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Problems in the management of urea cycle disorders

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Abstract

Several recent reviews describe the management of urea cycle disorders. There is much agreement on diet, alternative pathway therapy, maintenance of arginine and ornithine levels in acute and chronic management, sick day regimens, and some aspects of monitoring. However, differences remain in several areas, and physicians at most treatment centers have relatively little experience, because these disorders are rare. Early suspicion of the diagnosis of a urea cycle disorder, and prompt referral to a tertiary center is vital. Drug treatment using chronic administration of sodium benzoate has been abandoned by some centers, but the acceptability of phenylbutyrate is an issue for many patients. Using citrulline chronically is not always successful in recommended doses, and may result in an arginine level too low for maximum control. Appetite and nutrition problems are common. One major concern is the early identification and management of chronic catabolism, theoretically easy, but hard in practice. Biochemical measurement problems complicate monitoring, and there are disagreements about the optimum way of identifying OTC carriers. It is not always clear whom to treat. Within a kindred with an early onset phenotype, an asymptomatic newborn girl may need treatment for some undetermined time, but target values for monitoring are not clear. In late onset phenotypes, management of asymptomatic males identified by family screening is also difficult. Most centers do not have sufficient cases to solve these conundrums, some of which require further multicenter study. This paper examines the recommendations of a consensus conference on management, outlines some remaining problems, and incorporates in the text the points raised in open discussion during a session of a symposium held in Sydney in 2003 entitled "New Developments in Urea Cycle Disorders."

Keywords: Urea cycle; Ornithine transcarbamylase; Ammonia-scavengers; Sodium benzoate; Sodium phenylbutyrate; Carrier testing; Heterozygote; Nitrogen flux

Introduction

There have been several reviews and position statements about the diagnosis and management of urea cycle disorders (UCDs) [1,2]. In 2000 a consensus conference was held in Washington DC, to discuss and try to come to agreement about the management of patients with UCDs. The proceedings were published in January 2001 [3] and consist of a series of papers tackling various aspects of diagnosis and management. The publication opens with a number of recommendations reflecting the opinion of the 18 participants, all but two of whom were from the United States of America. The authors rightly point out that many of the relevant aspects have not been studied scientifically, and "...cannot be regarded as evidence-based medicine." Much of what is set out in this excellent document is well accepted by everyone in the field, but there are remaining concerns and points of contention.

The UCDs are individually quite rare, and many otherwise experienced metabolic physicians have managed far fewer than 50 patients with a UCD. Table 1 shows the numbers of patients diagnosed in New South Wales over a period of nearly 30 years. New South Wales has a population of 6 million, and there is a long-established central service for both the diagnosis and treatment of patients with inborn errors of metabolism. The 56 cases of primary UCD presenting clinically equate to an observed birth prevalence in New South Wales of 1:44,000, similar to that in British Columbia [4] but much lower than the estimate postulated by Brusilow and Maestri [5]. Also, as is found in most other centers, a high proportion (57%) of our patients had

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Table 1
Urea cycle defects diagnosed in New South Wales 1974 2003

Diagnosis	Number
Primary defects	
NAGS	1 (not proven)
CPS	5
OTC	32 (57%)
Early onset males	19
Late onset males	7
Female propositi	6
ASS	7
ASL	11
Arginase	1
Total	56
Overall incidence, primary UCDs	1:44,000 (95% CI
excluding asymptomatic	1:31,000 1:53,000)
OTC heterozygotes	
Secondary defects	
Lysinuric protein intolerance	4
HHH syndrome	0
Citrin deficiency (NICCD)	1

NAGS, *N* acetyl glutamate synthase deficiency; CPS, carbamoyl phosphate synthase deficiency; OTC, ornithine transcarbamylase deficiency; ASS, argininosuccinate synthase deficiency; ASL, argini nosuccinate lyase deficiency; HHH syndrome, hyperornithinemia, hyperammonemia, homocitrullinemia; and NICCD, neonatal intra hepatic cholestasis with citrin deficiency.

Total population (2003): 6 million; births: c.90,000 per annum.

symptomatic ornithine transcarbamylase (OTC) deficiency. These data are included to give a flavor of the small numbers of patients that will inform the thoughts and experience of most laboratory staff and treating physicians throughout the world.

During this satellite meeting on New Developments in Urea Cycle Disorders, the final session was devoted to a discussion of management problems. This paper attempts to review the recommendations of the 2001 consensus paper [3], highlight areas of uncertainty or contention that may exist, especially for people working "at the coal face," and incorporate points made during the prolonged discussion. Not all of the problems are answered!

Consensus paper review and discussion

Neonatal presentation

The 2001 consensus statement [3] has seven recommendations about the neonatal presentation of UCDs, and none seems controversial. These can be summarized as: (1) always consider the diagnosis of hyperammonemia in a sick neonate; (2) include a plasma ammonia measurement in a sepsis work-up; (3) respiratory alkalosis is a clue to a UCD; (4) all neonates with symptomatic hyperammonemia require rapid transfer to a tertiary referral center; (5) with the prior initiation of the administration of intravenous glucose and fluids; (6) if possible, collection of samples of plasma and urine for analysis; and (7) all feedings containing protein should be discontinued.

It cannot be too strongly emphasized that prompt referral should take precedence over everything else, and that while stabilization, with intubation and intravenous glucose and fluids is important before transport, blood and urine samples and placement of a central line can readily wait until arrival. As pointed out in the paper by Summar [6], specific treatment of hyperammonemia should begin before the precise diagnosis is made, so a short delay in finalizing the diagnosis is unimportant compared with any delay in initiating treatment. In New South Wales we have a single center for the investigation and treatment of patients with urea cycle disorders (and similar acutely presenting inborn errors of metabolism). Rapid transport is available from any part of the state, using a dedicated helicopter service. Urgent biochemical genetics testing is always available, and a firm diagnosis for all UCD disorders except *N*-acetyl glutamate synthase (NAGS) and carbamyl phosphate synthase (CPS) deficiencies is expected within 2 3 h of receipt of plasma and urine samples. The appropriate drugs and treatment protocols are stored in the intensive care unit, and a metabolic physician is on call, as is the renal team for dialysis. There is no doubt that a centralized service is vital for the treatment of rare and challenging disorders.

Acute hospital management

Here too, the consensus conference recommendations [3] are uncontroversial. They can be encompassed in three topics: (1) measure the plasma ammonia level; (2) start therapy with intravenous ammonia scavenging drugs; and (3) make preparation for dialysis.

The possibility of using ammonia-scavenging compounds in treatment was first discussed by Brusilow et al. [7], and subsequently protocols were developed which are still in use, with modifications, in most treatment centers. While there was no doubt about the wisdom of using ammonia-scavenging drugs, there was some discussion about the detail. The advisability of giving a priming infusion to a patient with acute decompensation but already on treatment was thought to be safe, provided that not more than 500 mg/kg/24 h of sodium benzoate and phenylacetate was given. This view, however, is not universal [8]. The general opinion on the safety of using intravenous sodium benzoate and phenylacetate in the presence of cerebral edema was that dialysis, which would be used in the presence of cerebral edema, would rapidly remove any excess of these compounds, and that there was no good reason not to use them. However, some participants felt that there was a danger of osmotic shifts because of the sodium load. The need to monitor potassium levels closely during

intravenous (as well as oral) therapy with ammonium scavengers was emphasized in discussion. The acute use of phenylbutyrate in argininosuccinate lyase (ASL) deficiency is not recommended in the "Brusilow" protocols [2], but is recommended in the consensus document [6] and was again supported at this meeting.

When to stop treating neonates was a subject of some discussion. Factors affecting the neurological outcome in UCDs are addressed elsewhere in this issue (Batshaw). European experience suggests that if the ammonia level at presentation has been above 400 μmol/L there is no normal cognitive outcome, [9] and devastating deficits can be expected if coma has lasted for more than 72 h. Any decision about the withdrawal, or non-institution, of treatment must of course be negotiated with the parents.

Blood sampling, laboratory testing, and the interpretation of results

Blood ammonia

The consensus conference recommendations [3] about ammonia measurement relate mainly to blood collection. This is appropriate, as with the usual methods of ammonia measurement, most inaccuracies are pre-analytical in origin. Currently the usual method in Australia is enzymatic, and the mean coefficient of variation among 60 laboratories using the enzymatic method during the first half of 2003 was <5.0%. The paper by Summar [6] suggests that an ammonia level of >150 μmol/L, together with a normal anion gap and blood glucose is a strong indication for the presence of a UCD. It must be very uncommon for a symptomatic *neonate* with a UCD to present with a plasma ammonia level of less than 200, and in our experience ammonia levels have always been above 300 μmol/L. A reported ammonia level of >150 μmol/L in a sick neonate is not uncommon, and frequently results from a combination of pre-analytic problems including a capillary collection.

Confirmation of diagnosis

In the consensus document, the confirmation of diagnosis of argininosuccinate synthase (ASS) and ASL deficiencies is said not to require an enzyme analysis, as "...the AA (amino acid) analysis is definitive and unambiguous" while this would be required for NAGS, CPS, and OTC deficiencies. It is true that a liver biopsy and enzyme analysis is required for the definitive diagnosis of CPS 1 and NAGS deficiencies, but the plasma amino acid findings, together with elevated urinary orotic acid is also definitive and unambiguous for hemizygous OTC deficiency.

OTC carrier detection

The detection of carriers of OTC deficiency is another matter, and the plasma amino acid profile can indeed be normal. Although DNA testing is recommended where possible, in approximately 20% of cases no mutation can currently be found [10]. Where DNA confirmation is not possible, the consensus document comes down in favor of the allopurinol-loading test [11], while noting that it "... is not completely sensitive or specific...." Indeed, Bonham et al. [12] found very poor specificity with the allopurinol load in sick children. We recently published our data using an improved protein-loading test [13]. The improved features of the test included ensuring an orotate-free load (containing no milk, which has a high orotate content) of 35 g protein/m^2 of body surface area, using a more specific method for measuring orotic acid, and measuring the rate of orotate excretion as a ratio of urinary excretion at 2–4 h/0–2 h after the load. Table 2 shows a summary of our results, including some previously published [13]. All 21 obligate heterozygotes had an unequivocally positive result. Among 14 family members who were possible heterozygotes, one, the mother of an affected male in whom no mutation could be found, showed an unequivocally positive result, and the remainder were negative. Six had later DNA confirmation of non-carrier status, one was the mother of an affected girl, and the remaining six were at 1:4 to 1:16 risk only. A protein load, given as voluntary oral intake of normal food, has appeared completely safe in our hands, as protein-averse subjects will refuse to take the whole load, and these invariably have a positive result to testing, but no, or trivial, adverse symptoms. The reliability of this improved test seems likely to be better

Table 2
Protein loading test results—adults

Subjects	Number	Observed ratio* 2–4 h/0–2 h	Comment
Healthy controls	18	0.8–1.39	Mean ± 4SD 0.64–1.85
Obligate carriers	21	2.28–17.8	Three had elevated fasting urinary orotate, and no load
Possible carrier	1	5.72	Mother of affected male
Possible carriers (family members)	13	0.8–1.2	Six—DNA excluded carrier status, one—mother of female propositus, six—at 1:4 to 1:16 risk

See, http://www.chw.edu.au/prof/services/biogen/ and follow prompts to "protein load test protocol" for details.
* Observed ratio—ratio of urinary orotic acid in timed urine collections from 0–2 to 2–4 h after a protein load.

than that of an allopurinol load, and is a more direct test of functional capacity of the liver in nitrogen disposal. Methods using ^{15}N-labeled urea and glutamine are not yet readily available [14,15].

Monitoring and laboratory tests

The 2001 consensus document [3,11] identifies plasma glutamine as a useful marker of metabolic control and suggests maintaining the level at <1000 μmol/L. The discussion first centered on the correlation between plasma glutamine and ammonia levels, which was said to be good in CPS and OTC deficiencies, but much less so in later disorders. Also, there is patient to patient variation, with some patients chronically running glutamine levels higher than 1000 μmol/L whilst remaining well. Elevated glutamine beyond the normal basal level for an individual patient was thought likely to be a harbinger of a hyperammonemic decompensation, although there appear to be no publications relating to this. Circulating glutamine may not correlate well with brain glutamine, and elevated levels may simply indicate an overload of nitrogen. The meeting's consensus was that in the absence of studies, glutamine levels above the patient's usual levels indicate the need for intervention. The possibility of glutamine monitoring by tandem mass spectrometry, using a blood-spot sample taken at home and sent by post to the laboratory was also raised [16].

The question was raised as to what time of day to measure plasma amino acids. Within the first hour or so after a meal plasma amino acids are elevated, and decline slowly to trough values at 3.5 h after the end of a meal. Citrulline levels differ, with a trough at 30 min after a meal, and a steady climb thereafter. It is also clearly important to relate blood sampling to the timing of doses of arginine or citrulline when adjusting medication. Monitoring of levels of ammonia scavengers was also discussed. A recently developed stable isotope dilution blood-spot assay for phenylbutyrate, phenylacetate and benzoate, demonstrates the immense variability in both peak and trough levels, not clearly related to weight or dosage [17]. This could be due in part to incomplete absorption, or to poor solubility of the medications, so that the dosages actually taken could vary. Several episodes of toxicity with unintended over-dosage have been recorded [18]. Home ammonia monitoring may be a useful strategy, but there was little experience of this.

Nutritional management

The broad recommendations of the 2001 consensus document [3] are not controversial. In essence they are: (1) a reduced protein intake, (usually less than the Recommended Daily Allowance), (2) giving essential amino acid supplements, (3) supplementation with minerals, vitamins, and trace elements, and (4) monitoring growth, hair, skin, nails, and biochemical indices of nutritional status. The document does not specifically recommend the involvement of a metabolic dietician, which in New South Wales we find essential. Some practical problems with nutrition include anorexia, and the management of nasogastric or gastrostomy tubes when these are necessary. A more difficult issue is the recognition of chronic catabolism.

In discussion, it was conceded that it was difficult to tell when branched-chain amino acids in a protein-restricted patient were too low, or whether they should be specifically supplemented as treatment with phenylbutyrate may selectively decrease them (see the paper by F. Scaglia et al. in this supplement). Monitoring of linear growth will detect a problem of chronic catabolism, but a deficit may occur at a late stage, possibly after growth in head circumference has slowed. Nutritional deficits must be detected at an early stage where possible. The role of carnitine was also canvassed. Low carnitine levels were reported to be uncommon in treated UCD patients and it was pointed out that carnitine administration in patients treated with sodium benzoate will favor the formation of benzoyl carnitine, and thus negate the benefit of the nitrogen scavenger potential of the benzoate.

Pharmacological management and chronic therapy

The consensus document [3] makes several recommendations, some of which have been discussed above. There is an emphasis on safety: double checking of written orders for pharmacological scavenging agents; monitoring of plasma levels; patients should have a written treatment protocol for acute management to present to the emergency room. For chronic therapy, the document recommends 4 times daily dosage of ammonia-scavenging drugs, linked to meals, to maximize the effect of ammonia removal. The recommendation for OTC and CPS deficiencies is for the use of oral citrulline rather than arginine, in combination with the ammonia scavengers [19]. Our experience with several patients with OTC is that divided doses of citrulline of 170 mg/kg/day as recommended often result in quite low levels of plasma arginine, near the bottom of the reference range, usually accompanied by elevated levels of glutamine. Additional supplementation with arginine lowers the glutamine into the normal range. Clearly this needs further investigation to determine whether there is a particular advantage in giving arginine or whether the recommended dosage of citrulline is far too low in some patients. The ideal level of plasma arginine during treatment is said to be between 50 and 200 μmol/L [20], but our experience is that levels below 90 μmol/L are not ideal for some

patients, and again there need to be studies to explore this. The use of benzoate and phenylbutyrate is much more complex. Some centers favor the chronic use of phenylbutyrate alone, without any benzoate. There was no further discussion about this, but an accompanying paper in this supplement spells out the pharmacokinetics of these medications.

The safety of phenylbutyrate in pregnancy was queried during the discussion. The consensus document recommends caution, as the safety of phenylbutyrate has not been determined. The discussants noted one published and two unpublished pregnancies where the offspring have been healthy, but one view was that sodium benzoate might be a better option, since we all ingest benzoate in soft drinks and other common foods, whereas sodium phenylbutyrate affects many metabolic and other functions. Another safety issue mentioned in discussion was the danger of phenylbutyrate tablets lodging in the esophagus and causing serious tissue damage. Patients must be instructed how to ensure complete swallowing.

Another aspect of long-term therapy that was discussed was the influence of menses on the exacerbation of hyperammonemia. Although there are no good published studies, there seems no doubt from individual cases that menses can adversely affect control in some patients. The mechanism is not clear but it was postulated that cytokine release might be involved. The menstrual periods were suppressed by both estrogen and progesterone separately in one patient, and both strategies led to marked improvement.

Long-term correction

The consensus document recommends consideration of liver transplantation for severe CPS and OTC deficiency patients, and for others whose disease is not well controlled by medical means. This is dealt with elsewhere in this publication.

Whom to treat?

There are issues regarding treatment for patients with less than the severe phenotype, or those detected when still asymptomatic, either through family studies or by newborn screening. This aspect was not covered in the consensus document, but attracted quite a lot of discussion. Asymptomatic neonates known to be OTC carriers may or may not need treatment. In New South Wales we think it wise to avoid any, even mild, hyperammonemia in the first two years of life, when the brain is growing rapidly, and accordingly we offer mild protein restriction and some oral arginine to known affected baby girls. During the discussion it was pointed out that only 15% of carrier females ever have symptoms, and almost all who do will have a severe mutation. However, mutation analysis is not useful for decisions about treatment because of the inherent mosaicism. It was thought helpful to measure glutamine and other amino acids, ammonia, and urinary orotic acid at a time of catabolic stress. So far there are no normative data for ^{15}N studies in babies, so this approach to decision-making about the need for treatment is not yet available.

Newborn screening by tandem mass spectrometry makes possible the diagnosis of UCDs in neonates, some of whom may be asymptomatic, which opens up new problems. In New South Wales, 10 patients with UCDs have been born during our tandem mass spectrometry screening program [21]. Four were known to be affected with OTC, in two of whom both treatment and the screening test were refused. The other two had levels of citrulline below our low cut-off level (i.e., a positive test result). One patient with severe ASL deficiency presented symptomatically before the screening test result was known, and one girl with OTC deficiency presented clinically at 5 months, but was not detected by screening. The remaining patients, all detected by newborn screening, had mild ASL deficiency, mild citrullinemia type I, citrullinemia type II, and apparent CPS deficiency (not yet confirmed), and all are receiving appropriate treatment or monitoring. The boy with mild ASL deficiency was quite well when first seen at 14 days of age, but had an elevated plasma glutamine level of 1313 μmol/L and a confirmed ammonia of 167 μmol/L. It could be that chronic mild hyperammonemia in the first year of life causes intellectual deficits. This boy has normal development at the age of 4 years, on protein restriction and arginine supplementation. None of our other mild but late-detected ASL patients had normal development at that age. It is too early yet to say what will be the overall effect of newborn screening for UCDs, and the sensitivity for detecting CPS and OTC deficiencies is not yet clear.

Summary

There are still many unsolved problems in the management of UCDs, and the small number of patients makes appropriate investigations difficult, but not impossible. Some of the most pressing problems are the optimization of medical treatment regimens and determination of the best methods of monitoring patients, including prediction of decompensations. In the longer term, advances in liver transplantation and hepatocyte therapy offer the best hope for UCD patients.

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