Clinica Chimica Acta 478 (2018) 171–181

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Perturbation of muscle metabolism in patients with muscular dystrophy in early or acute phase of disease: In vitro, high resolution NMR spectroscopy based analysis

Niraj Kumar Srivastava[a,b,*,1], Ramakant Yadav[c], Somnath Mukherjee[b], Neeraj Sinha[d]

[a] Department of Neurology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, U.P., India
[b] School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India
[c] Department of Neurology, UP University of medical sciences, Saifai, Etawah, UP 206130, India
[d] Center of Biomedical Research, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, U.P., India

ARTICLE INFO

Keywords:
Muscle
Metabolism
Tissue
Muscular dystrophy
Perchloric acid extract
Aqueous metabolites
NMR spectroscopy
Metabolomics
Metabolic alteration

ABSTRACT

Background: Muscular dystrophy is an inherited muscle disease, characterized by progressive muscle wasting and weakness of variable distribution and severity.

Methods: In vitro, high-resolution proton nuclear magnetic resonance (NMR) spectroscopy based analysis was performed on perchloric acid (PCA) extract of muscle specimens of patients suffering from various types of muscular dystrophies to identify alteration in hydrophilic low-molecular weight substances (aqueous metabolites) as compared to muscle of control subjects as well as in between the types of muscular dystrophy.

Muscle tissue specimens were obtained from Duchenne muscular dystrophy (DMD) [n = 11], Becker muscular dystrophy (BMD) [n = 12], facioscapulohumeral dystrophy (FSHD) [n = 9] and limb girdle muscular dystrophy (LGMD)-2B [n = 22]. Control muscle specimens [n = 40] were also taken.

Results: Concentration of branched chain amino acids (BCA), glutamine/glutamate (Gln/Glu), acetate (Ace) and fumarate (Fum) was decreased and His was increased in muscle tissue of DMD, BMD, FSHD and LGMD-2B patients as compared to control subjects. Alanine (Ala) was significantly reduced in BMD, FSHD and LGMD-2B patients as compared to control subjects. Tyrosine (Tyr) was present only in the muscle tissue of control subjects. Propionate (Prop) was present in muscle tissue of DMD, BMD, FSHD and LGMD-2B patients and was absent in muscle tissue of control subjects. Concentration of BCA and Prop is significantly reduced in patients with DMD as compared to BMD, but Glucose is significantly higher in patients with DMD as compared to BMD. Quantity of Glucose, His and Gln/glu are significantly higher in patients with DMD as compared to FSHD, but Prop is significantly reduced in patients with DMD as compared to FSHD. Concentration of Ala and His is significantly higher in patients with DMD as compared to LGMD-2B, but BCA, Glucose and Prop are significantly reduced in patients with DMD as compared to LGMD-2B. Concentration of His is significantly higher in patients with BMD as compared to FSHD. Concentration of His is significantly reduced and Glucose is higher in patients with LGMD-2B as compared to BMD. Glucose concentration is significantly reduced in patients with FSHD as compared to LGMD-2B. ROC curves supported the noticeable discrimination in between the patients with DMD and FSHD for the quantity of Gln/Glu, and patients with LGMD-2B and DMD for the quantity of Ala. Collectively, these findings showed the perturbation of muscle metabolism in muscular dystrophy.

Conclusions: The data of presented study may be used as supporting information for existing methods of the diagnosis for patients with muscular dystrophy.

Abbreviations: BCA, branched chain amino acids; Lac, lactate; Gln/glu, glutamine/glutamate; Ala, alanine; Ace, acetate; Suc, succinate; Cr/PCr, creatine/phosphocreatine; GPC/Car, glycerophosphocholine/ carnitine; Fum, fumarate; His, histidine; Tyr, tyrosine; Prop, propionate; TSP, 3,(trimethylsilyl) propionic,2, 2, 3, 3,d4 acid, sodium salt; DMD, Duchenne muscular dystrophy; BMD, Becker muscular dystrophy; FSHD, facioscapulohumeral dystrophy; LGMD,2B, limb girdle muscular dystrophy; PCA, perchloric acid; ROC, receiver operating characteristic

* Corresponding author at: School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India.
E-mail address: neeraj.sinha@cbmr.res.in (N.K. Srivastava).
[1] Present address.

https://doi.org/10.1016/j.cca.2017.12.036
Received 1 June 2016; Received in revised form 6 December 2017; Accepted 22 December 2017
Available online 24 December 2017
0009-8981/

1. Introduction

Few important features of muscular dystrophy are selective involvement, significant wasting and weakness of muscles. This is in contrast with other forms of myopathies, where the weakness is diffuse and comparatively more than wasting, and muscle enlargement is rare. Depending upon the age, progress, site of involvement and the inheritance pattern, several types of muscular dystrophy have been described. These include Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), facioscapulohumeral dystrophy (FSHD), myotonic dystrophy (DM) and limb girdle muscular dystrophy (LGMD)-2B [1].In muscular dystrophy, biochemical alterations may initially be limited and restrained, but later become widespread and associated with the gradual deterioration of the muscle tissue. The direct analysis of affected muscles, its chemical composition and enzyme activities, helps understand the causes and progression of muscular dystrophy [2]. Dreyfus et al. showed that rate of glycolysis of dystrophic muscles was much less as compared to normal muscle tissue and the degree of decrease in the rate of glycolysis was parallel to the state of disease progression. Assays of individual glycolytic enzymes showed that α-glucan phosphorylase and aldolase had low activity in dystrophic muscle [3]. DiMauro et al. reported that, in contrast to other glycolytic enzymes, muscle phosphorylase showed an earlier and more marked loss in activity in progressive muscular dystrophy as compared to the neurogenic diseases [4]. Studies by Vignos and Lefkowitz (1959) showed that the rate of glycolysis was low in the juvenile forms, but was essentially normal in the adult forms of muscular dystrophy [5]. Latter researchers also found low creatine kinase activity in juvenile muscular dystrophy and neurogenic atrophy of muscle compared to marginal changes in adult muscular dystrophy [6]. Heyck et al., Hooft et al. and Kleine et al. showed decrease in concentration of adenylate kinase and many other glycolytic enzymes [7,8]. The enzyme fructose 1,6,bisphosphatase (which controls the glycolytic process) was present in normal amounts in all the types of muscular dystrophy cases [9]. Another enzyme, AMP aminohydrolase (AMP deaminase) is markedly decreased in both dystrophic mouse muscle and in muscle from patients with Duchenne dystrophy even at an early stage, while in other muscle diseases a low concentration is seen only in some severely affected muscles [10,11]. These enzymes exist in far greater concentration in skeletal muscle than in other tissues, while its precise role is not yet clear, it may be important in the control of glycolysis in muscle [10,11].

This reports clearly indicate towards dysregulation of glycolytic and bioenergetics metabolic pathway in muscular dystrophy. In this regard, metabolomics based approach or analysis is very important in muscular dystrophy to understand the mechanism or development of new diagnostic methods or therapy. Metabolomics based analysis involves the quantification of the dynamic multivariate, metabolic response of an organism to a pathological event or genetic modification [12]. The concept of metabonomics has evolved over two decades of ^{1}H NMR spectroscopic analysis of the multi-component metabolic composition of biofluids, cells and tissues under different physiological and pathological conditions [13]. This technique is useful to investigate many diseases and disease models including inborn error of metabolism, classification of tumors, evaluation of transplant patients, detection of markers for neurodegenerative diseases and monitoring of drug overdose cases [14]. Proton NMR spectroscopic analysis is non, destructive, cost effective, and typically takes only a few minutes for each sample, requiring little or no sample pretreatment or reagents and is therefore bioanalytically more efficient as compared to the methods used to characterize either the genetic or proteomic composition of samples [14].

In vivo, ^{31}P NMR spectroscopy has been used to analyse the alteration of high, energy phosphate molecules in skeletal muscle. Increased ratio of Pi/PCr at rest was observed in patients with DMD and BMD indicating an impaired energy metabolism. A premature drop in the concentration of PCr was obtained during exercise in patients with BMD [15,16]. However, due to comparative shortage of phosphorous containing observable metabolites, it provides only limited and non-specific views of biochemical processes of cell.

^{1}H nuclei are present in the majority of the metabolites and provide a suitable method to reveal the metabolic profile of cells/tissue in normal and pathological conditions. In, vitro high-resolution proton NMR spectroscopy has been used to investigate the metabolites in body fluid and tissue extract to explore the muscle metabolism. In, vitro, ^{1}H NMR spectroscopy was also successfully applied on the mouse model of muscular dystrophy (mdx) as compared to control to observe the alteration in metabolic profiling [15,16]. Venkatasubramanian et al. studied the 2-dimensional proton NMR spectra of human muscle tissue extracts and documented differences in metabolite composition between normal and disease muscles [11]. Sharma et al. performed the analysis of perchloric acid (PCA) extract of the muscle tissue of the DMD and LGMD patients and found significant difference in the quantity of aqueous metabolites [15,16]. Above described studies afforded a ground to explore NMR spectroscopy for the observation of the biochemical changes in the muscle tissue of the patients with muscular dystrophy.

Study by Sharma et al. only performed the analysis on muscle PCA extract of DMD and LGMD-2B with limited metabolites [15,16]. We performed on the PCA extract of muscle tissue from DMD, BMD, FSHD and LGMD-2B patients as compared to normal subjects with large number of metabolites. The objectives of the present study were: (1) to identify the metabolically relevant substances in the PCA extracts of normal human skeletal muscle tissue as well as in DMD, BMD, FSHD and LGMD-2B patients using one and two-dimensional NMR spectroscopy, (2) estimation of the selected metabolites to observe any quantitative difference in muscle tissue of the patients with muscular dystrophy as compared to control subjects.

2. Material and methods

2.1. Tissue specimens

Muscle tissue specimens were obtained from patients with muscular dystrophy viz. DMD (n = 11; age, mean ± SD; 9.2 ± 1.4 y; all males), BMD (n = 12; age, mean ± SD; 21.4 ± 5.0 y; all males), FSHD (n = 9; age, mean ± SD; 25.2 ± 10.1 y; 7 males and 2 females) and LGMD,2B (n = 22; age, mean ± SD; 27.4 ± 7.0 y; 18 males and 4 females). The procedure of muscle biopsy was performed by senior residents of neurology department, Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, for the histopathological and immunohistochemical investigations. Muscle biopsy was performed from suitable location or site, such as quadriceps (rectus femoris and vastus laterails) in the leg, the biceps in the arm, deltoid, gastrocnemius and distal limb muscles. The weight of muscle tissue was taken in the range of 100 to 150 mg. Normal muscle specimens (n = 40; Mean age ± SD 21.0 ± 9.6 y; 30 males and 10 females) were obtained from paraspinal site of muscles from control subjects. Normal muscle specimens were obtained from the patients with critical neuro,-pathological conditions, who had undergone spinal surgery in the neurosurgery department of the institute. EMG (Electromyography) and CK (Creatine kinase) are two crucial laboratory examinations for neuromuscular diseases and performed on these patients. None of the single evidence of neuro-muscular diseases was found in these patients. In this regard, muscle specimens of these patients were appropriate for considering as a normal as compared to muscle of patients with various types of muscular dystrophy. Muscle specimens were collected directly in a sterilized tube and then immersed in liquid nitrogen for NMR spectroscopy based analysis. This study was approved by ethical committee of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow. Prior consent was obtained from the relatives of patients.