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ORIGINAL COMMUNICATION

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Lipoic (thioctic) acid increases brain energy availability and skeletal muscle performance as shown by in vivo ^{31}P-MRS in a patient with mitochondrial cytopathy

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Abstract A woman affected by chronic progressive external ophthalmoplegia and muscle mitochondrial DNA deletion was studied by phosphorus magnetic resonance spectroscopy (^{31}P-MRS) prior to and after 1 and 7 months of treatment with oral lipoic acid. Before treatment a decreased phosphocreatine (PCr) content was found in the occipital lobes, accompanied by normal inorganic phosphate (Pi) level and cytosolic pH. Based on these findings, we found a high cytosolic adenosine diphosphate concentration [ADP] and high relative rate of energy metabolism together with a low phosphorylation potential. Muscle MRS showed an abnormal work-energy cost transfer function and a low rate of PCr recovery during the post-exercise period. All of these findings indicated a deficit of mitochondrial function in both brain and muscle. Treatment with 600 mg lipoic acid daily for 1 month resulted in a 55% increase of brain [PCr], 72% increase of phosphorylation potential, and a decrease of calculated [ADP] and rate of energy metabolism. After 7 months of treatment MRS data and mitochondrial function had improved further. Treatment with lipoate also led to a 64% increase in the initial slope of the work-energy cost transfer function in the working calf muscle and worsened the rate of PCr resynthesis during recovery. The patient reported subjective improvement of general conditions and muscle performance after therapy. Our results indicate that treatment with lipoate caused a relevant increase in levels of energy available in brain and skeletal muscle during exercise.

Key words Mitochondrial cytopathy · Lipoate treatment · Brain bioenergetics · Muscle energy metabolism · Magnetic resonance spectroscopy

Introduction

Much progress has been made in the genetic, biochemical and clinical diagnosis of mitochondrial cytopathies [21], whereas little is known about the treatment of these disorders. In this heterogeneous group of diseases, mitochondrial DNA mutations are responsible for the malfunctioning of different respiratory enzymes which in turn result in a defective energy metabolism. The functional consequences of defective adenosine triphosphate (ATP) production are more severe in tissues with a high ATP turnover like the central nervous system [45], where even a small reduction in the efficiency of ATP biosynthesis may impair proper brain function. On the other hand, biochemical deficits of skeletal muscles mainly become functionally evident when energy demand is increased, i.e. during sustained muscle work, resulting in muscle weakness.

Attempts to increase the efficiency of energy production in these disorders have been made with different natural compounds such as vitamins K_3 and B_2, thiamine and

coenzyme Q [26, 37, 40], the latter with controversial results [10, 33]. In reports from the 1960s to the 1980s, children with mitochondrial dysfunction such as Leigh's encephalomyopathy [16, 18, 28] pyruvate dehydrogenase deficiency [31] and pyruvate carboxylase deficiency [30] showed clinical and biochemical improvement following the administration of lipoic acid. Lipoate is a coenzyme of pyruvate and α-ketoglutarate dehydrogenase multienzyme complexes [41]. In animal experiments, the administration of lipoate has been shown to facilitate glucose utilization [27] and glucose oxidation [44], leading to an increase of cell ATP production. The precise assessment of treatments is, however, very difficult in the clinical setting.

Phosphorus magnetic resonance spectroscopy (^{31}P-MRS) is currently the only non-invasive method available of assessing in vivo the functionality of mitochondria in both brain and skeletal muscle by measuring the cytosolic concentration of adenosine triphosphate [ATP], phosphocreatine [PCr], and inorganic phosphate [Pi] as well as pH [19]. Several ^{31}P-MRS studies of different mitochondrial cytopathies have, in fact, shown abnormalities of brain [8, 25, 35] and skeletal muscle [2, 4, 32] mitochondrial function even in the absence of clinical involvement [5–7, 17].

In the present study we used non-invasive ^{31}P-MRS to test the efficacy of oral lipoate treatment on the mitochondrial ATP production of brain and skeletal muscle in a patient with chronic progressive external ophthalmoplegia (CPEO) and mitochondrial DNA deletion.

Patients and methods

Case report and treatment

The patient was a 33-year-old woman with a negative family history for neurological disorders. She had been a thin, weak child with decreased resistance to physical exercise. She presented with progressive drooping of the eyelids during her twenties, as documented by pictures taken at 22 years. At approximately the same time, the patient presented bilateral impairment of eye movements with no double vision. Fundus oculi, visual acuity, hearing and heart function (including heart rate) were always normal. Neurological examination showed a short, thin woman. She had bilateral ptosis and asymmetrical palsy of oculomotion with normal pupillary reflexes. Mild muscle weakness (grade 4, range 0–5) was present in the proximal upper and lower limbs. Respiratory function was normal. Lactate level was normal at rest, but showed an abnormal peak after standard exercise on a cycle ergometer, returning to normal within 60 min. Pyruvate and ammonia and creatine kinase (CK) were normal. EMG evaluation disclosed myopathic features in deltoid and quadriceps muscles, nerve conduction studies of median and peroneal nerves were normal. Lipoic acid (Thioctacid; Asta Medica, Germany) was administered orally in coated capsules at a dose of 200 mg three times per day. MRS was performed before treatment and after 1 and 7 months of lipoate treatment.

Histology and genetic analysis

A biceps brachialis biopsy specimen was obtained when the patient was 30 years old and a quadriceps biopsy was performed 3 years later. For histochemical studies, specimens were processed as previously described [23, 34]. Part of both specimens was fixed in 2% glutaraldehyde and processed for electron microscopy (EM). Mitochondrial(mt) DNA analysis was performed on the quadriceps muscle sample, which had been snap frozen, and on peripheral blood. mtDNA from muscles and leucocytes of five aged-matched individuals was used as a control [42].

Phosphorus MRS

All spectroscopic measurements were performed according to the quantification and quality assessment protocols defined by the EEC Concerted Research Project on "Tissue Characterisation by MRS and MRI", COMAC-BEM II.1.3. [24].

Brain ^{31}P-MRS was performed on occipital lobes as reported previously [6, 7]. Briefly, spectra were acquired using a General Electric 1.5 T Signa system with a spectroscopy accessory by a surface coil positioned on the occipital region after brain imaging. The DRESS (depth-resolved surface-coil spectroscopy) localization technique [12] was used to avoid contribution to the signal by neck muscles, skin and other interposed tissues. Spatial co-ordinates were carefully recorded to repeat the examination precisely on the same region of interest. The stimulation-response sequence was repeated every 5 s. The flip angle in the selected volume was approximately 40°, and it was not necessary to introduce any correction for saturation effects due to repetition time. Four hundred free-induction decays (FIDs) were accumulated to obtain a signal-to-noise ratio of 9–12 for β-ATP. A computerised curve-fitting program was used to quantify the individual peaks of the spectrum [6–8]. Metabolite concentrations were calculated assuming a cytosolic [ATP] of 3 mM, a value found in human brain [11]. We do not have absolute data on ATP content in the brain of patients with mitochondrial cytopathies. Nevertheless, if cytosolic [ATP] were lower in our patient, cytosolic [ADP] would be even higher. Mitochondrial functionality was assessed by calculating [ADP] from the CK equilibrium [14], the relative rate of ATP biosynthesis (V/V_{max}) by the Michaelis and Menten equation [14], and the phosphorylation potential [43].

Muscle ^{31}P-MRS was performed on the dominant gastrocnemius by the pulse-and-acquire technique (repetition time of 5 s), first at rest, then during isokinetic exercise performed on a pneumatic ergometer [46], and finally during recovery from exercise. The rate of post-exercise recovery was calculated from the mono-exponential equation best fitting the experimental points and is reported as time constant (TC). Intracellular pH was calculated from the shift of Pi from PCr [39]. Control subjects were 12 healthy women aged 30–50 years. No athletes were included in the study. Control figures are presented as mean ± SD). Intra-individual variability was much lower (less than half) than inter-individual variability. However, we preferred to compare our patient's data with the mean values of matched controls to be more confident when assessing the meaning of metabolite changes. Hence, a variable is considered normal if it fell within the range of mean controls (± 2 SD).

Results

Morphology and genetic analysis

The deltoid muscle biopsy showed a mild myopathic pattern with several ragged red fibres on staining with succinate dehydrogenase (SDH) and Gomori trichrome preparations. No fibres with deficient cytochrome *c* oxidase and SDH reactions were seen on histochemistry. The quadriceps muscle biopsy showed mild myopathy with a

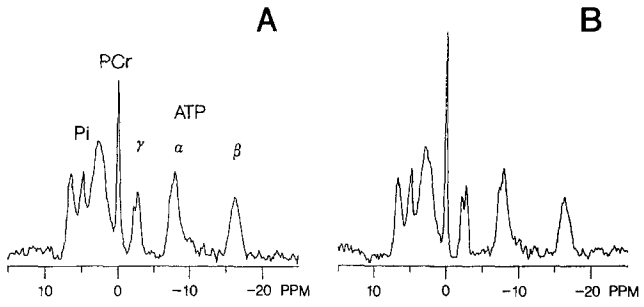

Fig. 1 ^{31}P-MRS spectra of occipital lobes in a patient with mitochondrial cytopathy before (**A**) and after 1 month of lipoate treatment (**B**). Phosphocreatine signal is increased after treatment (*PCr* phosphocreatine, *Pi* inorganic phosphate, *ATP* adenosine triphosphate). Phosphomonoester peak is located to the left of Pi; phosphodiester peak is located between the Pi and PCr peaks

slight increase of mitochondria. EM showed only minimal mitochondrial abnormalities and glycogen granules in both biopsy samples. mtDNA analysis showed a 3.3 kb deletion in approximately 20% of the mtDNA in the muscle but not in blood.

After lipoate treatment the patient reported a subjective improvement characterized by decreased pain and discomfort of the eyes and increased systemic muscle strength. Objectively, she only showed slight improvement of ptosis.

Phosphorus MRS

Figure 1 shows the ^{31}P-MR spectra of occipital lobes from the patient before and after 1 month of treatment with 600 mg lipoate daily (spectrum A and B respectively). [PCr] was very low in the patient before therapy as compared with normal volunteers (Table 1), while [Pi] and cytosolic pH did not show any significant difference from normal control subjects. The calculated mitochondrial values, i.e. [ADP], V/V_{max} [14], and the phosphorylation potential [43] were all remarkably abnormal (Table 1).

Treatment with lipoate resulted after 1 month in a large increase of [PCr] (+55%), which after 7 months further increased, reaching the normal range (defined by the 95% confidence limits of control mean). Lipoate therapy also resulted in a decrease of the calculated V/V_{max}, and in a rise of the phosphorylation potential, thus indicating a tendency to normalize the readily available energy levels (Table 1). [Pi] showed a slight increase after 7 months of therapy, while intracellular pH remained unchanged.

All ^{31}P-MRS data, i.e. [Pi], [PCr] and cytosolic pH of the patient's resting gastrocnemius muscle, did not differ from those of normal volunteers either before or after lipoate treatment (data not reported).

Figure 2 shows the MR spectra of working gastrocnemius muscles of the patient at the last level of exercise before and after lipoate treatment. The patient performed the required levels of in-magnet controlled steady-state work and repeated precisely the same work rate at each level of exercise in all sessions. By exercising at the same work rate, at the end of exercise the patient's [PCr] had decreased to 38%, 46%, and 45% of the resting value during the first, second and the third examinations respectively, i. e. before therapy, after 1 and 7 months of lipoate treatment, respectively. The kinetic changes that occurred with increasing work before and after therapy were evaluated by plotting the work rate versus the Pi/PCr ratio. The result

Table 1 ^{31}P-MRs data and mitochondrial function of the brain (occipital lobes) and calf muscle in a patient with mitochondrial cytopathy before and after therapy with lipoate, compared with 12 age-matched healthy women (data reported as average values (± SD)). Slope of transfer function [13] is reported as J/min/Pi/PCr unit. Muscle theoretical V_{max} was calculated from the Hanes plot of work-energy cost transfer function [14]. The rate of PCr initial recovery in the skeletal muscle is reported as the time constant (*TC*) of the monoexponential equation best fitting the experimental points (*ADP* adenosine diphosphate, *PCr* phosphocreatine, *Pi* inorganic phosphate, V/V_{max} relative rate of ATP biosynthesis)

	Before lipoate	After 1 month of therapy	After 7 months of therapy	Controls (n = 12)
Brain				
^{31}P-MRS values				
[PCr] (mM)	2.18	3.39	4.06	4.48 (± 0.29)
[Pi]	1.33	1.38	1.61	1.27 (± 0.15)
pH	7.03	7.02	7.04	7.03 (± 0.019)
Calculated mitochondrial values				
[ADP]	78	43	34	28 (± 2.9)
V/V_{max}	74	64	60	55 (± 2.1)
Phosphorilation potential	29	50	56	81 (± 7.3)
Muscle				
Working				
Slope of transfer function (J/min/Pi/PCr unit)	86	142	145	345 (± 41)
V_{max} (J/min)	125	145	150	618 (± 83)
PCr end of exercise (% of rest)	36	46	45	48 (± 5.2)
Initial post-exercise recovery				
TC PCr (s)	70	98	63	30 (± 3.96)
TC Pi (s)	57	85	49	34 (± 3.95)

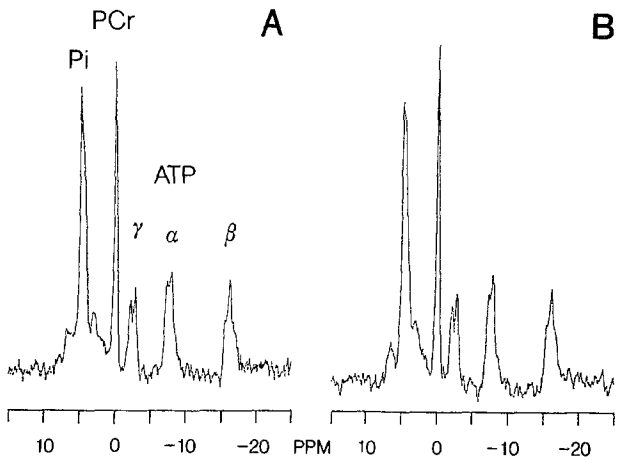

Fig. 2 ^{31}P-MRS spectra of working gastrocnemius muscle at the last level of exercise in our patient with mitochondrial cytopathy before (**A**) and after one-month lipoate treatment (**B**). After treatment PCr signal is higher, although the patient is exercising at the same rate. Peaks as in Fig. 1

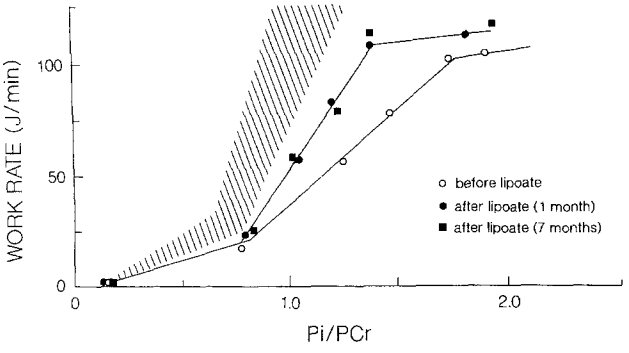

Fig. 3 Work-energy cost transfer function of calf muscles in our patient with mitochondrial cytopathy before (*open circles*), after 1 month (*closed circles*) and 7 months (*open squares*) after lipoate treatment. *Dashed area* represents the normal range defined as the 5–95% confidence interval

of this analysis was the characteristic plot shown in Fig. 3 (termed Chance "transfer function") [13]. The initial slope of transfer function was lower in our patient than in normal individuals , and it was increased by lipoate treatment, i.e. from 85 J/min/Pi/PCr unit before therapy to 142 and 150 J/min/Pi/PCr unit following 1 and 7 months of therapy, respectively. The transfer function was also linearized by a Hanes plot, which is known to be reliable for relatively low levels of V/V_{max} [14]. V_{max} was lower than in normal individuals before and after treatment, showing a small increase after lipoate treatment (Table 1). The rate of PCr re-synthesis after exercise was slower in the patient than in controls before lipoate treatment, and did not show any improvement after therapy.

Discussion

This patient affected by CPEO showed ^{31}P-MRS findings typical of mitochondrial cytopathies in both brain [8, 25, 35] and skeletal muscles [2, 4, 32]. Genetic, biochemical and histological features were also typical of mitochondrial abnormalities.

Brain MRs in our patient revealed decreased [PCr] and increased [ADP]. Stable and unstable states of metabolism can be recognized if the [ADP] is known [15, 20], a high [ADP] indicating that the tissue is operating under unstable conditions. Indeed, this was the case in our patient, who also showed a high V/V_{max} and a low phosphorylation potential.

Treatment with lipoate resulted in an increased energy reserve in the brain, as shown by an increased content of the PCr energy buffer and an increased phosphorylation potential (Table 1), the latter being a measure of the readily available energy in the cell [43]. Lipoate treatment also decreased the V/V_{max} as well as [ADP] (Table 1), indicating that after lipoate treatment the tissue was operating farther away from the asymptote and hence in a more stable metabolic condition. It is known that lipoate facilitates glucose uptake and oxidation [27, 41], thus increasing the cell availability of glycolytic ATP and pyruvate for mitochondrial oxidation. Brain relies only on glucose for its energy production; therefore, the beneficial effect of lipoate treatment on the overall rate of ATP biosynthesis can be attributed to a positive effect of lipoate on glucose uptake and utilization.

^{31}P-MRS features of resting gastrocnemius muscle in our patient were not significantly different from those of normal controls (data not reported). To assess muscle mitochondrial function more precisely, we also studied calf muscle during exercise by measuring the metabolic transitions from rest to full activity, i.e. from resting state 4 to fully activated state 3 of mitochondrial respiration, and vice versa. Under these stressful experimental conditions, a marked impairment of energy metabolism was shown by a low ability to perform work at comparable levels of metabolic activation (Fig. 3), and by a low rate of PCr post-exercise recovery (Table 1), the latter depending entirely on mitochondrial respiration [3], thus being looked upon as a very sensitive index of the functionality of this organelle.

Treatment with lipoate also had a positive effect on muscle energy metabolism, as shown by the increased initial slope of the work-energy cost transfer function (Fig. 3). The unavailability of glucose-6-phosphate as an oxidizable fuel resulting from glycogen depletion in normal individuals [38] or from a defective myophosphorylase activity in patients with McArdle's syndrome [1, 9, 22] greatly limits the oxidative capacity of muscle mitochondria. In contrast, it has been shown that infused glucose affects the working muscle in patients with McArdle's

syndrome [1], resulting in an increased exercise slope that represents improved mitochondrial activity. It is also known that lipoate stimulates glucose utilization in rat diaphragm in vitro [27] and in intact diabetic rats [36]. It seems reasonable to assume that lipoate treatment also increases glucose availability in human skeletal muscle cells. As a consequence, we measured increased mitochondrial activity during submaximal exercise due to increased availability of substrates that yield a high redox potential, and pyruvate is such a preferred substrate. At the same time, we found that the calculated maximal velocity of oxidative metabolism (V_{max}) showed only a small increase in our patient following lipoate treatment (Table 1). This is not surprising as the maximal rate of metabolism in the presence of excess substrates, i.e. our experimental conditions [29], is limited by the number of functioning mitochondria and of enzyme complexes of the respiratory chain rather than by substrate availability. A very similar result was reported by Argov et al. [1] in a patient with McArdle's syndrome after infusion of glucose.

Mitochondrial respiration, assessed by the rate of PCr post-exercise recovery, initially (after 1 month of therapy) was further slowed in our patient's calf muscles by the lipoate treatment (Table 1), although lipoic acid is a coenzyme of pyruvate and α-ketoglutarate dehydrogenase multienzyme mitochondrial complexes. To understand this result we must take into consideration that: (1) in skeletal muscles the glycolytic pathway is blocked, hence no pyruvate is produced, as soon as work is stopped; (2) muscle mitochondrial respiration during recovery from exercise relies only on the availability of fatty acid; and (3) lipoate administration reduces the oxidation of ^{14}C-labelled palmitate in intact diabetic rats [36]. Furthermore, we also must remember that if large amounts of lipoate are available in the cell there is competition for electrons with reduced NAD, being the redox potential of the lipoate/dihydrolipoate couple higher than that of NAD^+/NADH (–0.29 and –0.22 V, respectively, at pH 7) and this may act as an electron sink. The latter effect is possibly masked during submaximal muscle activity by the high rate of metabolism. This interpretation is also consistent with our MRS findings, showing a positive effect of lipoate on the availability of energy in the brain, a tissue relying mainly on glucose as oxidizable substrate, and where the rate of energy metabolism is constantly high.

Our present findings show for the first time the beneficial effects of lipoate treatment on brain and muscle energy metabolism in a patient with mitochondrial cytopathy matched by clinical improvement. Our results also indicate that the overall effect is mediated by increased activity of the glycolytic pathway, possibly due to increased glucose utilization or oxidation or both. Further studies are necessary to evaluate the therapeutic efficacy of lipoate treatment on other metabolic disorders, to understand whether it can be successfully used in other pathologies primarily or secondarily involving the functionality of the electron transport chain and ATP production.

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