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Plasma amino acids concentration in amyotrophic lateral sclerosis patients

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Summary. Previous investigations showed an impairment of amino acids (AA) metabolism in amyotrophic lateral sclerosis (ALS). It was hypothesized that excitatory AA may play an important role in the etiopathogenesis of this disease. The aim of the study was to determine plasma AA concentrations in ALS patients, and to examine the relationship between AA and the clinical state of ALS patients, the type of ALS onset and the duration of the disease. The study involved 20 ALS patients and 30 control group people. The AA analysis was performed by ion – exchange chromatography on an automatic AA analyser. The results showed significantly decreased concentrations of valine, isoleucine, leucine, tyrosine and aspartate in the plasma of the whole group of ALS patients compared to the control group, and a significantly decreased concentration of arginine in the patients with a long duration of ALS compared to the patients with a short duration. The clinical state of ALS patients significantly influenced only plasma alanine concentration. Other plasma AA concentrations were not significantly associated with clinical parameters of the disease. Our study confirms that metabolic abnormalities concerning AA exist in ALS patients. However, the normal plasma glutamate concentration observed in this study in the whole group of ALS patients compared to the controls does not exclude that this excitatory AA may play a role in neurodegeneration in ALS.

Keywords: Amyotrophic lateral sclerosis – Neurodegeneration – Plasma amino acids

Introduction

Amyotrophic lateral sclerosis (ALS) is the common variant of motor neurone disease affecting adults that usually strikes from mid to late life. The aetiology of this disease is poorly understood, but it is suggested that glutamatergic excitotoxicity is an important mechanism of neurodegeneration in ALS (Blin et al., 1991; Cluskey and Ramsden, 2001). There is evidence for abnormal amino acids (AA) metabolism in this disease. Previous studies demonstrated abnormalities in glutamate enzymes, tissue glutamate content, transporter proteins, and postsynaptic receptors (Rothstein, 1995), which may lead to glutamate excitotoxicity and degeneration of motoneurons.

The data found in the literature concerning not only plasma excitatory AA concentrations but also others plasma AA concentrations in the ALS patients is still controversial. It seems that abnormalities in AA metabolism may be the result of malnutrition observed in those patients. Therefore, we measured plasma AA concentrations in the ALS patients and examined if there is a relationship between AA concentrations and clinical parameters such as the clinical state of patients, the type of ALS onset, or the duration of the disease.

Material and methods

The study involved 20 ALS patients and 30 control group people. The ALS patients were diagnosed according to the El Escorial criteria of ALS (Brooks, 1994). The patients were divided into the groups depending on their clinical state; mild, moderate, severe and terminal (Riviere et al., 1998):

1. Mild – Mild deficit in only one of the three regions (speech, arm, leg); functionally independent in speech, upper extremity activities of daily living, and ambulation
2. Moderate – Mild deficit in all the three regions, or moderate to severe deficit in one region while the other two regions are normal or mildly affected
3. Severe – Patient needs assistance in two or three regions; speech is dysarthric and/or patient needs assistance in walking and/or patient needs assistance with upper extremity activities of daily living
4. Terminal – Non-functional use of at least two regions and moderate or non-functional use of the third region

The whole group of ALS patients was also divided into the groups of patients with a bulbar and a limb onset of the disease.

The mean duration of the disease was 18 months (3–48 months). The ALS patients were also divided into the groups according to the duration of ALS; one group of patients with a short (up to 12 months), and the other one with a long (over 12 months) duration of the disease. The duration of ALS was defined as the interval (in months) between the first signs of the disease and the hospitalization, during which blood was obtained for investigation.

The control group consisted of patients with lumbosacral disc disease. The disease was confirmed by computed tomography. The control and ALS group patients with other diseases known to affect the concentration of AA were excluded from this study. The exclusion was based on history, clinical examination and additional tests (biochemical test of blood, urine analysis, chest X-ray). The ALS patients had dysphagia and weight loss. The nutrition status was worse in the ALS patients than those in the control group. The characteristic of patients is presented in Table 1.

The study was approved by the Ethics Committee of Medical University in Lublin. Each ALS patient and control subject gave informed consent for entering the study.

The ALS and control group patients were fasting before the collection of their blood samples. The blood specimens were collected in heparinized tubes and centrifuged rapidly (within half an hour) before being stored at −20°C. The supernatant plasma was removed with a Pasteur pipette. Before the measurements 1 ml of each plasma sample was treated with 1 ml of 6% sulfosalicylic acid and centrifuged at a high speed. AA analysis was performed by the automated ion – exchange chromatography with five lithium – based buffers (Moore et al., 1958) on an Ingos AA 400 amino acid analyser (Ingos, Czech Republic).

For statistical analysis the Mann – Whitney U test, the ANOVA Kruskal – Wallis test, and Spearman rank correlation were used. The values were expressed in μmol/cm^3. p values ≤ 0.05 were considered significant.

Results

Our results show that concentrations of valine, isoleucine, leucine, tyrosine and aspartate were significantly decreased in the plasma of the whole group of ALS patients compared to the control group patients ($p \leq 0.05$). There were no significant differences in other plasma AA concentrations between the whole group of ALS patients and the control group patients ($p > 0.05$) (Table 2).

The plasma arginine concentration was significantly decreased in patients with a long duration compared to the patients with a short duration of ALS ($p = 0.043$). The difference in other plasma AA concentrations between the groups of ALS patients with a long and a short duration of the disease was not significant ($p > 0.05$).

The clinical state of ALS patients significantly influenced only plasma alanine concentration (p =

Table 2. Plasma AA concentrations [μmol/cm^3], and a comparative analysis between the group of ALS patients and control group

AA	Controls	ALS (total)	ALS vs Controls
valine	0.283 ± 0.08	0.237 ± 0.04	p = 0.018*
methionine	0.023 ± 0.00	0.019 ± 0.00	p = 0.08
glutamine	0.460 ± 0.13	0.526 ± 0.16	p = 0.12
isoleucine	0.091 ± 0.03	0.064 ± 0.02	p = 0.003*
leucine	0.175 ± 0.06	0.137 ± 0.03	p = 0.012*
phenylalanine	0.075 ± 0.02	0.074 ± 0.02	p = 0.95
tyrosine	0.080 ± 0.02	0.067 ± 0.02	p = 0.017*
aspartate	0.069 ± 0.01	0.061 ± 0.01	p = 0.05*
alanine	0.479 ± 0.09	0.457 ± 0.14	p = 0.50
taurine	0.099 ± 0.04	0.104 ± 0.04	p = 0.46
serine	0.161 ± 0.02	0.170 ± 0.03	p = 0.41
glutamate	0.199 ± 0.11	0.185 ± 0.12	p = 0.56
glycine	0.278 ± 0.05	0.297 ± 0.10	p = 0.89
lysine	0.243 ± 0.05	0.220 ± 0.04	p = 0.13
histidine	0.103 ± 0.01	0.097 ± 0.02	p = 0.20
citrulline	0.022 ± 0.02	0.029 ± 0.01	p = 0.16
arginine	0.154 ± 0.04	0.155 ± 0.06	p = 0.95
threonine	0.166 ± 0.04	0.148 ± 0.04	p = 0.09
ornithine + NH3	0.151 ± 0.04	0.164 ± 0.07	p = 0.83
ethanolamine	0.071 ± 0.01	0.071 ± 0.01	p = 0.96

The values are expressed as mean ± SD
* statistically significant, at least $p \leq 0.05$, Mann-Whitney U test

Table 1. The characteristic of patients

Group	Number of patients	Age (years)	Male/female
Control	30	57.84 ± 11.2	15/15
ALS – total	20	57.21 ± 12.5	11/9
ALS – short duration	11	58.27 ± 10.33	5/6
ALS – long duration	9	57.66 ± 11.60	6/3
ALS – bulbar onset	8	55.75 ± 10.74	2/6
ALS – limb onset	12	59.50 ± 11.16	9/3
ALS – mild clinical state	4	54.00 ± 12.78	4/0
ALS – moderate clinical state	8	57.75 ± 9.11	4/4
ALS – severe clinical state	5	57.80 ± 15.45	1/4
ALS – terminal clinical state	3	64.33 ± 4.61	2/1

Age of patients is expressed as mean ± SD

0.026). The highest plasma alanine concentration was found in the ALS patients with a terminal clinical state. However, there was no relationship between the clinical state of ALS patients and other plasma AA concentrations ($p > 0.05$).

There was also no difference in plasma AA concentrations between the groups of ALS patients with a bulbar and a limb onset of the disease ($p > 0.05$).

There was no significant correlation between plasma AA concentrations and clinical parameters of ALS, such as the clinical state of patients and the duration of the disease ($p > 0.05$).

Discussion

Our results confirm previous observations that plasma AA abnormalities exist in ALS patients. The study has shown significantly decreased plasma concentrations of valine, isoleucine, leucine, tyrosine and aspartate in the whole group of ALS patients compared to the controls, and a significantly decreased plasma concentration of arginine in the patients with a long duration of ALS compared to the patients with a short duration. The clinical state of ALS patients significantly influenced only plasma alanine concentration. Moreover, plasma AA concentration did not depend on a bulbar or a limb onset of ALS.

Previous studies showed an increase in blood glutamate, aspartate and glycine concentrations in the ALS patients compared to the controls (Iwasaki 1992, Babu 1998). Plaitakis et al. (1993) observed significant increase in plasma concentration of glutamate in ALS patients compared to controls, but glutamate and aspartate concentrations were significantly reduced in the spinal cord of ALS tissue. The authors suggested the presence of a generalized defect in the metabolism of excitatory AA in the ALS resulting in their altered distribution between intracellular and extracellular pools, and disturbances of excitatory transmission mediated by glutamate receptors that may cause degeneration of motor neurons. Rothstein et al. (1990) also demonstrated a significantly increased CSF glutamate and aspartate concentrations in ALS patients compared to controls, but there was no relationship between AA concentration and the age of ALS patients, their clinical state, or the duration of the disease. Spreux–Varoquaux et al. (2002) showed an increase in CSF glutamate concentration in ALS patients, and its positive significant correlation with a spinal onset of ALS. The authors suggested that an elevation in CSF glutamate concentration may be dependent on the intensity of the cell insult in the spinal cord. Niebroj – Dobosz et al. (1999) observed that in severely progressing ALS cases serum glutamate and aspartate concentrations were increased. Patten et al. (1978) showed that the severity of ALS correlated directly with serum aspartate and CSF alanine concentrations, and inversely with glutamate, aspartate and O-phosphoserine concentrations in the CSF. In our study the clinical state of ALS patients significantly influenced only plasma alanine concentration. In contrast to previous investigations, Perry et al. (1990) observed that concentrations of AA such as glutamate, aspartate, and glycine were normal in the plasma and CSF of ALS patients. Meier et al. (1988) also demonstrated that AA concentrations were not changed in the CSF of ALS patients. Camu et al. (1993) reported a decrease in plasma concentrations of neutral AA such as alanine, isoleucine, leucine, methionine and tyrosine, which was particulary striking in the bulbar type. The plasma glutamate concentration in the ALS patients did not differ from the control group patients, but it was significantly increased in the patients with a spinal onset, and significantly decreased in the patients with a bulbar onset of the disease. There was no significant correlation between AA concentrations and clinical parameters of the disease. Our results are similar to those demonstrated by Camu, but in our study there was no difference in the plasma AA concentrations depending on the type of ALS onset.

Progressively weakening muscles and dysphagia lead to weight loss and malnutrition, which could also be observed in our ALS patients. Previous studies showed that malnutrition is present in 16–50% of ALS patients, and is an independent prognostic factor for worsened survival (Silani et al., 1998, Desport et al., 2000).

Plasma AA concentrations are dependent on the protein content of the diet. Thus, malnutrition in the ALS patients may cause a decrease in their concentration. Similar to the changes in the concentration of the various AA in the body amino acid pool, the branched – chain AA (leucine, isoleucine and valine) profile is also known to be altered with the altered nutrition. It is known that protein deprivation leads to branched – chain AA pool size decrease (Lal and Chugh, 1995). This observation could help to explain the decreased plasma leucine, isoleucine and valine concentrations in the ALS patients demonstrated

in our study. However, there were no significant differences in the branched – chain and other AA concentrations between the group of ALS patients with a bulbar onset, suffering from early feeding deficit caused by difficulties in swallowing, and the group of ALS patients with a limb onset, in which swallowing is less affected in the early stage of the disease. These results suggest that malnutrition in both a bulbar and limb onset of ALS may be present and can support previous observations that the degree of malnutrition is not dependent on the forms of ALS onset (Desport et al., 1999). There is evidence that the branched – chain AA are the important regulators in the synthesis of body proteins. Moreover, it has been shown that these AA may also play a role in the normalization of the plasma AA profile. It has been shown that leucine and possibly other branched – chain AA are a significant source of energy for the skeletal muscle. The leucine inhibits protein degradation and enhances protein synthesis in muscle cells. It has also been suggested that leucine induces activity and synthesis of proteins involved in messenger RNA translocation to upregulate protein synthesis in the skeletal muscle (Holecek, 2002; Freund and Hanani, 2002). Thus, decreased plasma branched – chain AA concentrations observed in this study may influence energy status and protein synthesis in the skeletal muscle of ALS patients.

Our results showed that the degree of malnutrition, which may correlate with advance of ALS, does not significantly influence plasma AA concentrations, because only arginine concentration was significantly decreased in the patients with a long duration compared to the patients with a short duration of the disease. Previous studies conducted on the animal model showed a decreased extracellular arginine concentration when feeding animals with arginine free food. It was found that the elimination of arginine from the diet resulted in about 30% decrease in plasma arginine (Kean, 1967; Dillon et al., 2002). This result could explain a significantly decreased plasma arginine, observed in our study, in the ALS patients with a long duration of ALS, in which malnutrition occured more frequently, compared to the ALS patients with a short duration of the disease. On the other hand, an inverse relationship between nutritive value and argininase activity was observed. Nitric oxide (NO), attributed to participate in neurodegeneration in ALS, is synthesized from extracellular arginine (Liu et al., 2002). It was speculated that an increase in argininase activity may have a neuroprotective effect through inhibiting NO production (Mori and Gotoh, 2000).

Previous studies suggested that glutamate excitotoxicity may play an important role in neurodegeneration in ALS. The attention is paid to glycine because it enhances glutamate – mediated neurotransmission via influence NMDA receptors (Mayer, 1989; Plaitakis, 1991). Puka – Sundvall et al. (1995) showed that there is a synergistic effect between cysteine and glutamate. It is also suggested that an increase in extracellular glutamate concentration, observed in previous studies, may lead to inhibition intracellular transport of cysteine that causes an increase in cell vulnerability to free radical damage (Meister and Anderson, 1983). It seems that such mechanism of cell oxidative damage might play a role in motoneurons degeneration in ALS. However, our study does not confirm this hypothesis because the glutamate, glycine and cysteine concentrations were not changed in the plasma of ALS patients compared to the controls.

The study conducted on the animal model of ALS showed a progressive decrease in the immunoreactivity of the glial glutamate transporter GLT-1 in the ventral horn of lumbar spinal cord of SOD1 transgenic mice, but the glutamate concentration in the CSF of these mice was not altered. Based on this observation Bendotti et al. (2001) concluded that the loss of GLT-1 selectively occurs in the areas affected by neurodegeneration, and it is not associated with a CSF glutamate concentration increase. This result suggests that affecting tissue glutamate metabolism in ALS does not have to change CSF or plasma glutamate concentration. Thus, the normal plasma glutamate concentration observed in our study in the whole group of ALS patients compared to the controls, does not exclude that this excitatory AA may participate in neurodegeneration in ALS.

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