma tryptophan and tyrosine ratios to competing amino acids in relation to antidepressant response to citalopram and maprotiline: a preliminary study. Psychopharmacology 88:96–100

Muller N (1995) Psychoneuroimmunology: implications for the drug treatment of psychiatric disorders. CNS Drugs 4:125–140

Pardridge WM (1979) Tryptophan transport through the blood-brain barrier: in vivo measurement of free and albumin-bound amino acid. Life Sci 25:1519–1528

Perez-Cruet J, Chase TN, Murphy DL (1974) Dietary regulation of brain tryptophan metabolism by plasma ratio of free tryptophan and neutral amino acids in humans. Nature 248:693–695

Russ MJ, Ackerman SH, Banay-Schwartz M, Shindledecker RD, Smith GP (1990) Plasma tryptophan to large neutral amino acid ratios in depressed and normal subjects. J Affect Disord 19:9–14

Sainio E-L, Pulkki K, Young SN (1996) L-tryptophan: biochemical, nutritional and pharmacological aspects. Amino Acids 10:21–47

Seidel A, Arolt V, Hunstiger M, Rink L, Behnisch A, Kirchner H (1996a) Major depressive disorder is associated with elevated monocyte counts. Acta Psychiatr Scand 94:198–204

Seidel A, Arolt V, Hunstiger M, Rink L, Behnisch A, Kirchner H (1996b) Increased $CD56^+$ natural killer cells and related cytokines in major depression. Clin Immunol Immunopathol 78:83–85

Sluzewska A, Nowakowsky E, Gryska K, Machkiewicz A (1994) Haptoglobin levels in a chronic mild stress model of depression in rats before and after treatment. Eur Neuropharmacol P–1–18:302

Sluzewska A, Rybakowski JK, Laciak M, Mackiewicz A, Sobieska M, Wiktorowiz K (1995) Interleukin-6 serum levels in depressed patients before and after treatment with fluoxetine. Ann NY Acad Sci 762:474–476

Sluzewska A, Rybakowski J, Sobieska M, Wiktorowicz K (1996) Concentration and microheterogeneity glycophorms of α_1-acid glycoprotein in major depressive disorder. J Affect Disord 39:149–155

Smith SE, Pihl RO, Young SW, Ervin FR (1987) A test of possible cognitive and environmental influences on the mood lowering effect of tryptophan depletion in normal males. Psychopharmacology 91:451–457

Smith QR, Fukui S, Robinson P, Rapaport SI (1990) Influence of cerebral blood flow on tryptophan uptake into brain. In: Lubec G, Rosenthal GA (eds) Amino acids: chemistry, biology and medicine. Escom, Leiden, pp 364–369

Song C, Leonard BE (1994) An acute phase protein response in the olfactory bulbectomized rat: effect of sertraline treatment. Med Sci Res 22:313–314

Song C, Dinan T, Leonard BE (1994) Changes in immunoglobublin, complement and acute phase protein levels in the depressed patients and normal controls. J Affect Disord 30:283–288

Spitzer RL, Williams JBW, Gibbon M, First MB (1990) Structured Clinical Interview according to DSM-III-R. American Press, New York

Takikawa O, Yoshida R, Yasui H, Hayaishi O (1984) The relationship between plasma kynurenine and indoleamine 2,3-dioxygenase activity in the extrahepatic tissues. In: Schlossberger HG, Kochen W, Linzen B, Steinhart H (eds) Progress in tryptophan and serotonin research. Walter de Gruyter, Berlin, pp 517–520

Thase ME, Rush AJ (1995) Treatment-resistant depression. In: Bloom FE, Kupfer DJ (eds) Psychopharmacology: the fourth generation of progress. Raven Press, New York, pp 1081–1098

Walser M, Hill SB (1993) Free and protein-bound tryptophan in serum of untreated patients with chronic renal failure. Kidney Int 44:1366–1371

Young SN, Smith SE, Pihl R, Ervin FR (1985) Tryptophan depletion causes a rapid lowering of mood in normal males. Psychopharmacology 87:173–177

Yuwiler A, Oldendorf WH, Geller E et al. (1977) The effect of albumin binding and amino acid composition on tryptophan uptake into the brain. J Neurochem 28:1015–1023