CURRENT TOPIC

Ornithine carbamoyltransferase deficiency

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The death of Jesse Gelsinger on 17 September 1999 had major effects on the gene therapy community. It brought to a halt a gene therapy clinical trial at the Institute of Human Gene Therapy, University of Pennsylvania, USA and brought to a wider audience the potential clinical problems associated with this technology.¹ In addition a number of clinicians became aware of Jesse's genetic disorder, ornithine carbamoyltransferase deficiency (OCTD, McKusick 311250), for the first time. OCTD is the most common disorder of ureagenesis (prevalence 1/40 000) and is inherited as an X linked trait. The OCT gene is located on the short arm of the X chromosome (Xp21.1) and over 150 mutations have been found in OCTD families. There are no common mutations and defects include gross deletions, missense and splicing mutations, as well as small insertions and deletions.^{2 3} The importance of detecting mutations within families lies primarily with accurate carrier detection and prenatal diagnosis, as biochemical and enzymatic methods of detection are less reliable. In addition, as enzyme activity is not expressed in either amniocytes or chorion villus biopsy material, prenatal testing had to rely on invasive fetal liver biopsy until DNA methods became available. In certain families knowledge of the mutation may help with disease prediction.⁴

Although our understanding of OCTD has increased greatly over recent years our abilities to treat the severe variants of this disorder remain limited.

The urea cycle and OCT

Figure 1 shows a simplified version of the urea cycle.

Ammonia is an extremely toxic molecule and organisms have evolved a number of different ways of excreting this waste product of protein metabolism. Where water is abundant (for example, fish) ammonia is directly excreted via the gills. Where water intake is often very scarce (for example, birds and terrestrial reptiles) ammonia is first converted to uric acid and excreted via the kidneys, producing in the case of birds, the white crystalline droppings which are a common feature of urban architecture! In mammals, by a series of chemical reactions, ammonia is converted to a less toxic, more water soluble molecule, urea, via the urea cycle.

In addition to the important excretory function, in mammals and some lower organisms, the urea cycle is the route of de novo synthesis of the amino acid arginine, a role that assumes great importance when discussing therapy for urea cycle disorders.

As can be seen from fig 1 the urea cycle takes place partly within the cytosol and partly within the mitochondria. Each turn of the cycle consumes two molecules of nitrogen and one molecule of carbon dioxide. In return one molecule of urea is created and one molecule of ornithine is regenerated to stimulate another turn of the cycle. Beginning and ending with ornithine, the reactions of the cycle consume three equivalents of ATP and a total of four high energy nucleotide phosphates; urea is the only new compound generated by the cycle.

OCT is present within the mitochondrial matrix and has no regulatory significance. Ornithine arising in the cytosol is transported to the mitochondrial matrix where OCT catalyses the condensation of ornithine with carbamoyl phosphate to produce the amino acid citrulline. The energy for the reaction is provided by the high energy anhydride of carbamoyl phosphate. The product citrulline is transported to the cytosol where the remainder of the reactions of the urea cycle take place.

In patients with severe, neonatal onset OCTD, plasma citrulline concentrations are very low and often undetectable. The concentrations in less severe, late presenting or female

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Figure 1 The urea cycle. CPS, carbamoyl phosphate synthetase; OTC, ornithine transcarbamylase; AS, argininosuccinic acid synthetase; AL, argininosuccinic acid lyase; A, arginase; *waste nitrogen molecules.

Figure 2 Pyrimidine synthetic pathway.

heterozygotes are more variable and often not of diagnostic significance. In addition, accumulating carbamoyl phosphate spills into the cytosol stimulating pyrimidine biosynthesis (fig 2). As a consequence phosphoribosylpyrophosphate (PRPP) may be depleted, limiting flux through the orotate PRPP transferase reaction, resulting in orotate (orotic acid) accumulation and excretion into the urine. The combination of hyperammonaemia with low plasma citrulline and increased urinary orotic acid concentrations is thought to be pathognomonic of OCTD, but an identical biochemical picture occurs in ornithine aminotransferase deficiency presenting in the newborn period. In addition to helping with the primary diagnosis, levels of pyrimidine biosynthesis in OCTD can be used to try to detect female heterozygotes by the inhibition of orotidine monophosphate decarboxylase (OMP decarboxylase) with allopurinol (fig 2). In female heterozygotes there may be no ostensible biochemical abnormalities despite having an increased rate of pyrimidine biosynthesis within their OCTD hepatocytes. When OMP decarboxylase is inhibited by oxipurinol ribonucleotide (produced by allopurinol metabolism), OMP accumulates which leads to orotidine accumulation and orotidinuria, the degree of which serves to distinguish normal women from OCTD heterozygotes.⁵ This test is much safer than protein loading which can no longer be recommended as a screen for OCTD heterozygotes. The test, however, can give both false positive⁶ and negative results,⁷ and wherever possible heterozygosity should be confirmed by mutation analysis.

Hyperammonaemia and the brain

The effects of hyperammonaemia on cerebral function have recently been reviewed.⁸ Acute and chronic elevations in plasma ammonia are capable of producing devastating neurological

symptoms which are dependent on the age of the patient as well as the duration and severity of the hyperammonaemia. A number of different mechanisms have been proposed to explain this potent toxic effect. These include a direct effect on neurotransmission, interference with neuronal energy metabolism, and a direct effect on astrocyte function. Irrespective of the underlying cause the end result is a clinical disorder which will range from mild personality dysfunction to seizures, coma, and death.

Clinical presentation of OCTD

The hallmark of the severe form of OCTD in an affected male infant is a rapidly progressive metabolic encephalopathy presenting very soon after birth. The infant usually appears well at birth but on the second day of life develops irritability, feed refusal, and becomes increasingly lethargic. Blood gas analysis will often show a respiratory alkalosis at this stage. Clouding of consciousness increases and the baby becomes comatose and has an irregular respiratory pattern. Respiratory arrest and seizures may occur and without urgent resuscitation and treatment the infant will die before the end of the first week of life. Although this pattern of disease progression is very familiar to paediatricians managing metabolic patients it is still usually misdiagnosed as "sepsis" by neonatologists delaying the onset of treatment.⁹

Like most X linked disorders OCTD is a very heterogeneous disorder and male hemizygotes can present with a milder phenotype, presumably as a result of carrying less damaging genetic mutations. In these patients presentation can be in the first year of life or as late as middle age.¹⁰ In older, affected boys, behavioural disturbance often dominates,¹¹ but many patients will also have recurrent episodes of vomiting and failure to thrive. Despite our increasing knowledge of this mode of presentation the variant continues to be under recog-

nised because of this remarkable variation in clinical signs and symptoms.¹² The initial differential diagnostic list after admission to hospital often includes psychiatric illness, cerebellar ataxia, drug ingestion, "encephalitis", cyclical vomiting, and various food intolerances/allergies.

Female heterozygotes are even more difficult to diagnose, especially if there has been no previous positive family history. Considerable diagnostic confusion and delay can occur in this group of patients who may have protein aversion as their only apparent symptom. The phenotypic variability seen in these patients reflects both genetic heterogeneity and the random pattern of X inactivation in their hepatocytes. These patients may remain completely normal throughout life or develop the same degree of profound neurological impairment as seen in the male hemizygote with a severe neonatal onset. In large studies up to 20% of female heterozygotes will experience encephalopathic episodes and as the diagnosis is often not considered, a significant percentage (80%) will die.¹³ Parenteral nutrition,¹⁴ pregnancy,¹⁵ and initiation of sodium valproate therapy¹⁶ are specific risk factors in this group.

Irrespective of age any individual with an encephalopathic illness should have an urgent blood ammonia estimation. If this is increased in the presence of normal liver function, OCTD should be high on the list of possible diagnoses and further investigation should be undertaken. At the same time urgent measures should be taken to reduce the ammonia concentration down to the normal range as quickly as possible.

Making the diagnosis

The most important step is suspicion, but this needs to be quickly followed by urgent plasma ammonia estimation. At the same time samples for urine orotic acid and plasma amino acid concentrations are collected, but treatment is dictated by the ammonia concentration.

In the neonatal period a healthy, term neonate will have a plasma ammonia concentration of less than 50 $\mu\text{mol/l}$, but in growth retarded or premature infants a modest elevation up to 80 $\mu\text{mol/l}$ may be seen in otherwise healthy infants. In OCTD deficiency presenting in the newborn period ammonia concentrations are usually greater than 300 $\mu\text{mol/l}$ at presentation and often rise quickly thereafter.

Confusion often occurs when moderately increased plasma ammonia concentrations (for example, 90–150 $\mu\text{mol/l}$) are received back from the laboratory. Any illness can be associated with a raised plasma ammonia but the concentration in these circumstances is usually less than 170 $\mu\text{mol/l}$. If in doubt, the estimation should be repeated; if the values rises above 200 $\mu\text{mol/l}$ treatment should be instituted and further investigation pursued with vigour.

In older children plasma ammonia concentrations depend partly on protein intake. In normal children an upper limit of 50 $\mu\text{mol/l}$ is usual with an elevation up to 80 $\mu\text{mol/l}$ with non-specific illness. Concentrations above 100 $\mu\text{mol/l}$ require further investigation although

lower values may be significant if the patient has been on a low protein diet or intravenous fluids for several days. This can occur in patients with late presenting OCTD who may initially be thought to have a gastrointestinal illness. In most male patients with milder variants or symptomatic females heterozygous for OCTD, plasma ammonia concentrations will be above 150 $\mu\text{mol/l}$ during episodes of illness but may be normal at other times.

In OCTD orotic acid concentrations during acute episodes will be above the metabolic laboratories' reference range (normal <5 $\mu\text{mol/mol}$ creatinine), and in severe neonatal patients plasma citrulline concentrations will be low (normal 20–60 mmol/l depending on age). The diagnosis can be confirmed by enzyme assay on a liver biopsy specimen, but many clinicians proceed directly to DNA mutation studies now that these are readily available.

Diagnosis by enzyme or DNA analysis is essential if prenatal diagnosis is to be offered in the future.

The treatment of OCTD

The outcome in severely affected males is so poor that I think it is reasonable to question whether or not we should regard this variant as a treatable disorder. Although earlier diagnosis may be expected to give a better outcome even in those in whom the diagnosis is anticipated and who are managed prospectively, the results after severe neonatal presentation of OCTD are very poor with almost every survivor showing severe neurological impairment.^{9, 17} In OCTD infants presenting early in the neonatal period, who have been comatose for over 24 hours, and who have a blood ammonia above 1000 $\mu\text{mol/l}$, it may be kinder to let nature run its course rather than embarking on heroic therapy which will result in a survivor with very poor neurological and intellectual functions.

In patients presenting with less severe variants, aggressive therapy is indicated with the aim of reducing the concentration of blood ammonia as quickly as possible. This approach should also be undertaken in the management of metabolic decompensation in known patients as well as with the diagnostic episode. The standard long term therapy for OCTD is directed at reducing the requirement for urea biosynthesis by using a low protein diet and by increasing waste nitrogen excretion by alternative pathway therapy.¹⁸ As arginine becomes an essential amino acid in this disorder a supplement is required to replace that which is not synthesised (see Appendix). In the emergency situation this approach has to be augmented by other means of rapid ammonia removal. There have been no controlled studies of the various modalities employed to manage hyperammonaemia. Published studies are usually single centre and involve very small numbers of patients.^{19–25} Although often technically more difficult, the evidence that is available suggests that haemofiltration or haemodialysis may be more effective than peritoneal dialysis in the acute stages. Because of the intensity of therapy

that is needed to manage these patients, early transfer to a major centre with good metabolic back up is advocated.

In those patients who survive the initial hyperammonaemic insult, liver transplantation becomes an option.^{26–28} While this will not improve central nervous system damage pre-dating transplantation, it will prevent further episodes of hyperammonaemia. Successful transplantation leads to a reduction of daily medication and the ability to consume an unrestricted diet.

The outcome

As mentioned, large studies of outcome for the severe neonatal form of OCTD make depressing reading. Survival is much better in those male infants who present outside the neonatal period (presumed milder variants) and females (heterozygotes). In patients with a neonatal onset the usual outcome is death or severe developmental delay. The only way to improve this would be by early diagnosis, aggressive management of the hyperammonaemia, and early liver transplantation in survivors with good intellectual function.

In female heterozygotes, alternative pathway therapy (see Appendix) improves prognosis by reducing hyperammonaemic episodes and thus preventing further cognitive decline.²⁹

The future

At first sight OCTD would appear to be an attractive candidate for a gene therapy approach. The gene has been cloned, it is a relatively common disorder, there is a very good animal model (the sparse fur, *spf/Y* mouse) and current therapy is unsatisfactory. In addition the evidence that liver transplantation normalises the metabolic dysfunction suggests that a liver based gene transfer approach could be successful; this was confirmed in animal studies.³⁰ Initial experience in humans has seemingly ended in disaster, but lessons will undoubtedly be learned from Jesse Gelsinger's death; it must not be forgotten that gene therapy is a treatment in its infancy and is certain to improve. OCTD can be a devastating disorder and an innovative approach to therapy will be necessary if prognosis is to improve.

Appendix

Acute management of hyperammonaemia in OCTD with alternative pathway therapy and toxin removal via dialysis

- 1) Stop all exogenous protein.
- 2) Inhibit endogenous catabolism:
  - correct acid base balance
  - give maintenance fluids as 10–15% glucose (aim for blood glucose values of 7–10 mmol/l). Combine with continuous insulin infusion at 0.05–0.1U/kg/h.
- 3) Intravenous medication (table A1). Start if ammonia >180 µmol/l. Monitor NH₄⁺, sodium, potassium, acid–base, and glucose every four hours.
- 4) Dialysis for ammonia concentrations >400 µmol/l. Haemodialysis is preferable.
- 5) Oral medication in same dosage with low protein diet is the basis of long term treatment after the acute episode.

Table A1 Intravenous medication

	Sodium benzoate (mg/kg)	Sodium phenylbutyrate (mg/kg)	Arginine 10% (mg/kg (ml/kg))
Priming infusion*	250	250	200 (2)
Maintenance†	250	250	200 (2)

*Make up in 30 ml/kg of 10% dextrose and give over 90 min.

†Make up in 30 ml/kg of 10% dextrose and give as continuous infusion over 24 hours.

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