

ACTIVITIES OF ANTIOXIDANT DEFENSE ENZYMES IN THE BLOOD OF INDIVIDUALS WITH Leu144Phe MUTATION

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Summary: Activities of copper zinc superoxide dismutase (Cu,Zn SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and glutathione-S-transferase (GST) in the blood of familial amyotrophic lateral sclerosis patients with Leu144Phe mutation (FALS), asymptomatic carriers with Leu144Phe mutation and controls were studied. Activity of Cu,Zn SOD was significantly lower in the FALS patients and asymptomatic carriers than in controls ($p < 0.001$). In the FALS patients GSH-Px activity was lower ($p < 0.01$) and activity of GR was higher ($p < 0.001$) in comparison with controls. Canonical discriminant analyses provide statistical evidence that examined groups are different in the composition of antioxidant enzymes in blood and revealed that each component confers to observed difference. Our results suggest that oxidative stress is involved in pathogenesis of FALS and the activities of antioxidant enzymes are exposed to different kind of oxidative pressure in FALS patients, asymptomatic carriers and controls.

Key words: FALS, Leu144Phe mutation, antioxidant defense enzymes

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal progressive disorder characterized clinically by muscle wasting and weakness and pathologically by the relatively selective degeneration of the upper motor neurons in the motor cortex and lower motor neurons in the brain stem and spinal cord (1). Evidence of increased oxidative damage in sporadic form of ALS (SALS) and FALS (2) are not yet correlated with composition antioxidative defense and activities of antioxidant enzymes. Our previous results (3) showed that basic relations between the activities of antioxidant enzymes, as well as, their coordinated action against oxidative pressure in SALS patients were different com-

paring to healthy controls, and therefore suggests that antioxidant cocktail based on the activities of antioxidant enzyme correlation studies can be promising adjuvant treatment of ALS (4, 5).

In 5–10% of ALS patients there is a positive family history of the disease and in most of them the pattern of inheritance is autosomal dominant (6). More than 110 different mutations involving 50 of 153 amino acid residues have been reported in the gene encoding copper zinc superoxide dismutase (SOD1) of FALS patients (7). Subset of these is associated with a defect in gene encoding SOD1 account for approximately 20% of all FALS (8). Mase et al. (9) described the large family with a relatively not severe phenotype of FALS due to Leu144Phe SOD1 mutation. They found that the activity of Cu,Zn SOD in red and mononuclear cells was significantly reduced in three FALS patients (9). We described phenotype of the six Yugoslav families with the same mutation (10).

Since Cu,Zn SOD is only a part of a complex of antioxidative defense system, in this work we present results of determination the activities of antioxidant enzyme in erythrocytes and plasma of eleven FALS

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patients, five asymptomatic carriers and thirty-one matched healthy controls.

Material and Methods

Patients and controls

We examined the activities of antioxidant enzyme: copper zinc superoxide dismutase (Cu,Zn SOD, EC 1.15.1.1.), glutathione peroxidase (GSH-Px, EC 1.11.1.9.), catalase (CAT, EC 1.11.1.6.) and glutathione reductase (GR, EC 1.6.4.2.) in erythrocytes, as well as, the activity of glutathione-S-transferase (GST, EC 2.5.1.18) in the blood plasma of eleven Leu144Phe FALS patients (+/+) and five asymptomatic carriers with the same mutation without clinical signs of the disease (+/-) and control group (-/-) comprised of thirty-one age matched controls. ALS was diagnosed using standard criteria as suggested by the World Federation of Neurology, Subcommittee on Motor Neuron Disease (11). The clinical assessment using the Norris score was obtained in FALS patients (12). These examinations were provided in newly diagnosed ALS patients and at the time of the examinations, none of patients included in the study were receiving any medication. Serving as controls were a group of thirty-one age and sex matched patients with other neurological disorders (8 tension headache, 4 lumbar discus herniation, 19 healthy controls).

Biochemical procedures

Blood samples from ALS patients and controls were collected in heparinised glass tubes. The erythrocytes and plasma were separated by centrifugation (10 min, 5000 g, 4 °C). Aliquots of the three times washed erythrocytes were lysed by ice cold water. For Cu,Zn SOD estimation hemoglobin was removed by the method of Tsuchihashi (13), and the supernatant was used for the analysis. Erythrocyte Cu,Zn SOD was assayed by the method of Misra and Fridovich (14). CAT was assayed by the procedure of Beutler (15) and the activity was expressed in U/g Hb. GSH-Px was determined using t-butylhydroperoxide as a substrate (16), and the activity was expressed in $\mu\text{mol NADPH}/\text{min/g Hb}$. GR activity was assayed as suggested by

Glatzle (17) and expressed in $\mu\text{mol NADPH}/\text{min/g Hb}$. The hemoglobin in erythrocyte lysates was estimated by the method of Drabkin and Austin (18). GST activity was assayed in the plasma by the method of Habig (19), and expressed in nmol GSH/min/L plasma.

Statistical analyses

Statistical analyses were performed according to the protocols described by Hinkle et al (20) and Manley (21).

Results

Activities of SOD, CAT, GSH-Px GR and GST in blood of FALS patients, asymptomatic carriers and controls were determined.

The mean Cu,Zn SOD activity was significantly lower ($p < 0.001$) and GR activities was higher ($p < 0.001$) in FALS patients and in asymptomatic carriers in comparison with controls. Activity of GSH-Px was lower only in FALS patients than in controls ($p < 0.001$).

By canonical discriminant analysis it is possible to discriminate different categories (in this study analyzed groups) by the composition of the antioxidative defense components and find which component significantly confers this difference (22). In this way, FALS patients, asymptomatic carriers and controls were analyzed and a single statistically significant ($p < 0.001$) canonical discriminant function was obtained:

$$D1 = 0.99 \text{ Cu,Zn SOD} + 0.98 \text{ CAT} + 0.92 \text{ GSH Px} + 0.98 \text{ GST} - 0.93 \text{ GR } p < 0.001$$

According to the results of canonical discriminant function it might be concluded that all examined antioxidant enzymes in erythrocytes significantly confer to difference in antioxidant defense composition observed between compared groups. The results of this analysis showed that we are able to distinguish with certainty FALS patients, asymptomatic carriers and the controls.

Since canonical distribution of groups is presented in two-dimensional discriminant canonical space

Table I Activities of antioxidant enzymes in FALS patients, asymptomatic carriers and controls. Difference between groups was analysed by one-way analysis of variance (ANOVA) and post-hoc compared by Tukey's test for unequivocal number of examinees. Different letters (a, b) indicating presence of statistical significance between comparing groups.

	Leu144Phe FALS patients (+/+) (n=11)	Asymptomatic carriers (+/-) (n=5)	Controls (-/-) (n=31)
Cu,Zn SOD	2830 \pm 609 ^a	2857 \pm 538 ^a	3888 \pm 503 ^b
CAT	17.6 \pm 3.3	19.3 \pm 3.4	21.3 \pm 3.6
GSH-Px	14.7 \pm 3.7 ^a	17.7 \pm 2.3 ^{ab}	18.7 \pm 2.7 ^b
GR	5.2 \pm 0.9 ^a	5.6 \pm 0.7 ^a	4.2 \pm 0.9 ^b
GST	4.1 \pm 0.8	4.6 \pm 1.1	5.0 \pm 0.9

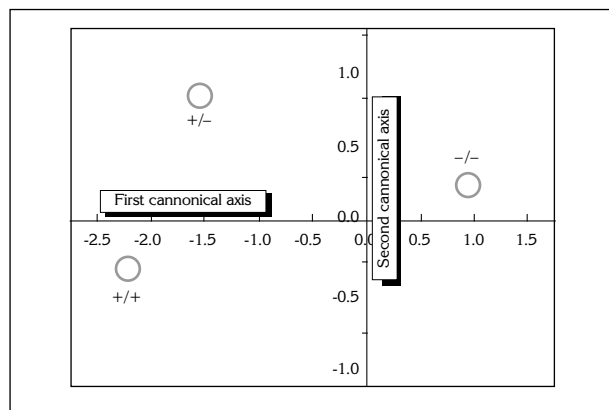


Figure 1 Two dimensional canonical discriminant space for the location of analyzed groups

(named as X and Y function), it is worth mentioning that left canonical space is for groups carrying mutation on Cu,Zn SOD, that is to say there are similarities between them comparing to controls with adequate Cu,Zn SOD protein.

Discussion

Our previous results in study of SALS patients showed that disease factors changed relations between antioxidant enzymes components and that antioxidant defense system in ALS patients could be characterized as different composed comparing to healthy individuals (3). From our present result, it is obvious that FALS patients have exchanged antioxidant enzymes relations in comparison with controls. Differences in response to

oxidative stress between groups of FALS patients and asymptomatic carriers may be the cause of observed viability in disease progression demonstrated for Leu144Phe mutation. Viability in the age of onset, disease progression, rate and initial signs of the disease, both within and between families has been demonstrated for the Leu144Phe mutation (5). Similar-viability in disease progression – was found among sporadic ALS patients.

Our results in this paper showed, that the Cu,Zn SOD activity is significantly lower also in asymptomatic carriers in comparison to controls. Cu,Zn SOD activity determined from the hemolysate of the FALS patients was significantly reduced when compared to the controls and this is in accordance with results of Mase et al 2001 (9). We noticed in FALS patients significantly increased of GR and decreased of GSH-Px and GST activities in comparison with controls. Since this phenomenon was not found in asymptomatic carriers, it could be attributed to specific oxidative factors induced in ALS pathophysiology.

Although Cu,Zn SOD activity is significantly decreased in asymptomatic carriers, it is not enough to induce significant changes in the activities of other antioxidant enzymes, but changes in the composition of antioxidant defence system in asymptomatic carriers are obvious. Pathological process in FALS patients is correlated with further changes in antioxidant defense composition.

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AKTIVNOST ANTIOKSIDACIONIH ZAŠTITNIH ENZIMA U KRVI LJUDI SA Leu144Phe MUTACIJOM

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Kratak sadržaj: U ovom radu ispitivana je aktivnost: bakar cink sadržavajuće superoksid dismutaze (Cu, Zn SOD), katalaze (CAT), glutation peroksidaze (GSH-Px), glutation reduktaze (GR) i glutation-S-transferaze (GST) u krvi pacijenata sa familijarnim oblikom amiotrofične lateralne skleroze (FALS) sa mutacijom Leu144Phe, asimptomskim nosiocima mutacije Leu144Phe i kontrola. Aktivnost Cu,Zn SOD je statistički značajno niža kod FALS pacijenata i asimptomskih nosioca mutacije Leu144Phe nego kod kontrola ($p < 0,001$). Kod FALS pacijenata aktivnost GSH-Px je niža ($p < 0,01$), a aktivnost GR je veća ($p < 0,001$) u poređenju sa kontrolnom grupom. Kanonijska diskriminantna analiza obezbeđuje statističku podršku uočene razlike u sastavu antioksidacionih zaštitnih enzima u krvi ispitivanih grupa i pokazuje nam da svaka komponenta značajno doprinosi toj razlici. Naši rezultati sugerišu da je oksidacioni stres uključen u patogenezu FALS i da su antioksidacioni zaštitni enzimi izloženi različitom oksidacionom pritisku kod FALS, asimptomskih nosioca mutacije Leu144Phe i kontrola.

Cljučne reči: FALS, Leu144 mutacija, antioksidacioni zaštitni enzimi

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