

## ORIGINAL RESEARCH ARTICLE

# Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy

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**Cytokine therapy for cancer or viral diseases is accompanied by the development of depressive symptoms in a significant proportion of patients. Despite the increasing number of studies on the neurotoxic effects of cytokines, the mechanisms by which cytokines induce depressive symptoms remain largely unknown. In view of the relationship between neurotransmitter precursors and mood, the present study aimed at assessing the relationship between serum concentrations of the amino acids tryptophan and tyrosine, major precursors of serotonin and norepinephrine respectively, and depressive symptoms in cancer patients undergoing cytokine therapy. Sixteen cancer patients eligible to receive immunotherapy with interleukin-2 and/or interferon-alpha participated in the study. At baseline and after one week and one month of therapy, depressive symptoms were assessed using the Montgomery–Asberg Depression Rating Scale (MADRS), and blood samples were collected for the determination of the large neutral amino acids (LNAA) (tryptophan, tyrosine, valine, leucine, isoleucine, phenylalanine) which compete for transport across the blood–brain barrier. Serum concentrations of tryptophan as well as the tryptophan/LNAA ratio significantly decreased between baseline, one week and one month of therapy. The development and severity of depressive symptoms, especially anorexia, pessimistic thoughts, suicidal ideation and loss of concentration were positively correlated with the magnitude of the decreases in tryptophan concentrations during treatment. These findings indicate that the development of depressive symptoms in patients undergoing cytokine therapy could be mediated by a reduced availability of the serotonin relevant amino acid precursor, tryptophan.**

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## Introduction

### *Cytokines and depression*

There are now several pre-clinical and clinical lines of evidence that support a relationship between immune activation and the development of depression: (1) activation of the immune system in laboratory animals through the administration of proinflammatory cytokines (eg, interleukin-1  $\beta$  (IL-1 $\beta$ ), tumor necrosis factor alpha (TNF- $\alpha$ )) or lipopolysaccharide, a potent cytokine-inducer, is accompanied by a wide range of behavioral effects referred to as ‘sickness behavior’ that resemble symptoms of depression; (2) clinical diseases with an inflammatory component (eg, influenza, herpes

virus, HIV) are associated with a high prevalence of depressive disorders; (3) depressed patients display evidence of activation of the immune response in comparison to healthy controls; (4) the use of cytokines as therapeutic agents in clinical trials is accompanied by neuropsychiatric changes in treated patients.<sup>1–3</sup>

Depressive symptoms frequently develop in patients receiving cytokines, mainly interleukin-2 (IL-2) and interferon-alpha (IFN- $\alpha$ ), for the treatment of cancer or viral diseases.<sup>4–8</sup> When severe, these symptoms require the adjustment of therapeutic schedules, and can even lead to interruption of treatment.<sup>5,9–11</sup> Despite the increasing number of studies demonstrating the neuropsychiatric effects of cytokines, the mechanisms by which cytokines induce depressive symptoms are still largely unknown.

### *Cytokines and neurotransmitter functions*

The depressive effects of cytokines could be due to their interference with the metabolism of serotonin and norepinephrine known to play a role in the pathophysiology of depression.

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siology of depressive disorders.<sup>12,13</sup> These neurotransmitters are synthesized within the brain from their precursors, the large neutral amino acids tryptophan (TRP) and tyrosine (TYR) respectively, the latter being also the precursor of dopamine. Other large neutral amino acids (LNAA), notably valine, phenylalanine, leucine and isoleucine affect precursor availability by competing with tryptophan and tyrosine for transport across the blood–brain barrier.<sup>14–16</sup> Therefore, the TRP/LNAA and TYR/LNAA ratios are considered in many studies as the peripheral indexes of TRP and TYR availability to the brain.<sup>14–16</sup> TRP and TYR levels and the TRP/LNAA ratio have been found to be significantly lower in depressed subjects than in healthy controls.<sup>12,16</sup>

Proinflammatory cytokines have profound effects on the metabolism of brain serotonin, dopamine, and noradrenaline in mice and rats.<sup>17,18</sup> Whereas the effects of acute administration of cytokines on the metabolism of neurotransmitters, notably serotonin, have been often described as stimulating in animal studies,<sup>19</sup> recent data indicate that cytokine-induced changes in the release of biogenic amines in the nucleus accumbens are specific to the cytokine administered (eg, IL-1 and IL-6 modestly increased extracellular 5-hydroxyindoleacetic acid (5-HIAA) from the nucleus accumbens in the rat whereas IL-2 did not).<sup>18</sup> More importantly, IFN- $\alpha$  administration reduced brain levels of serotonin and 5-HIAA in several regions of the rat brain.<sup>20</sup> In clinical studies, significant decreases in serum TRP concentrations have been noted in patients receiving IL-2 or IFN- $\alpha$ .<sup>21,22</sup> In another type of investigation, associations between plasma TRP concentrations and immune variables have been observed in psychiatric patients with major depression.<sup>23</sup> Taken together, these data point to the possibility that depressive symptoms in patients treated with cytokine therapy could be related to the actions of cytokines on monoamine metabolism. The aim of the present study was therefore to assess the relationship between the development of depressive symptoms and blood concentrations of the amino acid precursors of serotonin and norepinephrine in cancer patients receiving IL-2 or IFN- $\alpha$  therapy.

## Patients and methods

### Patients

Sixteen patients (eight males, eight females, mean age: 48.9 years (range: 19–76 years)) with renal cell carcinoma ( $n = 9$ ) or melanoma ( $n = 7$ ) participated in the present study. Patients were treated either with IFN- $\alpha$  alone or with IL-2, administered alone or in combination with IFN- $\alpha$ . The study was performed during the first 4 weeks of cytokine treatment. In patients treated with IFN- $\alpha$  alone ( $n = 9$ ), the cytokine was administered either intravenously at the dose of  $20 \times 10^6$  IU  $m^{-2}$   $day^{-1}$  during 5 consecutive days a week<sup>9</sup> ( $n = 7$ ) (IntronA, Schering Plough Corp, France) or subcutaneously three times a week at the dose of  $18 \times 10^6$  IU  $day^{-1}$  ( $n = 2$ ) (Roferon, Roche, France). In patients who received IL-2-based therapy ( $n = 7$ ), IL-2 (Proleukin,

Chiron Therapeutics, France) was administered either alone at the dose of  $18 \times 10^6$  IU  $day^{-1}$  during 5 consecutive days a week<sup>24</sup> ( $n = 5$ ) or in combination with IFN- $\alpha$  ( $n = 2$ ). In patients who received both IL-2 and IFN- $\alpha$ , IL-2 was administered in two injections per day during 5 consecutive days at the daily dose of  $18 \times 10^6$  IU  $m^{-2}$   $day^{-1}$  on the first and third weeks of therapy, and IFN- $\alpha$  (Roferon, Roche, France) was administered three times a week at the dose of  $6 \times 10^6$  IU  $day^{-1}$ .<sup>25</sup>

Several medications were used to control the side effects of cytokine therapy: (1) paracetamol was systematically administered to prevent fever and flu-like symptoms induced by cytokines; (2) ketoprofene was given to one patient who developed fever and associated symptoms despite paracetamol; (3) antiemetic medications (ondansetron, metopimazine, metoclopramide) were administered to six patients as needed. None of the patients included in the study displayed any current mood disorder, especially depressive disorder, before the initiation of cytokine therapy as assessed by a semi-structured interview (with the help of the MINI<sup>26</sup>) carried out at baseline. Patients treated with antidepressant drugs during the course of the study were excluded from the data analysis. All patients were adult and gave written informed consent. The study was approved by the local committee for the protection of patients in biomedical research (CCPPRB Bordeaux A).

### Methods

Clinical assessments and blood collections were carried out before the initiation of cytokine therapy (baseline) and at the end of the first week and first month of therapy. The Montgomery and Asberg Depression Rating Scale (MADRS),<sup>27</sup> a semi-structured standardized assessment of depressive symptomatology, was conducted at each clinical interview. The MADRS scale includes 10 items measuring several dimensions of depressive symptomatology such as apparent and reported sadness, inner tension, reduced sleep, reduced appetite, loss of concentration, lassitude, inability to feel, pessimistic thoughts and suicidal ideas. Blood for the assay of serum amino acids was collected on the morning of the neuropsychiatric evaluations around 9.00 am ( $\pm 60$  min) (always prior to cytokine administration). Sera were stored at  $-70^\circ\text{C}$  until thawed for assay. Serum amino acids were determined by means of an HPLC method as described previously.<sup>28,29</sup> The measurements included tryptophan and tyrosine, and the other large neutral amino acids, ie, valine, leucine, isoleucine and phenylalanine. All assays were done at the same time by operators blind to patients' characteristics.

### Data analysis and statistics

Because of the small size of each therapeutic group, only global analyses on the whole population of the study were performed for the data analysis. However, prior to performing these analyses, we checked by analysis of variance (ANOVA) that there were no main and interaction effects of the different modalities of

treatment on the serum amino acids concentrations during cytokine therapy. Mean differences in serum amino acids concentrations during treatment were assessed by ANOVA with time as a repeated measure factor. Multiple post-hoc comparisons using both conservative (Bonferroni corrected *t*-test) and powerful (Newman–Keuls) tests of significance were performed when appropriate. When significance was attained using both tests, only the Bonferroni test was reported. Homogeneity of variances was checked with the Bartlett test before performing ANOVA. Sphericity was checked using the Mauchly sphericity test. When necessary, a correction for sphericity was applied (Greenhouse–Geisser Adjustment). This correction was necessary only for the tyrosine/LNAA ratio. Correlations between changes in serum amino acids concentrations and MADRS scores were assessed by means of Bravais–Pearson moment products method. Correlations between changes in serum TRP and specific MADRS dimensions were assessed by non-parametric Spearman rank correlation procedure for discrete variables. All the probabilities were two-sided and significance was set at  $P \leq 0.05$ .

## Results

### 1. Changes in MADRS scores and serum amino acids concentrations during cytokine therapy

Depression scores on the MADRS scale significantly increased during the first month of cytokine therapy ( $F(2,30) = 5.67$ ;  $P < 0.01$ ) in the whole population under study. More precisely, MADRS scores were higher respectively after one week ( $P < 0.05$ ) and one month of cytokine therapy ( $P < 0.01$ ) compared to baseline (Table 1). Interestingly, as observed in a previous study,<sup>11</sup> MADRS scores at baseline were significantly correlated with the intensity of depressive symptoms at the end of the first month of cytokine therapy ( $r = 0.662$ ,  $P < 0.01$ , data not shown).

Serum concentrations of total TRP significantly changed during the first month of cytokine therapy ( $F(2,30) = 9.36$ ;  $P < 0.001$ ) (Table 1). At the end of the first week of therapy, serum TRP concentrations were significantly decreased compared to baseline ( $P < 0.01$ ) (33% reduction), and remained lower at the end of the first month of therapy compared to baseline ( $P < 0.01$ ) (32.3% reduction). In contrast, serum concentrations of total TYR remained stable during treatment ( $F(2,30) = 1.75$ ;  $P = 0.19$ ). Similarly, the sum of the large neutral amino acids (LNAA) decreased between baseline and one month of therapy ( $P < 0.05$ ), whereas this sum remained stable during treatment when TRP was excluded from the analysis (LNAA1). Interestingly, as observed for the serum concentrations of total TRP, the TRP/LNAA1 ratio significantly decreased during cytokine therapy ( $F(2,30) = 7.21$ ;  $P < 0.01$ ). At the end of the first week of therapy, the TRP/LNAA1 ratio was significantly lower compared to baseline ( $P < 0.01$ ) (29.2% reduction), and this decrease remained observable after one month of therapy ( $P < 0.05$ ) (25% reduction).

**Table 1** Temporal evolution of MADRS scores and serum tryptophan and tyrosine availability during the first month of IL-2 and/or IFN- $\alpha$  therapy in the whole population of the study

	Baseline	1 week	1 month
MADRS	6.2 (4.4)	9.9 (7.6) <sup>†</sup>	11.9 (7.8)**
TRP	51.3 (18)	34.3 (16)**	34.7 (16)**
TYR	62.9 (21)	56.6 (13)	56.2 (18)
LNAA	706 (195)	634 (157)	604 (143)*
LNAA1	655 (184)	600 (145)	569 (130)
LNAA2	643 (176)	578 (146)	548 (128)*
TRP/LNAA1 ( $\times 100$ )	7.9 (2.5)	5.6 (2)**	5.9 (2.1)*
TYR/LNAA2 ( $\times 100$ )	9.8 (1.9)	9.9 (1.5)	10.2 (2)

Note: All results are shown as mean (SD), in  $\mu\text{mol l}^{-1}$ .

TRP = tryptophan; TYR = tyrosine.

LNAA: sum of the large neutral amino acids (ie, TRP + TYR + phenylalanine + valine + leucine + isoleucine); LNAA1: sum LNAA excluding TRP (ie, TYR + phenylalanine + valine + leucine + isoleucine); LNAA2: sum LNAA excluding tyrosine (ie, TRP + phenylalanine + valine + leucine + isoleucine).

\*\*Significantly different from baseline with  $P$  value  $< 0.01$ ;

\*significantly different from baseline with  $P$  value  $< 0.05$ ;

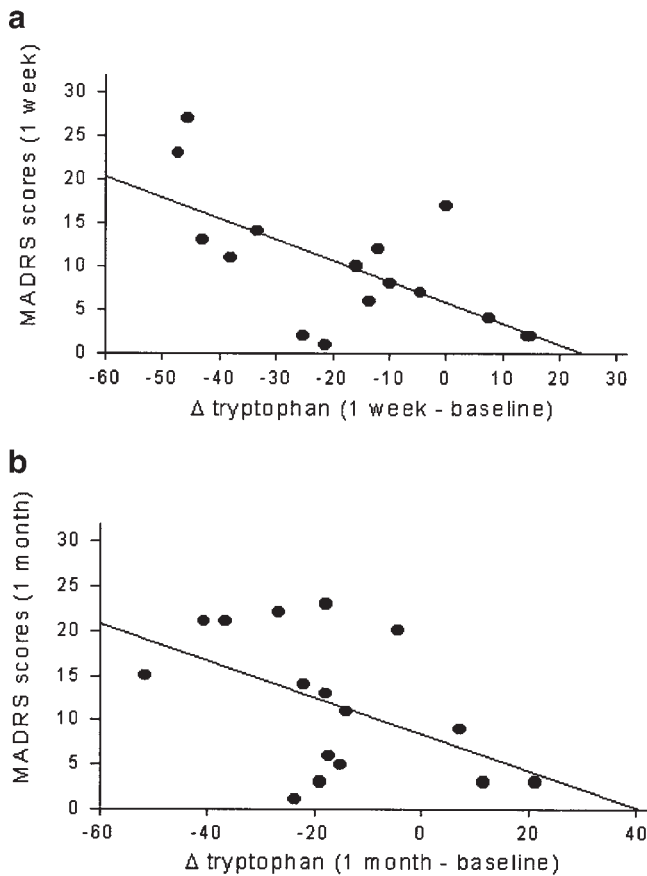
(Bonferroni corrected *t*-test performed after repeated measures ANOVA); <sup>†</sup>significantly different from baseline with  $P$  value  $< 0.05$  (Newman–Keuls test).

### 2. Relationships between amino acid concentrations and MADRS scores

As shown in Figure 1a, the magnitude of the MADRS scores at the end of the first week of therapy was significantly correlated with the magnitude of the decreases in TRP concentrations ( $r = -0.648$ ;  $P < 0.01$ ) between baseline and one week of therapy and tended to be correlated with the decreases in the TRP/LNAA1 ratio between the two times of evaluation ( $r = -0.469$ ;  $P = 0.06$ ). Similarly, the early increases in MADRS scores between baseline and the end of the first week of treatment were significantly correlated with the decreases in the serum concentrations of TRP during the same period of therapy ( $r = -0.489$ ;  $P = 0.05$ ). After one month of therapy, the magnitude of the MADRS scores was significantly correlated with the decreases in TRP concentrations between baseline and one month ( $r = -0.499$ ;  $P < 0.05$ ) (Figure 1b). In addition, the magnitude of the MADRS scores at one month of therapy tended to be correlated with the decreases in the TRP/LNAA1 ratio between baseline and one month of therapy ( $r = -0.407$ ;  $P = 0.11$ ).

### 3. Relationships between serum TRP changes and specific depressive symptoms

Spearman correlations were performed to investigate the possibility of a relationship between specific symptoms assessed in the MADRS scale and changes in TRP concentrations during cytokine therapy in the whole population under study. Results showed that the decreases in TRP concentrations during the first week



**Figure 1** Relationships between depressive symptoms and decreases in TRP concentrations during cytokine therapy. The magnitude of the MADRS scores at the end of the first week and first month of cytokine therapy was correlated with the magnitude of the decreases in TRP concentrations (a) between baseline and 1 week and (b) between baseline and 1 month.

of treatment were significantly correlated with reduced appetite (Spearman'  $\rho = -0.609$ ;  $P < 0.05$ ), pessimistic thoughts (Spearman'  $\rho = -0.574$ ;  $P < 0.05$ ), suicidal ideas ( $\rho = -0.533$ ;  $P < 0.05$ ) and loss of concentration ( $\rho = -0.495$ ;  $P = 0.05$ ) at the end of the first week of therapy. At the end of the first month of therapy, inner tension and suicidal ideas were associated with the magnitude of the decrease in TRP concentrations between baseline and one month ( $\rho = -0.533$ ;  $P < 0.05$  and  $\rho = -0.496$ ;  $P = 0.05$  respectively). In addition, the degree of loss of concentration experienced by patients at the end of the first month of cytokine therapy was significantly associated with the magnitude of the decrease measured in the TRP/LNAA1 ratio between baseline and one month ( $\rho = -0.563$ ;  $P < 0.05$ ).

## Discussion

Results from the present study indicate for the first time that the development of depressive symptoms during cytokine therapy is associated with significant changes in serum TRP concentrations. Concomitantly to increased MADRS scores, patients undergoing cyto-

kine therapy displayed significant decreases in serum TRP concentrations and in the TRP/LNAA1 ratio during the first month of treatment. Serum concentrations of the other large neutral amino acids, such as TYR, did not significantly change during cytokine therapy. In addition, the severity of depressive symptoms, as measured by the magnitude of the MADRS scores, was related to the magnitude of the decreases in the concentrations of TRP between baseline and each therapeutic measure (ie, respectively at the end of the first week and first month of therapy). Dimensional analyses revealed that decreases in TRP concentrations were more particularly associated with the development of anorexia, suicidal ideation, pessimistic thoughts, loss of concentration and inner tension during cytokine therapy. These findings can be interpreted to suggest that reduced access of the central serotonergic system to relevant amino acid precursors participate in the development of depressive symptoms during cytokine treatment.

As previously noted, TRP share the same transport system across the blood–brain barrier with the other large neutral amino acids, notably tyrosine, valine, leucine, isoleucine and phenylalanine.<sup>14–16</sup> In the present study, decreases in TRP concentrations during cytokine therapy in the absence of significant changes in the concentrations of the other large neutral amino acids, as indicated by the decrease in the TRP/LNAA1 ratio during treatment compared to baseline, are strongly suggestive of a reduced TRP availability to the brain. Many studies have already shown that acute TRP depletion, accomplished in humans by consuming a TRP-free drink, decreases brain serotonin levels and is associated with depressed mood, especially in women and in individuals with a personal or familial history of depression, and leads to depressive relapse in partially or recently remitted depressed patients.<sup>30–32</sup> The hypothesis that TRP depletion is involved in the mechanisms mediating the depressive effects of cytokine therapy is in accordance with the matched time course observed in the present study between the development of depressive symptoms and the decreases in serum concentrations of TRP. In addition, the association measured in this study between serum TRP concentrations and individual depressive symptoms, especially loss of appetite, pessimistic and suicidal thoughts, cognitive impairment and tension are in accordance with previous data indicating that decreases in TRP concentrations, or in its product serotonin, affect many neurovegetative or psychological functions related to mood such as appetite, cognition, anxiety, or suicidal ideation.<sup>33–36</sup>

There are at least two mechanisms, not necessarily mutually exclusive, by which cytokines may induce TRP depletion. One mechanism is that cytokines may indirectly affect TRP by decreasing food intake.<sup>37,38</sup> Because TRP levels are strongly modulated by dietary intake,<sup>30</sup> numerous studies are under way to investigate the possibility that ingestion of dietary precursors of amino acids could participate in the treatment of depression.<sup>13</sup> In the present study, patients who dis-

played the highest scores in the dimension of 'reduced appetite' of the MADRS scale after one week of treatment were those who also displayed the highest decreases in TRP concentrations between baseline and the end of the first week of treatment. There was, however, no correlation between appetite and TRP concentrations at one month of therapy. These preliminary findings point to the existence of a possible relationship between cytokine-induced anorexia and TRP depletion during the early stage of cytokine therapy. In the context of the present study, decreased appetite was evaluated only through the dimension of reduced appetite assessed by the MADRS scale. It would therefore be important for future investigations to use more specific measures of appetite and food intake to further assess the relationships between TRP depletion and dietary intake. However, the fact that only TRP concentrations decreased during cytokine therapy whereas the other large amino acids did not significantly change is not in favor of a role of variations in food intake in the cytokine-induced TRP depletion.

A second mechanism is that cytokines induce TRP depletion and consequently depressive symptoms by enhancing the activity of indoleamine 2,3-dioxygenase (IDO), the first enzyme in the kynurenine pathway that degrades and converts TRP to kynurenic and then quinolinic acid.<sup>39,40</sup> The induction of IDO results in the decreased local availability of TRP for immune cells. This pathway has physiological relevance since it is involved in the mechanisms of immunologic tolerance of the fetus.<sup>40</sup> Cytokines differ in their potency to induce IDO. Although interferon-gamma (IFN- $\gamma$ ) is one of the most potent inducers of IDO,<sup>41,42</sup> both IL-2 and IFN- $\alpha$  are also able to induce IDO activity and TRP degradation in peripheral blood mononuclear cells.<sup>43</sup> In the case of IL-2, the induction of IDO is probably indirectly mediated via the stimulation of IFN- $\gamma$ . Studies on cancer patients given IL-2, Type I or Type II IFN- $\alpha$  indicated that IFN could induce IDO which resulted in decreased serum TRP levels and increased urinary metabolites of the kynurenine pathway.<sup>21,22</sup> In addition, a recent study showed a significant decrease of the kynurenine/tryptophan quotient associated with inflammatory signs in hepatitis C patients undergoing IFN- $\alpha$  therapy.<sup>44</sup> In patients with immune-related diseases, such as immunodeficiency virus type I infection, increased levels of endogenous IFN- $\gamma$ , kynurenine and neopterin, a marker of cell-mediated immune response, were associated with increased degradation of TRP, suggesting the involvement of IDO.<sup>45</sup> In addition, significant associations were found between IDO-induced lower TRP and neurologic/psychiatric symptoms in this category of patients.<sup>46</sup> These data support the possibility that immunotherapy induces depressive symptoms through IDO induction and IDO-mediated TRP depletion, which clearly deserves further investigation.

In conclusion, the present results demonstrate for the first time a strong relationship between decreases in the peripheral amino acid precursor of serotonin and the development and intensity of depressive symptoms

in cancer patients receiving IFN- $\alpha$  or IL-2 therapy. Although these findings remain preliminary notably because of the small sample size of the population considered in this study, they provide new hypotheses that could be tested in future investigations about the pathophysiology of the neuropsychiatric effects of cytokines administered therapeutically to patients with cancer or chronic viral diseases. Moreover, these findings suggest that nutritional or pharmacological interventions to enhance serotonin availability could participate in the prevention of cytokine-induced depressive symptoms. This hypothesis is supported by recent data showing that the administration of the selective serotonin reuptake inhibitor, paroxetine, is associated with a significant reduced incidence of depressive disorders in patients undergoing IFN- $\alpha$  therapy.<sup>47</sup>

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