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CEREBROSPINAL FLUID ANGIOGENIN LEVEL IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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SUMMARY – Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease. It is suggested that angiogenin (ANG) may play a role in the pathomechanism of this disease. The aim of the study was to measure cerebrospinal fluid (CSF) ANG levels in patients with ALS. Twenty ALS patients and 15 control subjects were included in the study. CSF ANG levels were measured by ELISA. Study results showed that CSF ANG level did not differ between ALS patients and control group ($p>0.05$). There was no significant correlation between CSF ANG level and clinical state of ALS patients either ($p>0.05$). The present study conducted on CSF of patients with ALS did not confirm previous observation on the possible role of ANG in neurodegeneration in this disease.

Key words: *Amyotrophic Lateral Sclerosis, Neurodegenerative Diseases – Cerebrospinal Fluid; Angiogenesis inducing agents*

Introduction

Literature data suggest that angiogenic factors may play a role in the pathomechanism of amyotrophic lateral sclerosis (ALS). Angiogenin (ANG) is a member of the ribonuclease (RNase) superfamily which is implicated in angiogenesis[1]. This protein showed angiogenic activity in several experimental models and its expression may be stimulated by hypoxia[2]. ANG binds to endothelial cells and translocates to nucleolus where it binds to DNA. The cross-talk between ANG and protein kinase B/Akt signaling pathway may be essential for ANG-induced angiogenesis *in vitro* and *in vivo*[3]. Nuclear ANG influences proliferation of endothelial cells and is important for angiogenesis induced by other angiogenic factors. It was shown that the activities of another angiogenic factor, vascular endothelial growth factor (VEGF), and ANG are linked[4]. Olson *et al*.[5] report that ANG can be regulated *in vivo* as an acute phase protein, and may be implicated in tissue repair after inflammation or trauma. Huang *et al*.[6] observed that transplantation of ANG-overexpressing mesenchymal stem cells augmented cardiac function in an experimental model of chronic ischemia. Recently, it has been suggested that ANG may be a risk factor for ALS and this protein might be a therapeutic target for this disease[7,8].

The aim of the study was to measure cerebrospinal fluid (CSF) ANG levels in patients with ALS in comparison with control subjects.

Material and Methods

Twenty ALS patients (12 male and eight female), average age 57, range 38-80 years, were diagnosed according to the El Escorial criteria of ALS[9]. The clinical condition of study patients was measured by use of the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS)[10]. According to this scale, the ALS patients scored 2 to 35 points and were divided into two subgroups: 11 patients with a mild clinical state (over 25 points) and nine patients with a severe clinical state (up to 25 points).

The age-matched control group consisted of 15 patients (eight male and seven female) with tension-type headache.

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The study was approved by the Medical University Ethics Committee and performed in accordance with the ethical standards established in Helsinki Convention.

CSF samples from ALS patients and control subjects were collected into plastic tubes and stored at -70 °C until analysis. ANG levels were measured by the enzyme-linked immunosorbent method using commercial ELISA kit for human Angiogenin (R&D Systems, Minneapolis, USA) in accordance with the manufacturer's instructions.

On statistical analysis, the nonparametric Mann-Whitney rank sum test was used to examine differences between the groups. Correlation analysis was performed by using Spearman rank correlation. The values were expressed in pg/mL, as median and range. The values of $p<0.05$ were considered significant.

Results

Our study showed that CSF ANG levels did not significantly differ between ALS patients and control subjects (median 328.1, range: 207.9-450.0 pg/mL and median 285.6, range: 153.0-482.8 pg/mL, respectively; $p=0.48$). There were no significant differences in the CSF ANG levels between the subgroups of ALS patients with mild and severe clinical state either ($p=0.62$). There was no significant correlation between CSF ANG level and severity of the clinical state of ALS patients ($p=0.72$). The CSF ANG levels recorded in study patients are presented in Figures 1 and 2.

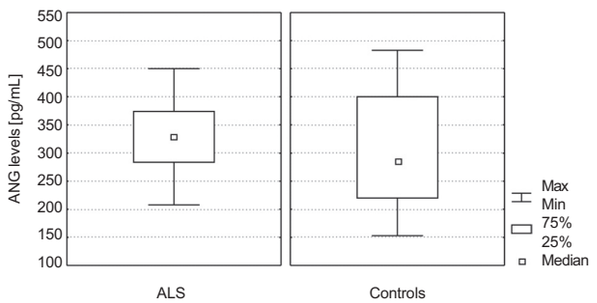

Fig. 1. Cerebrospinal fluid (CSF) angiogenin (ANG) level in patients with amyotrophic lateral sclerosis (ALS) and control subjects.

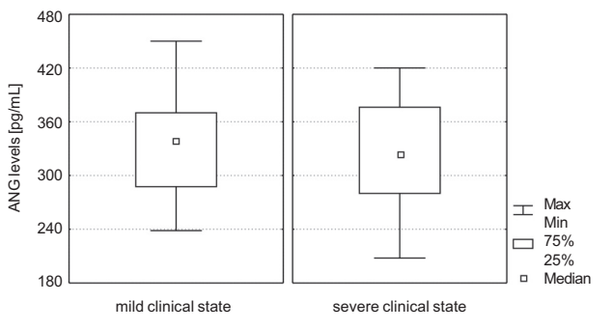

Fig. 2. Cerebrospinal fluid (CSF) angiogenin (ANG) level according to clinical state of patients with amyotrophic lateral sclerosis.

Discussion

Experimental investigation conducted on mice showed ANG-1 to be expressed in the developing nervous system during embryogenesis. ANG is strongly expressed in motor neurons in the spinal cord and dorsal root ganglia[11]. It suggests that ANG might play a significant role in motor neuron activity, and affected expression and/or function of this protein might be associated with ALS.

Recently, Greenway *et al*.[12,13] identified a novel mutation in ANG gene in patients with ALS, which may inhibit the function of this protein, and similar to VEGF, could influence the risk of ALS. On the other hand, Corrado *et al*.[14] report that ANG gene is not associated to ALS in the Italian population.

Cronin *et al*.[15] showed serum ANG levels to be elevated, especially in patients with spinal onset of ALS. In this recent study, CSF ANG level in patients with ALS was not different from that in controls. Because CSF ANG levels are not affected in ALS patients, it cannot be excluded that an increase in serum ANG levels, observed in the study by Cronin *et al*.[15], especially in patients with spinal onset of the disease, may be the result of muscle atrophy and is not associated with neurodegeneration within the central nervous system.

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Sažetak

RAZINA ANGIOGENINA U LIKVORU BOLESNIKA S AMIOTROFIČNOM LATERALNOM SKLEROZOM

J. Iłżecka

Amiotrofična lateralna skleroza (ALS) je neurodegenerativna bolest. Pretpostavlja se da bi angiogenin (ANG) mogao imati ulogu u patomehanizmu ove bolesti. Cilj ove studije bio je izmjeriti razine ANG u likvoru bolesnika s ALS. U studiju je bilo uključeno 20 bolesnika s ALS i 15 kontrolnih osoba. Razine ANG u likvoru mjerene su metodom ELISA. Ispitivanje je pokazalo kako nema razlike u likvorskoj razini ANG između bolesnika s ALS i kontrolne skupine ($p>0,05$). Isto tako nije bilo značajne korelacije između razine ANG u likvoru i kliničkog stanja bolesnika s ALS ($p>0,05$). U zaključku, ova studija provedena na likvoru bolesnika s ALS nije potvrdila prijašnja zapažanja prema kojim bi ANG mogao biti upleten u neurodegenerativne procese ove bolesti.

Ključne riječi: *Amiotrofna lateralna skleroza; Neurodegenerativne bolesti – likvor; Sredstva koja izazivaju angiogenezu*