Riboflavin-Responsive Trimethylaminuria in a Patient with Homocystinuria on Betaine Therapy

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Abstract A 17-year-old female patient with pyridoxine non-responsive homocystinuria, treated with 20 g of betaine per day, developed a strong body odour, which was described as fish-like. Urinary trimethylamine (TMA) was measured and found to be markedly increased. DNA mutation analysis revealed homozygosity for a common allelic variant in the gene coding for the TMA oxidising enzyme FMO3. Without changing diet or betaine therapy, riboflavin was given at a dose of 200 mg per day. An immediate improvement in her odour was noticed by her friends and family and urinary TMA was noted to be greatly reduced, although still above the normal range.

Gradual further reductions in TMA (and odour) have followed whilst receiving riboflavin. Throughout this period, betaine compliance has been demonstrated by the measurement of dimethylglycine (DMG) excretion, which has been consistently increased. Marked excretions of DMG when the odour had subsided also demonstrate that DMG was not the source of the odour.

This patient study raises the possibility that betaine may be converted to TMA by intestinal flora to some degree,

resulting in a significant fish odour when oxidation of TMA is compromised by *FMO3* variants. The possibility exists that the body odour occasionally associated with betaine therapy for homocystinuria may not be related to increased circulating betaine or DMG, but due to a common *FMO3* mutation resulting in TMAU. Benefits of riboflavin therapy for TMAU for such patients would allow the maintenance of betaine therapy without problematic body odour.

Introduction

Trimethylaminuria (TMAU) and its associated body odour (“Fish Odour Syndrome”) can be caused by lack of trimethylamine (TMA) N-oxidation by the hepatic enzyme flavin containing mono-oxygenase type 3 (*FMO3*) (Primary TMAU) (MIM 136132) (Humbert et al. 1970) (Lee et al. 1976) as well as excess TMA production by intestinal flora (Secondary TMAU) (Mitchell 1996) (Fraser-Andrews et al. 2003). More than 40 mutations have been described in the *FMO3* gene, which is located on chromosome 1, with clear genotype–phenotype correlation (Treacy et al. 1998; Zschocke et al. 2002) including combinations of allelic variants, which have been associated with mild or intermittent TMAU. Most common is the variant p.[Glu158Lys; Glu308Gly] which, in homozygous state, may affect between 1 in 100 and 1 in 20 of the population (Zschocke et al. 1999). Presentation may be limited to periods of high dietary choline and seafood as well as peri-menstrual periods in female patients.

Body odour has been reported as a side effect of betaine (trimethylglycine) administration (Wilcken and Wilcken 1997) when used in therapeutic doses for the treatment of pyridoxine non-responsive homocystinuria (cystathionine beta-synthase deficiency (MIM 236200). The description of

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the odour has been reported as fishy (Kraus and Kozich 2001) suggesting TMA, but could also be due to the demethylated metabolite of betaine, dimethylglycine (DMG). DMG has not only been reported at high concentrations in plasma and urine following betaine administration (Schwahn et al. 2003), but has been associated with a fish-like odour in a single case of DMG-dehydrogenase deficiency (Moolenaar et al. 1999).

Riboflavin (vitamin B2) administration has been associated with reduction of TMA excretion in some TMAU patients (unpublished observations) presumed to be due to increased FMO3 activity with riboflavin acting as a co-factor. Riboflavin responsiveness has previously been reported for some patients with multiple acyl-CoA dehydrogenase deficiency where riboflavin is a co-factor for the electron transfer flavoprotein-CoQ, which is vital for the activity of acyl-CoA dehydrogenases (Olsen et al. 2007). The case of TMAU presented here clearly demonstrates a response to riboflavin in lowering TMA excretion with concomitant reduction in body odour in a teenage girl receiving high-dose betaine therapy for homocystinuria.

Methods

TMA: Urinary measurement of TMA (with TMA-oxide by titanium chloride reduction) was achieved using alkalised samples heated in a headspace autosampler with gas chromatography–mass spectrometry (GCMS) of gaseous TMA. Stable isotope ratio of TMA to deuterated (d9-) TMA was used for quantitation (Treacy et al. 1995).

40 µg of d9-TMA-DCI internal standard was added to 2 ml of urine for TMA (free) analysis and 0.2 ml of urine for free TMA plus TMA-oxide (total) analysis. Following reaction with titanium chloride for the total TMA measurement duplicates of free and total TMA for each urine were cooled in ice and alkalised by addition of 0.6 g of KOH and 1 g of K₂CO₃. Calibration standards of TMA hydrochloride and TMA-oxide were taken through the method together with quality control samples at two levels.

Prepared samples were sealed in vials and loaded on to a headspace autosampler (HP7694 Agilent Technologies), which was linked through a heated line to a GCMS (Agilent 5973N) fitted with a 15 m fused silica column with no stationary phase. Samples were heated to 90°C for 40 min prior to injection. GCMS run time was 5 min during which TMA was monitored using the M-H ion (m/z 58) and d9-TMA with the M-D ion (m/z 68). Peak area ratios for both free and total TMA were used to calculate concentrations following calibration of the internal standard.

DMG: Urine with d2-DMG internal standard was analysed by lyophilisation and GCMS of tert-butyldimethylsilyl ester.

100 µl aliquots of urine (diluted to achieve a creatinine value of 0.1 mmol/L) were mixed with an aqueous solution containing 0.5 µg d2-DMG internal standard and 1 ml of methanol was added to aid lyophilisation. Vials were placed under a gentle stream of nitrogen for 45 min at 40°C. Dried samples were then derivatised by addition of 100 µl N-methyl-tert-butyldimethylsilyl-trifluoroacetamide (MTBSTFA) with 100 µl acetonitrile and heating to 80°C for 45 min. Tertiary butyl dimethylsilyl esters were injected on to a GC column (SGE BPX5 30m) using a 3 µl split injection. Quantitation was achieved by monitoring ions of m/z 160 and 162 for DMG and d2-DMG, respectively.

DNA: Patient genomic DNA was extracted from peripheral blood samples using Magnetic Separation Module I (Chemagen). Genomic DNA was amplified by PCR using Red Hot Taq polymerase (ABGene) with 3 mM MgCl₂.

Primers were designed to amplify each protein-coding exon and at least 25 bp of the intron/exon boundaries for exons 2–9 of the *FMO3* gene (Accession number NM_006894.5). Cycle sequencing was performed using standard M13 primers attached to the gene-specific primers with electrophoresis carried out on ABI3730 DNA analysers.

Case Report

SW was initially diagnosed with homocystinuria at 7 years of age after presenting with dislocated lenses. Her plasma total homocysteine (tHcys) was measured at 145 µmol/L (ref. < 16), which was partially responsive to pyridoxine (121 µmol/L). Further reduction of tHcys (59 µmol/L) was achieved by betaine therapy initially at three doses of 2 g per day, which was increased to two doses of 8 g per day by the time she was 15 years of age. Plasma methionine increased dramatically with betaine therapy from 57 to 1,445 µmol/L (ref. 8–47) indicating successful remethylation of homocystine. During teenage years plasma tHcys concentrations of more than 100 µmol/L gave cause for concern as linear growth came to an end, thus decreasing protein requirement and increasing protein catabolism. As a response to this change, betaine dosage was increased to 20 g per day, which unfortunately resulted in a lack of compliance (unused betaine was discovered at home) with resultant very high plasma tHcys results. Following clinical advice and family intervention, compliance improved and tHcys values started to normalise. At this new high dosage, however, a strong fishy body and breath odour was noticed by family and friends, which began to cause problems at school. The odour persisted when the betaine dose was reduced to 16 g per day. At 17 years of age, this serious social problem posed another threat to betaine compliance

Table 1 Urinary TMA and DMG $\mu\text{mol}/\text{mmol}$ creatinine. Plasma DMG $\mu\text{mol}/\text{L}$ before and after riboflavin administration

Days on riboflavin	Free TMA (ref. < 11)	Free/total TMA (ref. < 0.21)	DMG (ref. < 16)	Plasma DMG (ref. < 8)
0	392	0.93	1,471	272
28	77	0.36	1,076	216
77	37	0.27	1,017	
133	27	0.21	2,044	
223	22	0.17	1,935	
328	20	0.07	957	

and metabolic control of SW's homocystinuria. It was also reported that the odour was more noticeable around the time of menstruation. The family reported that the odour was directly related to betaine administration.

TMA was measured in a urine sample and found to be markedly increased at 392 mmol/mol creatinine (normal range 2–11). Her free TMA/total TMA (free TMA + TMA-oxide) ratio was also increased at 93% (normal range < 21%).

It was decided to not withdraw or modify betaine therapy or make any changes to her diet, but to try riboflavin at two doses of 100 mg per day.

Within days of riboflavin supplementation, SW's body odour had significantly improved and her family could hardly detect any body odour.

Urinary TMA was measured after 30 days and was found to have decreased to 77 (mmol/mol creat.) (Table 1) (36% free/total TMA). Further measurements of TMA showed continued reduction of excretion to 20 after 330 days on riboflavin (7% free/total TMA). SW has reported a residual fishy taste (breath odour only), however body odour is no longer a problem.

Urinary DMG measurements (Table 1) show the expected marked excretion consistent with betaine compliance ranging from 1.0 to 2.2 mol/mol creat. Plasma DMG was also measured and found to be 272 and 216 $\mu\text{mol}/\text{L}$ (ref. < 8) at the time of the first two urines collected. Importantly, these DMG values in our patient were maintained after the body odour subsided.

Mutation Analysis

Genomic DNA analysis by sequencing revealed a previously described common variant allele p.Glu158Lys; Glu308Gly in the homozygous state (Zschocke et al. 1999). Previous reports have associated this genotype with a mild or intermittent phenotype, most likely to only present with a significant odour when challenged by dietary

TMA precursors or during hormonal fluctuation such as just prior to menstruation.

Discussion

Side effects of betaine (Cystadane) have been well documented and include anorexia, agitation, depression, irritability, personality disorder, sleep disturbance, dental disorders, diarrhoea, glossitis, nausea, stomach discomfort, vomiting, urinary incontinence, hair loss, hives and abnormal skin odour (Orphan-Europe 2007). These undesirable effects have been classified as “uncommon” with a frequency of between 1 in 100 and 1 in 1,000 patients.

In a pilot study of betaine therapy for non-alcoholic steatohepatitis, however, four of the ten subjects experienced nausea, abdominal cramps, loose stools and body odour (Abdelmalek et al. 2001).

Choline (2-hydroxy-trimethylammonio-ethanol) has been shown to be readily converted to TMA-oxide when fed to rats (Norris and Benoit 1945). In experiments comparing urinary TMA-oxide produced after injection and feeding approximately 27% of fed choline was converted to TMA-oxide compared to 2% by injection. This bacterial route was not reproduced by betaine feeding, which resulted in less than 1% conversion. Previously, intestinal bacterial production of TMA from betaine had been described as “trace” (Wunsche 1940). Therefore, it is unsurprising that betaine has been disregarded as a source of TMA by enterobacterial action (Busby et al. 2004).

Conversion of betaine to TMA as part of methanogenesis has been reported for some bacterial species, notably *Clostridium sporogenes* (Naumann et al. 1983), *Desulfuromonas acetoxidans* (Heijthuisen and Hansen 1989), and *Haloanaerobacter salinarum* (Moune et al. 1999). Whether the dynamics of the pathways for these species could result in significant output of TMA, however, is not certain.

Our patient had no problematic side effects of betaine therapy from the age of 7 until 15 years. Her odour can be strictly correlated with TMAU and follows the course of the mild or intermittent form of this disorder, which is associated with the common allelic variant previously described. Female patients with this variant often suffer from a fish odour around the time of menstruation. The odour may then subside unless challenged with a significant dietary load (usually high-choline foods such as eggs, legumes, offal as well as seafood (TMA-oxide). Dietary loading with high-choline foods and marine fish has become a vital diagnostic tool, especially given that intermittent odour is a feature of some *FMO3* variants (Chalmers et al. 2003).

Our patient's presentation appears, therefore, to be strongly linked to the combined effects of betaine loading and a mild FMO3 deficiency caused by an underlying genetic condition other than her homocystinuria. The immediate reduction in TMA excretion when given 100 mg per day riboflavin without reducing betaine therapy demonstrates a mild phenotype, which would possibly present intermittently if loading with betaine was not ongoing.

Betaine compliance was demonstrated by a marked excretion of DMG, which remained elevated when the odour (and TMA excretion) was significantly reduced. Our patient's odour remained barely detectable following the establishment of riboflavin supplementation, although DMG excretion actually increased between 77 and 133 days after riboflavin. Plasma values were similar to those of the patient described with a fishy odour attributed to dimethylglycinuria (Moolenaar et al. 1999) with urinary values for our patient two- to fourfold greater than those of the reported dimethylglycinuria patient. Fish odour associated with increased DMG therefore appears to be inconsistent when taking into account our patient and other conditions, which result in increased DMG such as multiple acyl-CoA dehydrogenase deficiency (MADD) where no reports of odour have been cited (Burns et al. 1998) with excretion of DMG as marked as in the single reported case of DMG-dehydrogenase deficiency.

SW has continued to be betaine compliant even though a residual breath odour has been reported by her as the taste of fish. TMA excretion has continued to fall over the months since commencement of riboflavin and may eventually reach normal values. Without riboflavin administration, there is no doubt that taking high doses of betaine would have become difficult for this patient. Variable betaine compliance has been reported as an issue for the treatment of pyridoxine non-responsive homocystinuria (Singh et al. 2004). Side effects such as odour may have contributed to this lack of compliance and led to sub-optimal control of patients' metabolic state. Despite the routine listing of unusual body odour as an occasional side effect of betaine therapy (Orphan Europe 2007), the precise nature of the odour has not been described other than that of "fishy" (Kraus and Kozich 2001). Hypermethioninaemia from remethylation of homocystine by betaine has been associated not only with organ toxicity in rats and growth retardation in infants (Smolin et al. 1981) but also with a sulphurous, cabbage-like body odour (Påby et al. 1989). Therefore, a combination of odours may trouble some patients receiving betaine, both TMA and methionine contributing. Measurement of TMA, FMO3 genotyping and riboflavin therapy for some patients with homocystinuria may enable odour relief and promote compliance with high dosage betaine therapy and its life-saving benefits.

Synopsis

Betaine-related body odour may be due to trimethylaminuria and respond to riboflavin therapy without lowering betaine dosage.

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