Impaired oxidative metabolism in exercising muscle from ALS patients

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Abstract

The pathogenic mechanism of selective loss of motor neurones in amyotrophic lateral sclerosis (ALS) is still poorly understood. Recently, research evidence has suggested that mitochondrial dysfunction occurs in central nervous system as well as in peripheral tissues from ALS patients. The aim of our study was to indirectly investigate in vivo oxidative metabolism of exercising muscle in a case history of patients affected by ALS. To this purpose 11 patients, 8 male and 3 female, mean age \( \pm \) SD: 52.4 \( \pm \) 11.1 years, performed a bicycle incremental test for the assessment of lactate production. At rest, there was increased lactate concentration in patients: 2.77 \( \pm \) 0.79 vs. 1.48 \( \pm \) 0.49 mmol/l in normal controls (normal range: 0.67–2.47 mmol/l). Analysis of lactate curve during exercise showed a lactate production increase compared to controls. Furthermore, anaerobic lactate threshold was detected at 40–50% of the predicted normal power output, anticipated with respect to both normal subjects and non-ALS chronically denervated controls with comparable motor impairment (60–70%), suggesting that mitochondrial dysfunction can occur in exercising skeletal muscle from ALS patients.

Keywords: ALS; Mitochondria; Exercise; Lactate

1. Introduction

In amyotrophic lateral sclerosis (ALS), a disorder characterised by a degeneration of the anterior horn cells of the spinal cord and cortical motor neurons, pathogenetic mechanisms underlying motor neurone loss are still unclear. Current knowledge on this matter points out on the hypothesis that excitotoxicity contributes to selective motor neuron loss [1], mainly by alterations in synaptic glutamate turnover mechanisms [2,3]. Among other pathogenic hypotheses in ALS, also oxidative stress theory has been put forward [4,5]. It is mainly sustained by the discovery of cytosolic copper–zinc superoxide dismutase (SOD-1) gene mutations in 15–20% of familial ALS [6] and the observation, although not invariable, of increased generation of reactive oxygen species in ALS [7–10], a key step for abnormal peroxidation and nitrosylation of neuron macromolecules [11,12]. In both the abovementioned instances, the role of mitochondria appears intriguing but a body of data suggests it not to be irrelevant.

Observations on the implication of mitochondria in ALS include motor neuron morphological abnormalities in transgenic mice carrying Cu–Zn SOD mutations [13] and, in humans, morphological abnormalities and reduced cytochrome c oxidase activity in anterior horn motor neurons [14]. Also, there are evidences that mitochondrial dysfunction can occur in skeletal muscle of ALS patients, in which respiratory chain defects and decreased mitochondrial manganese SOD activity have been found [15,16]. Furthermore, there are reports in single cases of ALS on the occurrence of mtDNA alterations such as multiple deletions [17] or an out-of-frame mutation in cytochrome c oxidase subunit I gene [18].

The aim of the present study has been to provide further evidence on the implication of mitochondrial dysfunction in the pathogenesis of ALS, by indirectly investigating oxidative metabolism in exercising skeletal muscle. To do that, we assessed lactate kinetics, which is one of the main markers of in vivo mitochondrial function of contracting muscle, in ALS patients performing submaximal incremental exercise and compared the results to normal subjects as well as to non-ALS chronically denervated controls with comparable motor impairment.
2. Materials and methods

2.1. Patients

The study was performed on 11 patients, 8 male and 3 female, mean age ± SD: 52.4 ± 11.1 years. Definite (n = 6) or probable (n = 5) ALS diagnosis was made on the basis of El Escorial criteria [19]; three cases were affected by familial ALS and eight by the sporadic form. All patients presented a classic form of ALS, two subjects showed predominant second motor neurone signs, and another case revealed predominant bulbar involvement. The mean duration of illness was 13.8 ± 12.8 months.

Multifocal conduction blocks were excluded by electromyography. Cerebrospinal as well as serum levels of anti-GM1 anti-bodies were within the normal range. No mutation in the five exons of Cu–Zn SOD was detected by DNA SSCP technique.

In the selection of patients undergoing study protocol, it was cared that nobody of them had a history of cardiac or pulmonary disease. Furthermore, their forced vital capacity (FVC) was at least 70% of the predicted value and arterial pO₂, as detected by hemogasanalysis, within the normal range. Biochemical parameters, such as liver function tests and creatine kinase, were within the upper normal range. No patient had a history of diabetes or thyroid disease. All patients had a normal resting electrocardiogram.

Clinical characteristics of ALS patients are summarized in Table 1.

2.2. Exercise protocol

In order to evaluate aerobic metabolism in exercising muscle, all patients underwent to an incremental workload exercise on cycloergometer (Ergocard III, Esaote Biomedical). The Committee on Human Experimentation of our Institution approved the exercise study protocol. Each subject gave his informed consent after having been explained the purposes and procedures of the study. Patients, at least 3–4 h after a normal mixed-diet meal, were then invited to comfortably stay and relax in the exercise room for at least half an hour before the exercise, according to a protocol already used in our laboratory and reported elsewhere [21]. Briefly, all subjects performed a series of 3-min exercise bouts, at a pedalling rate of 60–70 rpm, interspaced with 2-min rest intervals, at increasing workloads. The exercise started at 10% of predicted normal maximal power output (pnPOmax), defined for each patients on the basis of his/her sex, age, weight and height [22]; and then, through successive increments of 10% of pnPOmax, brought to the highest work level at which cycling could be maintained for 3 min; this figure, when expressed in watts, was taken as the real maximum power output (rPOmax). The choice of such a protocol was based on the assumption that the exercise is mainly aerobic at the beginning of the test and then progressively anaerobic as the power output increases, due mainly to the recruitment sequence of slow and fast motor units [23].

Consecutive blood samples were collected from an antecubital vein for lactate dosage in the basal conditions, halfway during each resting period between successive exercise bouts, and during the recovery, 1, 15 and 30 min after the end of the exercise. Venous lactate levels were assessed spectrophotometrically on an ERIS Analyzer 6170 (Eppendorf Geratebau, Hamburg, Germany), reference values being 0.67–2.47 mmol/l. Anaerobic lactate threshold (LT) was defined as the exercise power output level at which the slope of the best-fit lactate curve begins to rise exponentially [1], and expressed as the percentage of the pnPOmax. Heart and ventilation rate as well as capillary hemoglobin O₂ saturation (Radiometer, Copenhagen, Denmark) were assessed under basal conditions and during exercise.

Nine untrained healthy volunteers, seven males and two females, mean age: 50.3 ± 5.4 years, as well as eight

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**Table 1**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex/age onset (yrs)</th>
<th>Disease duration (months)</th>
<th>Bulbar involvement</th>
<th>BMRC</th>
<th>O-T reflexes</th>
<th>ALS- FRS</th>
<th>FVC (%)</th>
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*Assessed on six pairs of lower limb muscles: hip, knee and ankle flexors/extensors (maximum score = 60).

*Osteo-tendon reflexes at lower limbs: +/– = increased/decreased. For the other abbreviations see the text.
patients affected by chronic denervating process (CD group), i.e. chronic inflammatory demyelinating polyneuropathy or multifocal motor neuropathy, with motor involvement of lower limbs comparable to ALS patients as from muscle BMRC score, age range: 47–63 years, were recruited as comparison groups for exercise testing and blood lactate measurements. Patients in CD group had not taken medication in the 3 months before the study while in all of them EMG analysis was characterized by the presence of discrete active muscle denervation in lower limbs in form of sharp waves and fibrillation and/or fasciculation potentials. The same criteria as for ALS patients were adopted to calculate pnPOmax in these two groups.

2.3. Data analysis

Goodness-of-fit models, in the sense of minimal square residuals, were utilised to analyse all curves. After Kolmogorov–Smirnov test had confirmed that our data did not show a Gaussian distribution, non parametric analysis was
selected. The Kruskal–Wallis test was utilised in order to estimate differences between patients and control subjects. Relationships between considered parameters were tested by the Spearman rank correlation test and a regression analysis. In all tests, we have considered a significance level of 0.5 %.

3. Results

rPOMax ranged from 50% to 80% of the pnPOMax in ALS patients, compared to 80–100% in controls and 60–80% in CD group.

In both ALS patients and CD group, mean lactate basal value was significantly higher than in normal controls (respectively, 2.77 ± 0.79 and 2.79 ± 1.29 mmol/l vs. 1.48 ± 0.49 mmol/l; Fig. 2). At rest, capillary hemoglobin O₂ saturation was within the normal range (95–98%) in all groups.

As effect of the exercise, both mean normalized peak lactate and its correspondent mean absolute value in ALS patients were significantly higher compared to CD group (respectively, 264 ± 36.4% vs. 196 ± 22.4%, of the basal lactate, Fig. 1, and 7.4 ± 1.8 mmol/l vs. 5.0 ± 1.1, Fig. 2), at 70% of the pnPOMax. For that level of exercise, these values in normal controls were 271 ± 14.2% and 3.7 ± 0.8 mmol/l, respectively.

When examining exercise lactate curves, LT was achieved at 40–50% of the pnPOMax (absolute LT lactate value: 4.9 ± 1.5 mmol/l) in ALS patients, compared to 60–70% in CD and normal control groups (absolute LT lactate values, respectively, 4.2 ± 0.7 and 2.5 ± 0.9 mmol/l; Figs. 1 and 2).

Finally, at the end of the recovery period from exercise, lactate values were still higher, even if not significantly than baseline, only in ALS patients: 3.6 ± 0.9 mmol/l, while they approached rest values in the two other groups of subjects.

Mean value of exercise maximum heart rate increased by 68% of the basal value in ALS group and 65% in CD group. No significant changes in capillary hemoglobin O₂ saturation throughout the exercise protocol were observed in all groups.

No correlation was found between rest or exercise lactate levels and the FRS and FVC scores or rPOMax.

4. Discussion

Although aetiopathogenesis of the neuronal death in ALS remains unclear, the hypothesis that mitochondria are involved in the degeneration process of motor neurons is gaining increasing evidence. Mitochondria are implicated in both necrotic and apoptotic cell death pathways [24]. In fact, functional loss of mitochondria respiratory chain integrity is the main determinant of oxygen radical-associated cell damage by leading to in excess production of reactive oxygen species (ROS) [25]. A recent editorial overview [26] underlines possible ways by which mitochondria involvement, primary or secondary occurring, can play a significant role in the pathogenesis of neuronal damage in ALS.

In human studies, evidences indicating mitochondrial dysfunction in sporadic ALS include finding of mitochondrial morphological abnormalities [27] and reduced cytochrome c oxidase activity [14] in anterior horn neurons. Data obtained in tissues different from central nervous system seem to indicate that this mitochondrial dysfunction in ALS is a more generalized one. In fact, impaired response to oxidative phosphorylation inhibitors has been observed in peripheral blood lymphocytes [28] and, overall, a number of morphological, biochemical and molecular mitochondrial abnormalities has been reported in skeletal muscle of ALS patients [15–18]. Consistent with the abovementioned data, the recent observation of reduced levels of membrane-associated mitochondrial manganese superoxide-dismutase in skeletal muscle biopsy of patients with sporadic ALS could reinforce the hypothesis of a mitochondrial related increased risk of cell exposure to ROS in this disease [16].

Taking these considerations into account, we designed this study in order to evaluate the oxidative metabolism of exercising muscle in ALS patients. As one of the main markers of in vivo functional mitochondrial impairment is excessive muscle lactate production during submaximal exercise, we studied venous lactate kinetics during incremental exercise. In this condition, the anaerobic lactate threshold (LT), the exercise workload or oxygen consumption at which the accumulation of lactate assumes an exponential trend, can be considered the main marker of switching on from aerobic to anaerobic muscle metabolism [23]. In our patients, we observed an abnormal accumulation of lactate both in rest condition and during the exercise, significantly higher than controls.

In the interpretation of these results, some considerations have to be taken into account. First of all, it has to be reminded that some previous works [29] have showed an increased lactate concentration at rest and during exercise in patients with non ALS-related chronic denervation; this was accompanied by an augmented utilization of glycolytic–glycolytic-borne fuels by denervated muscles, probably as consequence of muscle deconditioning and regional ischemia accompanying reduced physical activity of a denervating process. Despite a control group of spinal muscular atrophy patients, the same bias should be taken into account for histological data reported by Vielhaber et al. [15] in ALS muscle biopsy, indicating core-like appearance of myofiber cytochrome c oxidase defect. However in the present study, consistent with our conclusions, LT of ALS patients was achieved at workload of 40–50% of the pnPOMax, anticipated with respect to both normal and non-ALS chronically denervated controls: 60–70%, indicating a precocious activation of the anaerobic metabolism.
in these patients. Moreover, our data do not show any correlation in ALS patients between lactate levels and either their functional rating scales or the real maximal power output detected in increasing exercise, confirming that muscle deconditioning cannot per se justify the observed results of lactate kinetics.

Also, considering the differences of absolute lactate values between our ALS patients and age-matched controls, it is unlikely that the observed results can merely be explained on the basis of the ageing process, per se responsible in normal skeletal muscle of consequences such as sarcopenia and mtDNA common deletion accumulation [30]. Finally, although some ALS patients showed a degree of compromise of baseline respiratory function and we could not measure their partial oxygen gas pressure in arterial blood, the absence of significant modification in capillary hemoglobin O2 saturation, for the level of exercise that the patients could perform, leads us to reasonably exclude that the observed impairment of FVC played a major role in determining the present results.

In conclusion, our data are consistent with the occurrence of functional oxidative metabolic impairment in exercising muscle of ALS patients. This result is in agreement with the hypothesis that mitochondria function can be involved in ALS and at the same time reaffirms that further studies will be of considerable interest in attempting to understand the role of mitochondria in the pathogenesis of ALS.

References


