Molecular Genetics and Metabolism Reports 5 (2015) 12–14

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: http://www.journals.elsevier.com/molecular-genetics-and-metabolism-reports/

Urinary phenylacetylglutamine (U-PAGN) concentration as biomarker for adherence in patients with urea cycle disorders (UCD) treated with glycerol phenylbutyrate

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ARTICLE INFO

Article history:
Received 5 August 2015
Received in revised form 18 September 2015
Accepted 18 September 2015
Available online 27 September 2015

Keywords:
Urinary phenylacetylglutamine
Glycerol phenylbutyrate
Phenylbutyrate
Phenylacetate
Biomarker
Urea cycle disorder

ABSTRACT

Urinary phenylacetylglutamine (U-PAGN) concentrations in spot urine samples were analyzed as a dosing biomarker during glycerol phenylbutyrate (GPB) dosing in 68 healthy adults and 66 adult and pediatric patients with urea cycle disorders who participated in GPB clinical trials. Age- and body surface area (BSA)-specific 25th percentile cutoff points for spot U-PAGN concentrations (<~9000 μg/mL for <2 years old patients, <7000 μg/mL for >2 years with BSA ≤1.3 m^2, and <~5000 μg/mL for >2 years of age with BSA >1.3 m^2) were determined as an approach to identify patients for whom increased dosing and/or adherence to prescribed dosing should be assessed.

1. Introduction

Urea cycle disorders (UCDs) are manifested by the accumulation of toxic levels of ammonia in the blood and brain [1–3]. Patients whose symptoms are not adequately controlled with diet alone are generally treated with phenylacetic acid pro-drugs such as glycerol phenylbutyrate (GPB) and sodium phenylbutyrate, which lower ammonia by enhancing excretion of waste nitrogen in the form of urinary phenylacetylglutamine (PAGN) [4,5].

Recent studies indicate that blood ammonia and phenylbutyrate metabolite levels, including the plasma phenylacetate (PAA):PAGN ratio and the total daily urinary PAGN (U-PAGN) output, are useful biomarkers of GPB dosing [6,7]. However, measurement of daily U-PAGN output involves timed urine collections [7] which are cumbersome in adults and generally not feasible in children on an outpatient basis. In contrast, measurement of U-PAGN concentration can be readily performed on spot urine samples in patients of all ages.

The objective of this study was to evaluate the utility of morning spot U-PAGN concentrations as a marker of GPB dosing across all age groups of UCD patients.

2. Methods

A total of 500 spot urine samples, including 259 of which from the first morning void (fasting), were collected from 68 healthy adults and 66 UCD patients (44 adult and 22 pediatric patients <6 years of age). Samples were collected during dosing with GPB (RAVICTI®, Horizon Pharma) in one of three clinical studies: a blinded crossover thorough QTc study in healthy adults; a randomized, double-blind, active-controlled, crossover study in adult UCD patients [8] and a short-term, open-label, switchover study in UCD patients <6 years of age [9] (ClinicalTrials.gov NCT01135680, NCT00992459, and NCT01347073).

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http://dx.doi.org/10.1016/j.ymgmr.2015.09.003

The protocol and informed consent for each study were reviewed and approved by the Investigational Review Board of each participating institution and informed consent was obtained from all subjects prior to the initiation of any study procedures.

Healthy adults received daily GPB (1 mL = 1.1 g PBA) doses of 12 and 18 mL/day in the thorough QTc study (mean of 8.16 mL/m^2/day), whereas adult UCD subjects received a mean total daily dose of 12.1 mL/day (range = 0.57–13.95 mL/m^2/day) and pediatric UCD subjects received a mean daily dose of 5.1 mL/day (range = 0.92–12.63 mL/m^2/day). Mean daily GPB doses were higher for subjects with a body surface area BSA >1.3 m^2 (14.4 mL/day) than for those with a BSA ≤1.3 m^2 (5.1 mL/day), but mean GPB doses adjusted for BSA were similar for these two groups (7.86 and 7.17 mL/m^2/day, respectively).

Regression analyses were performed and Spearman rank-order correlations computed based on all 500 urine samples to define the relationship between overall dosing and U-PAGN concentration. To further examine the relationship between dose, age, and BSA so as to determine clinically useful cut off points to identify patients who may benefit from increased dosing and/or assessment of compliance, analyses were restricted to spot urine samples at the 24-h time points (i.e. equivalent to a first morning void after an overnight fast) obtained from patients >2 years of age (n = 259) but including all samples for patients <2 years of age (n = 57). The rationale is that the youngest patients were on frequent feeding schedules and morning spot samples cannot be reliably categorized as fasting or distinguished from other daily urine samples as is the case for older patients who feed less frequently. U-PAGN concentrations were measured by a validated liquid chromatography tandem mass spectrometry method as previously described [7].

3. Results

Regression analyses demonstrated a positive correlation between spot U-PAGN concentration and BSA-corrected total daily GBP dose and ($r = 0.13$, $p = 0.0036$) and negative correlations between spot U-PAGN and the total daily GBP dose (mL/day) ($N = 500$, $r = -0.155$, $p = 0.0005$) as well as with BSA ($r = -0.347$, $p < 0.0001$).

To define possible clinically useful cut offs, further analyses were carried out on morning (fasting) urine samples (259 of 500 samples) from healthy adults and UCD patients and over 2 years of age and from all samples in UCD patients <2 years of age. The mean, median and percentile values were calculated and the lower 25th percentile values determined for each category (Table 1). Mean (median) U-PAGN concentrations in samples obtained from subjects >2 years and with BSA ≤1.3 m^2 (N = 87) and those with BSA >1.3 m^2 (N = 172) were 15,488 (15,147) and 8959 (7574) μg/mL, respectively, and were highest in subjects <2 years old (N = 57) at 17,358 (17,288) μg/mL.

Table 1
Dose, no. of samples and spot U-PAGN concentrations by BSA and age.

	BSA > 1.3 m^2	BSA ≤ 1.3 m^2	2–5 years	<2 years
Dosing and number of urine samples	Mean total dose (mL/day)			
	14.38	5.09	5.8	3.2
	Daily dose (mL/m^2/day)			
	7.86	7.17	7.80	6.5
	Number of samples (all time points)			
	290	210	229	57
	Number of morning samples (24-h time point)			
	172	108	85	21
	U-PAGN concentration (μg/mL)			
	>2 years[a]		<2 years[b]	
	BSA > 1.3 m^2	BSA ≤ 1.3 m^2		
N	172	87	57	
Mean	8959	15,488	17,358	
Median	7574	15,147	17,288	
Minimum	642	119	348	
10th percentile	3538	1369	3200	
25th percentile	5038	6398	8996	
75th percentile	11,276	23,779	25,019	
90th percentile	15,994	30,629	28,295	
Maximum	60,960	43,372	44,298	

[a] Only samples taken in morning (fasting) were included.
[b] All available samples for subjects under 2 years were used.

4. Discussion

The principal objective of these analyses was to determine whether the relationship between U-PAGN concentration and GPB dose is sufficiently consistent as to allow U-PAGN concentration to serve as a useful dosing biomarker. They build on a previous report [7] demonstrating the utility of 24-h urinary PAGN output as a marker of drug mediated waste nitrogen excretion and extend this work in two respects. First, rather than dealing with 24 h U-PAGN output, the current analyses focused on urinary PAGN concentration taken from a single urine sample, which is more convenient to use in clinical practice. Second, whereas the prior report was restricted to UCD patients ages ≥6 years, the current analyses include UCD patients down to 2 months of age as well healthy adults. While urinary creatinine concentrations were not available for all samples and therefore U-PAGN concentrations were not normalized by the concomitant creatinine concentration to correct for hydration status, the vast majority of samples were taken in the morning when urine is maximally concentrated. Moreover, prior studies in adult UCD patients [8] showed no difference in the correlation between dose and either spot U-PAGN concentration or the ratio of the concentrations of U-PAGN to creatinine in a spot urine.

The finding that U-PAGN concentration varies inversely with BSA and/or age likely reflects the fact that pediatric UCD patients, who tend to be more severely affected and often receive proportionately more drug than adults, also exhibit slower PAA to PAGN conversion [10] and, therefore, more continuous PAGN output. In this regard, the results of the present analyses are also consistent with the findings previously reported by Monteleone et al. [10]. Based on population PK analyses and dosing simulations reported by Monteleone, there is a direct relationship between the PAA to PAGN conversion rate and BSA, an inverse relationship between BSA and plasma PAA levels, and that a BSA of ≤1.3 m^2, which includes children less than 2 years of age with the highest U-PAGN concentrations and corresponds closely to the division between children (<11 years) and teenagers/adults (≥12 years), was a better differentiator of systemic PAA exposure than the categories of 'adult vs. 'pediatric'.

Finally, these analyses suggest that spot U-PAGN concentrations falling into the lower end of the expected range may be useful in identifying patients who may be receiving insufficient drug. While various cutoff points could be used, a U-PAGN concentration falling into the bottom 25th percentile for age and BSA (<~9000 μg/mL for <2 years old, <~7000 μg/mL for >2 years with BSA ≤1.3 m^2, and <~5000 μg/mL for >2 years of age with BSA >1.3 m^2) represents a clinically conservative and reasonable cutoff. In this context, a U-PAGN concentration in the bottom 25th percentile accompanied by elevated ammonia may represent a useful clue to the treating physician in identifying patients who may benefit from an increase in dose, who are not compliant with the prescribed dosing and/or, particularly in the case of small children and infants, whose parents or caregivers are not effectively delivering the drug.

Disclosures

M Mokhtarani, DF Coakley and BF Scharschmidt are/were Horizon Pharma (former known as Hyperion Therapeutics, Inc.) employees and shareholders at the time of this study.

References

[1] S.W. Brusilow, N.E. Maestri, Urea cycle disorders: diagnosis, pathophysiology, and therapy, Adv. Pediatr. 43 (1996) 127–170.

[2] M.L. Summar, D. Dobbelaere, S. Brusilow, B. Lee, Diagnosis, symptoms, frequency and mortality of 260 patients with urea cycle disorders from a 21-year, multicentre study of acute hyperammonaemic episodes, Acta Paediatr. 97 (2008) 1420–1425.
[3] M. Tuchman, B. Lee, U. Lichter-Konecki, M.L. Summar, M. Yudkoff, S.D. Cederbaum, D.S. Kerr, G.A. Diaz, M.R. Seashore, H.S. Lee, R.J. McCarter, J.P. Krischer, M.L. Batshaw, Cross-sectional multicenter study of patients with urea cycle disorders in the United States, Mol. Genet. Metab. 94 (2008) 397–402.
[4] M.L. Batshaw, S. Brusilow, L. Waber, W. Blom, A.M. Brubakk, B.K. Burton, H.M. Cann, D. Kerr, P. Mamunes, R. Matalon, D. Myerberg, I.A. Schafer, Treatment of inborn errors of urea synthesis: activation of alternative pathways of waste nitrogen synthesis and excretion, N. Engl. J. Med. 306 (1982) 1387–1392.
[5] G.M. Enns, S.A. Berry, G.T. Berry, W.J. Rhead, S.W. Brusilow, A. Hamosh, Survival after treatment with phenylacetate and benzoate for urea-cycle disorders, N. Engl. J. Med. 356 (2007) 2282–2292.
[6] M. Mokhtarani, G.A. Diaz, W. Rhead, S.A. Berry, U. Lichter-Konecki, A. Feigenbaum, A. Schulze, N. Longo, J. Bartley, W. Berquist, R. Gallagher, W. Smith, S.E. McCandless, C. Harding, D.C. Rockey, J.M. Vierling, P. Mantry, M. Ghabril, R.S. Brown Jr., K. Dickinson, T. Moors, C. Norris, D. Coakley, D.A. Milikien, S.C. Nagamani, C. Lemons, B. Lee, B.F. Scharschmidt, Elevated phenylacetic acid levels do not correlate with adverse events in patients with urea cycle disorders or hepatic encephalopathy and can be predicted based on the plasma PAA to PAGN ratio, Mol. Genet. Metab. 110 (2013) 446–453.
[7] M. Mokhtarani, G.A. Diaz, W. Rhead, U. Lichter-Konecki, J. Bartley, A. Feigenbaum, N. Longo, W. Berquist, S.A. Berry, R. Gallagher, D. Bartholomew, C.O. Harding, M.S. Korson, S.E. McCandless, W. Smith, J. Vockley, S. Bart, D. Kronn, R. Zori, S. Cederbaum, N. Dorrani, J.L. Merritt 2nd, S. Sreenath-Nagamani, M. Summar, C. Lemons, K. Dickinson, D.F. Coakley, T.L. Moors, B. Lee, B.F. Scharschmidt, Urinary phenylacetylglutamine as dosing biomarker for patients with urea cycle disorders, Mol. Genet. Metab. 107 (2012) 308–314.
[8] G.A. Diaz, L.S. Krivitzky, M. Mokhtarani, W. Rhead, J. Bartley, A. Feigenbaum, N. Longo, W. Berquist, S.A. Berry, R. Gallagher, U. Lichter-Konecki, D. Bartholomew, C.O. Harding, S. Cederbaum, S.E. McCandless, W. Smith, G. Vockley, S.A. Bart, M.S. Korson, D. Kronn, R. Zori, J.L. Merritt 2nd, C.S.N. S, J. Mauney, C. Lemons, K. Dickinson, T.L. Moors, D.F. Coakley, B.F. Scharschmidt, B. Lee, Ammonia control and neurocognitive outcome among urea cycle disorder patients treated with glycerol phenylbutyrate, Hepatology 57 (2013) 2171–2179.
[9] W. Smith, G.A. Diaz, U. Lichter-Konecki, S.A. Berry, C.O. Harding, S.E. McCandless, C. LeMons, J. Mauney, K. Dickinson, D.F. Coakley, T. Moors, M. Mokhtarani, B.F. Scharschmidt, B. Lee, Ammonia control in children ages 2 months through 5 years with urea cycle disorders: comparison of sodium phenylbutyrate and glycerol phenylbutyrate, J. Pediatr. 162 (2013) 1228–1234 (1234 e1221).
[10] J.P. Monteleone, M. Mokhtarani, G.A. Diaz, W. Rhead, U. Lichter-Konecki, S.A. Berry, C. Lemons, K. Dickinson, D. Coakley, B. Lee, B.F. Scharschmidt, Population pharmacokinetic modeling and dosing simulations of nitrogen-scavenging compounds: disposition of glycerol phenylbutyrate and sodium phenylbutyrate in adult and pediatric patients with urea cycle disorders, J. Clin. Pharmacol. 53 (2013) 699–710.