Role of Superoxide Dismutase in the Pathogenesis of Amyotrophic Lateral Sclerosis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 4, pp. 460-462, April, 1999
Original article submitted May 21, 1998

Activity of cytosolic Cu^{2+}/Zn^{2+}-superoxide dismutase and total superoxide dismutase activity of the spinal fluid of patients with sporadic amyotrophic lateral sclerosis were measured. These parameters correlates with the form of this disease, its duration, and the severity of neurological disorders. Our findings indicate that free-radical processes are involved in the damage to motor neurons in this disease.

Key Words: *amyotrophic lateral sclerosis; oxidative stress; superoxide dismutase*

Oxidative stress is considered as the main mechanism of neuronal alterations in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Parkinson's disease, and Huntington's disease. Oxidative stress results from sharp activation of free-radical processes inducing accumulation of reactive oxygen metabolites $O_2^{\bullet}$, H_2O_2, $OH^{\bullet}$ lipoperoxides, and secondary products of their oxidation. Damage-inducing effects of free radicals are associated with inactivation of enzyme SH-groups, hydroxylation of nitrogenous bases, DNA fragmentation, intensification of lipid peroxidation (LPO), and destruction of biological membranes.

Insufficiency of the tissue antioxidant system may cause oxidative stress. Superoxide dismutase (SOD) that catalyzes dismutation of $O_2^{\bullet}$ with the formation of H_2O_2 and molecular oxygen is the key enzyme of the antioxidant system. There are three SOD isoenzymes in humans: cytosolic Cu^{2+}/Zn^{2+}-SOD (SOD1), mitochondrial Mn^{2+}-SOD (SOD2), and extracellular Cu^{2+}/Zn^{2+}-SOD (SOD3). These isoenzymes are coded by certain genes located on chromosomes 21q21, 6q27, and 4p, respectively.

Mutations in the SOD1 gene were revealed in 20-30% of patients with familial ALS and in 5-7% of patients with sporadic ALS [8]. Now, 47 point mutations in the SOD1 gene are identified [11]. No genetic defects in SOD2 and SOD3 were found. The majority of SOD1 genetic mutations induce a 35-70% decrease in enzyme activity in motor neurons and erythrocytes compared with the control levels [3,7]. The decrease in SOD1 activity in ALS affects the reaction of dismutation and leads to the accumulation of $O_2^{\bullet}$ and oxidative stress.

The decrease in SOD1 activity may result from mutations and oxidation of the enzyme by H_2O_2 and lipoperoxides during oxidative stress [5]. The inhibition of SOD1 promotes apoptosis in cultured spinal motor neurons [9]. However, the increase in SOD1 activity can not be considered as the compensatory reaction only, because its peroxidase activity increases in this case. This is manifested in H_2O_2 reduction and the formation of highly toxic $OH^{\bullet}$ [13].

The data on SOD activity in autopsy materials and biological fluids from patients with sporadic ALS are ambiguous. Activity of SOD1 was shown to be increased in motor neurons, astroglia, and erythrocytes of patients [1,10]. However, in other studies no significant differences from the control levels were revealed [2,4,7]. Some authors reported a decrease in SOD1 activity in erythrocytes and spinal fluid (SF) of patients with ALS [12].

Here we studied activity of cytosolic Cu^{2+}/Zn^{2+}-SOD1 in erythrocytes and total SOD activity in the SF of patients with sporadic ALS.

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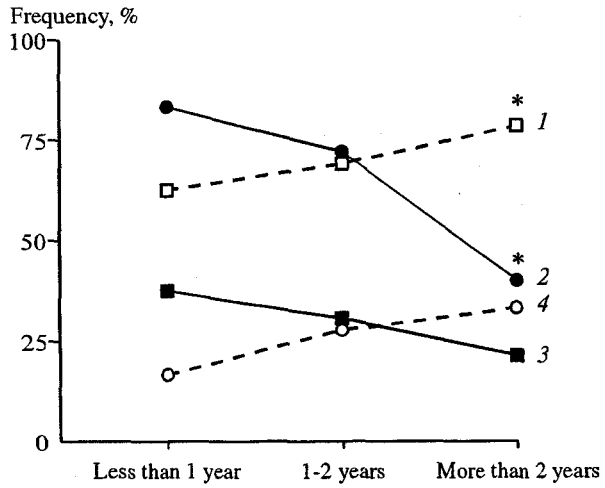

Fig. 1. Frequency distribution of patients with high (*1,2*) and low (*3,4*) SOD activities depending on the disease duration: SOD activities in the spinal fluid (*1,3*) and SOD1 activities in erythrocytes (*2,4*). *$p<0.05$ compared with patients with low SOD activity.

MATERIALS AND METHODS

We examined 88 patients (41 women and 47 men) aged 22-72 years (the average age was 51.2 years) with cervicothoracic (n=46), lumbosacral (n=13), and bulbar (n=29) forms of ALS. Mild and moderate alterations were observed in 41 patients, severe alterations were revealed in 47 patients. The mean history of ALC was 17 months (less than 1 year, 1-2 years, and 2-4 years in 46, 30, and 12 patients, respectively). Control group consisted of 50 healthy donors and 20 patients with acute surgical pathology. SF samples were taken during spinal anesthesia.

Whole blood samples (1.5 ml) were washed with 25 ml Hank's solution and centrifuged at 2000g and room temperature for 10 min. The erythrocyte pellet (1 ml) was suspended in 1 ml of deionized water and maintained in cold for 30 min to complete erythrocyte lysis. Heme was precipitated with 0.86 ml chloroform: ethanol mixture (3:5). SF samples (0.5-3 ml) were concentrated by ultrafiltration in a special cell (Amicon) using UM-10 membrane filters (Amicon) at 4 atm.

Activity of SOD in erythrocyte supernatants and SF concentrates was determined by the inhibition of epinephrine autoxidation. The concentration of adrenochrome formed was measured in a Perkin Elmer 555 spectrophotometer at 480 nm [6]. The enzyme quantity that induced a 50% inhibition of epinephrine autoxidation was taken as a unit of SOD activity. Data were analyzed statistically by Student's t test. Small samples were analyzed using nonparametric Wilcoxon—Mann—Whitney U-test. The frequency analysis was performed using antitrigonometric Fisher transformations.

RESULTS

Erythrocytic and total SOD activity in SF of patients with ALS significantly surpassed the control levels (Table 1). High erythrocytic SOD1 activity was found in patients with cervicothoracic and lumbosacral ALS. However, total SOD activity was increased in patients with all forms of the disease (Table 1).

The maximum activity of erythrocytic SOD1 was found in patients with ALS history less than 1 year (3.41±0.26 U/mg Hb). This activity then progressively decreased, while SOD activity in the SF increased ($p<0.05$) and attained the maximum in patients with ALS history more than 2 years (3.23±0.68 U/ml).

The frequency analysis demonstrated biochemical heterogeneity of ALS patients. Increased activity of erythrocytic SOD1 was found in 64% of patients, while SOD activity in the SF was increased in 73% of patients. High SOD1 activity was more often observed (83.3%) in patients with lumbosacral ALS characterized by longer history and more favorable prognosis and less frequently in patients with cervicothoracic and bulbar ALS. However, the greatest number of patients with high SOD activity in the SF had bulbar (81.8%) and cervicothoracic (75%) forms of ALS. As the diseases progressed, the number of patients with high SOD1 activity in erythrocytes and high SOD activity in the SF decreased and increased, respectively (Fig. 1). The frequency analysis of the distribution of patients in dependence on the severity of this

TABLE 1. SOD Activity in Patients with ALC ($M±m$)

Groups		SOD1 activity in erythrocytes, U/mg Hb	SOD activity in SF, U/ml
Control (n=50)		2.34±0.19	1.32±0.11
ALS	whole group (n=88)	2.91±0.24*	2.62±0.04*
	cervicothoracic form (n=46)	3.03±0.35*	2.83±0.54*
	lumbosacral form (n=13)	3.20±0.35*	2.62±0.35*
	bulbar form (n=29)	2.65±0.25	2.71±0.57*

Note. *$p<0.05$ compared with the control.

disease yielded similar results. Patients with mild and moderate forms of ALS had high SOD1 activities, while severe alterations were accompanied by an increase in the number of patients with high SOD activity in the SF (Fig. 2).

Thus, the changes in SOD activities in patients with ALS and their dependence on the form, duration, and severity of neurological disorders confirm the possibility that this disease can be accompanied by oxidative stress. The increase in cytosolic SOD1 should be considered as a protective reaction to enhanced $O_2^{\bullet}$ production in cells, while the increase in SOD activity in the SF probably reflects destructive process in motor neurons. In this case, the activation of extracellular SOD3 as the compensatory mechanism accompanying the decrease in intracellular SOD1 cannot be excluded. Low SOD1 activity in ALS patients can be associated with poor prognosis and results from SOD1 mutations or inactivation of the enzyme by free-radical compounds. The decrease in the activity of SOD1 with the development and generalization of the disease is a possible mechanism of ALS progression.

Authors are grateful to Prof. V. Z. Lankin for helpful discussion.

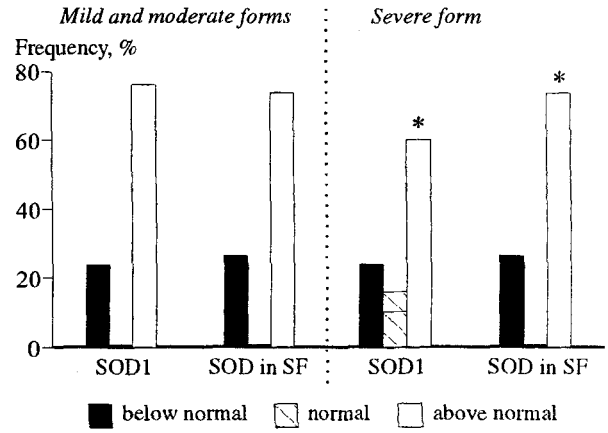

Fig. 2. Distribution of patients with high and low SOD activities depending on the severity of neurological disorders in patients with amyotrophic lateral sclerosis. *$p<0.05$ compared with the corresponding parameters in patients with mild and moderate alterations.

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