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Regular Article

Prenatal treatment of ornithine transcarbamylase deficiency

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ABSTRACT

Purpose of study: Patients with neonatal urea cycle defects (UCDs) typically experience severe hyperammonemia during the first days of life, which results in serious neurological injury or death. Long-term prognosis despite optimal pharmacological and dietary therapy is still poor. The combination of intravenous sodium phenylacetate and sodium benzoate (Ammonul®) can eliminate nitrogen waste independent of the urea cycle. We report attempts to improve outcomes for males with severe ornithine transcarbamylase deficiency (OTCD), a severe X-linked condition, via prenatal intravenous administration of Ammonul and arginine to heterozygous carrier females of OTCD during labor.

Methods used: Two heterozygote OTCD mothers carrying male fetuses with a prenatal diagnosis of OTCD received intravenous Ammonul, arginine and dextrose-containing fluids shortly before birth. Maintenance Ammonul and arginine infusions and high-caloric enteral nutrition were started immediately after birth. Ammonul metabolites were measured in umbilical cord blood and the blood of the newborn immediately after delivery. Serial ammonia and biochemical analyses were performed following delivery.

Summary of results: Therapeutic concentrations of Ammonul metabolites were detected in umbilical cord and neonatal blood samples. Plasma ammonia and glutamine levels in the postnatal period were within the normal range. Peak ammonia levels in the first 24–48 h were 53 mcmol/l and 62 mcmol/l respectively. The boys did not experience neurological sequelae secondary to hyperammonemia and received liver transplantation at ages 3 months and 5 months. The patients show normal development at ages 7 and 3 years.

Conclusion: Prenatal treatment of mothers who harbor severe OTCD mutations and carry affected male fetuses with intravenous Ammonul and arginine, followed by immediate institution of maintenance infusions after delivery, results in therapeutic levels of benzoate and phenylacetate in the newborn at delivery and, in conjunction with high-caloric enteral nutrition, prevents acute hyperammonemia and neurological decompensation. Following initial medical management, early liver transplantation may improve developmental outcome.

1. Introduction

The urea cycle is the final common pathway for the metabolism of waste nitrogen in humans, and defects in this pathway result in the accumulation of nitrogen as ammonia, glutamate, alanine and intermediates prior to the metabolic block [8,15]. Urea cycle disorders (UCDs) are among the most common inborn errors of metabolism with a cumulative incidence of 1:35,000 [21]. In UCDs, the conversion of ammonia to urea is dependent on six enzymes located in the liver [12]. Of the six disorders, ornithine transcarbamylase deficiency (OTCD) is the most common, with an estimated incidence of 1 in 56,500 [20]. OTCD is caused by mutations in the OTC gene on chromosome Xp11.4.

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The biochemical hallmarks of OTCD are elevated plasma ammonia, glutamine and alanine, and low levels of arginine and citrulline.

OTCD is particularly severe in hemizygous males. The clinical presentation of males with complete enzyme deficiency includes a short period of normal behavior followed by severe hyperammonemic encephalopathy in the neonatal period [20]. The prognosis in children with UCDs presenting in the neonatal period is generally poor; half of the children who survive neonatal-onset OTCD die before entering school and those who survive have a high incidence of developmental disabilities [7,17].

Therapeutic principles for neonatal UCDs are based on the creation of anabolism and activation of alternative pathways of nitrogen elimination via intravenous nitrogen-scavenging medications and arginine [8,9]. Hepatic and renal acyltransferases conjugate benzoate with glycine to form hippuric acid and phenylbutyrate with glutamine to form phenacetylglutamine, allowing urinary elimination of waste nitrogen [4]. However, the long-term prognosis for early-onset UCDs is still poor despite optimal dietary and medical management [1]. Liver transplantation (LT) has emerged as a definitive treatment for the risk of metabolic decompensation in UCDs, as the urea cycle as a whole is present only in the liver. LT restores the metabolic defect and results in eradication of risk for hyperammonemia, as well as the need for dietary restrictions and nitrogen-scavenging medications ([10,11], McBride et al. [16], [19,22,25]). LT may also improve neurocognitive outcome in children transplanted early in life [5,22].

In an attempt to improve the outcome of severe, neonatal UCDs, two mothers carrying fetuses with UCDs were treated shortly before birth with intravenous infusions of benzoate [6]. Prenatal maternal benzoate infusion appears to be safe and leads to therapeutic levels of benzoate in umbilical cord blood, as well as in the neonate, immediately after birth. Although information is limited, no side effects of sodium benzoate infusions were noted in the newborn; specifically metabolic acidosis, hypernatremia and hyperbilirubinemia were absent [6]. We now report the perinatal treatment of two mothers who harbor severe early-onset OTCD mutations and were carrying affected male fetuses with intravenous infusions of Ammonul and arginine. The newborns did not suffer from neonatal hyperammonemia or acute decompensation and have grossly normal development following LT.

2. Methods

Quantification of Ammonul metabolites (phenacetylglutamine, phenylbutyrate, phenylacetate and benzoate) was performed by ethyl acetate extraction followed by capillary gas chromatography-mass spectrometry with stable isotope dilution and selected ion monitoring from filter paper blood spots of maternal blood during delivery, cord blood and newborn whole blood immediately upon arrival to the NICU, after placing lines, and at 12 h of life.

Therapeutic levels of Ammonul metabolites were defined based on a previous report by Yu et al. Peak (1.5 h postdose) and trough (predose) concentrations of phenylbutyrate (PBA) and phenylacetate (PAA) were 220–551 μmol/l and 307–473 μmol/l, respectively, 1.5 h after 100–125 mg/kg doses of PBA. In all their patients, the predose concentration of PBA was < 26 μmol/l, and the predose concentration of PAA was < 170 μmol/l. The highest postdose PBA concentration measured was 1542.4 μmol/l, and the highest postdose PAA concentration was 664.7 μmol/l. The benzoic acid (BA) concentration in patients not taking BA was 12–62 μmol/l, which likely reflects values in healthy individuals [26]. Therapeutic peak levels after an oral dose of benzoate are considered to be 800–23,300 μmol/l [13]. We defined therapeutic target values in intravenous therapy based on these concentrations.

3. Case 1

An older brother of case 1 developed severe hyperammonemic encephalopathy on the 2nd day of life and subsequently died on the 4th day of life. Liver biopsy analysis during the autopsy exam revealed very low levels of OTC activity. Carrier testing on the mother revealed a loss in copy number encompassing 172 to 212 kb in the short arm of chromosome X, involving exon 1 to exon 9 of the OTC gene. Included in this copy number loss is the gene *RPGR* associated with X-linked retinitis pigmentosa. Prenatal diagnosis at 34 weeks of gestation via amniocentesis revealed an affected male fetus. The pregnancy was uneventful and obstetrics, neonatology and metabolic genetic teams prepared for spontaneous delivery. Upon arrival to the delivery room mother was treated with intravenous loading doses of Ammonul (5.5 g/m^2 over 90 min) and arginine (4 g/m^2 over 90 min). Intravenous maintenance doses were started immediately after the loading doses (Ammunol maintenance dose of 5.5 g/m^2 and arginine 4 g/m^2, both continuous infusion over 24 h). Intravenous dextrose 10% plus electrolytes was also initiated to decrease the risk of maternal decompensation. The mother's ammonia levels were measured during the delivery and were normal. No adverse effects of Ammonul infusion were observed in the mother or in the newborn, and there was no metabolic acidosis in either the mother or child. Labor lasted around 12 h and the maternal infusions were discontinued after the delivery. The ammonia, glutamine and alanine levels were normal at birth in the neonate. After delivery he was transferred to the neonatal intensive care unit (NICU) where treatment with high dextrose plus electrolytes infusion, intravenous arginine (200 mg/kg/d continuous infusion) and Ammonul (250 mg/kg/d continuous infusion) were immediately initiated. He was transitioned to oral sodium phenylbutyrate and citrulline at age 4 days and remained in the NICU for 33 days. Thereafter, he was managed as an outpatient with close monitoring of ammonia levels until orthotropic liver transplantation at age 3 months. While on a diet of 1.5 g/kg/day of protein, he experienced a single episode of hyperammonemia of 291 μmol/l (normal < 30) at age 5 weeks that resolved within 12 h after acute management with intravenous Ammonul and arginine. This episode was detected on routine ammonia level monitoring without any inciting event, and was not associated with neurological signs. Protein intake was decreased to 1.2 g/kg/day and he did not experience further significant hyperammonemic events. There is a history of mild speech delay in infancy, but he is currently 7 years old and has generally typical development.

4. Case 2

The maternal uncle of case 2 died in the first week of life due to OTCD. Genetic analysis showed a mutation in the OTC gene c.67C > T (p.R23X). The mother was confirmed to be a heterozygous carrier. Prenatal diagnosis at 18 weeks of gestation via amniocentesis revealed an affected male. The pregnancy was complicated by alcohol, tobacco and methamphetamine use until 9 weeks of gestation. The neonate was delivered spontaneously at term. We used the same treatment protocol as in Case 1, with Ammonul, arginine and intravenous dextrose infusions for the mother during labor, as well as serial laboratory monitoring. Labor lasted around 3 h. No side effects were observed in the mother. Mother's ammonia levels were measured during the delivery and were normal. There was one episode of hypoglycemia (glucose 21 mg/dL normal 70–100) immediately after delivery in the neonate that resolved with glucose infusion and did not recur. There was no metabolic acidosis in either the mother or child. The ammonia, glutamine and alanine levels were normal in the neonate. After delivery he was transferred to the NICU and treated as in Case 1. He was transitioned to oral sodium phenylbutyrate and citrulline at age 13 days, and was monitored in the hospital until orthotropic liver transplantation at age 5 months. He experienced two episodes of *Escherichia coli* urosepsis related to vesiculouretral reflux grade 2 before transplantation. His highest ammonia level was 234 μmol/l (normal < 30) during one of those episodes and it resolved within 8 h after treatment with IV dextrose. He was last evaluated at age 3 years, and had normal