

ORIGINAL PAPER

Rottraut Ille · Jürgen Spona · Michaela Zickl · Peter Hofmann · Theresa Lahousen
Nina Dittrich · Götz Bertha · Karin Hasiba · Franz Alfons Mahnert · Hans-Peter Kapfhammer

“Add-On”-therapy with an individualized preparation consisting of free amino acids for patients with a major depression

Published online: 1 April 2007

Abstract The efficacy of a deficit oriented add-on therapy with free amino acids in depressive patients treated with the antidepressant Remeron® was evaluated. About 40 in-patients were investigated by a randomised double-blind placebo-controlled study during 4 weeks. Plasma levels of 20 amino acids and measures of depression, suicidal behaviour and aggression were surveyed on admission and after a 4 weeks' therapy with Remeron® plus an individualized amino acid mixture or placebo. The preparation of the amino acid mixture was based on an aminogram and consisted of essential amino acids plus vitamins and trace elements as co-factors for the amino acid metabolism. Patients of the experimental group showed a significantly better improvement of depression and a higher responder rate than those of the placebo group. The results suggest that oral application of a deficit oriented amino acid mixture can improve the therapeutic outcome of an antidepressant. Furthermore, lacking side effects of the amino acids resulting also in a better patient compliance may improve the benefit/risk ratio.

Key words major depression · add-on-therapy · aminogram · individualized amino acid preparation

This study was supported by the Ludwig Boltzmann Gesellschaft and the “Amt der Steiermärkischen Landesregierung”.

Dr. R. Ille · Prof. Dr. J. Spona · Dr. M. Zickl
Ludwig Boltzmann Institute for Experimental Endocrinology
Vienna, Austria

Prof. Dr. P. Hofmann · Dr. T. Lahousen · Dr. N. Dittrich
Prof. Dr. G. Bertha · Dr. K. Hasiba · Dr. F. A. Mahnert
Prof. Dr. H.-P. Kapfhammer · Dr. R. Ille (✉)
University Hospital of Psychiatry
Auenbrugger Platz 31
Graz 8036, Austria
Tel.: +43-699-19542196
E-Mail: rottraut.ille@uni-graz.at

Introduction

Life time prevalence for a major depression amounts 12–17%, with women being affected two times more than men [37]. A newer epidemiological study from the USA shows a 12-months prevalence of 10–12% [25]. Pharmacologic therapy with antidepressants improving neurotransmitter deficiency is the most accepted treatment for all forms of moderate to severe depressions independent of their genesis. The antidepressant therapy has a variety of undesirable side-effects such as sedation, decrease of blood pressure, increase of weight, indigestion or sexual dysfunction. This often results in patients' poor compliance resulting in a break-up of medication with recurrence of depressive symptoms and increased suicidal risk [14, 24]. Previous investigations have shown that reduced plasma concentrations of amino acids, such as the serotonin precursors tryptophan and tyrosine are a good indicator for an insufficient availability of this transmitter in the brain [10, 13, 31, 51]. Patients with major depression showed lower absolute plasma concentrations of tryptophan and tryptophan/big neutral amino acid ratio in comparison to healthy persons [30, 44]. The results of Gronier et al. [20] suggest a defect of transport of L-tryptophan within the platelets of depressive patients. An experimentally induced reduction (“depletion”) of the tryptophan plasma levels to 70–90% of the basis concentration by application of an amino acid mixture without tryptophan led to a decrease of central availability of serotonin. In addition patients with major depression exhibited a stronger mood decrease than healthy persons [6, 16, 27, 47]. In contrast, persons having received a balanced amino acid mixture showed no change of mood [47]. Based on these results attempts were made to replace or supplement antidepressant therapy by a therapeutic increase of the tryptophan

levels primarily by means of an oral application of L-tryptophan [17, 52].

A backlash of such approaches occurred in 1989 when contaminations in tryptophan preparations caused an epidemic outbreak of the eosinophilic-myalgia syndrome in the USA and tryptophan was taken from the market by the FDA.

Recent investigations could corroborate earlier results and showed that tryptophan enhance the mood of depressive patients compared to placebo [45]. Levitan et al. [29] found that the mood of depressive patients after a 1-week therapy by fluoxetine plus 2–4 g tryptophan/d was enhanced superior than by a therapy with fluoxetine plus placebo. In spite of this, such approaches could not be established in psychiatric therapy schemes. One reason for this is the fact that the efficacy for moderate and severe depressions was not sufficient [46]. This also was true for the drug “Kalma” which has been registered since 1988. It is synthesized from L-tryptophan and used only in mild depressions and as “add-on” therapy on subjects on an antidepressant. Only high dosages of tryptophan were reported to increase mood and to improve insomnia but simultaneously increasing the rate of undesirable side effects such as eosinophilia myalgia syndrome, liver damage or development of a cataract. This treatment did not offer an advantage compared to conventional antidepressant therapy [49].

Only a few studies were done to examine plasma concentrations other than tryptophan. Kishimoto and Hama [26] reported that plasma levels of tyrosin were significantly lower in depressed patients than in controls and increased after the period of depression.

Plasma concentrations of taurine and lysine were increased for patients with a major depression [2, 33] whereas Tachiki et al. [50] showed decreased levels of taurine in depressed patients. Mathis et al. [32] found increased levels of valin, leucine and isoleucine in depressive patients.

Goldberg [19] could reduce the dose of amphetamines after an oral therapy with L-tyrosine for patients with a deficiency of norepinephrine. Sabelli et al. [41, 45] reported a successful therapy with phenylalanine of depressed patients compared to healthy controls. No differences in the therapeutic effects of the antidepressant imipramine and of DL-phenylalanine were noted when using the scores of the HAM-D scale [4].

Improvement of current therapies is an important issue in health policy. Previous findings on a relationship between deficiencies and increased plasma concentrations of some amino acids in depressed patients and on oral treatment with amino acids are very inconsistent. Furthermore, therapeutic use of a single amino acid may result in an imbalance of amino acids within the body as corroborated by the negative effects of the “depletion” experiments. Preliminary investigations have shown that plasma concentrations of the essential amino acids are correlated significantly. Therefore, an imbalance of amino acids

Table 1 Sociodemographic characters of subjects

	Total (n = 40)	Experimental group (n = 20)	Placebo group (n = 20)
Sex %			
Men	20.0	15.0	25.0
Women	80.0	85.0	75.0
Age M (SD)	46.4 (12.1)	48.9 (12.0)	43.8 (12.0)
Recurrent depression	70.0	75.0	65.0
Substance abuse %	17.5	20.0	15.0
Ideations %	55.0	50.0	40.0
Attempted suicide(s) %	27.5	20.0	35.0
Negative life events %	45.0	50.0	55.0
Melancholia %	55.0	50.0	45.0
mg Remeron® M (SD)	34.5 (12.3)	33.8 (9.6)	35.3 (14.8)

% = percentage, M = mean, SD = standard deviation

may be avoided by application of an acid preparation containing all essential amino acids based on individual deficits [8, 48].

This study was aimed at examining the effects of an individualized amino acid preparation on the improvement of symptoms of depression in patients with major depression.

Methods

■ Participants

About 48 in-patients (36 women, 12 men) out of a pool of 233 patients at the University Hospital of Psychiatry, Graz, Austria were included to the study. All patients gave informed consent to the study, which was approved by the institutional review board of the General and University Hospital of Graz.

Inclusion criteria were the diagnosis of major depression according to the criteria of DSM IV; [42] and indication of Remeron® (agent mirtazapine), applied with agitation and sleep disturbances being in the front of the depressive pathology. Mirtazapine is a potent antagonist of central $\alpha 2$ -adrenergic auto- and heteroreceptors, is an antagonist of both 5-HT₂ and 5-HT₃ receptors possibly preventing side effects associated with non-selective 5-HT activation and contributing also to the anxiolytic and sleep-improving properties of mirtazapine. Mirtazapine has minimal effects on monoamine reuptake and it enhances noradrenalin transmission. Blockade of presynaptic $\alpha 2$ noradrenergic autoreceptors leads to increased norepinephrine release [12].

Most patients suffered from a recurrent depression (Table 1), with 2–5 episodes in their history. The duration of the current episode was about 7–30 days before admission. For pretreated patients (82.5%) the previous antidepressant was tapered within 4 days and patients were changed to Remeron®.

Exclusion criteria were other psychotic disorders, pregnancy, cancer and all aminoacidopathies and an additional medication with another antidepressant than Remeron®.

Seven patients dropped out early after a change of the antidepressant due to incompatibility, ineffectiveness or a strong increase of weight, and one woman was excluded during the course of the study because of a later diagnosed co-morbidity of major depression with a distinct panic disorder. Thus, 40 patients were entered into the study. Twenty of the patients (17 women, 3 men) received Remeron® and a mixture of amino acids, another 20 patients (14 women, 6 men) were allotted to the placebo group receiving Remeron® plus placebo preparation. As an influence of nutrition on plasma concentration of several amino acids could be expected patients were matched respective to their hospital diet.

Table 2 Normal ranges of plasma concentrations ($\mu\text{mol/l}$) and mean plasma concentrations of the 20 amino acids in the experimental and the control group at the term of admission (T1) and after a 4-week therapy (T2)

	Normal ranges	T1		T2	
		Amino acids <i>n</i> = 20 <i>M</i> (SD)	Placebo <i>n</i> = 20 <i>M</i> (SD)	Amino acids <i>n</i> = 20 <i>M</i> (SD)	Placebo <i>n</i> = 20 <i>M</i> (SD)
Arginine (arg)	38–140	66.6 (16.7)	67.4 (27.7)	63.9 (16.1)	69.5 (16.6)
Histidine (his)	60–150	60.1 (10.1)	65.7 (12.0)	63.6 (11.0)	66.7 (11.6)
Isoleucine (ile)	35–150	53.7 (12.7)	61.1 (12.8)	57.9 (10.7)	60.4 (15.4)
Leucine (leu)	85–260	105.4 (22.7)	115.6 (24.6)	101.9 (20.2)	108.2 (25.4)
Lysine(lysin)	70–200	131.0 (32.6)	130.3 (42.0)	123.5 (18.9)	127.0 (33.6)
Methionine (met)	20–60	33.3 (7.4)	35.1 (7.2)	35.6 (7.5)	33.3 (6.9)
Phenylalanine (phe)	50–140	51.9 (9.8)	51.8 (7.6)	54.3 (9.8)	50.9 (7.7)
Threonine (thr)	80–250	109.8 (46.3)	103.5 (38.1)	98.1 (25.9)	101.2 (25.2)
Tryptophan (trp)	40–120	45.1 (9.0)	46.5 (6.4)	48.6 (9.1)	48.2 (8.8)
Valine(val)	180–480	179.4 (38.4)	193.0 (34.6)	190.7 (28.6)	191.3 (35.1)
Glycine (gly)	200–450	259.5 (83.5)	234.3 (105.7)	209.9 (82.7)	212.7 (67.2)
Serine (ser)	80–200	88.1 (20.3)	86.7 (29.5)	71.5 (17.7)	79.3 (20.9)
Taurin (tau)	40–200	54.8 (14.0)	54.4 (14.9)	81.2 (38.7)	49.5 (10.3)
Tyrosine (tyr)	38–87	57.1 (15.4)	55.3 (14.8)	55.6 (10.1)	59.8 (14.1)
Asparagine (asn)	35–150	36.1 (8.5)	34.8 (10.1)	31.7 (7.7)	35.1 (7.9)
Aspartic acid (asp)	5–30	4.8 (1.4)	4.4 (1.3)	5.4 (1.9)	4.6 (1.5)
Citrulline (cit)	10–50	24.8 (5.4)	24.1 (6.4)	25.5 (4.8)	27.3 (7.3)
Glutamic acid (glu)	45–150	52.6 (27.0)	51.6 (34.7)	74.8 (54.9)	56.3 (28.1)
Glutamine (gln)	550–1050	497.9 (103.5)	535.1 (234.1)	455.6 (77.2)	529.5 (222.8)
Ornithin (orn)	30–100	42.5 (13.6)	41.3 (16.3)	38.7 (12.0)	37.6 (11.6)
*trp/CAAx100		10.3 (1.9)	9.9 (1.4)	10.6 (1.7)	10.3 (1.5)

M = mean, *SD* = standard deviation

* CAA: sum from plasma concentrations of tyr, phe, val, ile, leu

Sociodemographic characters of patients are reported in Table 1.

■ Measurements and procedure

A randomized double-blind placebo-controlled design was used in the present study meaning that neither the patients nor the attending physicians or the nursing staff knew if the verum or the placebo was given. Fasting blood samples were taken the day after admission and after 4 weeks of therapy. Analyses of the plasma levels of 20 amino acids (aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, citrulline, tyrosine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, ornithin, lysine, taurine, arginine) were performed by HPCL (high pressure liquid chromatography) using a Hewlett Packard Series 1100 HPLC and a pre-column derivatisation [43]. Eight of these amino acids are essential (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan und valine), they cannot be produced by the human body and must be provided by nutrition. Data were standardized to internal and external norms. Internal norms were used for correcting potential losses during analysis and external norms for determination of calibration factors as not all amino acids show similar signal strength at similar concentration.

As controls of most previous studies are based on very small samples amino acid plasma concentrations of the patients were compared to normal ranges of amino acids published by Pangborn [40] previously. In this study healthy persons were characterized by nitrogen balance showing no defects of enzymes necessary for amino acid metabolism. This normal range was used by Bralley [8] in a similar study (Table 2) recently. Our experiences have shown that also patients being within the low 20% of normal range frequently report different disturbances of health, therefore we included these values for group comparison.

Severity of depression was measured by external rating using the “Hamilton Depression Scale” (HAM-D; [21]) being the most

widely used observer-rating scale used for the evaluation of drug trials in depression [35], and by a self-report using the “Beck Depression Inventory” (BDI; [3]) on the day of admission and after 4 weeks of treatment. According to DSM IV one of the characteristics of a major depression episode is suicidal behaviour being an additional risk of this affective disorder. Therefore we additionally recorded the value at the scale “auto-aggression” of the “Fragebogen zur Erfassung der Aggressivitätsfaktoren” (FAF; [22]) being a reliable indicator for suicidal behaviour [23]. The benefit/risk ratio of the medicinal treatment was estimated by the “Clinical Global Impression-Test” (CGI; [38]). Patients were treated by 30–60 mg/d Remeron® (mean dosage 34 mg \pm 12.3) depending on individual requirement and either an amino acid preparation or placebo. Subjects of the experimental group were administered a free form amino acid mixture formulated according to measured plasma levels. This consisted of a base formulation containing the Recommended Daily Allowance (RDA) doses of 8 essential and 2 semi-essential amino acids (arginine and histidine) in pharmaceutical grade and free form. Additional amounts of specific amino acids were added to this mixture if the amino acid was below an optimized reference range as described by Bralley [8] previously.

In addition, the amino acid preparation contained the vitamins β -carotene, C, E, B1, B2, B6, B12, folic acid, pantothenic acid, nicotinamide, biotine and additionally zinc, magnesium and selenium. These vitamins and trace elements were dosed according to their RDAs and are obligatory as co-factors for the metabolism of the amino acids. The amino acids used were of herbal origin predominantly. The mixture was prepared and administered to the patients 5 \pm 2 days after the aminogram has been figured. Patients of the experimental group got a dose of 5 g of the amino acid mixture three times/day 15 min before the principal meals as a water soluble powder. Patients of the control got the same portion of a placebo mixture, which was comparable to the verum as to taste and appearance.

After 4 weeks of therapy amino acid plasma concentrations and psychometric measures were analysed once more.

Table 3 Mean values of the psychometric tests for the experimental and the control group at the term of admission (T1) and after a 4-week therapy (T2)

	T1		T2	
	Amino acids <i>n</i> = 20	Placebo <i>n</i> = 20	Amino acids <i>n</i> = 20	Placebo <i>n</i> = 20
BDI <i>M</i> (SD)	26.9 (10.2)	21.7 (11.4)	9.9 (9.2)	12.7 (11.2)
HAM-D <i>M</i> (SD)	26.7 (5.9)	20.7 (3.6)	7.7 (5.0)	12.2 (8.7)
Auto-aggressions	6.6 (1.5)	5.4 (2.1)	5.6 (2.0)	5.0 (2.1)
CGI	5.4 (0.8)	5.1 (0.8)	3.1 (1.1)	3.4 (0.8)

M = mean, SD = standard deviation

* Age- and gender-related norm values (stanines) [22]

■ Data analysis

For comparison of groups, two-factor between subject ANOVAs, mixed factorial ANOVAs, independent means *t*-tests, Wilcoxon signed-rank tests, McNemar tests and Friedman tests were calculated, and chi-square tests for testing frequency distributions. Alpha level significance was set at 0.05 for all statistical tests. For correction of multiple testing a bonferroni correction was done.

Results

Frequency distributions of the demographic parameters did not differ between groups (Table 1). On admission patients of the experimental group showed higher values of depression (HAM-D: $t = -3.87$, $p = 0.000$) than patients of the placebo group (Table 3). Patients with suicidal behaviour (ideations or earlier suicide attempts) scored higher for auto-aggression (7.1 ± 1.2 vs. 5.1 ± 1.8 , $t = -4.03$, $p = 0.000$).

The average plasma levels of the 20 amino acids and proportion of patients with levels in the range of the lower 20% and below the normal range did not differ between groups at the term of admission (Tables 2, 4). Average dosage of Remeron® (Table 1) did not differ between groups ($p < 0.10$). Controlling for the dosage of Remeron® we could find a significant effect of therapy as a decrease of scores on the HAM-D-scale ($F = 11.39$, $p = 0.002$) and a significant interaction between therapy and group allocation ($F = 18.28$, $p = 0.000$). After therapy the patients of the experimental group showed lower depression scores than those in the placebo group (Table 3). The mean difference between depression values before and after therapy (experimental group: 19.0, placebo group: 8.6) was higher in the amino acid group than in the placebo group ($t = -4.31$, $p = 0.000$) indicating a greater therapy effect in patients of the experimental group. Proportion of responders (reduction in the HAM-D Rating Scale >50%) differed between groups (experimental group: 66.7%, placebo group: 33.3%; $\chi^2 = 9.23$, $p = 0.002$) but proportion of remitters (score on HAM-D-scale after therapy <7) was not different (experimental group: 64.7%, placebo group: 35.3%; $\chi^2 = 2.56$, $p = 0.110$). A significant therapy \times group interaction ($F = 6.14$, $p = 0.018$) could also be shown for the self-reported depression on the BDI

(Table 3). No dosage dependent effect of Remeron® could be observed.

Patients after therapy also showed lower scores for auto-aggression ($F = 6.33$, $p = 0.016$) but there was no difference between groups. Patients with suicidal behaviour showed higher values of auto-aggression than those without suicidal behaviour even after therapy (6.4 ± 1.2 vs. 4.2 ± 2.2 , $t = -4.00$, $p = 0.000$). Values on the CGI were slightly lower after treatment than on admission ($F = 5.03$, $p = 0.031$) suggesting an improvement of the disease but there was no difference between groups.

Mean baseline plasma levels of aspartic acid and glutamine were below the normal range for both groups, additionally valine within the experimental group and asparagine within the placebo group (Table 2). Histidine, valine and glutamic acid were below the normal range especially frequent for patients of the experimental group, serine, asparagine and aspartic acid and glutamine for patients of the placebo group and glutamine for patients of both groups but the two groups did not differ respective to frequencies of low-level amino acids (Table 4). After therapy mean concentration of asparagine in the experimental group and of aspartic acid in the placebo group were below normal range, besides plasma level of serine for both groups (Table 2). Glycine and asparagine were below the normal range for most patients of the experimental group, aspartic acid for patients of the placebo group, and serine and glutamine for patients of both groups (Table 4). Additionally, a high proportion of patients of both groups showed levels of most amino acids being in the lower 20% of the normal range at both measuring times. Also after therapy the two groups did not differ respective to frequencies of low-level amino acids, and no changes could be demonstrated between baseline and measurement after therapy. Only for taurine more patients of the experimental group had levels within the norm range ($\chi^2 = 12.00$, $p = 0.001$) (Table 4). Plasma levels of serine were lower after therapy than before ($F = 9.04$, $p = 0.005$). There was an interaction between therapy and group allocation for taurine ($F = 9.13$, $p < 0.05$). After therapy plasma levels of taurine were higher in patients of the experimental group than in these of the placebo group. For all the other amino acids no changes and no differences

Table 4 Proportion of patients with deficient amino acid plasma concentrations at the term of admission and after a 4-week therapy

	T1				T2			
	Amino acids		Placebo		Amino acids		Placebo	
	<i>n</i> = 20		<i>n</i> = 20		<i>n</i> = 20		<i>n</i> = 20	
	-2	-1	-2	-1	-2	-1	-2	-1
arg	5	25	0	40	0	25	0	30
his	50	45	20	60	35	55	30	50
ile	10	55	0	40	0	60	0	55
leu	15	60	10	60	20	55	10	70
lysin	0	15	5	10	0	5	0	15
met	5	15	0	15	0	5	5	15
phe	45	55	40	55	30	60	35	65
thr	35	15	15	60	30	45	20	45
trp	20	70	15	80	20	60	25	55
val	55	35	40	45	35	60	40	55
gly	25	25	40	20	55	25	45	35
ser	35	55	50	15	65	35	50	40
tau	10	75	15	70	0	50	15	80
tyr	10	15	5	30	0	20	5	15
asn	40	55	60	35	70	30	45	55
asp	40	55	60	40	35	65	60	35
cit	0	5	0	15	0	5	0	0
glu	50	15	45	40	45	10	45	20
gln	75	15	70	5	100	0	70	10
orn	15	45	25	45	12	60	25	60

-1: in the range of the lower 20% of the normal range

-2: below the normal range

depending on group allocation were found after therapy. Baseline values of “auto-aggression” showed a weak correlation to the levels of valine ($r = -0.29$, $p = 0.074$) and methionine ($r = -0.28$, $p = 0.079$). After therapy we only found significant correlations between psychic parameters and amino acid plasma concentrations within the placebo group: Values on the BDI were correlated to levels of isoleucine ($r = 0.65$, $p = 0.002$), leucine ($r = 0.70$, $p = 0.001$) and glutamine ($r = 0.60$, $p = 0.005$), values of HAM-D were slightly correlated to levels of leucine ($r = 0.45$, $p = 0.048$), and values on the CGI were correlated to levels of isoleucine ($r = 0.61$, $p = 0.004$) and leucine ($r = 0.70$, $p = 0.001$). No therapy or group effect could be shown for relation between tryptophan and the big neutral amino acids (tyrosine, phenylalanine, valine, isoleucine and leucine).

Discussion

Therapy with the antidepressant Remeron® and additional application of amino acids or placebo reduced depression scores of the “Hamilton Depression Scale” and of the scale “auto-aggressions” and slightly decreased the values of the “Clinical Global Impression-test” which should give a global impression of patients’ health. Patients who had reported suicidal behaviour (ideations and/or attempted suicides) scored higher on the scale “auto-aggression” indicating that this therapy could not decrease suicidal

risk sufficiently. A therapeutic effect of the amino acids could be shown on the basis of both depression scales. Patients, which had received the amino acid mixture scored lower on these scales than patients of the placebo group despite starting with significant higher depression scores. This more unfavourable start data of patients of the experimental group was a consequence of the randomised classification of patients for the two groups. The proportion of variance defined by the interaction between therapy and group allocation was 33.1% (HAM-D). The difference between the depression scores of patients of the two groups after therapy was 4.5 points, which means a medium effect size of 0.63 [7]. The effect size for the self-rating scale (BDI) was 14.2% (2.8 points of difference) showing that patients themselves observed a smaller therapeutic effect than the attending psychiatrists. Patients of the experimental group also more frequently responded to the therapy showing a high response rate of 67%. As Remeron® is a very efficient antidepressant an additional effect of the amino acid application means a therapeutic benefit.

Analyses of amino acid plasma levels showed eight amino acids being most frequently deficient on admission as follows phenylalanine, aspartic acid, asparagine, tryptophan, histidine, valine, serine and glutamine whereas phenylalanine, histidine and valine are essential amino acids. Histidine and valine are indicators for an increased demand of amino acids in stress or during diseases since proteolysis exceeds protein synthesis under such unfavourable

conditions. As amino acids cannot be stored in the body a continuous supply by diet is necessary. If deficiencies occur during increased stress or diseases availability of amino acids is not sufficient for muscles, neurotransmitters in brain and hormone synthesis [9, 28]. To prevent such deficiencies in the course of the depressive episode the additional supply of amino acids is the basis of our therapeutic approach. Depressions are connected with a deficiency of the neurotransmitters serotonin and norepinephrine in particular. Phenylalanine, which was lowered in 90% of the patients at least serves as source for norepinephrine. Tryptophan being lowered in 90% of the patients, too, is the precursor for the neurotransmitter serotonin, which is not available in sufficient amounts in depressed subjects showing the typical symptoms like depressed mood and sleeplessness. Decreased levels of tryptophan also may result in increased aggressiveness [15]. This is important for therapy of depressed patients as high values of auto-aggression are an indicator for suicidal risk [23]. A comparison of the levels of tryptophan and the neutral amino acids with control groups of other studies [30, 31, 34] showed that levels of our patients were significantly lower but were in accordance with the values of the corresponding experimental groups (patients with a major depression respectively women before delivery). This also concerned the ratio of total tryptophan to neutral amino acids, confirming the results of Cowen et al. [11]. We could show a positive relation between values of depression and the plasma levels of leucine and isoleucine, two of these neutral amino acids. They are transported to the brain by the same transport system than tryptophan and therefore compete for the same carrier proteins [39]. Hence, this relation represents also a better indicator for availability of serotonin than the absolute plasma concentration of tryptophan [18].

Therapeutic effect of Remeron® and the optimized amino acid preparation or placebo were not related to a change of amino acid plasma levels or a decrease of proportion of patients with lowered plasma levels. Neither the antidepressant Remeron® nor the amino acid preparation led to verifiable increase of amino acid concentrations. This was contrary to our expectations but antidepressants may result in different effects on changes and displacements of the amino acid plasma concentrations during course of medication. Thus, Mauri et al. [33] reported that a therapy with fluvoxamine had no effect on tryptophan concentrations. Whereas Bralley et al. [8] found an improvement of symptoms of the “chronic fatigue syndrome” related to an increase of amino acid plasma concentrations after a 3 months application of an amino acid mixture.

In the present study amino acid application could only be performed over a period of 4 weeks because of the time limit of the stay at the hospital. But an

effect and a stabilization of the amino acid levels may be expected after 4–5 weeks at the earliest. Furthermore, it was reported previously that amino acid serum levels rise after amino acid supplementation but decline rapidly due to their metabolism [9].

Taurine was the only amino acid with an increase after therapy in patients of the experimental group. This may be due to the better resorption of taurine compared to the other amino acids. The serine levels were even lower for both groups after therapy than at baseline. A possible explanation is that serine is a very reactive amino acid with high concentrations in all cell membranes. The supply of amino acids could result in an increase of metabolism with an increased consumption of amino acids. As the additionally supplied amino acids will be reabsorbed and metabolised very rapidly a relative deficiency of serine can be observed [9]. As certain amino acids are starting substances of other amino acids their concentrations are not independent. Our data show movements of concentrations between certain amino acids between the two times of measurement. Therefore, in addition to a deficiency of amino acid plasma concentrations a loss of balance may be important for the development of a depressive episode. The importance of balance is also indicated by the “depletion studies” with tryptophan whereas amino acid mixtures without tryptophan resulted in a depressive mood [6, 16, 47]. Also the technical information of the manufacturer firm of “Kalma” with the agent L-tryptophan includes the passage to avoid nutrition poor of proteins when taking this drug for preventing an imbalance of amino acids.

We found no correlation between severity of depression and amino acid plasma concentrations before or after therapy. This is according to the results of Altamura et al. [2]. Whereas Moller et al. [36] showed positive correlations between levels of tryptophan, tyrosine and the neutral amino acids ratio and the values on the HAM-D after therapy with citalopram and maprotiline.

Our results show an improvement of the antidepressant pharmacological therapy by application of an amino acid mixture in addition to the antidepressant Remeron®. The intake of Remeron® resulted in an increase of appetite and weight for some patients with change of antidepressant and a dropout of study. The amino acid mixtures showed no side effects resulting in a better compliance and an increase in the benefit/risk ratio. A restriction of using amino acids concerns depressed patients treated with monoamine oxidase inhibitors, which may lead to an overload with tryptophan/serotonine.

Decreased concentrations of amino acids being the starting substances for neurotransmitters are only one reason for the development of a depression. Availability of neurotransmitters in the brain are also restricted by dysfunctions of their transfer between the nerve cells, e.g. due to low density of receptors in the

brain. Further influencing factors are the transfer of amino acids into the cell (cellular availability of amino acids) and the biosynthesis of final products from the precursors. Therefore, it is not clear how much of the amino acids attain to the brain with macrobiotic supply. Furthermore, individual optimum of amino acid concentrations within normal range is unexplained. Indeed, this is a problem concerning all reference ranges in medicine. Limitations of our results are also conditioned by the fact that there may be an interaction of effects between Remeron® and the amino acids. Although placebo-controlled trials with antidepressants are accepted to be essential [1] the effect of amino acids being expected was not strong enough allowing a comparison without using the antidepressant.

Further studies are necessary to find out whether for mild to moderate depressed patients a dosage reduction of antidepressant with add-on of amino acids or even a mono-therapy with amino acids may be an efficient antidepressant therapy.

References

- Adam D, Kasper S, Moller HJ, Singer EA (2005) Placebo-controlled trials in major depression are necessary and ethically justifiable: how to improve the communication between researchers and ethical committees. *Eur Arch Psychiatry Clin Neurosci* 255:258–260
- Altamura C, Maes M, Dai J, Meltzer HY (1995) Plasma concentrations of excitatory amino acids, serine, glycine, taurine and histidine in major depression. *Eur Neuropsychopharm* 5:71–75
- Beck AT, Ward CH, Medelson M, Mock F, Erbaugh F (1961) An inventory for measuring depression. *Arch Gen Psychiat* 4:561–571
- Beckman H, Athen D, Olteanu M, Zimmer R (1979) DL-Phenylalanine versus imipramine: a double-blind controlled study. *Arch Psychiatr Nervenkr* 227:49–58
- Beckman H, Strauss MA, Ludolph E (1977) DL-Phenylalanine in depressed patients: an open study. *J Neural Transm* 41:123–124
- Booij L, Van der Does W, Benkelfat C, Bremner JD, Cowen PJ, Fava M, Gillin C, Leyton M, Moore P, Smith KA, Van der Kloot WA (2002) Predictors of mood response to acute tryptophan depletion. A reanalysis. *Neuropsychopharmacol* 27:852–861
- Bortz J, Döring N (2002) *Forschungsmethoden und Evaluation für Human- und Sozialwissenschaftler*. Springer, Berlin
- Bralley JA, Lord RS (1994) Treatment of chronic fatigue syndrome with specific amino acid supplementation. *J Appl Nutrition* 46:74–78
- Braverman ER (2003) *The healing nutrients within*. Basic Health Publications, North Bergen
- Charney DS (1998) Monoamine dysfunction and the pathophysiology and treatment of depression. *J Clin Psychiat* 59:11–14
- Cowen PJ, Parry-Billings M, Newsholme EA (1989) Decreased plasma tryptophan levels in major depression. *J Affect Disord* 16:27–31
- De Boer T (1996) The pharmacologic profile of mirtazapine. *J Clin Psychiatry* 57:19–25
- Delgado PL, Miller HL, Salomon RM, Licinio J, Krystal JH, Moreno FA, Heninger GR, Charney DS (1999) Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. *Biol Psychiat* 46:212–220
- Demyttenaere K, Haddad P (2000) Compliance with antidepressant therapy and antidepressant discontinuation symptoms. *Acta Psychiatr Scand* 403:50–56
- Dougherty DM, Moeller FG, Bjork JM, Marsh DM (1999) Plasma L-tryptophan depletion and aggression. *Adv Exp Med Biol* 467:57–65
- Evans L, Golshan S, Kelsoe J, Rapaport M, Resovsky K, Sutton L, Gillin JC (2002) Effects of rapid tryptophan depletion on sleep electroencephalogram and mood in subjects with partially remitted depression on bupropion. *Neuropsychopharmacol* 27:1016–1026
- Farkas T, Dunner DL, Fieve RR (1976) L-Tryptophan in depression. *Biol Psychiat* 11:295–302
- Fernstrom JD, Wurtman RJ (1972) Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science* 178:414–416
- Goldberg I (1980) L-Tyrosine in depression. *Lancet* 16:364
- Gronier B, Azorin JM, Dassa D, Jeanningros R (1993) Evidence for a defective platelet L-tryptophan transport in depressed patients. *Int Clin Psychopharmacol* 8:87–93
- Hamilton M (1976) Hamilton depression scale. In: Guy W (ed) *ECDEU Assessment Manual for Psychopharmacology*. Rev. Ed. Rockville, Maryland, pp 179–192
- Hampel R, Selg H (1975) FAF. Fragebogen zur Erfassung von Aggressivitätsfaktoren. Hogrefe, Göttingen
- Huber H, Ille R, Zapotoczky HG (2000) Suicidal ideation, suicidal risk and aggressiveness: a comparative study of clinical and non-clinical subjects. *Eur Arch Psychiatry Clin Neurosci* 250:23
- Keller MB, Hirschfeld RM, Demyttenaere K, Baldwin DS (2002) Optimizing outcomes in depression: focus on antidepressant compliance. *Int Clin Psychopharmacol* 17(6):265–271
- Kessler RC, McGonagle KA, Zhao S, et al. (1994) Life time and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. *Arch Gen Psychiatry* 51:8–19
- Kishimoto H, Hama Y (1976) The level and diurnal rhythm of plasma tryptophan and tyrosine in manic-depressive patients. *Yokohama Med Bull* 27:89–97
- Klaassen T, Riedel WJ, Deutz NE, Van Someren A, Van Praag HM (1999) Specificity of the tryptophan depletion method. *Psychopharmacol Berlin* 141:279–286
- Laidlaw SA, Berg RL, Kopple JD, Naito H, Walker WG, Walser M (1994) Patterns of fasting plasma amino acid levels in chronic renal insufficiency: results from the feasibility phase of the modification of diet in renal disease study. *Am J Kidney Dis* 23:504–513
- Leviton RD, Shen JH, Jindal R, Driver HS, Kennedy SH, Shapiro CM (2000) Preliminary randomized double-blind placebo-controlled trial of tryptophan combined with fluoxetine to treat major depressive disorder: antidepressant and hypnotic effects. *J Psychiatry Neurosci* 25:337–346
- Lucca A, Lucini V, Piatti E, Ronchi P, Smeraldi E (1992) Plasma tryptophan levels and plasma tryptophan/neutral amino acids ratio in patients with mood disorder, patients with obsessive-compulsive disorder, and normal subjects. *Psychiat Res* 44:85–91
- Maes M, Ombelet W, Verkerk R, Bosmans E, Scharpé S (2001) Effects of pregnancy and delivery on the availability of plasma tryptophan to the brain: relationship to delivery-induced immune activation and early post-partum anxiety and depression. *Psychological Medicine* 31:847–858
- Mathis P, Schmitt L, Benatia M, Granier F, Ghisolfi J, Moron P (1988) Plasma amino acid disturbances and depression. *Encephale* 14:77–82
- Mauri MC, Ferrara A, Boscato L, Bravin S, Zamberlan F, Alecci M, Invernizzi G (1998): Plasma and platelet amino acid concentrations in patients affected by major depression and under fluvoxamine treatment. *Neuropsychobiology* 37:124–129

34. Molina JA, Jimenez FJ, Vargas C, Gomez P, De Bustos F, Orti-Pareja M, Tallon-Barranco A, Benito-Leon J, Arenas J, Enriquez-de-Salamanca R (1998) Cerebrospinal fluid levels of non-neurotransmitter amino acids in patients with Alzheimer's disease. *J Neural Transm* 105:279–286
35. Moller HJ (2001) Methodological aspects in the assessment of severity of depression by the Hamilton Depression Scale. *Eur Arch Psychiatry Clin Neurosci* 251:13–20
36. Moller SE, De Beurs P, Timmerman L, Tan BK, Leijnse-Ybema HJ, Stuart MH, 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
37. Möller HJ, Laux G, Kapfhammer HP (2003) *Psychiatrie und Psychotherapie*. Springer, Berlin
38. National Institute of Mental Health (1976) 12-CGI. Clinical global impressions. In: Guy W (ed) *ECDEU Assessment Manual for Psychopharmacology*. Rockville, Maryland, pp 217–222
39. Oldendorf WH, Szabo J (1976) Amino acid assignment to one of three blood–brain barrier amino acid carriers. *Am J Phys* 230:94–98
40. Pangborn JB (1986) Nutritionally correct amino acid ranges: urine and plasma. Technical Memorandum 1, Biostatistics
41. Sabelli HC, Fawcett J, Gusovsky F, Javaid JL, Wynn P, Edwards J, Jeffries H, Kravitz H (1986) Clinical studies on the phenylethylamine hypothesis of affective disorder: urine and blood phenylacetic acid and phenylalanine dietary supplements. *J Clin Psychiat* 47:66–70
42. Saß H, Wittchen HU, Zaudig M (1996) *Diagnostisches Manual psychischer Störungen DSM-IV*. Hogrefe, Göttingen
43. Schuster R (1988) Determination of amino acids in biological pharmaceutical plant and food samples by automatic pre-column derivatisation and HPLC. *J Chromatogr* 431:271–284
44. Shaw DM, Tidmarsh SF, Johnson AL, Michalakeas AC, Riley GJ, Blazek R, Francis AF (1978) Multicompartmental analysis of amino acids: II. Tryptophan in affective disorder. *Psychol Med* 8:487–494
45. Shaw K, Turner J, Del Mar C (2002) Are tryptophan and 5-hydroxytryptophan effective treatments for depression? A meta-analysis. *Aust NZ J Psychiat* 36:488–491
46. Shaw K, Turner J, Del Mar C (2002) Tryptophan and 5-hydroxytryptophan for depression. *Cochrane Database Syst Rev* 2002:CD003198
47. Smith KA, Fairburn CG, Cowen PJ (1997) Relapse of depression after rapid depletion of tryptophan. *Lancet* 349:915–919
48. Spona J (1998) Substituieren—aber individuell. *J Geriatr Gerontol* 4:18–19
49. Stieler W, Stadler R (1991) Eosinophilie-Myalgie-Syndrom nach Einnahme von L-Tryptophan. *Z Hautkr* 66:808–811
50. Tachiki KH, Hendrie HC, Kellams J, Aprison MH (1977) A rapid column chromatographic procedure for the routine measurement of taurine in plasma of normals and depressed patients. *Clin Chim Acta* 75:455–465
51. Young SN (1993) The use of diet and dietary components in the study of factors controlling affect in humans: a review. *J Psychiatr Neurosci* 18:235–244
52. Young SN, Pihl RO, Ervin FR (1988) The effect of altered tryptophan levels on mood and behaviour in normal human males. *Clin Neuropharmacol* 11:207–215