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Original article

Urinary prostaglandin metabolites as Duchenne muscular dystrophy progression markers

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Abstract

Background: Patients with Duchenne muscular dystrophy (DMD) exhibit increased prostaglandin D_2 (PGD_2) expression in necrotic muscle and increased PGD_2 metabolites in their urine. In mouse models, inhibiting PGD_2 production suppresses muscle necrosis, suggesting a possible intervention through PGD_2-mediated activities.

Objective: We investigated the involvement of PGD_2 and its potential use as a marker of pathological progression in DMD.

Methods: Sixty-one male children with DMD and thirty-five age-matched controls were enrolled in the study. DMD patients were divided into "ambulant" and "non-ambulant" groups, which were further subdivided into "steroid" and "non-steroid" therapy groups. Levels of the PGD_2 metabolite tetranor-PGDM (t-PGDM) and creatinine were measured in both spot and 24-hour urine samples, with comparisons between groups made according to geometric mean values.

Results: DMD patients had significantly higher levels of creatinine-corrected t-PGDM in spot urine samples as compared with the control group. Additionally, both ambulant and non-ambulant DMD groups had significantly higher levels of t-PGDM as compared with controls, with no significant difference in t-PGDM levels observed between steroid and non-steroid groups. Moreover, total creatinine excretion in 24-hour urine samples was significantly lower in DMD patients as compared with controls, and although DMD patients had lower muscle mass than controls, their overall levels of t-PGDM did not differ significantly from those in the non-ambulant and control groups.

Conclusion: PGD_2 might help explain the progression and symptomatic presentations (e.g., ambulatory difficulty) associated with DMD, suggesting it as a useful pathological marker and use of a selective PGD_2 inhibitor as a potential treatment modality.

Keywords: Ambulatory function; Duchenne muscular dystrophy; Inflammation; Prostaglandin D_2; Steroid therapy; Tetranor-PGDM

1. Introduction

Duchenne muscular dystrophy (DMD) is an X-linked hereditary disorder, where mutations of the dystrophin gene at locus Xp21.2 lead to the formation of dysfunctional dystrophin protein on the inside surface of muscle

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cell membranes [1]. This genetic disease presents in approximately 1 in 3500 live male births [2] or between 10.71 and 27.78 per 100,000 live male births [3]. Affected individuals usually experience progressive muscle atrophy and weakening as their muscle fibers degenerate and undergo necrosis. Inflammatory cells play a role in the muscle injury associated with DMD patients, as do prostaglandins, such as prostaglandin D_2 (PGD_2) [4].

PGD_2 is produced from prostaglandin H_2 (PGH_2) by prostaglandin D synthase and subsequently metabolized into tetranor-PGDM (t-PGDM). While lipocalin-type prostaglandin D synthase is expressed in the central nervous system, hematopoietic-type prostaglandin D synthase (HPGDS) is expressed in both necrotic muscle cells and inflammatory cells, such as mast cells and T-cells [5,6]. HPGDS expression has been observed in the muscles of DMD patients during the early stages of the disease, when muscle tissues begin to undergo necrosis [7]. Compared with age-matched healthy subjects, patients with DMD excrete increased urinary PGD_2 and t-PGDM [8,9], a trend that is especially striking in patients who are $\geq$8 years of age [10].

Inhibiting PGD_2 production reduces urinary t-PGDM excretion and suppresses muscle necrosis in a DMD *mdx* mouse model [11], suggesting that PGD_2-mediated inflammation is involved in DMD pathology. Although Nakagawa et al. [10] used spot urine samples to specifically analyze creatinine-normalized levels of t-PGDM, few studies have examined the total level of urinary t-PGDM using 24-hour urine collection in DMD patients. Additionally, although some studies show that urinary t-PGDM excretion increases with patient age [10], few studies have addressed whether differences in excretion are associated with differences in motor function.

This study investigated the role of PGD_2 in DMD by measuring PGD_2 metabolites in spot and 24-hour urine samples and examining how this correlates with patients walking or ambulatory ability. Our goal was to determine the involvement of PGD_2 in DMD, its potential use as a marker of disease progression, and its efficacy for manipulation in developing a therapeutic modality for DMD.

2. Methods

2.1. Patients

Sixty-one (61) male children aged between 5 and 15 years and diagnosed with DMD were recruited for this study and assigned to analytic groups according to their ability to walk (ambulant vs. non-ambulant: n = 50 vs. 11) and whether or not they were undergoing steroid therapy (steroid vs. non-steroid: n = 56 vs. 5). Thirty-five (35) age-matched male children with other diseases, including epilepsy (n = 27), attention-deficit hyperactivity disorder (n = 6), cerebellar atrophy (n = 1), and hereditary neuropathy (n = 1), were enrolled in the control group.

2.2. Ethical considerations

Informed consent was obtained from the parents of all patients (for both the DMD and control groups) after explaining the nature and purpose of the study. Ethical approval was obtained from the Institutional Review Board of the National Center of Neurology and Psychiatry, Tokyo, Japan (approval no. A2013-120).

2.3. Sample collection and analyses

In the urine collection phase, both spot and 24-hour urine samples were collected from 35 of the children (20 ambulant DMD, 5 non-ambulant DMD, and 10 controls), whereas only spot urine samples could be collected from the remaining 61 children (30 ambulant DMD, 6 non-ambulant DMD, and 25 controls). We measured the urinary content of the PGD_2 metabolite t-PGDM, the prostaglandin E_2 (PGE_2) metabolite tetranor-PGEM (t-PGEM), which is synthesized from the same substrate, and creatinine (Cre). The urinary t-PGDM and t-PGEM concentrations were determined using high-performance liquid chromatography-tandem mass spectrometry, while the stable isotopes of each substance measured were utilized as internal standards.

The resulting measurements were used to calculate t-PGDM and t-PGEM levels, which were normalized against creatinine levels in the spot urine samples (respectively depicted as "t-PGDM/Cre" and "t-PGEM/Cre" in the figures), as well as total creatinine, t-PGDM, and t-PGEM excretion in the 24-hour urine samples. t-PGDM/Cre values in the spot urine samples from patients in the DMD group were compared with those of the control group. After two-way analysis of variance, these values were also compared for patients in the ambulant and non-ambulant groups against those of patients in the control group. The same comparison was also made for patients in the steroid group against those in the non-steroid group. Additionally, t-PGEM/Cre values from patients in the DMD group were compared with those in the control group. After two-way analysis of variance, t-PGEM/Cre values were also compared for patients in the ambulant and non-ambulant groups against those from patients in the control group. The same comparison was made for patients in the steroid group against those in the non-steroid group.

Creatinine excretion values were also compared between the DMD and control groups, the ambulant and non-ambulant groups, and the control group for the 24-hour urine samples. Additionally, t-PGDM and

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