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13C and 31P NMR for the diagnosis of muscular phosphorylase-kinase deficiency

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RÉSUMÉ

Afin de continuer à développer et préciser les applications médicales de la spectroscopie RMN in vivo, il faut étudier le plus grand nombre possible de cas bien caractérisés. Nous avons étudié ici une forme purement musculaire de déficit en phosphorylase-kinase (PK) par RMN du phosphore 31 et du carbone 13. Les altérations observées sont en accord avec et augmentent nos connaissances physiopathologiques, par exemple concernant l' activation de la phosphorylase et PK. Par ailleurs, la combinaison d' altérations observées en 31P et 13C est différente de celle retrouvée dans d' autres myopathies métaboliques cliniquement semblables et pourrait être utilisée pour le diagnostic différentiel.

Mots-clé: RMN, myopathies, déficits enzymatiques, phosphorylase-kinase musculaire, RMN du phosphore 31, RMN du carbone 13.

ABSTRACT

To further develop and specify the range of medical applications of in vivo NMR spectroscopy for the study of myopathies, it is necessary to study the largest number of well characterized cases. We here report on the 31P and 13C NMR study of a purely muscular form of phosphorylase-kinase (PK) deficiency. Abnormalities were observed that agree with and increase our pathophysiological knowledge, in particular on the activation of phosphorylase and PK. Also, the abnormalities are different from those found in other clinically similar metabolic myopathies and could be used for the differential diagnosis.

Keywords: Nuclear Magnetic Resonance (NMR), muscle diseases, enzyme deficiency, muscular phosphorylase-kinase, phosphorus 31 NMR, carbon 13 NMR.

INTRODUCTION

Phosphorus 31 (31P) and carbon 13 (13C) NMR spectroscopy have already proven their usefulness for the diagnosis and for pathophysiological studies of various muscular diseases [1-7, for instance]. They are included as part of the routine diagnostic tools in some clinical centers investigating neuromuscular diseases, and in some cases they can yield a precise diagnosis. In order to further develop and specify their range of application in myopathies, it is necessary to study the largest possible number of well characterized cases.

We here report on the 31P and 13C NMR study of a purely muscular form of phosphorylase-kinase (PK) deficiency.

Phosphorylase-kinase is a key enzyme of glycogen metabolism, which activates glycogenolysis by activating phosphorylase. Various types of PK deficiency exist, for instance depending on what tissues are biochemically affected and on symptoms. The purely muscular form is a rare disease, usually with a clinical pattern of metabolic myopathy (essentially consisting of exercise intolerance). Its diagnosis is heavy and often overlooked or largely delayed since symptoms can be mild, the disease is rare, and therefore not thought of, and the diagnosis goes through the assay of a large number of enzyme activities and needs to be performed on muscle samples. A non-invasive test would therefore be wellcome.

The aim here was to investigate whether 31P and 13C NMR could be useful for the diagnosis of muscular PK deficiency.

METHODS

31P-NMR. Spectra of the flexor digitorum muscles of the forearm were recorded at rest, during exercise and during recovery at 2 T with a 3 cm diameter surface coil [1]. The relative concentrations of the phosphate metabolites (like phosphocreatine (PCr), inorganic phosphate (Pi) and phosphomonoesters (PME)) were obtained by peak integration and the intracellular pH from the chemical shift of Pi. Metabolite concentrations were normalized to [PCr]+[Pi]+[PME]. The exercise consisted of handgrips repeated every second for 7.5 min, with a graded increase in strength. The exercise was such that PCr, which was used as an internal biochemical control of the relative degree of exercise intensity, decreased to less than 40% of its rest value at the end of the exercise.

13C-NMR. Ten minute proton-decoupled 13C spectra of calf muscles were obtained with two concentric single-loop surface coils (7 cm diameter for 13C, 11 cm for proton). Spectra were acquired with a 180° pulse at the coil center repeated every 200 ms and with a 40 ms acquisition time (2 Watt average power). The glycogen content was evaluated from the ratio of the glycogen carbon 1 peak at 100.5 ppm to that of the creatine peak at 157 ppm, taken as a reference [2, 3].

Subject. The 33 year old patient suffered from exercise intolerance since chilhood. An increased glycogen storage was found in the muscle both with the PAS histological staining and by electron microscopy. The final diagnosis relied on the absence of PK enzyme activity on a skeletal muscle biopsy sample. All other screened enzymes involved in glycogenolysis or glycolysis were normal.

RESULTS

The 13C spectra showed an abnormally high muscle glycogen content (glycogen/creatine= 6.8 vs. 2.0 ± 0.7 for normals ; range 0.4 to 4 for 42 non-glycogenosis diseases and 8 normal subjects).

The muscle 31P NMR exercise test showed a delayed decrease in pH during exercise and the pH only decreased to 6.96 at the end of exercise (6.35 ± 0.19 for normals, n=20). This acidification also remained too moderate at the beginning of recovery (6.8 vs. 6.2 ± 0.2 for normals). No alcalosis was found that lasted for the whole duration of the exercise. No pathological accumulation of PME was observed.

DISCUSSION

The abnormalities observed in PK-deficiency (delayed and reduced acidification, glycogen storage) agree with our pathophysiological knowledge. The modifications in the acidification pattern can be interpreted as a delayed and decreased intracellular accumulation of lactic acid and suggest a defect in the activation of glycogenolysis. However, they also suggest that glycogenolysis and thus phosphorylase can still be partially activated, even though with a delay, in the absence of PK activity.

As far as diagnosis is concerned, the observed increase in glycogen content allows to diagnose a muscular glycogenosis [3]. Other kinds of muscular glycogenosis can be excluded by the 31P NMR exercise test [1]: the presence of a decrease in pH and the absence of alcalosis lasting during the whole exercise allow

to eliminate other deficiencies affecting glycogenolysis (a debrancher enzyme deficiency or a phosphorylase deficiency (McArdle's disease)); the absence of a pathological accumulation of PME allows to eliminate a deficiency affecting glycolysis.

So, abnormalities are observed by 31P and 13C NMR spectroscopy. They are different from those found in other clinically similar metabolic myopathies and could be used for the differential diagnosis. They are strongly suggestive of a PK deficiency: none of the large number of other kinds of patients studied showed such a combined pattern of 31P and 13C abnormalities.

NMR spectroscopy can thus be very useful for the diagnosis of muscular PK deficiency, which is heavy and often delayed or overlooked by classical methods.

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