

SERUM SOLUBLE OX40 IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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SUMMARY – Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease. Data from the literature show that systemic immune activation plays a role in ALS. OX40 (CD134) is member of the tumor necrosis factor receptor family and is expressed selectively on activated T lymphocytes. The aim of the study was to measure serum soluble OX40 (sOX40) levels in patients with ALS. The study included 25 ALS patients and 15 control subjects. Serum sOX40 levels were determined by the enzyme-linked immunosorbent method. Study showed that sOX40 levels were significantly decreased in serum of ALS patients compared with controls ($P=0.05$). There was no significant correlation between serum sOX40 levels and clinical parameters of ALS such as severity of the ALS patient clinical state and duration of the disease ($P>0.05$). In conclusion, decrease in serum sOX40 levels in patients with ALS suggests that this cytokine may be implicated in the pathomechanisms of this disease.

Key words: *Amyotrophic lateral sclerosis; Neurodegeneration; Neuroprotection; Serum; Soluble OX40*

Introduction

Data from the literature show that systemic immune activation plays a role in amyotrophic lateral sclerosis (ALS)¹. According to Alexianu *et al.*², the up-regulation of proinflammatory factors during early presymptomatic stages as well as the expansion of immune activation as the disease progresses in mutant SOD transgenic mice, an animal model of ALS, suggest that immune-inflammatory mechanisms could contribute to the disease progression.

OX40 (CD134) is member of the tumor necrosis factor (TNF) receptor family and is expressed selectively on activated T lymphocytes. The interaction with OX40 ligand (OX40 L) delivers costimulatory signals to T cells³. This cytokine regulates survival and functions of depletion-resistant T cells⁴. It is known that T cells play an important role during inflammation⁵.

The OX40/OX40 L interaction induces auto-immune-like diseases⁶. Blockade of the interaction between OX40 and OX40 L inhibited immune responses in both mouse and nonhuman primate models of allergic inflammation⁷. According to Tateyama *et al.*⁸ OX40 is expressed on activated T cells, which may play an important role in autoimmune diseases such as polymyositis and granulomatous myopathy. A study conducted by Xiaoyan *et al.*⁹ showed that freshly isolated CD4+ T-cells from patients with myasthenia gravis expressed OX40 to a greater extent than cells from healthy individuals. Moreover, CD134 is up-regulated in the central nervous system (CNS) of patients with multiple sclerosis¹⁰. OX40 may have a proinflammatory function during immune activation and is expressed on a variety of leukocytes within the body¹¹. The OX40 receptor is expressed on autoreactive CD4+ T cells isolated from the site of inflammation in rats with clinical signs of experimental autoimmune encephalomyelitis¹². OX40 can also control proliferation, survival, and production of cytokines^{13,14}.

The aim of the study was to measure soluble OX40 (sOX40) levels in serum samples from patients with

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ALS and to investigate whether there is a relationship between the concentration of sOX40 and clinical parameters of the disease.

Material and Methods

Twenty-five (14 male/11 female) ALS patients (average age 57, range 34-74 years) were diagnosed according to the El Escorial criteria of ALS¹⁵. The clinical condition of patients was measured by the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS)¹⁶. According to this scale, ALS patients scored from 8 to 36 points and were divided into two subgroups: those with a mild clinical state (over 25 points), 15 patients; and those with a severe clinical state (up to 25 points), 10 patients. The average duration of the disease was 16 months (3 months to 8 years). The ALS patients were also divided into two subgroups according to the duration of ALS (14 patients with short duration ≤ 12 months/11 patients with long duration >12 months) and according to type of ALS onset (15 patients with limb-onset/10 patients with bulbar-onset).

The age-matched control group consisted of 15 (8 male/7 female) patients with lumbosacral disk disease. The study was approved by the Medical University Ethics Committee and performed in accordance with the ethical standards established in Helsinki.

Serum samples from ALS patients and control subjects were collected into plastic tubes and centri-

fuged rapidly. The samples were stored at -70 °C until analysis. The sOX40 levels were measured by the enzyme-linked immunosorbent method aided commercial ELISA kit for human sCD134 (OX40) (Bender MedSystems Diagnostics GmbH, Vienna, Austria) in accordance with the manufacturer's instructions.

On statistical analysis, 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. P values ≤ 0.05 were considered significant.

Results

Study results showed that serum sOX40 levels were significantly decreased in the overall ALS patient group compared with controls ($P=0.05$). The sOX40 levels were also significantly decreased in patients with bulbar onset of ALS compared to controls ($P=0.02$). There were no significant differences in serum sOX40 levels between the subgroups of ALS patients according to their clinical state, type of ALS onset and disease duration ($P>0.05$). The median values of serum sOX40 levels and comparative analysis between subgroups are presented in Table 1.

Discussion

Data from the literature show that chronic inflammation is associated with neurodegenerative diseases,

Table 1. Serum soluble OX40 (sOX40) levels and comparative between-group analysis

Group	sOX40 level (pg/mL) Median (range)	Comparison
Control	1300 (747-1607)	Control <i>vs.</i> ALS total $P=0.05^*$
ALS – total	1149 (464-1711)	
ALS – short duration	1119 (464-1711)	ALS short <i>vs.</i> long duration $P=0.44$
ALS – long duration	1241 (622-1412)	
ALS – bulbar onset	1129 (471-1458)	ALS bulbar <i>vs.</i> limb onset $P=0.29$
ALS – limb onset	1241 (464-1711)	
ALS – mild clinical state	1129 (464-1711)	ALS mild <i>vs.</i> severe clinical state $P=0.56$
ALS – severe clinical state	1231 (622-1458)	

Data are expressed as median and range; ALS = amyotrophic lateral sclerosis; *statistical significance $P\leq 0.05$; Mann-Whitney rank sum test

including ALS^{17,18}. Zhang *et al.*¹⁹ observed activated monocyte/macrophages in serum of patients with ALS and their activation was related to the rate of ALS disease progression. Immunohistochemical studies demonstrated the presence of T-cell lymphocytes in the spinal cord of ALS patients. According to Engelhardt *et al.*²⁰, T-helper cells were observed in the proximity to corticospinal tract degeneration, while T-helper and T-suppressor cytotoxic cells were found in ventral horns. Graves *et al.*²¹ revealed the inflammation in ALS spinal cord and brain to be mediated by activated macrophages, mast cells and T cells.

Holmoy states that the inflammatory process in ALS involves infiltration of T cells and activation of antigen presenting cells co-localizing with motor neuron damage in the brain and spinal cord²². The role of T cells in this process is unclear. Probably, T cells may damage motor neurons by the cell-cell contact or cytokine secretion, or contribute indirectly to motor neuron damage through activation of microglia and macrophages. It cannot be excluded that T cell infiltration may be secondary process to the death of motor neurons. On the other hand, there is evidence that T cell responses may play a neuroprotective role in ALS.

Shi *et al.*²³ observed a significant positive correlation of T cell percentage with the ALS progression rate. A systemic low-grade inflammation was detected in patients with ALS and was correlated with their degree of disability²⁴. Moreover, elevation of inflammatory markers such as TNF-alpha, interferon-gamma (IFN- γ) and nitric oxide was demonstrated in serum of ALS patients compared to normal controls²⁵.

According to Troost *et al.*²⁶, the majority of many diffusely scattered lymphocytes seen in the anterior and lateral corticospinal tracts and anterior horns belonged to the suppressor/cytotoxicity T cell subset. The circulating autoreactive lymphocytes of patients with ALS were isolated and activated *in vitro*. It was observed that autoreactive cells may induce differentiation of mesenchymal stem cells to neuronal stem cells and this is a protective physiologic mechanism to nerve tissue repair²⁷.

It was observed that T cell-dependent immunity in the CNS is beneficial for neuroprotection and neurogenesis. The expression of OX40 L in microglia provides a molecular basis for the maintenance of T cell survival, expansion of T cells and increased secre-

tion of growth factors, which may contribute to the protective effect in the CNS²⁸. Beers *et al.*²⁹ showed in a model of chronic neurodegeneration that activation of glia did not predict glial function, and that the presence of CD4+ T cells induced neuroprotection by modulating the trophic/cytotoxic balance of glia.

There are no studies published in the literature concerning OX40 in ALS. The present study showed that sOX40 levels in serum of patients with ALS were decreased compared with controls. Banerjee *et al.*³⁰ investigated the role of T cell immunity in human G93A superoxide dismutase 1 (SOD1) transgenic mice and in ALS patients, and demonstrated dysfunction and deficits of T cells. Because OX40 can influence T cells, which have a potential protective role in immune/inflammatory reactions in the CNS, it cannot be excluded that this cytokine is implicated in neurodegeneration in ALS. In conditions associated with the activation of immune/inflammatory mechanisms, the sOX40 levels would be increased. Contrary, the present study showed that this cytokine levels were decreased in serum of patients with ALS compared to controls. It cannot be excluded that the lower serum level of this cytokine may be the result of its higher activity in the immune-inflammatory process within the CNS. It was observed that the study parameter of OX40 serum concentration need not reflect its concentration within the organs³¹.

References

1. KAWAMATA T, AKIYAMA H, YAMADA T, *et al.* Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. *Am J Pathol* 1992;140:691-707.
2. ALEXIANU ME, KOZOVSKA M, APPEL SH. Immune reactivity in a mouse model of familial ALS correlates with disease progression. *Neurology* 2001;57:1282-9.
3. TAYLOR L, SCHWARZ H. Identification of a soluble OX40 isoform: development of a specific and quantitative immunoassay. *J Immunol Methods* 2001;255:67-72.
4. KROEMER A, XIAO X, VU MD, *et al.* OX40 controls functionally different T cell subsets and their resistance to depletion therapy. *J Immunol* 2007;179:5584-91.
5. CAVANAQH MM, HUSSELL T. It is wise to target the late costimulatory molecule OX40 as a therapeutic target? *Arch Immunol Ther Exp (Warsz)* 2008;56:291-7.
6. MURATA K, NOSE M, NOLHLOVU LC, *et al.* Constitutive OX40/OX40 ligand interaction induces autoimmune-like diseases. *J Immunol* 2002;169:4628-36.

7. WANG YH, LIU YJ. OX40-OX40L interactions: a promising therapeutic target for allergic diseases? *J Clin Invest* 2007;117:3655-7.
8. TATEYAMA M, FUJIHARA K, ISHII N, *et al.* Expression of OX40 in muscles of polymyositis and granulomatous myopathy. *J Neurol Sci* 2002;194:29-34.
9. XIAOYAN Z, PIRSKANEN R, MALMSTROM V, *et al.* Expression of OX40 (CD134) on CD4+ T-cells from patients with myasthenia gravis. *Clin Exp Immunol* 2006;143:110-6.
10. CARBONI S, ABOUL-ENEIN F, WALTZINGER C, *et al.* CD134 plays a crucial role in the pathogenesis of EAE and is upregulated in the CNS of patients with multiple sclerosis. *J Neuroimmunol* 2003;145:1-11.
11. WEINBERG AD, MONTLER R. Modulation of TNF receptor family members to inhibit autoimmune disease. *Curr Drug Targets Inflamm Allergy* 2005;4:195-203.
12. KALEEBA JAR, OFFNER H, VANDENBARK AA, *et al.* The OX40 receptor provides a potent co-stimulatory signal capable of inducing encephalitogenicity in myelin-specific CD4+ T cells. *Int Immunol* 1998;10:453-61.
13. SO T, LEE SW, CROFT M. Immune regulation and control of regulatory T cells by OX40 and 4-1BB. *Cytokine Growth Factor Rev* 2008;19:253-62.
14. MOUSAVI SF, SOROOSH P, TAKAHASHI T, *et al.* OX40 costimulatory signals potentiate the memory commitment of effector CD8+ T cells. *J Immunol* 2008;181:5990-6001.
15. BROOKS BR. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. *J Neurol Sci* 1994;124 Suppl:96-107.
16. GORDON PH, MILLER RG, MOORE DH, ALSFRS-R. Amyotroph Lateral Scler Other Motor Neuron Disord 2004;5:90-3.
17. McGEER PL, McGEER EG. Inflammation and the degenerative diseases of aging. *Ann N Y Acad Sci* 2004;1035:104-16.
18. TILLEUX S, HERMANS E. Neuroinflammation and regulation of glial glutamate uptake in neurological disorders. *J Neurosci Res* 2007;85:2059-70.
19. ZHANG R, GASCON R, MILLER RG, *et al.* Evidence for systemic immune system alterations in sporadic amyotrophic lateral sclerosis (sALS). *J Neuroimmunol* 2005;159:215-24.
20. ENGELHARDT JI, TAJTI J, APPEL SH. Lymphocytic infiltrates in the spinal cord in amyotrophic lateral sclerosis. *Arch Neurol* 1993;50:30-6.
21. GRAVES MC, FIALA M, DINGLASAN LA, *et al.* Inflammation in amyotrophic lateral sclerosis spinal cord and brain is mediated by activated macrophages, mast cells and T cells. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2004;5:213-9.
22. HOLMOY T. T cells in amyotrophic lateral sclerosis. *Eur J Neurol* 2008;15:360-6.
23. SHI N, KAWANO Y, TATEISHI T, *et al.* Increased IL-13-producing T cells in ALS: positive correlations with disease severity and progression rate. *J Neuroimmunol* 2007;182:232-5.
24. KEIZMAN D, ROGOWSKI O, BERLINER S, *et al.* Low-grade systemic inflammation in patients with amyotrophic lateral sclerosis. *Acta Neurol Scand* 2009;119:383-9.
25. BABU GN, KUMAR A, CHANDRA R, *et al.* Elevated inflammatory markers in a group of amyotrophic lateral sclerosis patients from northern India. *Neurochem Res* 2008;33:1145-9.
26. TROOST D, Van den OORD JJ, VIANNEY de JONG JM. Immunohistochemical characterization of the inflammatory infiltrate in amyotrophic lateral sclerosis. *Neuropathol Appl Neurobiol* 1990;16:401-10.
27. MOVIQLIA GA, VARELA G, GAETA CA, *et al.* Autoreactive T cells induce *in vitro* BM mesenchymal stem cell transdifferentiation to neural stem cells. *Cytotherapy* 2006;8:196-201.
28. WANQY, LIM, SONQM, *et al.* Expression of OX40 ligand in microglia activated by IFN-gamma sustains a protective CD4(+) T-cell response *in vitro*. *Cell Immunol* 2008;251:86-92.
29. BEERS DR, HENKEL JS, ZHAO W, *et al.* CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. *Proc Natl Acad Sci USA* 2008;105:15558-63.
30. BANERJEE R, MOSLEY RL, REYNOLDS AD, *et al.* Adaptive immune neuroprotection in G93A-SOD1 amyotrophic lateral sclerosis mice. *PLoS ONE* 2008;3(7):e2740.
31. ŠIRANOVIĆ M, KOVAČ J, GOPČEVIĆ S, *et al.* Human soluble TREM-1: lung and serum levels in patients with bacterial ventilator associated pneumonia. *Acta Clin Croat* 2001;50:345-9.

Sažetak

OX40 TOPLJIV U SERUMU KOD BOLESNIKA S AMIOTROFIČNOM LATERALNOM SKLEROZOM

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SUMMARY – Amiotrofična lateralna skleroza (ALS) je neurodegenerativna bolest. Literaturni podaci pokazuju da sistemsko aktiviranje imuno sustava ima ulogu kod ALS. OX40 (CD134) pripada obitelji receptora faktora tumorske nekroze (TNF), a izražen je selektivno na aktiviranim T limfocitima. Cilj studije bio je izmjeriti razine OX40 topljivog u serumu (sOX40) kod bolesnika s ALS. U studiju je bilo uključeno 25 bolesnika s ALS i 15 kontrolnih osoba. Razine sOX40 u serumu mjerene su metodom ELISA. Rezultati su pokazali da su razine sOX40 značajno snižene u serumu bolesnika s ALS u usporedbi s kontrolnim osobama ($P=0,05$). Nije bilo značajne korelacije između serumskih razina sOX40 i kliničkih parametara ALS, kao što su težina kliničkog stanja bolesnika s ALS i trajanje bolesti ($P>0,05$). U zaključku, serumske razine sOX40 kod bolesnika s ALS ukazuju na to da bi ovaj citokin mogao biti upleten u patomehanizme ove bolesti.

Ključne riječi: *Amiotrofična lateralna skleroza; Neurodegeneracija; Neuroprotekcija; Serum; Topljivi OX40*

