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Limitations of galactose therapy in phosphoglucomutase 1 deficiency

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ABSTRACT

Introduction: Phosphoglucomutase 1 deficiency (PGM1 deficiency) has been identified as both, glycogenosis and congenital disorder of glycosylation (CDG). The phenotype includes hepatopathy, myopathy, oropharyngeal malformations, heart disease and growth retardation. Oral galactose supplementation at a dosage of 1 g per kg body weight per day is regarded as the therapy of choice.

Results: We report on a patient with a novel disease causing mutation, who was treated for 1.5 years with oral galactose supplementation. Initially, elevated transaminases were reduced and protein glycosylation of serum transferrin improved rapidly. Long-term surveillance however indicated limitations of galactose supplementation at the standard dose: 1 g per kg body weight per day did not achieve permanent correction of protein glycosylation. Even increased doses of up to 2.5 g per kg body weight did not result in complete normalization.

Furthermore, we described for the first time heart rhythm abnormalities, i.e. long QT Syndrome associated with a glycosylation disorder.

Mass spectrometry of IGFBP3, which was assumed to play a major role in growth retardation associated with PGM1 deficiency, revealed no glycosylation abnormalities. Growth rate did not improve under galactose supplementation.

Conclusions: The results of our study indicate that the current standard dose of galactose might be too low to achieve normal glycosylation in all patients. In addition, growth retardation in PGM1 deficiency is complex and multifactorial. Furthermore, heart rhythm abnormalities must be considered when treating patients with PGM1 deficiency.

1. Introduction

Phosphoglucomutase 1 deficiency (PGM1 deficiency) has been defined as a glycogenosis and congenital disorder of glycosylation (CDG) related to a great variety of biochemical and clinical symptoms. Phenotypical characterization is still incomplete, pathomechanisms are only partly understood and although therapeutic approaches have been proposed, further detailed research and investigation are indispensable.

By converting glucose-1-phosphate into glucose-6-phosphate, phosphoglucomutase 1 (PGM1) enables the liver to release glucose from glycogen in order to maintain glucose homeostasis (Fig. 1). In the reverse reaction, PGM1 catalyzes glucose-6-phosphate conversion into glucose-1-phosphate for glycogen biosynthesis and is related to the UDP-galactose pool needed for protein N-glycosylation [1]. PGM1 also

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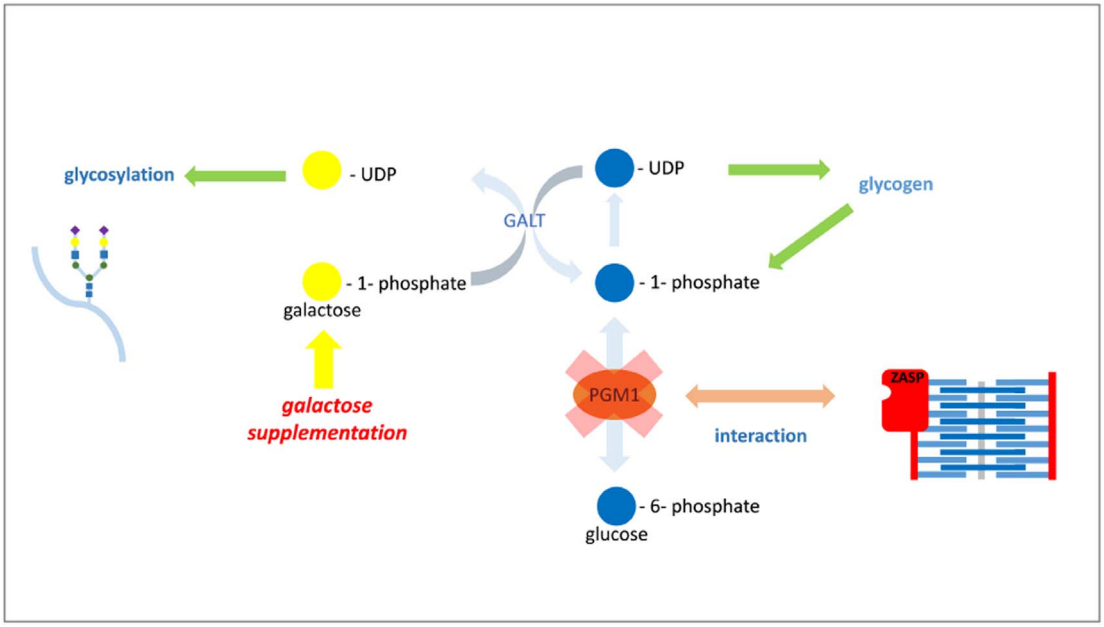

Fig. 1. Schematic representation of phosphoglucomutase 1 (PGM1) key role in metabolism and protein interaction.
In PGM1 deficiency, malfunction of the enzyme causes a wide range of clinical symptoms such as hepatopathy, uvula bifida, myopathy, cardiomyopathy and growth retardation [1].
PGM1 serves as a binding factor to the ZASP in heart muscle cells. The deficient enzyme causes dilated cardiomyopathy [2].
In PGM1 deficiency, glucose-6-phosphate (blue) cannot be generated from glycogen, which causes hypoglycemia and exercise- induced rhabdomyolysis [3].
By galactose supplementation (yellow) the PGM1 pathway is bypassed and the formerly deficient protein glycosylation is compensated [1].

interacts with *Z*-band alternatively spliced PDZ motif protein (ZASP) in heart muscle cells [2].

In this paper, we report on a patient with a novel *PGM1* mutation receiving galactose supplementation expanded by temporary uridine intake and additional growth hormone therapy. Results of this first long-term surveillance reveal the positive effects and limitations of galactose supplementation in PGM1 deficiency.

Mass spectrometry data on subunits of the growth hormone complex provide first insights into the glycoprotein profile of this PGM1 deficient patient and give explanations on therapeutic limitations.

2. Methods

2.1. Informed consent and ethical approval

Informed consent of the parents was obtained. Ethical approval was granted by the local Ethics Committee.

2.2. Glycosylation assays

Isoelectric focusing (IEF), as well as immunoprecipitation followed by sodium dodecyl sulfate-polyacrylamide-gel electrophoresis (SDS-PAGE) were performed as described elsewhere [1,4].

High performance liquid chromatography (HPLC) of serum transferrin was carried out following the manufacturer's protocol employing the “CDT in serum - HPLC” kit from Chromsystems (Gräfelfing, Germany).

Electrospray ionization mass spectrometry (ESI-MS) was performed as described previously [5].

2.3. Cells and tissues

Patient's leukocytes were obtained and isolated as outlined before [1].

2.4. Enzyme assays and PGM1 western blotting

A modified Beutler test using dried blood spots on Guthrie heel-prick test cards was used. Specific phosphoglucomutase 1 activity profile was gained by spectrometric measurement of the enzyme activity in extracts of leucocytes as stated elsewhere [1].

Investigation of phosphoglucomutase 1 expression by western blotting was carried out as described elsewhere [1] using different antibodies (Ab 55,616 and Ab 188,869; Abcam, Cambridge, UK).

2.5. Genetic analysis

Sanger Sequencing of *PGM1* was performed on the patient's and the parents' genomic DNA.

Genomic DNA obtained from patient's leucocytes was isolated and analyzed for long-QT syndrome (LQTS) gene mutations, in particular the LQT 1–3, 5–7 and 8 subforms (ion channel genes: *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, *KCNE2*, *HCNJ2*, *CACNA1C*) as previously described [6]. The complete coding sequences were analyzed.

2.6. IGF ternary complex analysis

2.6.1. Affinity purification and western blotting of serum IGFBP-3

IGFBP-3 was isolated from 2.5–5 mL serum samples by immunoaffinity chromatography on columns of anti-IGFBP-3 IgG (R-100, prepared in-house) immobilized onto agarose (Affi-Gel 10 (Bio-Rad #1536046)). Bound IGFBP-3 was eluted with 0.1 M acetic acid, 0.5 M NaCl, pH 2.8. Samples were further purified on a 4.6 × 250 mm C18 column (Jupiter 5 µm, 300 Å; Phenomenex 00G-4053-E0), using a 15–60% gradient of acetonitrile in 0.1% trifluoroacetic acid over 30 min at 1.5 mL/min.

Western blotting was performed as previously described [7].

2.7. IGFBP-3 mass spectrometry

Purified IGF-BP3 was reduced with dithiothreitol followed by carbamidomethylation, and then digested with a mixture of trypsin and