Altered Lactate Kinetics from Exercising Muscle in Hereditary Spastic Paraplegia

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Abstract

Hereditary spastic paraplegia (HSP) is a group of familial neurodegenerative disorders characterized by progressive lower limb spasticity and weakness due to degeneration of corticospinal axons. These disorders are classified both genetically, according to the mode of inheritance, and clinically, as pure and complicated forms. Recently the discovery that paraplegin, the defective protein in autosomal recessive HSP-SPG7, localizes in mitochondria allowed to foster the hypothesis that some mitochondrial dysfunctions can play a pathogenic role in HSP.

The aim of our study was to indirectly evaluate oxidative metabolism in contracting muscle, by assessing the anaerobic lactate threshold in 7 patients (5 M and 2 F, mean age 48.0±13.9 yrs) affected by HSP, both autosomal dominant or sporadic, during an incremental bicycle exercise.

Analysis of venous lactate curve showed that lactate levels were significantly higher than in controls (peak normalised lactate: 378.8 vs. 271%, p<0.01). Furthermore, an early threshold of lactate was detected only in HSP patients.

Even if other factors such as chronic spasticity or muscle deconditioning have to be taken into account in the interpretation of our data, these results suggest possible occurrence of mitochondrial involvement in skeletal muscle of HSP patients.

Key words: exercise test, lactate, mitochondria, paraplegin.

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Hereditary spastic paraplegia (HSP) is a group of familial neurodegenerative disorders characterized by progressive lower limb spasticity. These disorders are classified both genetically, according to the mode of inheritance, as autosomal dominant, autosomal recessive and X linked, and clinically, as pure and complicated forms [7, 8]. The complicated form is characterized by the association of progressive lower limb spasticity and other neurological abnormalities such as optic neuropathy, retinopathy, extrapyramidal disturbances, dementia, ataxia, ichthyosis, mental retardation and deafness. Also sporadic cases of spastic paraplegia can be included in HSP group, when other possible causes of pyramidal system involvement are ruled out.

There is evidence that HSP can be, at least in some instances, a mitochondrial dysfunction [19]. This is the case of the autosomal recessive SPG7 form in which a mutation of a nuclear gene encoding for a mitochondrial protein has been reported [5]. In fact, the product of the SPG7 gene is a protein of 795 amino acids, called paraplegin, which is highly homologous to a class of yeast ATP-dependent zinc metalloproteases [5] and has been demonstrated to specifically localize into the mitochondria in immunofluorescence transfected COS-7 cells [16]. Furthermore, analysis of muscle biopsies demonstrated that individuals with paraplegin mutation show typical signs of mitochondrial involvement suggesting the presence of impaired oxidative phosphorylation in these cases [5, 16].

Therefore aim of this study was to evaluate in patients affected by HSP oxidative metabolism in exercising muscle by the assessment of blood lactate kinetics during an incremental workload exercise.

Materials and Methods

The study was performed on seven patients affected by HSP, age range 31-63 yrs, mean age and standard deviation 48±13.9, 5 male and 2 female. Diagnosis was
Exercise lactate in Hereditary Spastic Paraplegia

clinically and neurophysiologically performed according to the accepted criteria [4, 10] after having excluded other possible causes for spastic paraplegia. Three patients were affected by the dominant form of HSP, while the other four were sporadic cases.

Neurological examination of HSP patients revealed normal upper-extremities muscle tone and strength. In the lower extremities, muscle hypertonicity and weakness were present in 7 patients and in 5 patients respectively. Deep tendon reflexes could be brisk in the upper extremities, but were pathologically increased in the lower extremities in all patients. Ankle or knee clonus and extensor plantar responses were present in 5 patients. Impaired vibration sense was detected in 1 patient. No urinary incontinence was reported. Pes cavus, nystagmus, dysarthria and dysdiadochokinesia were present in 2 patients (Table 1). Gait was always markedly abnormal with typical spastic scissoring and circonduction.

The clinical criteria for inclusion of these patients in the study were the following:

1) Mild degree of neurological state: the patient could manage an autonomous life (Activity of Daily living score 2 for each item [21]) and would theoretically be able to perform the test exercise proposed (MRC and Ashworth modified scores respectively 4 [3] and 2 [2, 21]).
2) Absence of cardiac and respiratory involvement, as assessed by means of ECG and cardiac ultrasound scan, chest X-ray and spirometric testing.
3) Absence of joint or bone deformities.
4) Body weight never above 20% of theoretical anthropometric value.

Exercise Protocol

An electrically braked pedal-rate bicycle ergometer (Bik, Elettronica Trentina, Italy) was used to have subjects performing a series of 3-min exercise bouts starting at a minimum pedaling rate of 60-70 revolutions per minute and increasing the workload after 2-min rest interval. Such bout duration was chosen to obtain steady-state blood lactate levels [23]. The predicted normal maximal power output (pnPO\textsubscript{max}) was provisionally defined for each patient on the basis of his/her sex, age, weight and height. The exercise started with a first bout at 10% of the pnPO\textsubscript{max} and then, through successive increments of 10% of pnPO\textsubscript{max}, was brought to the highest work level at which cycling could be maintained for 3 min; this figure was at least 60% of pnPO\textsubscript{max} and, when expressed in watt, was taken as the actual or real maximum power output (rPO\textsubscript{max}). The choice of such a protocol was based on the assumption that 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.

Apart from heart and respiratory function indexes, blood lactate was assessed spectrophotometrically on an ERIS Analyzer 6170 (Eppendorf Geratebau, Hamburg, Germany) under basal conditions and during exercise from an antecubital vein, at each interbout interval.

In order to determine the lactate threshold (LT) for each case, we calculated the exercise power output level at which the slope of the best-fit lactate curve began to rise exponentially. Blood levels of lactate were compared to those from a group of age and sex matched normal volunteers assessed by the same exercise protocol performed up to 70% of their pnPO\textsubscript{max}, a level of exercise corresponding to LT in normals.

Statistical Analysis

Goodness-of-fit models, in terms of minimal square residuals, were utilized to fit all curves of lactate. After a Kolmogorov-Smirnov test confirmed that the data did not present a Gaussian distribution, non-parametric analysis was selected. In particular, the Mann-Whitney test was utilized in order to estimate differences between patients and normal subjects. In all tests, we have considered a significance level of 0.5%.

Results

Mean rest values of lactate in HSP patients was 2.32±0.19 vs. 0.89±0.10 mmol/L in controls (p<0.05).

Table 1. Clinical features in HSP patients (n=7).

<table>
<thead>
<tr>
<th>SIGNS</th>
<th>N. PATIENTS</th>
</tr>
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<tbody>
<tr>
<td>Spasticity of the lower limbs</td>
<td>7</td>
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<tr>
<td>Weakness in the lower limbs</td>
<td>5</td>
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<tr>
<td>Deep tendon hyperreflexia</td>
<td>7</td>
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<td>Clonus</td>
<td>5</td>
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<tr>
<td>Extensor plantar reflexes</td>
<td>5</td>
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<tr>
<td>Impaired vibration sense</td>
<td>1</td>
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<td>Urinary incontinence</td>
<td>0</td>
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<tr>
<td>Pes cavus</td>
<td>2</td>
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<tr>
<td>Nystagmus (horizontal)</td>
<td>2</td>
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<tr>
<td>Dysarthria</td>
<td>2</td>
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<td>Dysdiachokinesia</td>
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Figure 1. Lactate curve in HSP patients and controls (normalised mean values and standard deviation).
Exercise lactate in Hereditary Spastic Paraplegia

In the HSP patients the rPO\textsubscript{max} ranged 60 to 70% of the pnPO\textsubscript{max}. Mean lactate values, normalized to rest values, were significantly higher in HSP patients than in controls from the 3\textsuperscript{rd} step of the exercise (30% of pnPO\textsubscript{max}) onward (p<0.05 at 30% of the pnPO\textsubscript{max}, p<0.01 from 40 to 70%).

As expected in controls the slope of the lactate curve reached an exponential trend at the 70% of pnPO\textsubscript{max}.

In HSP patients, we could observe a steady state level at 60% of pnPO\textsubscript{max} followed by a second sharp increment of lactate values, the LT, in the successive step. Furthermore in HSP patients an “early threshold” was achieved at exercise level as high as 30% of pnPO\textsubscript{max} (Fig. 1).

Differently from controls, lactate did not recover to basal values at 30 minutes after the cessation of exercise.

When analyzing the single cases, only one case differed from the average of HSP patients in showing a lactate curve similar to that of controls.

Discussion

Hereditary spastic paraplegia is a genetically heterogeneous group of neurodegenerative disorders, which are classified, according to clinical criteria, as pure and complicated forms. The clinical features of the pure form consist of progressive weakness and spasticity of the lower limbs. The complicated forms are characterized by the presence of additional neurological and non-neurological symptoms such as mental retardation, peripheral neuropathy, amyotrophy, ataxia, retinitis pigmentosa, optic atrophy, deafness and ichthyosis. The genetic heterogeneity is based on different patterns of inheritance, so that autosomal dominant, autosomal recessive and X-linked forms can be distinguished. Even if linkage analysis studies have assigned the gene locus in several forms of both autosomal and recessive HSP, only in autosomal recessive HSP-SPG7 the gene product, a protein of 795 amino acids, the paraplegin, highly homologous to a class of yeast ATP-dependent zinc metalloproteases, has been characterized. The functional role of paraplegin is still unknown but there are evidences that this protein can be involved in mitochondrial functioning. In fact it has been demonstrated that paraplegin localizes in mitochondria, where it could deserve both proteolytic and chaperon-like activities [5]. This supports the hypothesis that mitochondria involvement can be a keystone in the pathogenesis of HSP.

As one of the main markers of in vivo functional mitochondrial impairment is excessive muscle lactate production during submaximal exercise [1, 15], we studied venous lactate kinetics during incremental exercise by assessing the anaerobic lactate threshold, the exercise workload or oxygen consumption at which the accumulation of lactate assumes an exponential trend [13, 14]. In our patients we observed an abnormal accumulation of lactate during the exercise, significantly different from controls, starting from 30% of the pnPO\textsubscript{max}. Nevertheless the LT was achieved at work load of 60-70% of the pnPO\textsubscript{max} comparable to normal controls. The association of increased lactate values and normal LT can indicate that at this level of exercise an impaired removal of lactate can occur. Interestingly, in HSP patients, we also observed a significant increment of mean lactate precociously with respect to the LT, at the exercise interstep 30-40% of the pnPO\textsubscript{max}. This increment, may be the expression of an “early threshold”, has previously been observed in normal subjects [13, 17, 20] and has been attributed to additional recruitment of glycogenolitic fibres at low levels of muscle contraction [6, 18] during incremental exercise [12]. This threshold makes it possible to distinguish between patients and controls.

In conclusion these results generally support the hypothesis that mitochondrial impairment can occur in exercising muscle in HSP patients. However, in the interpretation of our data, other factors have to be taken into account, with particular reference to the deconditioning effect of disuse [22] and to the consequences of chronic spasticity on structural and functional phenotypic expression of skeletal muscle [11]. Investigative approaches, including muscle biopsies, aimed to deeply characterize morphologic and functional aspects of skeletal muscle in HSP patients can further shed lights on pathophysiology of motor impairment in these patients.

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Exercise lactate in Hereditary Spastic Paraplegia


