

Mitochondrial Superoxide Dismutase Activity in Amyotrophic Lateral Sclerosis

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Abstract: *Background:* Evidence suggests that mitochondrial dysfunction and oxidative stress may be involved in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). Some studies show the presence of altered anti-oxidative defence enzyme activity in the blood of ALS patients. It has also been demonstrated that a superoxide-dismutase-1 (SOD1) enzyme fraction is located in the mitochondria.

Objective: To Study the correlation between SOD1 activity and clinical data in ALS-patients.

Patients and Methods: SOD1, SOD2 and aconitase activity was analysed in mitochondria of platelets from nine sporadic-ALS (SALS) patients and nine healthy-controls. The biochemical data was compared and correlated with clinical data from the ALS-patients.

Results: Mitochondrial SOD1 activity was lower in ALS patients than in healthy controls, and much lower in more serious cases of ALS associated with respiratory insufficiency, weight-loss, low scores on the ALSFRS scale and the elderly.

Conclusion: Mitochondrial SOD1 activity may be involved in the clinical evolution of SALS.

Keywords: Amyotrophic lateral sclerosis, antioxidant enzymes, mitochondrial dysfunction, oxidative stress, superoxide-dismutase.

INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is the most common variant of motor neuron diseases affecting adults. The cause of ALS is not fully understood and several major and not mutually exclusive mechanisms of neurodegeneration may be involved. Only 10% of cases can be attributed to one specific molecular defect, such as a mutation in the SOD1-gene. However, oxidative stress is considered to be the common mechanism causing motor neuron death [1].

Studies of reactive oxygen species (ROS) generation and oxidative protein damage have been reported in both familial (FALS) and sporadic (SALS) forms of the disease [2]. SOD1 is a ubiquitously-expressed antioxidant metalloprotein that catalyzes the dismutation of superoxide to molecular oxygen and hydrogen peroxide. Recent research has demonstrated that a fraction of SOD1 is also located in mitochondria [3]. Mitochondria are the main source of energy and also the major source of ROS, and trigger the intrinsic pathway of apoptotic cell death. Several evidences suggest that mitochondrial dysfunction may be involved in the pathogenesis of ALS by metabolic impairment or by apoptosis [4-6]. However in ALS, oxidative stress and mitochondrial dysfunction may not be restricted only to the nervous system.

Some studies suggest the presence of altered antioxidative defence enzymes (ADE) activity in blood samples from ALS patients [7-9].

Our objective has therefore been to assess a possible alteration in mitochondrial ADE activity in blood samples from ALS patients. For this purpose, we determined mitochondrial SOD1 (Cu/Zn-SOD), SOD2 (Mn-SOD), TSOD (SOD1+SOD2) activities, SOD1-presence, and also aconitase activity since it is involved in mitochondrial energy metabolism. This study was carried out on mitochondria obtained from platelets.

- A) **Sample collection:** Blood samples (50 ml) were taken from 9 ALS patients and 9 healthy controls, after obtaining the informed consent. ALS patients were recruited at random, prospectively, within a preliminary study. Inclusion criteria were diagnosis of definite-ALS and the exclusion criteria were the presence of other consumptive diseases like cancer, systemic infectious or autoimmune diseases [10-11]. Controls were chosen paired by age and sex.
- B) **Clinical variables:** Clinical variables were recorded, with close attention to those variables relevant to the prognosis (age, time from onset, bulbar or spinal onset) and disease severity criteria, such as respiratory insufficiency (RI: PaO₂ <60 and/or PaCO₂>50 mmHg), weight loss (body mass index< 18.5), and ALSFRS scale [12-13].

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- C) Biochemical procedures: Platelet-rich plasma was extracted and centrifuged. Washed-platelets were submitted to hypo-osmotic shock, and the mitochondria released were isolated by differential centrifugation [14]. Proteins were determined by Bradford assay [15]; SOD-activity was analysed as described by Ukeda *et al.* [16]; and also aconitase activity [17]; Analysis of mitochondrial SOD1-presence and SOD1 electrophoretic migration, under partially denaturing SDS-PAGE conditions, were carried out by western-blot analysis [18]. The COX (Complex IV) subunit Vb was used as a mitochondrial protein loading control.
- D) Statistical Analyses: SPSS 6.1.2 was used to analyse and compare mean enzyme activity levels (Mann-Whitney test). Enzyme activity levels in ALS patients were analysed for correlation with the clinical variables.

RESULTS

A) Demographic and Clinical Variables (Table 1)

There were 9 ALS patients (7 male and 2 female) between 33-74 years of age (mean 57.6 ± 15), and 9 healthy controls (7 male and 2 female) between 31-80 years of age (mean 59.2 ± 16), with no significant difference in the sex and age of the two groups. Two of the ALS patients had the bulbar onset form of the disease and the remainder the spinal onset form. Disease severity: 5 had respiratory insufficiency and 4 weight loss, and the scores on the ALSFRS were between 13-28 points. The time since onset of the disease was between 8-96 months, and 3 patients had died when the study was performed.

B) Enzyme Activities (Table 2).

Mitochondrial TSOD activity was lower in the 9 ALS patients (335-554 mU/mg; mean 442 ± 74) than in the 9 healthy controls (740-995 mU/mg; mean 913 ± 82). The difference was due to a decrease in mitochondrial SOD1

activity with significantly lower values in ALS patients (20-148 mU/mg; mean 81 ± 52) than in controls (387-500; mean 439 ± 36 ; $p < 0.001$, M-W).

Aconitase-activity was lower in the 9 ALS-patients than in the healthy controls but less significant (136-405 mU/mg; mean 269 ± 124 ; $p < 0.05$, M-W) and was also related with age ($p < 0.05$, Spearman).

C) SOD1: Western-Blot and Denaturing Electrophoresis

Despite the differences observed in mitochondrial SOD1 activity between healthy controls and ALS patients, we found no significant difference in the level of SOD1 enzyme presence by western-blot. The COX (Complex IV) subunit Vb. was used as a mitochondrial protein loading control (Fig. 1). In addition, the partially denaturing SDS-PAGE electrophoresis of crude mitochondrial extract in the 9 ALS patients showed that mitochondrial SOD1 retained its association into homodimeric subunits.

D) SOD1 Activity and ALS Severity

In ALS patients, SOD1 activity was lower in cases 5, 6, 8 and 9, with more than one criterion of severity, such as respiratory insufficiency and weight loss (20-49 mU/mg; mean 30 ± 13 ; $p < 0.01$, M-W) and in patients (cases 5 and 6) with the bulbar onset form of ALS (20-25 mU/mg; mean 22.5 ± 3.5 ; $p < 0.05$, M-W). SOD1 activity values correlated with a low score (< 19) on the ALSFRS scale in the 9 ALS patients (20-148 mU/mg; mean 81 ± 52 and 13-28 ALSFRS; mean 20 ± 6 ; $p < 0.05$, M-W).

A relationship was also observed with age for aconitase, though less significant (136-405 mU/mg; mean 269 ± 124 ; $p < 0.05$, Spearman).

DISCUSSION

A significant decrease in SOD1 activity in mitochondria from ALS-patient platelets was found in this study. As others, we believe that this decrease in mitochondrial SOD1 activity could be involved in the presence of systemic oxida-

Table 1. Demographic and Clinical Variables

H-Controls			ALS Patients								
N°	Sex ¹	Age, Years	N°	Sex ¹	Age, Years	Time, Months	Survival, Months	Onset Form	RI ²	Weight Loss	ALS FRSS ³
1	M	65	1	M	60	36	Alive	Spinal	No	No	28
2	M	47	2	M	44	49	Alive	Spinal	No	No	28
3	M	74	3	M	71	8	Died, 11	Spinal	Yes	No	14
4	M	31	4	M	33	45	Alive	Spinal	No	No	20
5	F	69	5	F	71	12	Alive	Bulbar	Yes	Yes	16
6	M	65	6	M	64	12	Died, 14	Bulbar	Yes	Yes	14
7	M	37	7	M	37	16	Alive	Spinal	No	No	28
8	M	65	8	M	65	96	Alive	Spinal	Yes	Yes	21
9	F	80	9	F	74	26	Died, 29	Spinal	Yes	Yes	13
M SD		59.2 ± 16	M SD		57.6 ± 15	33.3 ± 27.8					20.2 ± 6.4

Sex¹: M: male, F: female; RI²: Respiratory insufficiency; ALSFRS³: ALS-Functional Rating Scale.

Table 2. Enzymatic Activities

Healthy Controls					ALS Patients				
N°	Aconitase mU/mg	SOD1 mU/mg	SOD2 mU/mg	TSOD mU/mg	N°	Aconitase mU/mg	SOD1 mU/mg	SOD2 mU/mg	TSOD mU/mg
1	535	450	520	970	1	400	135	302	437
2	216	430	310	740	2	397	148	340	488
3	260	460	490	950	3	136	85	400	485
4	413	387	443	830	4	389	123	431	554
5	355	420	575	995	5	165	25	335	360
6	420	400	500	900	6	189	20	357	377
7	535	480	490	970	7	405	120	389	509
8	650	500	400	900	8	136	49	286	335
9	205	425	540	965	9	202	25	410	435
M SD	398 ± 155	439 ± 36	474 ± 79	913 ± 82	M SD	268 ± 124	81 ± 52	361 ± 49	442 ± 74

W-B SOD1-presence in controls and ALS patients

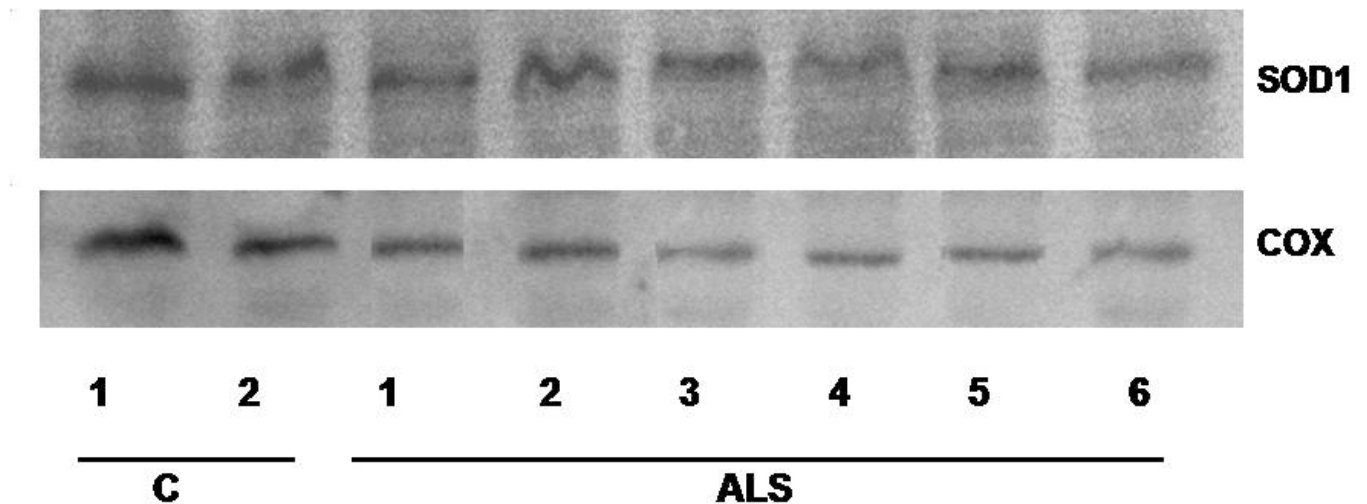


Fig. (1). Healthy controls and ALS patients with lowest activity were chosen. The COX (Complex IV) was used as a mitochondrial protein loading control.

tive stress, affecting the antioxidant enzyme potential of the blood cells [8-9]. This finding could be relevant in the pathogenesis of the disease since it could promote a mitochondrial oxidative stress that would lead to cell death by apoptosis, which is involved in both sporadic and familial ALS [2, 5-6]. SOD1-activity may also decrease due to a critical oxidation of thiol-groups [19]. In this preliminary study, we cannot demonstrate whether this decrease in mitochondrial SOD1 activity in ALS patients is a cause or a consequence of oxidative stress [20-21], nevertheless we believe that it corresponds to the latter since these are patients in terminal clinical situations.

We have also found a correlation between the decrease in SOD1 activity and the severity criteria of ALS (ALSFRS scale, respiratory insufficiency or weight loss), and with age. Some authors also suggest a relationship between the progression rate of ALS and reduced SOD1 activation [22].

Others consider the existence of chronic hypermetabolism in ALS occurring in parallel with a decrease in free fat mass, and both hypoxaemia and weight loss may increase oxidative pressure as the disease progresses [23]. Like other authors, we believe that narrow concept of ALS as a selective motor neuron disease is developing into a more general concept, in which this disease might be considered a multisystem disorder with selective vulnerability of motor neurons [24].

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