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Orthotopic Liver Transplantation for Urea Cycle Enzyme Deficiency

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Abstract

Hyperammonemia, abnormalities in plasma amino acids and abnormalities of standard liver functions were corrected by orthotopic liver transplantation in a 14-day-old boy with carbamyl phosphate synthetase-I deficiency and in a 35-yr-old man with argininosuccinic acid synthetase deficiency. The first patient had high plasma glutamine levels and no measurable citrulline, whereas citrulline values were markedly increased in Patient 2. Enzyme analysis of the original livers showed undetectable activity of carbamyl phosphate synthetase-I in Patient 1 and argininosuccinic acid synthetase in Patient 2. Both patients were comatose before surgery. Intellectual recovery of patient 1 has been slightly retarded because of a brain abscess caused by *Aspergillus* infection after surgery. Both patients are well at 34 and 40 mo, respectively, after surgery. Our experience has shown that orthotopic liver transplantation corrects the life-threatening metabolic abnormalities caused by deficiencies in the urea cycle enzymes carbamyl phosphate synthetase-I and argininosuccinic acid synthetase. Seven other patients—six with ornithine transcarbamylase deficiency and another with carbamyl phosphate synthetase-I deficiency—are known to have been treated elsewhere with liver transplantation 1½ yr or longer ago. Four of these seven recipients also are well, with follow-ups of 1½ to 5 yr. Thus liver transplantation corrects the metabolic abnormalities of three of the six urea cycle enzyme deficiencies, and presumably would correct all.

It was proposed in 1975 by Dr. Leon Rosenberg of Yale University that liver transplantation would allow somatic correction of diseases caused by deficiency of any of the six urea cycle enzymes (Personal communication, 1975). The liver enzymes responsible for biosynthesis of urea from its principal precursors (ammonium and glutamine) are *N*-acetylglutamate synthetase, carbamyl phosphate synthetase-I (CPS-I), ornithine transcarbamylase (OTC), argininosuccinic acid synthetase (ASS), argininosuccinase and arginase (1). *N*-acetylglutamate synthetase deficiency has been reported in one man (2). OTC deficiency is an X chromosome-linked disorder, and the other 4 deficiencies are autosomal recessive traits. High plasma ammonium and glutamine levels are thought to cause astrocyte swelling, brain edema and

secondary neuron injury (3), with variable neurological or behavioral consequences that may be acute or chronic. We report here the amelioration of such complications after liver transplantation in a 14-day-old white boy with CPS-I deficiency and a 35-yr-old Japanese man with ASS deficiency.

CASE REPORTS

Case 1

An apparently normal, full-term male child of first-cousin parents became bradycardic and apneic shortly after birth on May 18, 1988, requiring endotracheal intubation. By postpartum day 2, he was unresponsive to pain stimuli, although results of a computed tomographic scan of the head were normal. Serum ammonia level on day 6 was 387 $\mu\text{mol/L}$, total bilirubin was 202 $\mu\text{mol/L}$ (direct bilirubin = 27.4 $\mu\text{mol/L}$) and serum transaminases were normal. Peritoneal dialysis on day 7 reduced serum ammonia level from 514 to 160 $\mu\text{mol/L}$. With the diagnosis of CPS-I urea cycle enzyme deficiency, the patient was treated with an intravenous infusion of arginine, sodium benzoate and sodium phenylacetate supplemented with Intralipid and 5% dextrose (4), and tube feeding with polyxose and protein-free Mead Johnson formula 80056 (Mead Johnson, Evansville, IN). After transfer to the University of Pittsburgh, orthotopic liver transplantation (OLT) was performed with a newborn cadaver liver on June 2, 1988, at 14 days of age. Biliary drainage was performed by choledochojejunostomy (Roux-en-Y anastomosis). The surgery and subsequent immunosuppression with cyclosporine and prednisone were performed with standard techniques (5).

Severe rejection beginning in postoperative wk 3 necessitated augmented steroid dosage and the addition of OKT3. An *Aspergillus* brain abscess was treated successfully with anti-biotics, open drainage and, ultimately, ventriculoperitoneal shunt. Bacteremia and systemic cytomegalovirus infection further complicated the course, necessitating hospitalization for 71/2 mo (6). Subsequently, the child lived at home without special domiciliary care. Growth and development have been uninterrupted during the 40 mo of follow-up, with a weight gain from 2.5 to 20 kg on an unrestricted diet. Normal physical and intellectual milestones have been delayed, presumably because of the cortical injury caused by the brain abscess, meningitis and/or hydrocephalic complications. Liver function is normal, and most the recent blood ammonia level after 37 mo was 34 $\mu\text{mol/L}$ (normal < 39 $\mu\text{mol/L}$).

Case 2

A 35-yr-old Japanese man was the second son of healthy first-cousin parents. He had a normal brother and sister and was the father of a healthy daughter. He was ostensibly normal until three episodes of temporary altered consciousness and behavioral aberrations occurred in January, April and May 1987. After initial psychiatric hospitalization, he was transferred to Kurume University, where his serum ammonium and serum citrulline levels were 264 and 445 $\mu\text{mol/L}$, respectively (normal, < 39 and < 52 $\mu\text{mol/L}$). On diagnosis of ASS deficiency, he was prescribed sodium benzoate, arginine and branched amino acid infusion, with restriction of oral protein intake. During the following 18 mo, he had four major episodes of serum ammonium elevation, with transient bouts of stage IV coma, which were successfully treated by hemodialysis. Because of deterioration and stage III and IV coma necessitating ventilator support, he was flown in November 1988 to the University of Pittsburgh, where OLT was performed on December 23, 1987, with biliary drainage by duct-to-duct anastomosis.

Preoperative total serum bilirubin of 425 $\mu\text{mol/L}$ fell to normal within a few days. A computed tomographic scan of the head in November 1988 had revealed cortical atrophy. Post-operatively, complete recovery of consciousness and removal from ventilator support required several weeks, but convalescence was otherwise uncomplicated under cyclosporine-steroid

therapy (5). The patient was discharged from the hospital after 44 days and has been at home since then. Recovery of neuromotor and intellectual capabilities required many months, but eventually the patient returned to his previous position as vice president of a construction company. Liver function is normal 34 mo postoperatively. Blood ammonia levels have been 7 to 21 $\mu\text{mol/L}$ (normal < 39 $\mu\text{mol/L}$).

BIOCHEMICAL METHODS

Amino Acids

Plasma amino acids in patient 1 were measured 9 days after birth (5 days before transplantation) by an automated amino acid analyzer. At 34 mo after OLT, amino acids were determined by column chromatography (7). Amino acid separation was performed by a method modified from Hamilton (8). In Patient 2, the plasma amino acids were measured in the absence of treatment, 5 to 7 mo before transplantation, with a automated amino acid analyzer. The examinations were repeated 3, 4, and 20 mo after OLT.

Liver Enzymes

The removed native livers were immediately cut into small pieces, frozen with liquid nitrogen and stored at -800°C until the analyses of urea cycle enzymes. CPS-I and OTC were measured in Patient 1 in the Department of Human Genetics of the Yale University School of Medicine. Determinations were with a small-scale adaptation of the method of Nuzum and Snodgrass (9). The liver tissues from Patient 2 were analyzed for CPS-I, OTC, ASS and AS activity in the Department of Biochemistry of Kagoshima University, Kagoshima, Japan, using a previously described method (10).

RESULTS

Plasma Amino Acids

The pretransplant plasma in Patient 1 had elevated levels of glutamine. Citrulline was undetectable, and the other measured amino acids were relatively normal (Table 1). Plasma amino acids 34 mo after surgery were normal. Citrulline levels became detectable.

The plasma amino acids in Patient 2 before and after transplantation are summarized in Table 2. The extremely high preoperative levels of citrulline and the modest elevations of arginine were corrected at all measured postoperative times. However, ornithine level (normal = 40 to 100 $\mu\text{mol/L}$) was slightly elevated at 3 mo (153 $\mu\text{mol/L}$) and at 20 mo (124 $\mu\text{mol/L}$) postoperatively.

Tissue Studies

On light microscopy, the native liver of patient 1 had only minor, nonspecific abnormalities. Similarly, electron microscopic studies were normal, except for nonspecific elongation of the mitochondria. CPS-I activity was undetectable in the homogenate of this liver. OTC activity was normal when compared with that of a control liver sample (Table 3).

The native liver of patient 2 was enlarged, with cholestasis and massive steatosis and hemosiderosis. Periportal fibrosis, but no cirrhosis, was present. The liver homogenate was devoid of ASS activity, but exhibited significant CPS-I, OTC, and AS activity (Table 4).

DISCUSSION

The diagnosis of urea cycle enzyme deficiency and the specific enzyme involved were established with certainty in our two cases. Both had hyperammonemia and plasma amino acid

abnormalities, which permitted the presumptive diagnosis of the enzyme deficiency. The diagnoses were confirmed by analysis of the subsequently excised livers. To our knowledge, the 2-wk-old infant with CPS-I deficiency is the youngest patient ever to undergo successful liver transplantation. The 36-yr-old man is the only patient with ASS deficiency treated thus far with liver transplantation. Although both patients were comatose at the time of liver replacement, their lethal metabolic abnormalities were promptly and permanently corrected; this has been achieved with numerous other liver-based inborn errors (11).

In addition to these two patients, seven others with urea cycle deficiencies are known to have been treated with liver transplantation before March 1990 (Table 5). Six of the seven had OTC deficiency (12,13). The seventh was a 20-mo-old boy with CPS-I deficiency (14). Postoperatively, this child had undetectable citrulline in the plasma, prompting Tuchman to recommend citrulline supplementation postoperatively (14). This treatment was not provided in our patient with CPS-I deficiency during his more-than-3-yr survival. The finding of citrulline in the plasma of our patient suggests that in the human, unlike the rat (15,16), citrulline originates in the liver and in the gut. It is also noteworthy that the amino acid profile was not completely corrected by liver transplantation in Patient 2. Plasma ornithine level remained slightly high as late as 20 mo after surgery.

The cumulative experience in our two cases and in the seven others elsewhere establishes the effectiveness of liver transplantation for at least three urea cycle deficiencies. When the operation is successful, the metabolic consequences are corrected to an extent not approached by attempts to activate alternative pathways of waste nitrogen synthesis and excretion or by restriction of dietary protein intake. However, the use of liver transplantation for somatic correction may prove to be only a step toward enzyme replacement. Such therapy with measures other than whole-liver or even isolated hepatocyte transplantation is not hard to envision with recent advances in understanding of the molecular basis for these diseases. Virus-mediated transfer of human ASS has already been accomplished in rodents (17, 18), suggesting that the introduction of a functional complementary DNA into hepatic or nonhepatic autologous cells could be a practical therapeutic possibility in the near future.

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Table 1

Plasma amino acids in patient 1 before and after OLT

Amino acids	Before OLT ^a		After OLT ^b	
	Normal range	As of 5/27/88	Normal range	As of 4/12/91
Aspartic acid	1-17 ^a	16	0-4	<10
Glutamic acid	1-85	72	0-150	30
Glutamine	337-673	909	320-870	650
Proline	51-271	177	30-400	150
Glycine	87-323	251	90-430	250
Citrulline	10-34	ND	0-70	10
Alpha-amino- <i>N</i> -butyric acid	4-32	34	0-40	40
Valine	78-326	86	70-350	190
Ornithine	22-94	59	0-120	50
Arginine	15-115	24	10-130	30

ND = not detectable.

^a Measured by an automated amino acid analyzer.

^b Measured by column chromatography.

^c All data expressed as $\mu\text{mol/L}$.

Table 2

Plasma amino acids in patient 2 before and after OLT

Amino acids	Normal range	Before OLT (5/31/88)	After OLT (8/25/90)
Aspartic acid	3,9-6,7 ^a	<3	4.5
Glutamic acid	14-59	57	51
Glutamine	560-890	641	612
Proline	110-310	98	190
Glycine	180-370	116	199
Citrulline	22-52	445	47
Alanine	280-650	229	424
Valine	180-370	165	204
Ornithine	40-100	65	124
Arginine	70-160	207	98

^a All data expressed as $\mu\text{mol/L}$.

Table 3

Urea cycle enzymes in patient 1

Enzyme	Patient 1	Normal range (sample control)
CPS-I	<0.005 ^a	0.26-1.0
OTC	28.7	19-38

^a All data expressed as μmol/hr/mg protein.

Table 4

Urea cycle enzymes in patient 2

Enzyme	Patient	Normal liver (sample control)
AS	293 ^a	453
ASS	2	300
CPS-I	37	58
OTC	4,337	7,359

AS = argininosuccinase.

^a All data expressed as $\mu\text{mol/hr/mg}$ protein.

Table 5

Urea cycle enzyme deficiencies treated more than 11/2 yr ago with OLT

Patient	Center	Date of OLT	Age at OLT	Enzyme deficiency	Alive/dead	OUTCOME		Reference
						Mental state	Reference	
1	Yale University	8/86	21mo	OTC	Alive	Normal and in school	1; personal communication of 8/91 ^a	
2	Lille, France	2/88	5yr	OTC	Alive	Normal	13; personal communication of 8/91	
3	University of Pittsburgh	6/88	14 days	CPS-I	Alive	Slightly retarded	This report	
4	Boston Children's Hospital	8/88	18mo	OTC	Alive	Normal	Personal communication of 8/91	
5	University of Minnesota	10/88	20mo	CPS-I	Died of pneumonia after 18 mo	-	14	
6	University of Pittsburgh	12/88	35yr	ASS	Alive	Normal	This report	
7	University of Chicago	10/89 ^b	20mo	OTC	Died 5/26/90	-	12	
8	University of Chicago	11/89	21 mo	OTC	Died 11/17/89	-	Personal communication of 4/91	
9	Riley Children's Hospital, Indianapolis, IN	2/90	28mo	OTC	Alive	Retarded	Personal communication of 8/91	

^aDr. Wayne Flye of Washington University, St. Louis, MO.

^bLiver fragment.