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Clinical Study

Functional relevance of mitochondrial abnormalities in sporadic inclusion body myositis

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ABSTRACT

Cytochrome c oxidase (COX)-deficient fibers and multiple mitochondrial DNA (mtDNA) deletions are frequent findings in sporadic inclusion body myositis (s-IBM). However, the functional impact of these defects is not known. We investigated oxygen desaturation during exercise using the forearm exercise test, accumulation of lactate during exercise using a cycle ergometry test and mitochondrial changes (COX-deficient fibers, biochemical activities of respiratory chain complexes, multiple mtDNA deletions by long-range polymerase chain reaction) in 10 patients with s-IBM and compared the findings with age and sex-matched normal and diseased controls (without mitochondrial disorders) as well as patients with mitochondrial disorder due to nuclear gene defects resulting in multiple mtDNA deletions (MITO group). The mean age of the s-IBM patients was 68.2 ± 5.7 years (range: 56–75). Patients with s-IBM had statistically significantly reduced oxygen desaturation (ΔsO_2) during the handgrip exercise ($p < 0.05$) and elevated peak serum lactate levels during cycle ergometry compared to normal controls ($p < 0.05$). The percentage of COX-deficient fibers in s-IBM and MITO patients was significantly increased compared to normal controls ($p < 0.01$). Five out of nine s-IBM patients had multiple mtDNA deletions. Thirty-three percent of s-IBM patients showed an increased citrate synthase content and decreased activities of complex IV (COX). The biochemical pattern of respiratory chain complexes in patients with s-IBM and MITO was similar. Histopathological analysis showed similar changes in s-IBM and MITO due to nuclear gene defects. Functional tests reflecting mitochondrial impairment suggest a contribution of mitochondrial defects to disease-related symptoms such as fatigue and exertion-induced symptoms.

1. Introduction

Mitochondrial changes are common features in sporadic inclusion body myositis (s-IBM) [1–7]. In s-IBM, aggregation of proteins is associated with their inadequate disposal and is thought to provoke endoplasmic reticulum stress, proteasome inhibition, decreased lysosomal degradation, and mitochondrial abnormalities in myofibers [8–9]. In s-IBM the small deletions in mitochondrial DNA (mtDNA) are clonally expanded and seen segmentally along the muscle fibers as abnormal mitochondria with deficient cytochrome c oxidase (COX) activity [2]. The frequency of COX-deficient fibers was found to range from 1.2 to 8.6% [5–7], whereas multiple mtDNA deletions are detected in most s-IBM patients [2–6]. Analysis of the deletion breakpoints of mtDNA deletions in s-IBM revealed similarities to what is found in normal aging [5,10]. However, the frequency of mtDNA changes in s-IBM seems to be low and is not considered to lead to a prominent impairment of overall oxidative metabolism in skeletal muscle [11]. Therefore the contribution of these oxidative disturbances to symptoms such as fatigue and weakness is not clear.

We measured the biochemical activity of respiratory chain enzymes and performed two functional tests of oxidative metabolism [12] in patients with s-IBM and sex and age-matched individuals.

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2. Patients and methods

2.1. Experimental protocols

2.1.1. Patients

Ten patients with clinical and light and electron microscope confirmed s-IMB were included. All the patients met the Griggs's classification criteria for definite s-IBM and the consensus criteria of the European Neuromuscular Centre [13]. They even exceeded the criteria, as ultrastructural confirmation of the diagnosis was achieved. For comparison, a diseased control group included age-matched patients with a genetically defined myopathy (without mitochondrial disorders), either facioscapulohumeral muscular dystrophy (n = 4) or myotonic dystrophy type 1 (n = 3). For histological, biochemical and molecular genetic comparison, available biopsy material from five age-matched patients with multiple mtDNA deletions with a genetically confirmed nuclear defect, i.e. MITO (*PEO1* [n = 4], *POLG1* [n = 1]) were also analysed (''MITO'' group). The normal controls were age-matched healthy volunteers (functional test, n = 9; histological, biochemical and molecular genetic analysis, n = 20).

Written consent for participation in the study was obtained from all subjects. The study was approved by the Ethics Committee of Martin-Luther University of Halle (Saale), Germany. None of the patients regularly took steroids, valproic acid, acetylsalicylic acid, or biguanides.

2.1.2. Non-ischemic forearm exercise test

All subjects performed standardized tests under aerobic, non-ischemic exercise conditions with intermittent isometric contractions of 0.5 Hz using a custom-built dynamometer connected to a screen to monitor the workload. Maximal contraction force (MCF) was assessed as the highest of three brief maximal hand grip efforts 30 minutes before the exercise started. Blood samples were collected from the median cubital vein of the exercising arm. Two protocols were applied: the high intensity exercise protocol consisted of contractions at 80% MCF for 3 minutes and the low intensity exercise protocol consisted of contractions at 30% MCF for 15 minutes. Blood was collected before exercise and at minute 1, 2, and 3 during exercise in Test 1 and every second minute until minute 15 during exercise in Test 2. There was a resting period of 30 minutes before and between both tests [12].

Workload over time (performance in Newton-seconds) was defined as the area under the curve, calculated using the dynamometer software. The lowest value of oxygen saturation and partial pressure during exercise was chosen.

2.1.3. Cycle ergometry

All subjects underwent exercise with constant workload at 30 W on a cycle ergometer for 15 minutes (Ergometer TX1, Kettler, Germany). Blood samples for serum lactate analysis were collected from the median cubital vein after a 30 minute rest in the supine position before the test, at 5, 10, and 15 minutes after starting cycling, and at 15 minutes after finishing the exercise and resting [12].

2.1.4. Blood analysis

Venous blood was immediately analyzed for lactate, oxygen saturation and partial pressure (Radiometer, Copenhagen, Denmark).

2.2. Biochemical and histochemical analysis

2.2.1. Histochemical staining

Biopsy samples were available for analysis from nine patients (in one patient the diagnosis of s-IBM had been confirmed histopathologically, but was not available for us in the study). These were screened for mitochondrial changes by light microscopy using modified Gomori trichrome stain (ragged red fibers [RRF]), COX/succinate dehydrogenase double staining (COX-deficient fibers) and succinate dehydrogenase (ragged blue fibers). All muscle fibers visible in the whole cross sectional area were counted (400–1800 fibers).

2.2.2. Biochemical analysis

The activities of rotenone-sensitive nicotinamide adenine dinucleotide (NADH):coenzyme Q1 oxidoreductase (respiratory chain complex I), succinate dehydrogenase (complex II), succinate:COX (complex II+III), COX (complex IV) and citrate synthase in nine patients were determined using standard methods as described previously [14,15]. Enzyme activity was expressed as a percentage of citrate synthase activity. The activities of the respiratory chain complexes were measured in skeletal muscle biopsies of nine patients with s-IBM, 20 age-matched normal controls and five age-matched MITO patients. The age-matched normal controls were defined as having myalgia and exertion induced complaints but no paresis or muscle atrophy, normal creatine kinase levels, normal electromyography, and only mild non-specific changes on muscle biopsy. The number of patients in the MITO group could not be increased because more samples from patients fulfilling the criteria for this group were not available.

2.3. Genetic analysis

Presence of multiple mtDNA deletions in s-IBM patients (n = 9), MITO patients (n = 5) and normal controls (n = 20) was analyzed by long-range polymerase chain reaction as described previously [16].

2.4. Statistical analysis

Data are given either as mean ± one standard deviation (SD) or median ± one SD. The cut-off reference value for the normal range was based on the mean ± one SD for lactate in normal controls, and the mean – two SD for oxygen saturation and partial pressure in normal controls.

Normal distribution was tested using the Kolmogorov–Smirnov test. Chi-squared test was used to compare parameters. Kruskal–Wallis one way analysis on ranks for non-normally distributed samples was performed to compare the three patient cohorts. *Post hoc* analysis with all pairwise multiple comparison procedures was done using Dunn's method. $p < 0.05$ was considered to be statistically significant (SigmaStat version 9.0, Systat Software, San Jose, CA, USA).

3. Results

3.1. Clinical data

The mean age at biopsy of the nine s-IBM patients was 64.9 ± 5.5 years (range: 56–72), and at the time the functional tests were performed the mean age of the 10 s-IBM patients was 68.2 ± 5.7 years (range: 56–75); and the duration of the disease at that time ranged from 5 to 14 years (mean: 8.6 ± 3.2 years). In the functional tests, the age of the diseased controls was non-significantly lower (Table 1, 2), and in the groups with analysed biopsies the MITO patients were non-significantly younger (Table 3).

Table 1
Results of the non-ischemic forearm exercise tests in sporadic inclusion body myositis patients, diseased controls and normal controls

	s-IBM (n = 10)	Diseased controls (n = 6)[a]	Normal controls (n = 9)	*p* value
Male/Female	7/3	2/4	7/2	
Age, years	68.2 ± 5.7 (56–75)	57.8 ± 6.9 (50–67)	67.0 ± 6.3 (55–73)	n.s. (0.06)
MCF, Newton-seconds	110 ± 80 (27–307)	226 ± 148 (49–462)	442 ± 119 (230–549)	<0.01[b]
Body mass index	27.0 ± 4.8 (20.9–35.8)	24.8 ± 3.4 (19.1–28.7)	29.3 ± 3.6 (22.2–33.9)	n.s.
Forearm circumference, cm	20.1 ± 3.1 (16.5–27.0)	20.9 ± 1.9 (19.0–24.5)	23.5 ± 1.5 (20.5–25.0)	<0.05[c]
Test 1: 80% MCF 3 minutes				
Workload, kN/s	4.5 ± 1.8 (1.7–7.2)	11.4 ± 6.7 (4.0–22.0)	19.2 ± 5.3 (11.9–25.9)	<0.001[c]
ΔsO_2 (%)	21.3 ± 7.6 (9.3–33.1)	26.1 ± 9.8 (10.1–35.4)	33.9 ± 5.7 (5.6–39.5)	<0.01[c]
ΔpO_2 (kPa)	1.8 ± 0.6 (1.0–2.6)	1.5 ± 0.7 (0.7–2.3)	2.1 ± 1.1 (1.0–3.7)	n.s.
Test 2: 30% MCF 15 minutes				
Workload, kN/s	7.7 ± 1.9 (5.8– 10.6)	17.1 ± 12.4 (3.3–37.0)	35.0 ± 14.2 (14.2–53.4)	<0.01[c]
ΔsO_2 (%)	22.9 ± 8.6 (7.8–35.7)	33.9 ± 15.4 (14.4–54.1)	35.7 ± 12.2 (20.7–59.0)	<0.05[b]
ΔpO_2 (kPa)	1.5 ± 0.6 (0.5–2.5)	2.4 ± 1.1 (0.7–3.4)	2.6 ± 1.4 (1.0–4.9)	n.s.

Values are presented as mean ± one standard deviation (range).
[a] Only six out of seven diseased controls underwent the non-ischemic forearm exercise.
[b] Sporadic inclusion body myositis *versus* diseased controls and diseased controls *versus* normal controls.
[c] Sporadic inclusion body myositis *versus* normal controls.
MCF = maximal contraction force, n.s. = not significant, s-IBM = sporadic inclusion body myositis.

Table 2
Serum lactate levels in patients with sporadic inclusion body myositis, diseased controls and normal controls following cycle ergometry at 30 watts for 15 minutes

	s-IBM (n = 10)	Diseased controls (n = 7)	Normal controls (n = 8)[a]	*p* value
Male/Female	7/3	2/5	5/3	
Age at examination, years	67.9 ± 6.1 (56–75)	58.3 ± 6.4 (50–67)	66.9 ± 6.5 (55–76)	n.s.
Resting serum lactate, mmol/l	1.5 ± 0.3 (1.0–2.2)	1.4 ± 0.5 (0.8–2.2)	1.9 ± 0.6 (0.9–2.2)	n.s.
Peak serum lactate, mmol/l	3.2 ± 1.3 (1.4–5.3)	2.1 ± 0.7 (1.2–3.4)	2.2 ± 0.8 (1.2–3.5)	<0.05

[a] Data were available for only eight out of nine normal controls.
n.s. = not significant, s–IBM = sporadic inclusion body myositis.

Table 3
Comparison of patients with sporadic inclusion body myositis, age-matched normal controls and patients with genetically confirmed mitochondrial disorder

	s-IBM (n = 9)	MITO (n = 5)	Normal controls (n = 20)	*p* value
Male/Female	6/3	1/4	12/8	
Age at biopsy, years	64.9 ± 5.5 (56–72)	58.8 ± 5.9 (52–68)	65.1 ± 6.0 (51–73)	n.s.
COX-deficient fibers,%	7.1 ± 5.2 (1.5–19)	4.6 ± 5.5 (1.0–14)	0.75 ± 0.41 (0–1.1)	<0.01[a]
Multiple deletions	5 (56)	5 (100)	0 (0)	

Data are given as mean ± one standard deviation (range) or number (%).
[a] Kruskal–Wallis one way analysis on ranks, *post hoc* analysis of normal controls *versus* MITO, s-IBM.
COX = cytochrome c oxidase, MITO = mitochondrial disorder, n.s. = not significant, s-IBM = sporadic inclusion body myositis.

3.2. Experimental protocols

3.2.1. Non-ischemic forearm exercise test

The MCF was significantly lower in patients with s-IBM compared to normal controls and diseased controls (Table 1). Additionally, the workload in both protocols (30% and 80%) was significantly lower in s-IBM compared to normal controls.

There was no difference in the oxygen desaturation upon high and low intensity exercise between normal controls and diseased controls. In contrast, s-IBM patients showed a significantly reduced oxygen desaturation (ΔsO_2) compared to normal controls. When the oxygen partial pressure was used as a measurement for oxygen desaturation only a non-significant trend for reduced oxygen desaturation in s-IBM patients in the low intensity exercise protocol was observed. If the lower limit (– two SD) of the ΔsO_2 was used as a cut-off value, one in ten s-IBM patients had a diminished oxygen desaturation in the low intensity exercise test, but none of the normal or diseased controls did (cut-off: <11.3%). Applying this criterion, five out of ten patients with s-IBM had a diminished oxygen desaturation in the high intensity exercise test (cut-off: <22.5%), but none of the normal controls did and only one in six diseased controls did.

3.2.2. Cycle ergometry

Resting serum lactate was normal in all 10 patients with s-IBM (Table 2). The peak serum lactate was above the upper limit + one SD of the normal controls (i.e. >3.3 mmol/L) in four out of ten patients with s-IBM, but only in one patient each from the normal controls and the other myopathy patients. Patients with s-IBM had significantly elevated peak serum lactate levels ($p = 0.045$). Post-exercise serum lactate remained elevated (i.e. >2.2 mmol/L) in five out of ten patients with s-IBM (data not shown).

3.2.3. Histochemical staining

Eight out of nine patients with s-IBM had clearly increased numbers of COX-deficient fibers. Muscle biopsy of one patient was not available for analysis. There was no statistically significant difference in the percentages of COX-deficient fibers between patients with s-IBM and MITO. However, both patient groups had significantly higher numbers of COX-deficient fibers than normal controls ($p < 0.01$; Table 3). In three of the nine s-IBM biopsies, no RRF were observed. The number of RRF in the other six biopsies ranged from one to seven per cutting surface and never exceeded

1%. The tendency of presence of RRF was significantly lower than the number of COX-negative fibres.

3.3. Biochemical activities of the respiratory chain enzymes

Among the s-IBM patients, three out of nine had decreased activities of complex IV (COX) (Fig. 1). All s-IBM patients had a normal protein concentration, but one of the patients with decreased activity of complex IV (COX) had a low citrate synthase activity reflecting a low number of mitochondria. The activities of complex I and II+III were within the normal range in all s-IBM patients. Three out of nine patients with s-IBM showed an increased citrate synthase activity suggesting an increased number of mitochondria. Three out of five patients with MITO, but none of the normal controls, had decreased activities of complex IV (COX). The citrate synthase activity was increased in two out of five patients with MITO. In patients with s-IBM and MITO, the citrate synthase activities were significantly higher compared to normal controls ($p < 0.05$). However, the power of multivariate analysis was observed to be low ($\pi > 0.8$).

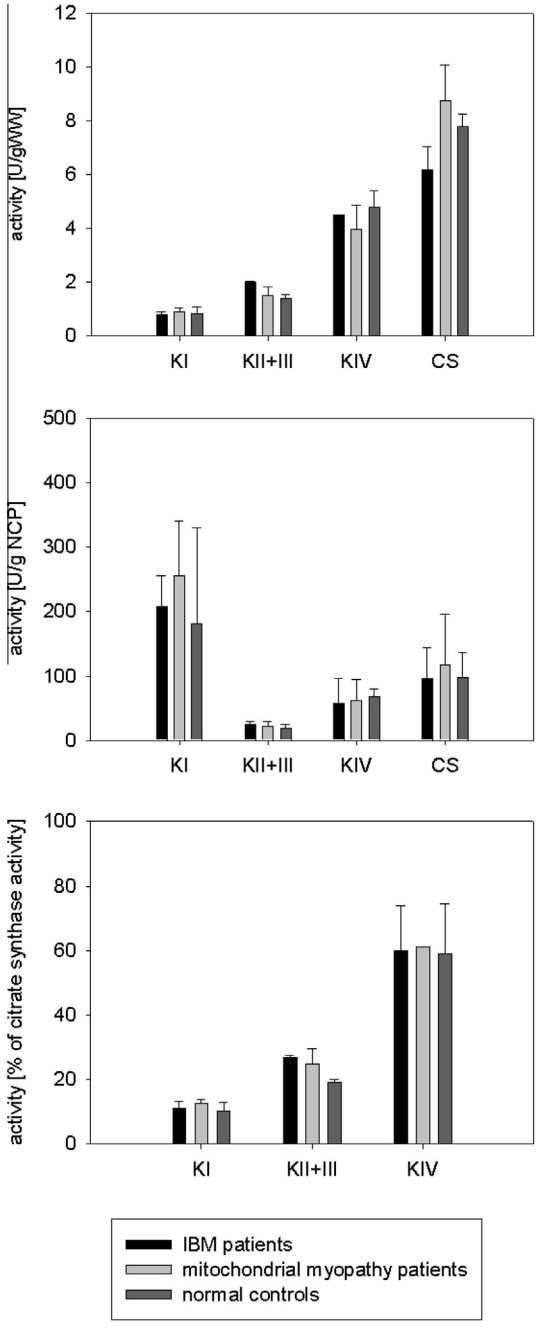

Fig. 1. Comparison of biochemical activities of respiratory chain complexes in patients with sporadic inclusion body myositis (s-IBM; n = 9), age-matched normal controls (n = 20) and patients with mitochondrial myopathy (n = 5). Bar charts represent the mean ± one standard deviation of activities of nicotinamide adenine dinucleotide:coenzyme Q1 oxidoreductase (KI), succinate dehydrogenase (KII), succinate:cytochrome reductase (KII + III), cytochrome c oxidase (KIV) and citrate synthase (CS) in the three different cohorts. NCP = non-collagenous protein, WW = tissue wet weight.

3.4. Genetic analysis

Multiple mtDNA deletions were observed in five of nine patients with s-IBM, five of five MITO patients and none of the normal controls (Table 3).

4. Discussion

In patients with clinically and histopathologically confirmed s-IBM, the different functional exercise protocols applied in the present study resulted in both a decreased oxygen desaturation (in the forearm exercise test) and increased serum lactate production (in cycle ergometry) compared with normals controls and (as a trend) diseased controls suggesting impaired mitochondrial oxidative capacity.

The very frequent occurrence of multiple deletions of the mtDNA in the skeletal muscle and an increased number of COX-deficient fibers in skeletal muscle biopsies are well known histopathological features and epiphenomena in s-IBM [1–7]. The number of COX-deficient fibers increases with age [10]. In the present study we were able to confirm these observations, as five patients with s-IBM had multiple deletions. The biochemical activities of the respiratory chain enzymes in the skeletal muscle in s-IBM showed similar alterations to those seen in MITO due to nuclear defects (i.e. decreased activities of complex IV [COX] and increased citrate synthase activities). Additionally, the number of COX-deficient fibers in s-IBM and MITO were both similarly increased and clearly exceeded the number seen in age-matched controls.

Due to impaired functioning of the respiratory chain, uptake of available oxygen from the blood during exercise is reduced in the skeletal muscle of patients with mitochondrial disorders. This effect has been demonstrated using near-infrared spectroscopy in patients with mitochondrial myopathies but not in patients with s-IBM to our knowledge [17]. Impaired oxygen desaturation as a surrogate marker of disturbed oxidative capacity in patients with MITO was previously described using hand dynamometer exercise protocols [12,18–20]. In the present study, low intensity exercise led to increased serum lactate accumulation (and its persistence for at least 15 minutes after the end of the exercise) and decreased oxygen desaturation in patients with s-IBM (the latter was also observed in short term high intensity exercise) very similar to observations in patients with mitochondriopathies. These statistically significant differences in s-IBM compared to age-matched controls were even found despite the low number of participants. The low oxygen desaturation in patients with s-IBM is probably not due to the low workload and low MCF. In a previous study using the same test protocols, it was shown that oxygen desaturation is independent of the degree of paresis [12]. The present study, analysing normal and diseased controls, confirmed this observation.

Dysfunctions in the energy-producing mitochondria are believed to cause muscle weakness, exercise intolerance and fatigue. Despite being common, the latter two symptoms are difficult to assess clinically. According to a database review of patients with mitochondriopathies in which 20% of patients reported exercise

intolerance, COX-negative fibers were frequently present in subjects with exercise intolerance. However, lactate levels could not be used to predict this symptom [21]. In a questionnaire survey, children with mitochondrial disorders and their parents complained of fatigue being the greatest burden [22]. In contrast, the impact and frequency of exercise intolerance and fatigue is not mentioned in several large series of patients with s-IBM, [23–27]. Recently a clinical study addressed this topic, suggesting that high interval training improved aerobic work capacity in patients with both mitochondrial myopathies and s-IBM without exacerbating symptoms of fatigue [28].

In summary, functional tests suggest that in addition to dystrophic changes leading to skeletal muscle atrophy, mitochondrial defects in patients with s-IBM might contribute to exertion induced complaints and fatigue.

Conflicts of Interest/Disclosures

The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

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