EXTENDED REPORTS

Impaired redox status and cytochrome *c* oxidase deficiency in patients with polymyalgia rheumatica

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Abstract

Objective—To evaluate redox status and muscular mitochondrial abnormalities in patients with polymyalgia rheumatica (PMR).

Methods—Prospective evaluation of deltoid muscle biopsy in 15 patients with PMR. Fifteen subjects matched for age and sex, with histologically normal muscle and without clinical evidence of myopathy, were used as controls. Cryostat sections of muscle were processed for conventional dyes, cytochrome *c* oxidase (COX), usual histochemical reactions, and Sudan black. A total of 300–800 fibres was examined in each case. Blood lactate, pyruvate, and lactate/pyruvate ratio were determined in all patients.

Results—Ragged red fibres were found in eight patients with PMR and accounted for 0–0.5% of fibres. Focal COX deficiency was found in 14 (93%) of 15 patients and in nine (60%) of 15 controls. COX deficient fibres were more common in patients with PMR (range 0–2.5%; mean 0.9%) than in controls (range 0–1.2%; mean 0.3%) (paired *t* test, *p*=0.001). Seven (47%) of 15 patients had high blood lactate levels (1.50–2.60 mmol/l) or high blood lactate/pyruvate ratios (22–25).

Conclusions—PMR is associated with mitochondrial abnormalities not solely related to the aging process.

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patients with PMR has been reported for years,^{11 12} but some authors of reference textbooks consider that there are no significant pathological changes in muscle.^{13–15} Two studies reported a high incidence of both biochemical and morphological mitochondrial abnormalities and suggested that PMR could be a form of late onset mitochondrial myopathy.^{16 17} However, the biochemical pattern of respiratory chain enzyme impairment was heterogeneous, and age matched control groups were absent¹⁶ or not clearly defined.¹⁷ On the other hand, ³¹P nuclear magnetic resonance spectroscopy did not detect alterations of muscle energy utilisation.¹⁸ Two recent studies by Miro *et al*^{19 20} failed to detect any evidence of mitochondrial dysfunction associated with PMR. Our results differ. We report the prospective evaluation of redox status and muscular mitochondrial abnormalities in a series of patients with PMR.

Methods

PATIENTS

We prospectively included patients with untreated, newly diagnosed PMR according to the criteria of Bird *et al*.²¹ Subjects matched for age and sex, with histologically normal deltoid muscle and without muscle weakness or fatigue, or any clinical evidence of myopathy, were used as controls. They had been referred to us for muscle biopsy to investigate isolated myalgias. After careful clinical and histological evaluation, all of these patients were considered not to have a mitochondrial cytopathy.

STUDY DESIGN

All patients with PMR participating in the study had a deltoid muscle biopsy, and their blood lactate and pyruvate levels were determined. In all patients, these procedures were performed at the time of diagnosis and before the onset of steroid treatment. The study was approved by the ethics committee of Henri-Mondor Hospital and each patient gave their informed consent.

MUSCLE BIOPSIES

Muscle samples were obtained by open biopsy and immediately frozen in isopentane cooled with liquid nitrogen (–160°C) and stored at –80°C. Cryostat sections of muscle were processed for conventional dyes, cytochrome *c* oxidase (COX, respiratory chain complex IV),

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Increasing attention has been focused on acquired mitochondrial dysfunction.¹ A variety of conditions have been recognised to be associated with mitochondrial abnormalities, including normal aging,² neurodegenerative disorders,³ myocardial ischaemia,⁴ idiopathic inflammatory myopathies,^{5 6} and muscular or hepatic disorders related to antiretroviral nucleoside analogues.^{7 8}

Polymyalgia rheumatica (PMR) is characterised by early stiffness, proximal myalgias, and weakness usually affecting people over the age of 55. Raised serum inflammatory markers and rapid responses to corticosteroid treatment are also typical of this syndrome of unknown cause and pathogenesis.^{9 10} The presence of mitochondrial abnormalities at muscle biopsy in

Table 1 Characteristics of 15 patients with polymyalgia rheumatica

Patient	Age/sex	RRF (%)	COX negative fibres (%)	Lipid excess	Type 2 fibre atrophy	Other pathological findings
1	56/F	0	0	0	++	—
2	58/M	0	0.5	+	0	Moth eaten fibres: 2.5%
3	61/F	0.3	0.3	+	++	Necrotic fibres: 1.5%
4	65/F	0.2	0.3	0	+	—
5	67/F	0.3	0.3	0	+	—
6	68/F	0	0.3	+	+	—
7	68/M	0	1.0	0	+	—
8	68/F	0.4	1.0	0	++	Moth eaten fibres: 6.7%
9	68/M	0.2	1.0	0	++	—
10	71/F	0	0.4	++	+	—
11	76/M	0	2.2	0	0	—
12	80/F	0	2.5	0	+	—
13	82/F	0.2	1.0	+	+	Moth eaten fibres: 3.8%
14	83/M	0.5	1.2	0	+	Moth eaten fibres: 3.7%
15	85/M	0.2	0.9	+	++	—

RRF = ragged red fibres; 0 = absent; + = mild; ++ = moderate.

succinate dehydrogenase (part of complex II), double stain for COX and succinate dehydrogenase,²² and the usual histochemical reactions, as previously described.²³ Type 2 fibre atrophy, characterised by a reduction in the diameter of the type 2 fibres, was recognised when: (a) the mean diameter of type 2 fibres was smaller than that of type 1 fibres; (b) most type 2 fibres were atrophic; and (c) atrophic type 1 fibres were absent or rare.²⁴ Lipid excess was assessed with Sudan black. Fibres were classified as “moth eaten” when multifocal loss of oxidative enzyme activity and disruption of the intermyofibrillar network was present.²⁴

Cryostat sections were evaluated under 100 × magnification, 300–800 fibres being considered in each case. Ragged red fibres were evaluated on Gomori trichrome. COX oxidase activity was classified as present or absent, and the number of COX negative fibres was determined. Necrotic fibres were excluded from the count of COX negative fibres considered in statistical analysis. Fibres with segmental enzyme deficiency were considered positive.

ASSESSMENT OF REDOX STATUS: DETERMINATION OF LACTATE AND PYRUVATE

Blood samples were collected by direct venepuncture after an overnight fast. The collected blood samples were immediately added to an equal volume of 1 mol/l chilled perchloric acid. The solution was mixed vigorously and centrifuged at 2000 *g* for 10 minutes at 4°C. Lactate was measured in the supernatant with a Roche model 640 Lactate Analyzer, specific for L-lactate (Roche, Basel, Switzerland). Pyruvate was measured in the supernatant with a kit from Boehringer (Mannheim, Germany). The optimised method depended on the oxidation of NADH in the presence of lactate dehydrogenase at an optimal pH of 7.0, as described.²⁵

Reference values for blood lactate and lactate/pyruvate ratio were 0.51–1.47 mmol/l and 6.5–18.7 (normal values (means (2SD) obtained in our laboratory from 20 subjects aged > 55 in whom the diagnosis of mitochondrial disorder could be excluded; ranges 0.62–1.40 mmol/l and 7–19, absence of correlation with age, $r=-0.29$, $p=0.22$ and $r=0.17$, $p=0.47$, respectively; P. Chariot, unpublished data). These values did not differ from the normal

Table 2 Redox status of patients with polymyalgia rheumatica: blood lactate and pyruvate concentrations and lactate/pyruvate ratio

Patient	Lactate (mmol/l)	Pyruvate (mmol/l)	Lactate/pyruvate (molar ratio)
1	0.80	0.063	13
2	0.53	0.045	12
3	0.90	0.060	14
4	0.90	0.069	13
5	2.60*	0.180	14
6	1.53*	0.082	19
7	2.60*	0.104	25*
8	0.58	0.067	9
9	1.70*	0.122	14
10	1.80*	0.222	8
11	1.00	0.060	17
12	0.80	0.086	9
13	2.61*	0.220	12
14	0.66	0.050	13
15	1.50*	0.069	22*

Blood lactate and lactate/pyruvate ratio were considered normal if lower than 1.50 mmol/l and 20.

*Abnormal values.

range observed in a younger population.²⁶ We considered that blood lactate and lactate/pyruvate ratio were normal if lower than 1.50 mmol/l and 20.

STATISTICAL ANALYSIS

Differences between groups were assessed using Student's paired *t* test. Correlations were evaluated using Spearman's correlation coefficient. All tests were two sided and conducted at the 0.05 significance level.

Results

A total of 15 patients (age range 56–85 years; mean 70) with PMR, and 15 controls matched for age and sex were included. Seven of 15 patients with PMR underwent temporal artery biopsy. None had histological signs of giant cell arteritis. Tables 1 and 2 give the main results.

PATHOLOGICAL RESULTS

Ragged red fibres were found in eight patients with PMR and accounted for 0–0.5% of fibres (fig 1). Mild lipid excess was found in six (40%) of 15 patients. Focal COX deficiency was found in 14 (93%) of 15 patients (fig 1, table 1) and in nine (60%) of 15 controls. Individual percentages of COX deficient fibres in controls were 0 (six of 15), 0.2 (three of 15), 0.3 (three of 15), 0.7 (one of 15), 0.9 (one of 15), and 1.2 (one of 15). A quantitative analysis showed that COX deficient fibres were more

Figure 1 Mitochondrial abnormalities in patients with polymyalgia rheumatica. (A) Ragged red fibres (asterisks) in patient 14 (modified Gomori trichrome, $\times 200$); (B) focal cytochrome c oxidase deficiency in patient 9 (cryostat section, double stain for cytochrome c oxidase and succinate dehydrogenase, $\times 450$); (C) moth eaten fibres and cytochrome c oxidase negative fibres in patient 14 (cryostat section, cytochrome c oxidase, $\times 320$).

common in patients with PMR (range 0–2.5%; mean 0.9%) than in controls matched for age and sex (range 0–1.2%; mean 0.3%) (paired *t* test, $p=0.001$). The percentage of COX deficient fibres in patients with PMR correlated with age ($r=0.70$; $p=0.003$). Other histological abnormalities included mild to moderate type 2 fibre atrophy, observed in 13 of 15 patients, and moth eaten fibres, found in four patients and accounting for 3.7–6.7% of fibres (fig 1). Ragged red fibres, lipid excess, type 2 fibre atrophy, moth eaten fibres, and necrotic fibres were absent in all 15 controls.

REDOX STATUS

In seven (47%) of 15 patients, we found high blood lactate levels (1.50–2.60 mmol/l) or high blood lactate/pyruvate ratios (22–25) (table 2). Lactate levels and lactate/pyruvate ratios did not correlate significantly with the percentages of COX deficient fibres.

Discussion

In this study, we observed mitochondrial abnormalities with ragged red fibres, COX deficiency, and lipid excess in patients with PMR. The finding of impaired redox status also suggests mitochondrial dysfunction. As ragged red fibres and COX deficiency can occur in the course of normal aging,^{5 27} the use of age matched control patients was important to establish the significance of our results. Our results showed that COX deficiency in patients with PMR could not be solely the result of the aging process.

To evaluate mitochondrial dysfunction, we assessed redox status and histochemical reaction for COX. Both tools have proved sensitive in the detection of acquired mitochondrial disorders.^{5 22 28 29} Miro *et al*²⁰ did not find decreased oxidative phosphorylation enzyme activities in polarographic and spectrophotometric studies. However, a focal enzyme deficiency affecting a few percent of cells can easily remain undetected by these methods performed on muscle homogenates, as previously shown in other acquired mitochondrial disorders.³⁰

The percentage of COX deficient fibres in patients with PMR was about 1%—that is, the same order of magnitude as those reported previously.^{20 31} Percentages found in controls were also similar to those already observed in elderly subjects.^{5 27} In the study by Uddhamar *et al*³¹, the absence of obvious differences between patients and controls for COX evaluation could be explained by both the lower number of patients and the lower precision of their evaluation compared with ours, as only 100–150 fibres were evaluated in each case.

The difference between our results from COX histochemical evaluation and those of Miro *et al*²⁰ is more puzzling. Although not individually matched for age and sex, patients and controls seemed to be comparable in the study by Miro *et al*.²⁰ The same clinical diagnostic criteria for PMR were used in that study and ours. The muscle biopsied was, however, different: deltoid muscle in our study and quadriceps in the Spanish study. As the shoulders are more commonly affected than the thighs in patients with PMR,³² examination of the deltoid muscle could be more sensitive than examination of the quadriceps for detecting abnormalities related to PMR. Another possible explanation is the biological heterogeneity of the syndrome, which may be in accordance with the pronounced heterogeneity of the mitochondrial abnormalities reported by Harlé *et al*¹⁷ in patients with PMR. This hypothesis may be strengthened by the results on redox status in our study: both lactate concentrations and lactate/pyruvate ratio varied greatly from one patient to another and did not correlate with the percentage of COX negative fibres.

Evaluation of redox status had not been reported in PMR; mild or moderate increases in blood lactate or lactate/pyruvate ratio were common (seven of 15 patients). Although these findings do not indicate that PMR can be considered a late onset mitochondrial myopathy,³³

as suggested by Harlé *et al.*,¹⁷ such abnormalities are similar to those encountered in patients with mitochondrial complications of treatment with antiretroviral nucleoside analogue.²⁸ The pathogenesis of mitochondrial abnormalities in PMR is still unclear: a role for inflammation itself cannot be excluded, and a role for inflammatory mediators in mitochondrial dysfunction has already been suggested.³⁴ However, although evidence of mitochondrial abnormalities has been reported in idiopathic inflammatory myopathies,^{5, 35} the intensity of tissue inflammation did not correlate with mitochondrial dysfunction.⁵

In PMR, involvement of dysfunctioning mitochondria in the production of proinflammatory cytokines such as interleukin 1 and tumour necrosis factor α is conceivable. It has been shown that tumour necrosis factor α can bind a 60 kDa receptor at the level of the inner mitochondrial membrane,³⁶ a finding reminiscent of previous immunoelectron microscopic studies showing interleukin 1 β accumulated in mitochondria of human monocytes³⁷ and interleukin 1 α bound to mitochondrial membranes in zidovudine myofibres.³⁸

The clinical significance of mitochondrial abnormalities is an important issue. We have no definite response. Firstly, this study showed a significant association between PMR and mitochondrial abnormalities, but a causal relation is still not established: we do not know whether the symptoms are related to the destruction of mitochondria. Secondly, although the percentages of COX deficient fibres observed in patients with PMR could be considered too small to justify the patients' myalgias, a number of patients with zidovudine myopathy, an acquired mitochondrial myopathy caused by long term treatment with zidovudine, present with myalgias and have less than 5% of COX negative fibres in the skeletal muscle.²³ Thirdly, in normal people, a variable number of natural COX negative mitochondria are mixed with COX positive mitochondria. The number of COX deficient mitochondria must reach a threshold level to transform the cell phenotype from COX positive to COX negative.³⁹ In patients with PMR, it is conceivable that muscle cells with abnormally high numbers of COX negative mitochondria are much more common than fibres with COX negative phenotypes. Fourthly, as the effect of steroid treatment on mitochondrial function is still not known, a response to steroids cannot be considered an argument for or against the clinical importance of mitochondrial abnormalities in patients with PMR.

In this study, mitochondrial abnormalities were commonly found, which is in keeping with previous studies that showed COX deficient muscle fibres in most patients over 50.^{5, 27, 40} Age related mitochondrial dysfunction is still a debated question.^{2, 41} Possible mechanisms involve accumulated mitochondrial DNA mutations,^{4, 42} accumulated nuclear recessive somatic mutations,⁴³ or involvement of both nuclear and mitochondrial factors.⁴⁰ This study also confirms the existence of common histological abnormalities in PMR such as type

2 fibre atrophy and moth eaten fibres, as previously mentioned.^{11, 44, 45}

We conclude that impaired redox status and mitochondrial histochemical abnormalities that are not solely related to the aging process are common events in PMR. The clinical relevance of these findings, which could not be addressed by our study, remains to be clarified.

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