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Effect of Riluzole on serum amino acids in patients with amyotrophic lateral sclerosis

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Objectives – There is evidence that an imbalance between glutamatergic and inhibitory neurotransmission may contribute to selective neurodegeneration in amyotrophic lateral sclerosis (ALS). The efficacy of Riluzole in prolonging the survival of patients with ALS has been demonstrated in two large controlled trials. It is believed that Riluzole is a glutamate antagonist, but the exact mode of its action is not known. Data on the effects of Riluzole treatment on excitotoxic amino acid levels in serum are not available. *Material and methods* – We prospectively studied 17 patients with ALS (diagnosed according to the El Escorial criteria), who received long-term treatment with Riluzole (100 mg/day). The subjects were evaluated at baseline (before treatment) and after 6, 12 and 18 months on drug. Assessments included the functional status of the patients and serum levels of amino acids. Analysis of the serum amino acids was performed using high performance liquid chromatography techniques at baseline, and after 6, 12 and 18 months of the treatment. *Results* – At baseline, glutamate, GABA and total amino acid concentration in serum of the ALS patients, mainly in those with severe course of the disease, were increased. During the first 6 months of Riluzole treatment there was a significant decrease of glutamate and total amino acids, afterwards the values returned to the initial high values, or even an 'overshooting' in their levels appeared. We did not observe a similar effect of Riluzole on glutamate and other amino acids in patients with less advanced ALS. *Conclusions* – It is suggested that the positive clinical effect of Riluzole in ALS patients may be related, at least partly, to its influence on amino acid metabolism in neural tissues.

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Introduction

One of the hypotheses of amyotrophic lateral sclerosis (ALS) indicates that glutamate accumulates at neuronal synapses to toxic concentrations and causes neurons to die (1, 2). Although this hypothesis is not commonly accepted, there were several trials with drugs which were able to modulate the glutamatergic system (3–6). The results of these trials are divergent. The opinion, however, is that antiglutamate drugs are able to slow down the progression of ALS, and extend the time to tracheostomy or death (2, 3, 7–11).

The aim of this study was to determine whether the clinical effect could be correlated with concentrations of serum amino acids in ALS patients.

Materials and methods

Patients

Seventeen patients with ALS were examined. All were sporadic cases without a family history of ALS. The diagnosis was based on WFN El Escorial criteria (12). All except one were of spinal origin. At the time of examination all patients were ambulatory. The clinical status, including muscular

strength, respiratory function, subjective assessment of symptoms and global clinical impression of the patients, was monitored. Nine patients developed severe impairment in swallowing, speech, breathing, walking or upper limbs motor function within 2 years. In eight patients severe clinical symptoms appeared after at least 2 years. The characteristics of the patients are presented in Table 1. Riluzole treatment (100 mg/day) lasted 12 months in seven patients, and in 10 patients it was continued up to 18 months. All patients gave their consent prior to their inclusion in this study. Normal values of the amino acids in serum were determined in healthy volunteers (10 males, 10 females), aged 27–65 years.

Biochemical analysis

Blood specimens, taken in the morning after an overnight fast, were centrifuged after clotting at 3000 r.p.m. for 10 min. The samples were stored at –72°C until chromatography. Before chromatography the samples were mixed with acidified methanol and after 20 min at 4°C they were centrifuged for 10 min at 15,400 *g* and 4°C (13). Analyses of amino acids were performed by high performance liquid chromatography (HPLC) technique (14, 15). Reverse phase chromatography coupled with a switch to precolumn derivatization, employing the fluorescent *ortho*-phthaldialdehyde (16) reconstituted by β-mercaptoethanol, and a gradient system of 0.05 M phosphate buffer (pH 5.7) and methanol (13). A fluorescent detector with excitation at 330 nm and emission at 408 nm was used. The HPLC system consisted of a Solvent Delivery System model 2800 (Bio-Rad, Hercules, CA, USA), Mobile Phase Conditioner M-3222 (Bio-Rad), Fluorescence Detector Fluor 304 (Linear, Fremont, CA, USA), Hyundai Delux Scan 15 PC Computer, Value Chrom™ Chromatography Software and a reverse-phase column Bio-Sil C18 HL 90-5S (150 × 4.6 mm, particle size 5 μm, Bio-Rad). Standard amino acids (Sigma, St Louis, MO, USA) were run separately in duplicate in concentrations in a range between 25 and 550 pM per injection.

Table 1 Clinical characteristics of ALS patients

Age (years)	46 ± 10
Sex	12 M, 5 F
Site of onset	16 L, 1 B
Disease duration (months)	21 ± 13
Norris score (0–120 points)	82 ± 23
FVC (percentage of normal value)	93 ± 17
Course of the disease	9 S, 8 Mo

M, male; F, female; L, limb; B, bulbar; FVC, forced vital capacity; S, severe; Mo, moderate; mean ± SD.

Statistical analysis

The variances, comparison of means and significance analysis were performed by the Wilcoxon's and Student's *t*-tests. The possible correlations between the amino acid concentration in serum and the age of the patients, duration of the disease, clinical course of the disease (severe vs moderate), and the Norris scale were evaluated by calculating the Pearson's coefficient.

Results

At the beginning of the trial the majority of the 17 ALS patients had elevated levels of glutamate ($P < 0.02$), GABA ($P < 0.0001$) and total amino acids ($P < 0.002$) (Table 2). After 6 months of Riluzole administration, a significant decrease of glutamate ($P < 0.02$) and total amino acids ($P < 0.0002$) was observed. Both glutamate and total amino acids returned to the initially high concentrations, and even an 'overshooting' in their levels after 18 months of Riluzole administration was noted ($P < 0.02$) (Figs 1 and 2). When divided into subgroups (severe vs less advanced ALS), it appeared that the most pronounced changes in the amino acid content in serum at baseline were present in patients with severe rapidly progressing ALS (Table 2). In the moderate subgroup changes in serum amino acids were less pronounced or even not present. In the rapidly progressing ALS, after the first 6 months of

Table 2 Concentration of amino acids (μM/l) in the serum of ALS patients and controls at baseline (before treatment)

	n	Aspartate	Glutamate	GABA	Glycine	Total amino acids
ALS patients	17	22.4 ± 26.3	119.9 ± 125.2[b]	53.9 ± 54.4[f]	100.4 ± 67.4	1795.5 ± 484.7[d]
severe ALS	9	36.3 ± 30.2[a]	216.5 ± 94.8[d]	8.8 ± 5.3[c]	61.5 ± 62.2[b]	2027.1 ± 377.1[e]
moderate ALS	8	6.8 ± 3.8[b]	11.1 ± 5.1[f]	104.6 ± 34.3[f]	144.1 ± 42.9	1535.1 ± 477.6
Normal controls	20	9.8 ± 2.6	41.2 ± 12.3	1.5 ± 0.8	120.1 ± 18.8	1248.4 ± 189.7

n = number of individuals, values are means ± SD.
[a] $P < 0.03$, [b] $P < 0.02$, [c] $P < 0.003$, [d] $P < 0.002$, [d] $P < 0.0005$, [e] $P < 0.0002$, [f] $P < 0.0001$.

Riluzole treatment there was a significant decrease of glutamate ($P < 0.004$) and total amino acid concentrations ($P < 0.004$). After 12–18 months, however, glutamate and total amino acids returned to or even exceeded their initial elevated levels.

No correlation between the amino acids concentrations and the patients age, duration of the disease and the Norris score was found.

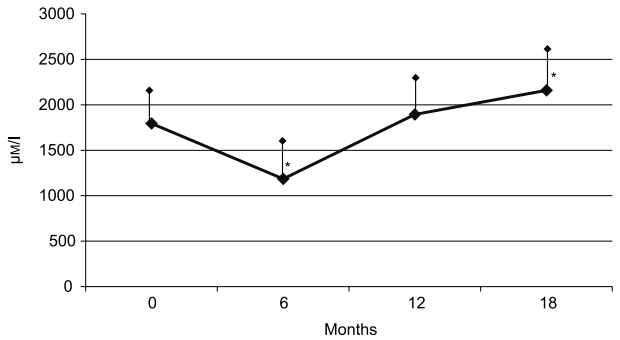

Figure 1. Mean concentration of amino acids (µM/l) in serum of ALS patients in course of Riluzole treatment. *Significant, when compared with baseline values.

Discussion

The excitatory amino acids, acting as neurotransmitters, represent the major substances for neuronal communication in brain and spinal cord. Glutamate and aspartate in the body fluids have been inversely correlated with duration and severity of ALS (17), the disability score (17, 18), and also the type of the disease, either of spinal or bulbar onset (19). Our previous data indicate on a possible involvement of glutamate and aspartate, especially in rapidly progressing ALS cases (20). No age, duration of the disease, and clinical impairment dependence is also reported (21–23).

Among drugs that are supposed to modulate the glutamatergic system, Riluzole (2-amino-6-trifluoromethoxy benzothiazole) has been proposed (24, 25). The mechanism(s) of its action, however, is not clear. It may be related to presynaptic inhibition of glutamate release in the central nervous system (24–26). Postsynaptically it interferes with the effect of excitatory amino acids by blocking the voltage-dependent sodium channels (3, 7), or G-protein dependent signal transduction processes

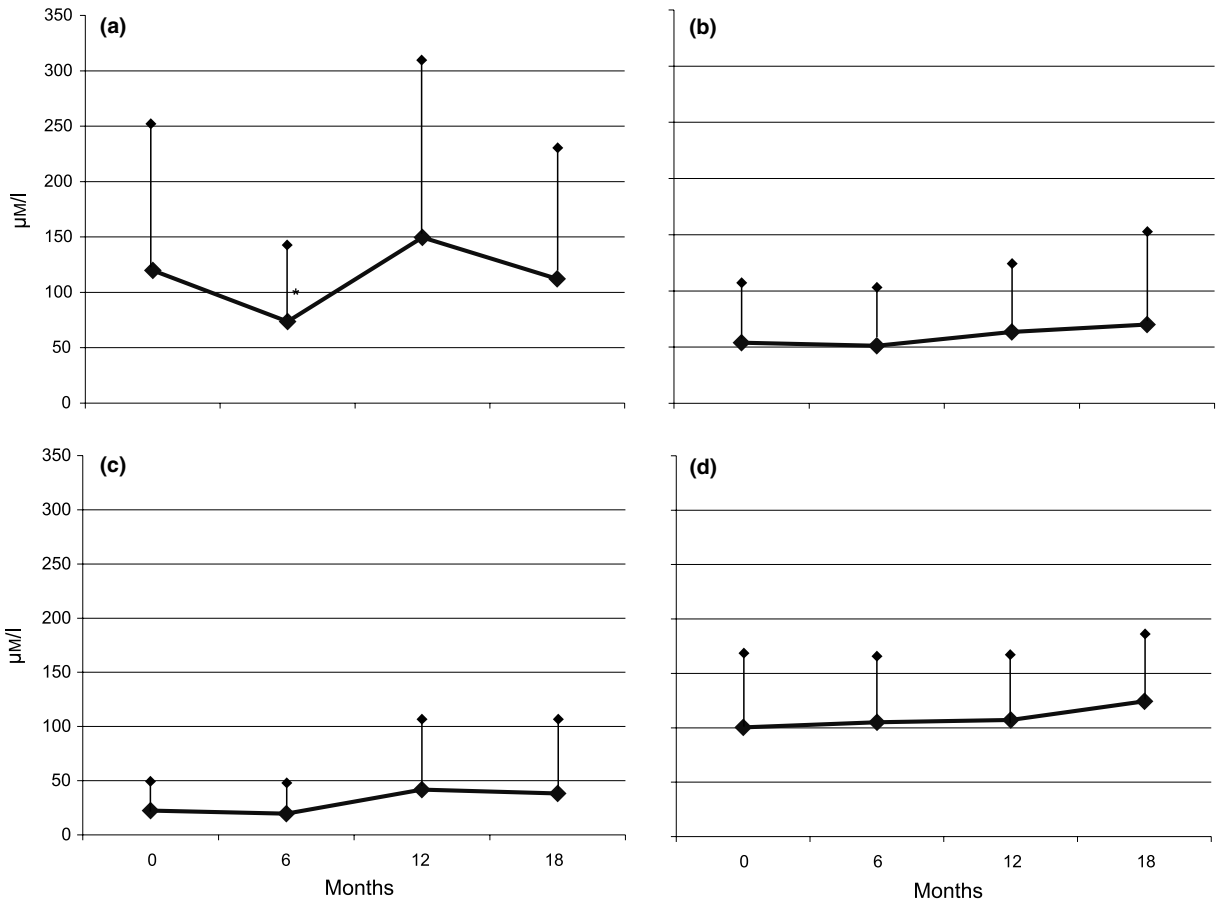

Figure 2. Mean concentration of amino acids (µM/l) in serum of ALS patients in course of Riluzole treatment: (a) glutamate, (b) GABA γ-amino-butyric acid, (c) aspartate, (d) glycine. *Significant, when compared with baseline values.

(27, 28) and preventing excess stimulation of *N*-methyl-D-aspartate receptors and massive calcium influx (29). An increase of high-affinity glutamate uptake in spinal cord synaptosomes (30), and protective effect on dopamine neurons (31) was also noted. *In vivo* Riluzole has neuroprotective, anticonvulsant and sedative properties (28). It acts also as an antioxidant and the antioxidant action of Riluzole could result in preserving glutamate transporters (32). *In vitro* Riluzole partially prevents the neuronal degeneration produced by CSF of ALS patients and protects cultured neurons from anoxic damage and toxic effects of inhibitors of glutamate uptake (28, 33).

In a controlled trial it appeared that Riluzole slowed down the progression of ALS and may improve survival and/or time to tracheostomy (3, 7–10, 34–36). The effect of Riluzole is time related, and clinically evident effect is present from 12 to 21 months of the treatment (7). It is also suggested that Riluzole lowers the rate of deterioration of muscle function (7), which is, however, not confirmed by a larger trial (34).

Our present data indicate that Riluzole induced transient changes in glutamate concentration in ALS cases, especially those with rapidly progressing disease (severe ALS). Several questions remain unanswered: (i) why the influence of Riluzole on amino acids level is time limited and appears during the first months of the treatment, (ii) why it appears mainly in patients with severe ALS, and (iii) why aspartate does not follow the changes of glutamate. There is no reasonable explanation for these questions as yet. One may only speculate that Riluzole acts either directly on the enzymes of glutamate metabolism, and/or indirectly on their activators/inhibitors. The observed effect of Riluzole on total amino acids concentration in serum of ALS patients, on the other hand, is probably the consequence of a transient positive effect of this drug on metabolism of tissues, including neural tissues.

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