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Cerebrospinal fluid caspase - 8 levels in patients with amyotrophic lateral sclerosis – a preliminary report

Stężenie kaspazy-8 w płynie mózgowo-rdzeniowym chorych na stwardnienie boczne zanikowe – doniesienie wstępne

Joanna Iłżecka, Katarzyna Hanus-Atras

Department of Neurological Rehabilitation, Medical University, Lublin, Poland

Summary

Purpose: There are evidences that apoptosis plays a role in pathomechanism of amyotrophic lateral sclerosis (ALS). Caspase - 8 is implicated in Fas - mediated apoptosis pathway. The aim of the study was to investigate cerebrospinal fluid (CSF) caspase - 8 levels in patients with ALS.

Material/methods: The measurement of caspase-8 was performed by the enzyme-linked immunosorbent method using commercial ELISA kit.

Results: The study showed that caspase - 8 level was significantly increased in CSF of the whole group of patients with ALS comparing to the control group ($p < 0.05$). The correlation between CSF caspase – 8 and severity of clinical status of ALS patients was statistically significant ($p = 0.02$).

Conclusion: The results indicate that caspase - 8 is implicated in pathomechanism of ALS.

Streszczenie

Cel: Istnieją dowody, że apoptoza odgrywa rolę w patomechanizmie stwardnienia bocznego zanikowego (SLA). Kaspaza - 8 uczestniczy w drodze apoptozy, której mediatorem jest receptor Fas. Celem pracy było określenie stężenia kaspazy-8 w płynie mózgowo-rdzeniowym (CSF) chorych na SLA.

Material/metoda: Stężenie kaspazy-8 oznaczano metodą immunoenzymatyczną ELISA.

Wyniki: Badanie wykazało, że stężenie kaspazy-8 jest istotnie podwyższone w CSF całej grupy chorych na SLA w porównaniu z grupą kontrolną ($p < 0.05$). Obserwowano istotną korelację pomiędzy stężeniem kaspazy-8 a ciężkością stanu klinicznego chorych na SLA ($p = 0.02$).

Wnioski: Wyniki badań wskazują, że kaspaza-8 może uczestniczyć w patomechanizmie SLA.

Key words: amyotrophic lateral sclerosis, apoptosis, caspase - 8, cerebrospinal fluid, neurodegeneration

Słowa kluczowe: stwardnienie boczne zanikowe, apoptoza, kaspaza-8, płyn mózgowo-rdzeniowy, neurodegeneracja

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease. There are evidences that programmed cell death, termed apoptosis, is implicated in pathomechanism of ALS and motor neuron degeneration [4,13]. The activation of apoptosis markers in ventral horn motor neurons during early presymptomatic stages of ALS in a transgenic mouse model has been observed [19].

Data from the literature showed that caspases play a key role in the mechanism of apoptosis. Kong et al. [6] reported

that extracellular nucleotides mediate neuronal apoptosis through activation of P2X(7) nucleotide receptors and their downstream signaling pathways involving C-jun N-terminal kinase 1 (JNK1), extracellular signal-regulated kinase (ERK) and caspases 8/9/3. In Fas apoptotic pathway, tumor necrosis factor alpha (TNF-alpha) acts on receptor ligands yielding to caspase - 8 activation [10]. According to Wang et al. [18] TNF-alpha is able to induce apoptosis via two distinct caspase - 8 activation pathways that are differentially regulated by cIAP1/2 and c-FLIP. Thus, caspase - 8 is implicated

as an initiator caspase in death receptor -induced signaling to apoptosis and plays a role in Fas - induced cell death. Fas stimulation induces the binding of caspase - 8 to a death-inducing signaling complex, leading to its autocatalytic cleavage and the formation of a caspase - 8 homodimer, which is released into the cytosol where mediates the apoptotic signaling cascade. However, caspases have also non-apoptotic function [8]. Activated caspases are involved in T-cell proliferation, cell cycle regulation and in the cellular differentiation [14]. It was observed that caspase - 8 plays a role in embryonic development, monocyte differentiation, T and B cell proliferation, and the activation of NF-kappaB [15]. A deletion of caspase - 8 in bone - marrow cells caused arrest of hemopoietic progenitor functioning, and in cells of the myelomonocytic lineage, its deletion led to arrest of differentiation into macrophages and to cell death [5].

Raoul et al. [12] showed that Fas - triggered death of normal embryonic motoneurons requires transcriptional upregulation of neuronal NOS and involves FADD/caspase-8 cascade but only in motoneurons. Moreover, motoneurons from transgenic mice overexpressing ALS – linked SOD1 mutants had increased susceptibility to activation of this pathway.

The aim of the study was to measure the cerebrospinal fluid (CSF) caspase - 8 levels in the patients with ALS and to investigate whether there is a relationship of this caspase with clinical parameters of the disease.

Material and methods

Twenty (11 male/ 9 female) ALS patients (average age 58, range 38-71 years) took part in the study. The ALS was diagnosed according to the El Escorial criteria of ALS [1]. There were 15 patients with clinically definite ALS (the presence of upper motor neuron as well as lower motor neuron signs in the bulbar region and at least two spinal regions, or the presence of upper motor neuron signs in two spinal regions and lower motor neuron signs in three spinal regions) and 5 patients with clinically probable ALS (upper motor neuron and lower motor neuron signs in at least two regions with some upper motor neuron signs rostral to (above) the lower motor neuron signs) according to these criteria. The clinical conditions of the patients were assessed by the Amyotrophic Lateral Sclerosis Functional Rating Scale [ALSFERS] [3]. According to this scale the ALS patients scored from 0 to 35 points. They were divided into the two subgroups: those with a mild clinical conditions (over 25 points according to ALSFERS) – 10 patients, and those with a severe clinical status (up to 25 points according to ALSFERS) – 10 patients. The patients with the ALS were also divided according to the type of the disease onset (12 patients with a limb-onset and 8 patients with a bulbar-onset). The average duration of ALS was 18 months (3 months – 5 years). According to duration of the disease the two subgroups of patients were isolated (with a short duration \leq 12 months – 12 patients and with a long duration $>$ 12 months – 8 patients). The control group consisted of 20 (10 male/ 10 female) patients with tension –

type headache. The average age in the control group was 52 (28-70) years. The study was approved by the Ethics Committee of Medical University and performed in accordance with the ethical standards established in Helsinki.

CSF samples were collected into the plastic tubes, centrifuged rapidly and stored at -70° until the analysis was performed. Caspase - 8 levels were measured by the enzyme-linked immunosorbent method using the commercial ELISA kit for human Caspase-8 (Bender MedSystems, Austria) in accordance with the manufacturer's instructions. The non-parametric Mann-Whitney rank sum test was used to examine the differences between the groups because the data was not normally distributed. The correlation analysis was performed by using the Spearman rank correlation. The caspase - 8 values are expressed in ng/mL, as median and range. P values $<$ 0.05 were considered significant.

Results

The study showed that the caspase - 8 levels are significantly increased in the CSF of the whole group of patients with ALS compared with those from the control group ($p < 0.05$), and significantly increased in the CSF of patients with severe clinical status compared to patients with mild clinical status ($p < 0.05$). The caspase-8 levels are significantly increased in the CSF of patients with limb-onset of ALS ($p < 0.05$) but not in bulbar-onset of ALS ($p > 0.05$) compared with controls, and significantly increased in the CSF of patients with severe clinical status of ALS ($p < 0.05$) but not in patients with mild clinical status of ALS ($p > 0.05$) compared with controls. The difference in the CSF caspase - 8 levels between subgroups of ALS patients according to the division of their type of ALS onset and duration of the disease was not significant ($p > 0.05$).

The median values of the CSF caspase - 8 levels, and a comparative analysis between subgroups are presented in Table 1 and in Figures 1-4.

The correlation between CSF caspase - 8 and severity of clinical status of ALS patients was statistically significant ($p = 0.02$).

Discussion

Data from the literature showed that adult motor neuron apoptosis is mediated by nitric oxide and Fas death receptor linked by DNA damage and p53 activation [9]. *Shin et al.* [16] observed that the proapoptotic proteins Fas, Fas - associated death domain, caspase - 8, and caspase-3 are increased in motor neurons from ALS mice. Moreover, *Tokuda et al.* [17] demonstrated that caspases, including caspase - 8 are present in nonactive forms in the spinal cords of wild - type mice during the early stage of the disease. In transgenic mice the caspases are present in their active forms. During the advanced stage of the disease, when paralysis is present, the active caspases levels are increased.

Casha et al. [2] showed that Fas deficiency reduces apoptosis, spares axons and improves function after spinal cord

injury that suggest that inhibition of the Fas pathway may be a neuroprotective therapy after spinal cord injury. Moreover, the inhibition Fas pathway may be protective strategy for motoneurons in ALS. Petri et al. [11] observed that motor neurons from ALS – mice have an increased sensitivity to Fas – induced cell death via this pathway, and a loss of Fas ligand – function improves survival in G93A – transgenic ALS mice. Locatelli et al [7] performed in vitro and in vivo small interfering RNA-mediated interference by silencing the Fas receptor on motoneurons that carry the superoxide dismutase – 1 (SOD1) – G93A mutation, and observed a significant reduction in Fas expression at messenger RNA and protein level. Treated motoneurons demonstrated an increase in survival. Moreover, treated mice showed a significant reduction in Fas and Fas mediators, neuronal nitric oxide synthase, and caspase - 8.

It was observed that sera from 26% of patients with sporadic ALS induced in vitro apoptosis of a human neuroblastoma cell line. In mixed cultures of rat embryonic brain and spinal cord cells, the sera from ALS patients induced the apoptosis of a subpopulation of motoneurons. Yi et al. [20] concluded that these results are compatible with the hypothesis that autoimmune mechanisms related to anti-Fas autoantibodies participate in the pathomechanism of ALS. Sengun et al. [15] showed that anti-Fas antibodies are elevated in serum of patients with ALS, but it was no correlation between the antibody levels and the length or stage of the disease.

There is no data from the literature concerning CSF caspase - 8 levels in patients with ALS. The study showed that CSF caspase – 8 levels are increased in these patients. This indicates that caspase -8 may be implicated in apoptotic motor neuron death in ALS. In addition, our findings are compatible with observations made on animal and cellular models of ALS, described above.

Conclusions

1. Caspase-8 may be implicated in the pathomechanism of ALS and may influence neurodegeneration.
2. Caspase-8 levels may be a marker of clinical status severity in patients with ALS.

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Adres Autorów:

Samodzielna Pracownia Rehabilitacji Neurologicznej
Uniwersytet Medyczny
Lublin
e-mail: ilzecka@onet.pl

(Praca wpłynęła do Redakcji: 2010.06.01)

(Praca przekazana do opublikowania: 2010.07.12)