Serum cathepsin L concentration in patients with amyotrophic lateral sclerosis – a preliminary report

Stężenie katepsyny L w surowicy krwi chorych na stwardnienie boczne zanikowe – doniesienie wstępne

Joanna Iłżecka

Department of Neurological Rehabilitation, Medical University of Lublin

Summary

Introduction - Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the selective loss of motor neurons in the cortex, brainstem, and spinal cord. So far there is no specific biochemical markers for this disease. Protein aggregates and apoptosis may play a significant role in the mechanism of cell death in ALS. Cathepsin L influences both intracellular proteolysis and apoptosis. Objective - The aim of the study was to measure serum cathepsin L concentration in patients with ALS. Methods - Twenty patients with ALS and 15 patients included in the reference group took part in the study. The measurement of cathepsin L was performed with the enzyme-linked immunosorbent method. Results – The study showed that serum cathepsin L concentrations were significantly increased in the group of ALS patients compared with those of the reference group (p<0.05). Conclusions - Data indicates that cathepsin L may play a significant role in the mechanism of neurodegeneration in ALS. It is possible that the increase of protease levels may accelerate motor neuron death.

Streszczenie

Wstęp – Stwardnienie boczne zanikowe (SBZ) jest chorobą neurodegeneracyjną, charakteryzującą się selektywną utratą neuronów ruchowych w obrębie kory mózgu, pnia mózgu i rdzenia kręgowego. Dotychczas nie wykryto biochemicznych markerów specyficznych dla tej choroby. Agregaty białkowe i apoptoza mogą odgrywać istotną rolę w mechanizmie śmierci komórek w „stwardnieniu bocznym zanikowym” (SBZ). Katepsyna L wpływa na oba mechanizmy: proteolizę wewnątrzkomórkową i apoptozę. Cel pracy – ocena stężenia katepsyny L w surowicy krwi u chorych na SBZ.

Metoda – W badaniu uczestniczyło 20 chorych na SBZ i 15 osób z grupy odniesienia. Stężenie katepsyny L oznaczano metodą immunoenzymatyczną.

 Wyniki – Badanie wykazało istotne podwyższenie stężenia katepsyny L w surowicy krwi chorych na SBZ w odniesieniu do grupy odniesienia (p<0.05).

Wnioski – Wyniki wskazują, że katepsyna L może odgrywać istotną rolę w mechanizmie neurodegeneracji w SBZ. Możliwe jest, że wzrost stężenia tej proteazy może przyspieszać śmierć neuronów ruchowych.

Key words: amyotrophic lateral sclerosis, cathepsin L, serum

Słowa kluczowe: stwardnienie boczne zanikowe, katepsyna L, surowica krwi

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective loss of motor neurons in the cortex, brainstem, and spinal cord. Patients have progressive wasting and weakness of limb, bulbar, and respiratory muscles, and die on average within 3 years of symptom onset. The incidence of ALS is about 1-2 per 100 000 [1]. There is no laboratory tests which could diagnose ALS. So far there are no specific biochemical markers for this disease. There is some evidence that both protein aggregates and apoptosis may be implicated in cell death in ALS [2]. Cathepsin L is a cysteine protease which play a role in apoptosis and also in intracellular proteolysis, and extracellular matrix remodeling. It was demonstrated that lysosomal cathepsins, including cathepsin L, induce mitochondrial apoptosis via the activation of the bid pathway [3, 4]. The oxidative stress, one of the probable mechanisms of cell death in ALS, can induce apoptosis through increase in protease cathepsin D activity [5]. Moreover, it is known that cathepsin D degradation may be regulated by cathepsin L that influences Fas-induced apoptosis [6]. Recently, it was showed that cathepsin L, present in secretory vesicles, functions as a key protease for proteolytic proces-
sing of proneuropeptides into active neuropeptides that are released to mediate cell-cell communication in the nervous system for neurotransmission [7].

The aim of the study was to measure cathepsin L concentrations in the serum of patients with ALS, the diagnostic assessment of the cathepsin L value in this disease, and to investigate whether there is a relationship of this enzyme with the clinical parameters of ALS.

Patients, material and methods
Twenty (13 males/ 7 females) ALS patients took part in the study. The average age of ALS patients was 55 (25-72) years. The patients were diagnosed according to the El Escorial criteria of ALS [8]. The clinical status of patients was evaluated by using Amyotrophic Lateral Sclerosis Functional Rating Scale [ALSFRS] [9]. According to this scale the ALS patients obtained from 14 to 36 points and they were divided into subgroups with mild (above 25 points) and with severe (up to 25 points) clinical status. The patients were also divided into subgroups according to type of ALS onset (limb-onset/bulbar-onset) and they were also divided into subgroups according to duration of ALS (short duration ≤ 12 months/long duration > 12 months). The average duration of the disease was 19 months (3 months – 7 years).

Table I
The characteristics of patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>15</td>
</tr>
<tr>
<td>ALS - total</td>
<td>20</td>
</tr>
<tr>
<td>ALS – bulbar-onset</td>
<td>5</td>
</tr>
<tr>
<td>ALS – limb-onset</td>
<td>15</td>
</tr>
<tr>
<td>ALS - short duration (≤12 months)</td>
<td>14</td>
</tr>
<tr>
<td>ALS - long duration (&gt;12 months)</td>
<td>6</td>
</tr>
<tr>
<td>ALS - mild clinical status</td>
<td>12</td>
</tr>
<tr>
<td>ALS - severe clinical status</td>
<td>8</td>
</tr>
</tbody>
</table>

N – number of patients

The reference group consisted of 15 (7 males/ 8 females) patients (age-matched) with radicular syndrome. The study was approved by the Ethics Committee of Medical University and performed in accordance with the ethical standards established in Helsinki. The characteristics of patients is presented in Table I.

The measurement of cathepsin L was performed with the enzyme-linked immunosorbent method using commercial ELISA kit for human cathepsin L (Bender MedSystems Diagnostics GmbH, Vienna, Austria) in accordance with the 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 ng/mL as mean ± SD. P values < 0.05 were considered significant.

Results
The study showed that cathepsin L concentrations were significantly increased in the serum of patients with ALS compared with those of the reference group (p<0.05). However, there were no significant differences in serum cathepsin L concentrations between subgroups of ALS patients divided according to their clinical status, type of ALS onset and duration of the disease (p>0.05).

The mean values of serum cathepsin L concentrations and a comparative analysis between groups are presented in Table II.

There was no significant correlation between cathepsin L concentration and severity of clinical status of ALS patients or duration of the disease (p>0.05).

Discussion
The study showed increased cathepsin L concentrations in the serum of patients with ALS compared with those of the reference group. However, the increase of cathepsin L concentration was not dependent on the clinical parameters of the disease, such as severity of the clinical status of ALS patients, type of ALS onset, and duration of the disease. In the recent study, the reference group consisted of patients with radicular syndrome, but not healthy persons. Liu et al. [10] measured serum cathepsin L concentration in patients with coronary heart disease and in controls without coronary heart disease (healthy persons). The cathepsin L concentration in the control group in their study was lower than in reference group in the recent study and was 3.9±0.2 ng/ml.

Table II
Serum cathepsin L concentrations and a comparative analysis between the groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>cathepsin L concentration [ng/mL] mean ± SD</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>5.02 ± 2.62</td>
<td>control vs ALS total</td>
</tr>
<tr>
<td>ALS – total</td>
<td>8.26 ± 3.75</td>
<td>p=0.01*</td>
</tr>
<tr>
<td>ALS – bulbar-onset</td>
<td>7.88 ± 4.44</td>
<td>ALS bulb vs limb onset</td>
</tr>
<tr>
<td>ALS – limb-onset</td>
<td>8.38 ± 3.66</td>
<td>p=0.69</td>
</tr>
<tr>
<td>ALS - short duration</td>
<td>8.29 ± 3.87</td>
<td>ALS short vs long duration</td>
</tr>
<tr>
<td>ALS - long duration</td>
<td>8.33 ± 3.83</td>
<td>p=0.74</td>
</tr>
<tr>
<td>ALS - mild clinical status</td>
<td>6.91 ± 3.26</td>
<td>ALS mild vs severe state</td>
</tr>
<tr>
<td>ALS – severe clinical status</td>
<td>10.27 ± 3.72</td>
<td>p=0.07</td>
</tr>
</tbody>
</table>

* p - statistically significant, at least p<0.05; Mann-Whitney U-test
This suggests that serum cathepsin L concentration could be also significantly higher in ALS patients compared with healthy controls.

Previous study revealed that cathepsin L is implicated in degradation of proteoglycan aggregates [11]. Moreover, the experimental investigation performed on cultured cortical neurons demonstrated that beta–amyloid, a neuropathological hallmark of Alzheimer’s disease, induces cathepsin L activity resulting in its cytosolic expression [12]. Banati et al. [13] observed that cathepsin L is strongly expressed in activated microglia and concluded that release of this protease from microglia results in the destruction of central nervous system tissue. Additionally, the increase in pro-cathepsin L expression in activated microglia in the brain of Alzheimer’s disease cases [14] was showed. It is known that the activation of microglia and protein aggregates are also a pathological change in ALS. The expression of cathepsins, among other cathepsin L, was increased in the spinal cord in ALS transgenic mice. Cathepsin B was also expressed by glial fibrillary acid protein positive astrocytes [15]. Offen et al. [16] found major changes in the expression of mRNA in different genes including the increase of cathepsins in postmortem spinal cord specimens of ALS patients. Li et al. [17] showed that activation of autophagy and the abnormal distribution of cathepsin L may be responsible for dopamine neuron death, involved in the pathogenic cascade event for the development of other neurodegenerative disorder – Parkinson’s disease. Data from the literature revealed that cathepsin L plays also a role in Huntington’s disease. This protease may affect N-terminal mutant huntingtin processing and levels of cleavage products which form inclusions and cell death [18]. Ferrucci et al. [19] suggest that the defect in autophagy system is responsible for the accumulation of intracellular protein aggregates within affected motor neurons in ALS.

Results from this study indicate that cathepsin L may be implicated in mechanisms of motor neuron death in ALS. It cannot be excluded that an increase in cathepsin L concentration, observed in this study, may be caused by the involvement of this protease in apoptosis in ALS. It seems that an increase in cathepsin L expression and/or level can accelerate motor neurons damage resulting in their degeneration. Recent data demonstrate that neuroproteases, including cathepsin L, play an important role in the production of peptide neurotransmitters, and in the production of toxic peptides in major neurodegenerative diseases, including ALS [20]. Mori et al. [21] suggest that the formation of TAR-DNA binding protein-43 (TDP-43) inclusions in ALS may be linked to the content of cystatin C, a cysteine protease inhibitor, involved in protein degradation in spinal motor neurons. The authors concluded that perturbations in endogenous levels of cystatin C in neuronal and glial cells may be a part of the neurodegenerative processes in ALS. Hook et al. [22] showed that cysteine protease inhibitors reduce brain beta-amyloid in Alzheimer’s disease, and may constitute a useful therapy in this disease. Because protein aggregates are a common hallmark for different neurodegenerative diseases it cannot be excluded that such therapy may have a beneficial effect also in ALS.

References


Zaakceptowano do publikacji: 30.05.2012

Address for correspondence:
dr hab. n. med. Joanna Iłżecka
20-050 Lublin, Szerokie 6B,
tel/fax: 81 7502434
e-mail: ilzecka@onet.pl