Tissue plasminogen activator level in serum of patients with amyotrophic lateral sclerosis – a preliminary report

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Summary
Aim: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, in which excitotoxicity plays an important role. Tissue plasminogen activator (tPA) induces neurodegeneration mediated by excitotoxicity. The aim of the study was to measure levels of tPA in serum of patients with ALS.

Material and methods: The measurement of tPA was performed by the enzyme-linked immunosorbent method using commercial ELISA kit.

Results: Results showed that levels of serum tPA are significantly increased in the group of ALS patients of severe clinical state compared with those of control group, and compared with ALS patients of mild clinical state (p<0.05).

Conclusion: It cannot be excluded that an increase in tPA levels in serum of patients with ALS could influence neurodegeneration.

Key words: amyotrophic lateral sclerosis, excitotoxicity, microglia, neurodegeneration, serum, tissue plasminogen activator

Introduction
There is an evidence that excitotoxicity may play an important role in neurodegeneration in amyotrophic lateral sclerosis (ALS) [4]. Tissue plasminogen activator (tPA) is a serine protease which plays a significant role in the functioning of central nervous system. It is implicated in neurite outgrowth but also it is an important inducer of neurodegeneration mediated by excitotoxicity [9, 17]. The tPA is a modulator of glutamatergic neurotransmission. It has been shown to act as a key activator of neuronal mitogen – activated protein kinase pathways via the N-methyl-D-aspartate (NMDA) receptor [7]. The tPA can potentiate calcium signaling following activation of glutamate-binding NMDA receptor (NMDAR). It has been hypothesized that tPA may cleave the NR1 subunit of the NMDAR and potentiate NMDA-induced calcium influx [14]. In the adult central nervous system, tPA is expressed at the mRNA and protein levels in many types of neurons, in particular in thalamus, cortex of cerebellum, pontine nuclei, neocortex, limbic system, and medulla oblongata [18]. The tPA converts plasminogen into plasmin that leads to degeneration of extracellular matrix and activation of microglia [10, 20]. Additionally, activated microglia can induce secretion of...
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tPA which enhances damage of extracellular matrix resulting in neuronal death [13,16]. Previously, it was observed that microglial activation can play a significant role in induction of motor neuron injury in ALS [15].
The aim of the study was to measure levels of tPA in serum of patients with ALS and to investigate whether there is a relationship of this molecule with clinical parameters of the disease.

Material and methods
Twenty (12 male/ 8 female) ALS patients with an average age of 58 (42-70 years) were diagnosed according to the El Escorial criteria of ALS. The clinical state of patients was measured by using Amyotrophic Lateral Sclerosis Functional Rating Scale [ALSFRS]. According to this scale the ALS patients obtained from 4 to 32 points and they were divided into two groups: with mild clinical state (above 25 points) and with severe clinical state (up to 25 points). The patients were also divided into two groups according to type of ALS onset (limb-onset/ bulbar-onset) and they were also divided into two groups according to duration of ALS (short duration ≤ 12 months/ long duration > 12 months). The average duration of the disease was 16 months (4 months – 3 years).
The control group consisted of 15 (7 males/ 8 females) patients (matched age) with tension – type headache. The study was approved by the Ethics Committee of Medical University and performed in accordance with the ethical standards established in Helsinki. The characteristic of patients is presented in Table I.

Venous blood samples were collected into plastic tubes at the time of clinical assessment. Blood samples were obtained from ALS patients and controls and immediately centrifuged (within half an hour). The supernatant plasma was removed with a Pasteur pipette and stored at -20°C until analysis. All samples were obtained with an identical procedure.
The measurement of tPA was performed by the enzyme-linked immunosorbent method using commercial ELISA kit for human tPA (Bender MedSystems Diagnostics GmbH, Vienna, Austria) in accordance to manufacturer’s instructions. The Mann–Whitney U test was used to examine the differences between the groups. The correlation analysis was performed by using Spearman’s correlation coefficient. The values are expressed in pg/mL as median and range. P values < 0.05 were considered significant.

Results
Results showed that levels of serum tPA are significantly increased in the group of ALS patients of severe clinical state

Table I
The characteristic of patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
</tr>
<tr>
<td>ALS – total</td>
<td>20</td>
</tr>
<tr>
<td>ALS – bulbar-onset</td>
<td>6</td>
</tr>
<tr>
<td>ALS – limb-onset</td>
<td>14</td>
</tr>
<tr>
<td>ALS – short duration (≤12 months)</td>
<td>15</td>
</tr>
<tr>
<td>ALS – long duration (&gt;12 months)</td>
<td>5</td>
</tr>
<tr>
<td>ALS – mild clinical state</td>
<td>10</td>
</tr>
<tr>
<td>ALS – severe clinical state</td>
<td>10</td>
</tr>
</tbody>
</table>

N – number of patients

Table II
Serum tPA levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>serum tPA level [pg/mL] median (range)</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>688.0 (413.0 – 3407.0)</td>
<td>Control vs ALS total p=0.12</td>
</tr>
<tr>
<td>ALS – total</td>
<td>1018.5 (514.0– 3447.0)</td>
<td>Control vs bulbar onset p=0.28</td>
</tr>
<tr>
<td>ALS – bulbar-onset</td>
<td>2016.0 (514.0-3447.0)</td>
<td>Control vs limb onset p=0.12</td>
</tr>
<tr>
<td>ALS – limb-onset</td>
<td>1568.0 (612.0-3266.0)</td>
<td>ALS bulbar vs limb onset p=0.17</td>
</tr>
<tr>
<td>ALS – short duration</td>
<td>825.0 (514.0-3447.0)</td>
<td>Control vs short duration p=0.57</td>
</tr>
<tr>
<td>ALS – long duration</td>
<td>2350.0 (612.0-3250.0)</td>
<td>Control vs long duration p=0.06</td>
</tr>
<tr>
<td>ALS – mild clinical state</td>
<td>744.0 (514.0-1253.0)</td>
<td>ALS short vs long duration p=0.14</td>
</tr>
<tr>
<td>ALS – severe clinical state</td>
<td>2982.5 (714.0-3447.0)</td>
<td>Control vs mild state p=0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control vs severe state p=0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ALS mild vs severe clinical state p=0.001*</td>
</tr>
</tbody>
</table>

Data is expressed as median (range)
*p — statistically significant, at least p<0.05; Mann-Whitney U test
compared with the control group, and compared with ALS patients of mild clinical state (p<0.05). There were no significant differences in levels of serum tPA between total group of ALS patients and controls, and between groups of ALS patients divided according to type of ALS onset, or duration of the disease (p>0.05). The median values of serum tPA, and a comparative analysis between groups are presented in Table II.

The frequency of elevated tPA levels in the investigated groups of patients was as following: in patients with bulbar-onset of ALS – 67%, in patients with limb-onset of ALS – 86%, in patients with short duration of ALS – 80%, in patients with long duration of ALS – 80%, in patients with mild clinical state of ALS – 60%, and in patients with severe clinical state of ALS – 100%.

There was a significant positive correlation between serum tPA levels and severity of clinical state of ALS patients (p=0.023).

Discussion

The study showed increased serum tPA levels in ALS patients of severe clinical state compared with controls, and compared with those of mild clinical state. This suggests a significant role of this protease in neurodegeneration in ALS. It was shown that Purkinje neuron degeneration in nervous mutant mice is mediated by a metabolic pathway involving excess tPA [6]. The tPA mediates neurotoxic effects on central nervous system cells as well alters blood-brain barrier permeability which induces neurodegeneration [11]. Villarán et al. [22] observed that intranigral injection of tPA induced blood-brain barrier disruption, inflammatory process and degeneration of the dopaminergic system of the rat. The plasminogen activator system can modulate inflammatory and degenerative events in the central nervous system through the effects of tPA on fibrinolysis and cell adhesion/migration [2].

It was revealed that the activity and expression of tPA in spinal neurons are increased after spinal cord injury in rats [21]. Kozai et al. [5] found a significant induction of tPA in activated astrocytes following L4/5 root injury. Experimental investigation showed that inactive tPA influences activation of microglia, however proteolytically – competent protein can induce neurodegeneration [12]. It was also observed that microglial tPA triggers neuronal apoptosis in vitro [3]. Medina et al. [8] showed that tPA influences a catalytic-independent activation of the extracellular regulated kinase pathway which is mediated by NMDAR, G proteins and protein kinase C, resulting in affected phosphorylation and apoptosis in primary hippocampal neurons. It was suggested that tPA inhibitors might have a neuroprotective role in neuronal cell death [19]. Demestre et al. [1] showed that purified plasminogen from both ALS patients and healthy controls, evoked electron-dense motoneurone degeneration. The cytopathology comprised disruption and distension of Nissl body rough endoplasmic reticulum, cytoplasmic polyribosomal proliferation, and significant Ca (2+) enhancement in mitochondria.

Conclusions

Data from the study suggests that tPA may be implicated in pathophysiology of ALS. It cannot be excluded that an increase in tPA levels in serum of patients with ALS could influence neurodegeneration.

References:


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