Molecular Genetics and Metabolism xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

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

Elevated plasma dihydroorotate in Miller syndrome: Biochemical, diagnostic and clinical implications, and treatment with uridine

John A. Duley [a,*], Michael G. Henman [b], Kevin H. Carpenter [c], Michael J. Bamshad [d], George A. Marshall [e], Chee Y. Ooi [f,i], Bridget Wilcken [g], Jason R. Pinner [h]

[a] School of Pharmacy and Mater Research Institute, The University of Queensland, Brisbane, QLD 4102, Australia
[b] Department of Pathology, Mater Health Services, Brisbane, QLD 4101, Australia
[c] NSW Biochemical Genetics Service, The Children's Hospital at Westmead, Disciplines of Genetic Medicine & Child and Adolescent Health, The University of Sydney, NSW 2145, Australia
[d] Department of Pediatrics, University of Washington, Division of Genetic Medicine at Seattle Children's Hospital, Seattle, WA 98195, USA
[e] Department of Pathology, Mater Health Services, Brisbane, QLD 4101, Australia
[f] School of Women's and Children's Health, University of NSW, Sydney Children's Hospital, Sydney, NSW 2031, Australia
[g] Department of Medical Genetics, Sydney Children's Hospital, University of Sydney, NSW 2031, Australia
[h] Department of Medical Genomics, Royal Prince Alfred Hospital, The University of Sydney, NSW 2050, Australia
[i] School of Medicine, University of NSW, Sydney Children's Hospital, Sydney, NSW, 2031, Australia

ARTICLE INFO

Article history:
Received 13 May 2016
Received in revised form 13 June 2016
Accepted 13 June 2016
Available online xxxx

Keywords:
Dihydroorotate-dehydrogenase
Miller syndrome
POADS
Mitochondria
Screening

ABSTRACT

Background: Miller syndrome (post-axial acrofacial dysostosis) arises from gene mutations for the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH). Nonetheless, despite demonstrated loss of enzyme activity dihydroorotate (DHO) has not been shown to accumulate, but paradoxically urine orotate has been reported to be raised, confusing the metabolic diagnosis.
Methods: We analysed plasma and urine from a 4-year-old male Miller syndrome patient. *DHODH* mutations were determined by PCR and Sanger sequencing. Analysis of DHO and orotic acid (OA) in urine, plasma and blood-spot cards was performed using liquid chromatography-tandem mass spectrometry. In vitro stability of DHO in distilled water and control urine was assessed for up to 60 h. The patient received a 3-month trial of oral uridine for behavioural problems.
Results: The patient had early liver complications that are atypical of Miller syndrome. *DHODH* genotyping demonstrated compound-heterozygosity for frameshift and missense mutations. DHO was grossly raised in urine and plasma, and was detectable in dried spots of blood and plasma. OA was raised in urine but undetectable in plasma. DHO did not spontaneously degrade to OA. Uridine therapy did not appear to resolve behavioural problems during treatment, but it lowered plasma DHO.
Conclusion: This case with grossly raised plasma DHO represents the first biochemical confirmation of functional DHODH deficiency. DHO was also easily detectable in dried plasma and blood spots. We concluded that DHO oxidation to OA must occur enzymatically during renal secretion. This case resolved the biochemical conundrum in previous reports of Miller syndrome patients, and opened the possibility of rapid biochemical screening.
© 2016 Elsevier Inc. All rights reserved.

Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; CYP, cytochrome-P450; Cyt C, cytochrome C; DHO, dihydroorotic acid; DHODH, DHO dehydrogenase; EDTA, ethylenediamine-tetraacetic acid; FMN, flavin mononucleotide; GGT, gamma-glutamyl transferase; LC-MS/MS, high performance liquid chromatography-tandem mass spectrometry; OA, orotic acid; POADS, post-axial acrofacial dysostosis; UMP, uridine monophosphate; UQH2, reduced ubiquinone.

* Corresponding author at: School of Pharmacy, PACE Building, Cornwall Street, Woolloongabba, Brisbane, 4102, QLD, Australia.
E-mail addresses: jduley@pharmacy.uq.edu.au (J.A. Duley), michael.henman@mater.org.au (M.G. Henman), kevin.carpenter@health.nsw.gov.au (K.H. Carpenter), mbamshad@u.washington.edu (M.J. Bamshad), george.marshall@mater.org.au (G.A. Marshall), keith.ooi@unsw.edu.au (C.Y. Ooi), bridget.wilcken@health.nsw.gov.au (B. Wilcken), jason.pinner@sswahs.nsw.gov.au (J.R. Pinner).

1. Introduction

Dihydroorotate dehydrogenase (DHODH, *OMIM #126064*) oxidises dihydroorotate (DHO) to orotic acid (OA) during pyrimidine de novo synthesis. The enzyme is located on the inner mitochondrial membrane, with flavin mononucleotide as the electron acceptor. Thereafter, electrons are passed to ubiquinone (coenzyme Q10) then cytochrome C and oxygen as final electron acceptors (Fig. 1).

DHODH directly links carbamoyl-phosphate metabolism to pyrimidine nucleotide synthesis and to the production of energy via mitochondrial oxidative phosphorylation [1].

http://dx.doi.org/10.1016/j.ymgme.2016.06.008
1096-7192/© 2016 Elsevier Inc. All rights reserved.

Miller syndrome, also known as Genee-Wiedemann or Wildervanck-Smith syndromes (exhibiting post-axial acrofacial dysostosis, POADS) was the first Mendelian disorder whose genetic basis was identified using whole exome sequencing, when it was shown to be caused by mutations in the *DHODH* gene [2]. However, despite the description of a number of *DHODH* mutations that have been demonstrated to cause loss of enzyme activity in vitro or in silico [3], DHO has not been shown to accumulate, at least not in urine: in the only two cases where metabolic analyses of Miller syndrome patients have been described, urinary OA was raised but DHO absent. This is the reverse of the predicted metabolite pattern, and has presented a conundrum for metabolic diagnosis.

We report here the presence of grossly raised concentrations of DHO in the plasma and urine of a patient with Miller syndrome. DHO was also easily detectable in dried blood and dried plasma on filter paper. OA was elevated in urine but it was undetectable in plasma. We examined the distribution of DHO between plasma and blood cells. We propose that loss of DHODH activity leads to the systemic accumulation of DHO, which is converted to OA during renal excretion, by either residual DHODH activity or an alternative oxidative enzyme mechanism.

2. Methods

2.1. Patient

Our 4-year-old patient was born prematurely at 27/40 weeks, in Sydney, with bilateral absence of the 5th rays of his hands, cleft palate and a peri-membranous ventricular septal defect. He had prominent eyes, a short nose with upturned nares, a low nasal bridge, high columella, a deeply grooved philtrum, prominent infraorbital creases, single palmar creases, broad thumbs and short, broad halluces as well as under-riding 4th toes (hypoplastic 4th rays on X-ray) (Fig. 2). There was no contributing family history and his parents were non-consanguineous. His neonatal course was complicated by respiratory distress syndrome, heart failure and cholestasis. The ventricular septal defect closed spontaneously. He had uncomplicated repairs to a bilateral inguinal hernia and cleft palate at 13 months.

He developed bilateral radioulnar synostosis and has myopic astigmatism corrected with glasses. His height and weight continue around the third percentiles. He has had mild speech delay and some behavioural problems. His facial features are similar to other reported cases, including sparse eyebrows, almond shaped eyes with up-slanting palpebral fissures, malar hypoplasia, long philtrum, small mouth, and low-set, normally-shaped ears. He has unusual cruciform palmar creases. Our case thus has the characteristic features of Miller syndrome, including cleft palate, congenital heart disease, oligodactyly and consistent dysmorphic features.

His neonatal cholestasis was initially thought to be due to total parenteral nutrition. Due to persistence of cholestasis despite cessation of parenteral nutrition and normalisation of oral feeds, he was extensively investigated but no aetiology was identified. An intraoperative cholangiogram and liver histopathology excluded biliary atresia. Liver biopsy revealed unusual generalised feathery degeneration of hepatocytes, mild fibrosis and cholestasis. Immunohistochemical staining for familial cholestatic diseases was negative, and typing of his alpha-1-antitrypsin, PiMM, was normal.

The conjugated hyperbilirubinaemia resolved at 6 months of age while receiving ursodeoxycholic acid, but the patient's liver parameters remained abnormal, with fluctuating elevated transaminases (AST 87–228 U/L, reference range: 10–50; ALT 132–433 U/L, ref.: 0–45; GGT 78–263 U/L, ref.: 0–45) and progressive hepatomegaly (9.5 cm). Albumin levels have been normal. He continued treatment with ursodeoxycholic acid 250 mg bd. Following approval from the local Human Research Ethical Committee, he was provided with a 3-month trial of oral uridine, 100 mg/kg/day, as a dietary supplement.

2.2. Patient samples

Blood and plasma (EDTA anticoagulant) and urine samples were initially collected in Sydney during October 2013, for biochemical diagnosis of the patient. The uridine trial began with baseline samples ($t = 0$ on Fig.4) being taken in mid-April 2015, approximately 80 weeks after diagnosis and 2 weeks before commencement of uridine. Samples were then collected routinely until the end of July 2015. Blood samples were cooled, and plasma and urine were frozen then immediately

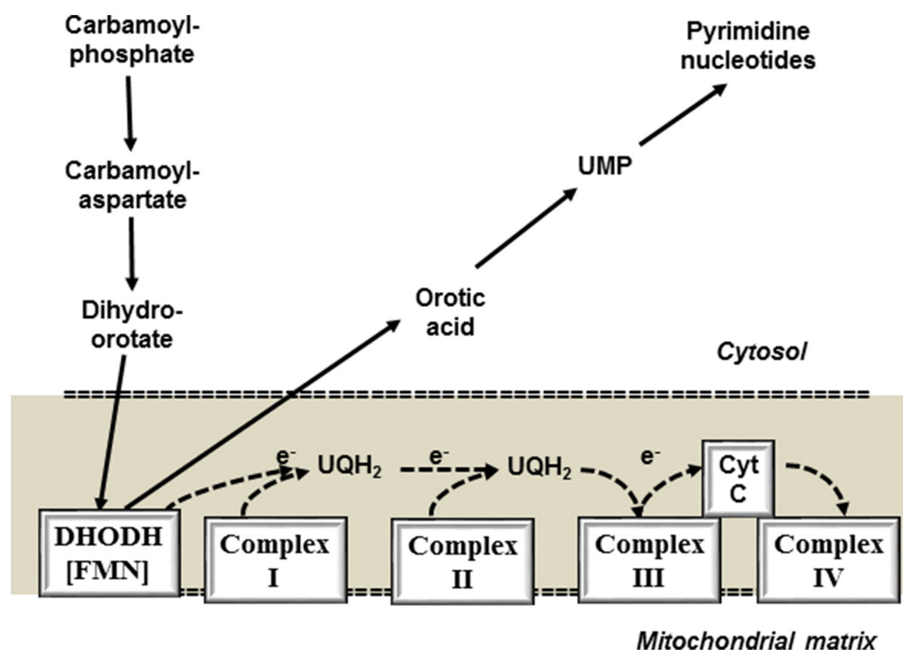

Fig. 1. Diagrammatic representation of cell cytosol and mitochondrial membrane showing dihydroorotate dehydrogenase (DHODH) located on the inner mitochondrial membrane, passing electrons (e^-) from flavin mononucleotide (FMN) coenzyme to reduce ubiquinone (UQH_2), ultimately reducing cytochrome C (Cyt C) and thus feeding into the oxidative phosphorylation complex which produces energy. UMP: uridine monophosphate.

Please cite this article as: J.A. Duley, et al., Elevated plasma dihydroorotate in Miller syndrome: Biochemical, diagnostic and clinical implications, and treatment with uridine, Mol. Genet. Metab. (2016), http://dx.doi.org/10.1016/j.ymgme.2016.06.008

Download English Version:

https://daneshyari.com/en/article/5513991

Download Persian Version:

https://daneshyari.com/article/5513991

Daneshyari.com