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Effect of creatine monohydrate in improving cellular energetics and muscle strength in ambulatory Duchenne muscular dystrophy patients: a randomized, placebo-controlled ^{31}P MRS study☆,☆☆

Bidisha Banerjee[a], Uma Sharma[b], Krithika Balasubramanian[b], M. Kalaivani[c], Veena Kalra[a], Naranamangalam R. Jagannathan[a,*]

[a]Department of Pediatrics, All India Institute of Medical Sciences, New Delhi 110029, India
[b]Department of Nuclear Magnetic Resonance, All India Institute of Medical Sciences, New Delhi 110029, India
[c]Department of Biostatistics, All India Institute of Medical Sciences, New Delhi 110029, India

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Abstract

Randomized, placebo-controlled single blinded study was carried out to evaluate the effect of oral creatine supplementation on cellular energetics, manual muscle test (MMT) score and functional status in steroid-naive, ambulatory boys suffering with Duchenne muscular dystrophy (DMD; *n*=33). Eighteen patients received creatine monohydrate (Cr; 5 g/day for 8 weeks), while 15 received placebo (500 mg of vitamin C). Phosphorus metabolite ratios were determined from the right calf muscle of patients using phosphorus magnetic resonance spectroscopy (^{31}P MRS) both prior to (baseline) and after supplementation of Cr or placebo. In addition, metabolite ratios were determined in normal calf muscle of age and sex matched controls (*n*=8). Significant differences in several metabolite ratios were observed between controls and DMD patients indicating a lower energy state in these patients. Analysis using analysis of covariance adjusted for age and stature showed that the mean phosphocreatine (PCr)/inorganic phosphate (Pi) ratio in patients treated with Cr (4.7; 95% CI; 3.9–5.6) was significantly higher (*P*=.03) compared to the placebo group (3.3; 95% CI; 2.5–4.2). The mean percentage increase in PCr/Pi ratio was also more in patients <7 years of age compared to older patients after Cr supplementation indicating variation in therapeutic effect with the age. In the placebo group, significant reduction in PCr/Pi (*P*=.0009), PCr/t-ATP (*P*=.05) and an increase in phosphodiester (PDE)/PCr ratios was observed after supplementation. Further, in the placebo group, patients <7 years showed reduction of PCr/t-ATP and Pi/t-ATP compared to older patients (>7 years), after supplementation. These results imply that the significant difference observed in PCr/Pi ratio between the Cr and the placebo groups after supplementation may be attributed to a decrease of PCr in the placebo group and an increase in PCr in the Cr group. Changes in MMT score between the two groups was significant (*P*=.04); however, no change in functional scale (*P*=.19) was observed. Parents reported subjective improvement on Cr supplementation versus worsening in placebo (*P*=.02). Our results indicated that Cr was well tolerated and oral Cr significantly improved the muscle PCr/Pi ratio and preserved the muscle strength in short term. However, this study provides no evidence that creatine will prove beneficial after long-term treatment, or have any positive effect on patient lifespan.

Keywords: Duchenne muscular dystrophy; Creatine monohydrate; Placebo; Phosphorus magnetic resonance spectroscopy; Cellular energetics; Muscle strength

1. Introduction

Duchenne muscular dystrophy (DMD) is X-linked muscle degenerating disease that affects one in 3300 live male births and characterized by the absence of a cytoskeletal protein dystrophin [1]. The deficiency of dystrophin causes membrane abnormalities that affect stretch activated ion channels of the sarcolemma and intracellular calcium ion level regulation [2,3]. Since calcium homeostasis is critical to many aspects of muscle function [4], alterations in calcium ion concentration

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* Corresponding author. Department of NMR and MRI Facility, All India Institute of Medical Sciences, New Delhi 110029, India. Tel.: +91 11 2659 3253; fax: +91 11 2658 8663.
E-mail address: jagan1954@hotmail.com (N.R. Jagannathan).

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results in the activation of many pathophysiological processes [2,3]. There is currently no effective treatment for this disease. Replacement of dystrophin gene through gene transfer techniques have been reported; however, these are still under development [5]. Corticosteroid treatment provides temporary clinical benefit but is associated with side-effects that need periodic monitoring [6].

DMD is also associated with many nutritional deficits [7] and supplementation of nutrients was shown to protect muscle weakness in an animal model (mdx mice) [8]. Impaired function of the creatine phosphokinase (CPK)/phosphocreatine (PCr)/creatine (Cr) system was reported to be associated with the impaired muscle function [9,10]. Phosphorus (^{31}P) magnetic resonance spectroscopy (MRS) studies on skeletal muscle of DMD patients reported low PCr/inorganic phosphate (Pi) and PCr/ adenosine triphosphate (ATP) ratios [11,12] indicating altered Cr metabolism/kinetics [10,13]. In vitro studies of dystrophic muscle and in vivo studies in patients with muscular dystrophy demonstrated reduced trapping of Cr [13,14] and creatinuria [13]. A significant reduction in the concentration of total Cr was demonstrated in dystrophic muscle of DMD patients together with the changes in several metabolic processes using in vitro proton MRS [15]. These studies have led to the hypothesis that oral supplementation of nutrients might limit muscle degeneration or even restore muscle function in neuromuscular diseases [9].

In normal healthy subjects, Cr supplementation for 2 weeks was shown to improve the muscle PCr store [16] and performance in high-intensity intermittent exercises [17]. The effect of drug therapy by measuring the changes in cellular energetics as indicated by the PCr/Pi ratio was documented using phosphorus magnetic resonance spectroscopy (^{31}P MRS) [18]. Few trials of oral Cr supplementation in DMD patients demonstrated positive effect; however, the use of different dosages, duration and outcome measures made the interpretation difficult [19–23]. Reduced Pi/PCr, α-ATP/PCr and phosphodiester (PDE)/PCr were reported after Cr supplementation in a case study of a 9 year child with DMD [19]. In a randomized, double-blind, placebo-controlled crossover trial of oral Cr in boys with DMD and Becker's muscular dystrophy, statistically significant increase in maximum voluntary contraction was demonstrated on ergometry in the Cr group [21]. A recent randomized, double blind placebo controlled trial did not demonstrate a statistically significant effect of Cr on the muscle strength though a disease modifying effect of Cr was noticed in younger patients (<7 years age) [23].

Thus in spite of extensive research using various therapeutic agents, no satisfactory long-term advantages have been demonstrated. Therefore, a controlled randomized trial is required. Thus, the objectives of the present study were: (a) to understand the metabolic activity of dystrophic muscles of patients compared to normal age matched controls, and (b) to study the effect of oral Cr supplementation on the muscle tissue energetics in comparison to placebo using ^{31}P MRS and the clinical change by manual muscle strength and function.

2. Patients and methods

2.1. Patients and controls

Randomized, single-blinded, placebo-controlled, parallel group prospective study was conducted. Only one of the experimenter (B.B.) who was involved in recruitment, randomization and treatment of patients was aware of the treatment group allocation. Criteria for eligibility of patients to be included in this study are shown in the flowchart (Fig. 1). Of the 44 patients assessed with DMD, 33 (age range, 3–12 years) were ambulatory and steroid-naïve. Patients were excluded if found to be non-ambulatory, on steroids or with renal dysfunction as evidenced by raised serum urea and creatinine. On randomization, 18 patients were included in the Cr group and 15 in the placebo group. The study was approved by the Institute Ethics Committee, and written informed consent was obtained from parent/guardian of all patients and controls.

Diagnosis of DMD was based on a compatible clinical history (progressive proximal muscle weakness starting in early childhood with pseudohypertrophy of calves) and muscle biopsy suggestive of DMD (features of dystrophy like degeneration, regeneration, "opaque" hypertrophic fibers, fiber size variation, replacement of muscle by fat and fibrous tissue) and absence of immunohistochemical staining for dystrophin including antibodies to rod, amino and carboxy terminus. A baseline renal function test (serum urea and creatinine), CPK, chest X-ray and electrocardiogram were obtained in all subjects. Multiplex polymerase chain reaction of the DMD gene deletion was performed in willing patients. Eight healthy (including normal muscle strength and CPK) age-matched male siblings of these children were recruited as controls. All the normal controls underwent physical including neurological examination and serum CPK evaluation. In addition, a detailed history, reports of the various examination and investigations carried out were recorded for controls and patients.

2.1.1. Intervention

The Cr group received oral creatine monohydrate (Coach's formula™, 99% pure creatine) 5 g/day in three divided doses taken with water 30 min–1 h after meals for 8 weeks. They were advised to take about 3–4 extra glasses of water everyday during the study period and to report back in case of fever, diarrhea or vomiting (to ensure good hydration). The placebo group received 500 mg of Vitamin-C (Celin™) as placebo per day for 8 weeks. Vitamin C was chosen as placebo since it has been found to have no effect on intracellular calcium levels [24]. Lowering of calcium is one of the pathological mechanisms leading to cell injury in DMD. Both the groups (those receiving creatine or placebo) were advised to participate in standard physiotherapy