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Arginase deficiency manifesting delayed clinical sequelae and induction of a kidney arginase isozyme

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Abstract. Deficiency of liver arginase (AI) is characterized clinically by hyperargininemia, progressive mental impairment, growth retardation, spasticity, and periodic episodes of hyperammonemia. The rarest of the inborn errors of urea cycle enzymes, it has been considered the least life-threatening, by virtue of the typical absence of catastrophic neonatal hyperammonemia and its compatibility with a longer life span. This has been attributed to the persistence of some ureagenesis in these patients through the activity of a second isozyme of arginase (AII) located predominantly in the kidney. We have treated a number of arginase-deficient patients into young adulthood. While they are severely retarded and wheelchair-bound, their general medical care has been quite tractable. Recently, however, two of the oldest (M.U., age 20, and M.O., age 22) underwent rapid deterioration, ending in hyperammonemic coma and death, precipitated by relatively minor viral respiratory illnesses inducing a catabolic state with increased endogenous nitrogen load. In both cases, postmortem examination revealed severe global cerebral edema and aspiration pneumonia. Enzyme assays confirmed the absence of AI activity in the livers of both patients. In contrast, AII activity (identified by its different cation cofactor requirements and lack of precipitation with anti-AI antibody) was markedly elevated in kidney tissues, 20-fold in M.O. and 34-fold in M.U. Terminal plasma arginine (1500 μmol/l) and ammonia (1693 mmol/l) levels of M.U. were substantially higher than those of M.O. (348 μmol/l and 259 μmol/l, respectively). By Northern blot analysis, AI mRNA was detected in M.O.'s liver but not in M.U.'s; similarly, anti-AI crossreacting material was observed by Western blot in M.O. only. These findings indicate that, despite their more long-lived course, patients with arginase deficiency remain vulnerable to the same catastrophic events of hyperammonemia that patients with other urea cycle disorders typically suffer in infancy. Further, unlike those other disorders, an attempt is made to compensate for the primary enzyme deficiency by induction of another isozyme in a different tissue. Such substrate-stimulated induction of an enzyme may be unique in a medical genetics setting and raises novel options for eventual gene therapy of this disorder.

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Introduction

Hyperargininemia, due to deficiency of liver arginase (AI; L-arginine urea-hydrolase; EC 3.5.3.1) which catalyzes the hydrolysis of arginine to ornithine and urea, is the rarest of the inborn errors of the urea cycle. It has also been considered the least life-threatening in that it does not typically present with the catastrophic neonatal hyperammonemic crises characteristic of the other disorders. Experience to date with a limited number of patients would seem to indicate that this feature is less prominent, less precipitate, and far less life-threatening than is seen in deficiency states involving the other four urea cycle enzymes. This difference has been ascribed to the unique isozyme distribution of human arginase. While the principal ureagenic enzyme activity (AI) is most abundant in normal mammalian liver (Herzfeld and Raper 1976; Gasiorowska et al. 1970; Spector et al. 1982) and absent in the patients (Cederbaum et al. 1979; Spector et al. 1983; Michels and Beaudet 1978), studies of rat and human tissues have revealed a second form of arginase in several other organs, most prominently in the kidney. Although this second isozyme (AII) exhibits some similar kinetic and physicochemical properties, it can be distinguished from AI immunochemically, electrophoretically, and biochemically (Spector et al. 1982, 1983; Carvajal and Cederbaum 1986). In addition, it is localized to the mitochondrial matrix, whereas AI is cytoplasmic (Grody et al. 1986).

The milder clinical course and much longer life span in arginase deficiency as compared to the other urea cycle

disorders has thus been attributed to the persistence of some ureagenesis in these patients through the activity of isozyme AII. Indeed, we have evidence from tissue assays in a previous patient, who succumbed following an unusual clinical course marked by liver fibrosis, that AII levels may even be elevated in the condition (Grody et al. 1989). Whatever the mechanism, the typical course of the disorder is marked not by early metabolic crisis but by progressive mental impairment and growth retardation, as well as a progressive spasticity, which is also unique to arginase deficiency among the urea cycle defects (Cederbaum et al. 1979; Brusilow and Horwich 1989; Bernar et al. 1986). While there may be episodes of hyperammonemia later in life, typically brought on by the anorexia associated with minor upper respiratory infections, they are usually readily reversed with intravenous fluids and sodium benzoate (Batshaw et al. 1982).

Severe inborn errors of metabolism are usually neonatal or pediatric diseases, and little is known of the long-term natural history of those, such as arginase deficiency, that are compatible with an extended lifespan given adequate care. We have had the opportunity to treat and study a number of these patients well into young adulthood. While moderately to severely retarded and spastic to varying degrees, their general medical care has been quite tractable, punctuated only by occasional hospitalizations for exacerbation of seizure activity or hyperammonemic stupor brought on by the aforementioned infection-associated anorexia. The decrease in exogenous protein intake induces a catabolic state resulting in an increased endogenous nitrogen load, though the imbalance had always been promptly responsive to treatment. Recently, however, two of our oldest patients underwent rapid deterioration that was refractory even to drastic therapeutic measures and ended in massive hyperammonemia, coma, and death. A clinical, enzymatic, and postmortem study of these patients points up some of the perilous and sudden long-term sequelae that may appear in even the more "benign" inborn errors of metabolism, along with some unusual compensatory mechanisms.

Case reports

Patient 1

Patient M.U. was a 20-year-old Hispanic male whose first symptoms were a Reye-like syndrome and development of early spasticity at age 3 (see Table 1 for summary of clinical history). Arginase deficiency was diagnosed at age 6, and he and his affected sister have been the subject of earlier reports (Cederbaum et al. 1979, 1982; Spector et al. 1983). By this time he was already severely mentally retarded, with an IQ of 15, and began to exhibit seizure activity, which was controlled with phenobarbital. He was placed on a low-protein diet, consisting of 0.65 g/kg/day of essential amino acids, low-protein fruits and vegetables, and Mead Johnson formula 80056 for calorie supplementation (Cederbaum et al. 1982). Oral sodium benzoate was administered at 250 mg/kg/day. On this therapy, his ammonia levels generally were maintained in the low-normal range, glutamine levels were low, and orotic acid levels were slightly high. Nevertheless, over the years he had multiple admissions to UCLA Medical Center for altered mental status and exacerbation of seizure activity secondary to hyperammonemia brought on by anorexia associated with minor respiratory infections. These incidents had been treated relatively easily by institution of intravenous fluids and sodium benzoate. The latest admission began in much the same way, but despite the usual therapy, the patient suffered an aspiration pneumonia, with plasma ammonia levels rising to a peak of 1,693 μmol/l, and he lapsed into coma punctuated by focal motor seizures uncontrolled by intravenous anticonvulsants. The patient was pronounced brain-dead on day 6 of admission, and ventilation and pressor support was withdrawn by parental consent.

Table 1. Clinical characteristics of the two patients

	Patient M.O.	Patient M.U.
Ethnicity	Ashkenazi Jewish	Mexican American
Family history	–	+
Age at first symptoms	3	3
Age at diagnosis	5	6
Age at death	22	20
Age when therapy began	10	8
Previous acute hyperammonia	+	+
Previous severe hyperammonia	–	+
Response to long-term therapy	+	+
Retardation	Severe	Severe

Patient 2

M.O. was a 22-year-old Caucasian Jewish male who began showing growth and psychomotor retardation at age 3, and presented with status epilepticus at age 5 (Table 1). Diagnosis of arginase deficiency was made at that time, and this patient too has been reported previously (Cederbaum et al. 1977; Spector et al. 1983). He was subsequently maintained on a low-protein diet and oral sodium benzoate as described for patient 1, along with phenytoin for his seizure disorder. Mental retardation and spasticity had been prominent features. Like M.U., he had multiple hospital admissions for hyperammonemia brought on by diminished food intake, but these were easily treated. His last admission occurred after he was found stuporous; he became comatose within 24 h. Plasma ammonia peaked at 259 μmol/l on the day of admission. Marked cerebral edema was observed by CT scan and did not resolve despite mannitol therapy and hemodialysis, eventually culminating in herniation of the brainstem.

Materials and methods

All diagnostic and therapeutic clinical procedures, including postmortem examination, were performed at UCLA Medical Center and conformed to the guidelines of our institutional review board. Postmortem tissues were obtained within 30 min after death and frozen in liquid nitrogen. Arginase activity in red blood cells and tissue samples was determined using our previously described modification (Spector et al. 1980) of the urease method of Schimke (1964). Distinguishing of arginase AI from AII activity was achieved by immunoprecipitation studies using rabbit anti-human liver arginase (AI) antibody, as described previously (Spector et al. 1983). Plasma ammonia levels were determined in the UCLA Clinical Laboratories on a DuPont ACA automated analyzer. Plasma amino acids were assayed at Children's Hospital of Los Angeles by standard methods (Hammond and Savory 1976). Genomic DNA for Southern blot analysis was isolated antemortem from peripheral blood leukocytes by standard methods (Kunkel et al. 1977). Restriction enzyme digestion, agarose gel electrophoresis, capillary blotting onto nylon membranes (Hybond-N, Amersham Corp., Arlington Heights, Ill.), and hybridization with a ^{32}P-labeled, 1,400-bp (base pair) human liver arginase cDNA probe (Dizikes et al. 1986) was performed as described (Gatti et al. 1984). RNA isolation in guanidinium from frozen liver tissue, and subsequent Northern blot analysis using formaldehyde gel electrophoresis, were performed by published methods (Chirgwin et al. 1979; Lehrach et al. 1977; Goldberg 1980).

Results

Clinical laboratory studies

The key laboratory values for both patients during their terminal episodes are shown in Table 2. M.U.'s peak ammonia level was higher than that of M.O. by almost an order of magnitude. Likewise, the single plasma arginine level obtained an M.U. was 2- to 5-fold higher than those of M.O. Peak arginine levels for both patients were markedly in excess of normal (9.5-fold and 4.2-fold, respectively). Both patients had urea nitrogen levels in the subnormal to low normal ranges.

Postmortem examination

In both cases, postmortem examination revealed severe global cerebral edema and aspiration pneumonia, with the proximate causes of death being necrotizing bronchopneumonia (M.U.) and uncal herniation (M.O.). Infarction of the pituitary and ischemic changes in the liver were other findings noted in M.U.

Enzyme assays

Assayed arginase activities in selected frozen postmortem tissue samples of both patients are shown in Table 3. Both patients were clinically deficient in AI activity as assayed in red blood cells (data not shown; reported previously in Cederbaum et al. 1977, 1979) and liver. Arginase activity in kidney, however, identified as the AII isozyme by its failure to immunoprecipitate with anti-AI antibody, was markedly elevated: 10-fold above normal in M.O. and 17-fold above normal in M.U. In fact, since half of the activity in the normal kidney control values is contributed by AI (Spector et al. 1983), which these patients are missing, the specific fold increase in AII activity in M.O. and M.U. was actually double these values, or 20-fold and 34-fold, respectively. The extent of increase appears to parallel the two patients' terminal plasma arginine levels (Table 2), and the peak terminal values were far in excess of any previously observed in these patients. AII levels in brain were also elevated as compared to control, but those in liver were not (Table 3). Normal control levels were based on multiple tissue samples obtained at surgery from adults on regular ad libitum protein intakes aside from the usual N.P.O. period prior to surgery.

Table 2. Clinical laboratory values during terminal hyperammonemic crisis in two patients with arginase deficiency[a]

Day of admission	Plasma ammonia (μmol/l)		Plasma arginine (μmol/l)		Urea nitrogen (mmol/l)	
	Patient M.O.	Patient M.U.	Patient M.O.	Patient M.U.	Patient M.O.	Patient M.U.
−5			348			
1	259	256			1.8	2.5
2	176	1,693	660	1,500	1.1	2.8
3	128	337			1.4	1.4
4		544				2.1
5		791				3.6

[a] Reference ranges: ammonia 11–30 μmol/l; arginine 42–159 μmol/l; urea 2.8–7.1 mmol/l

Table 3. Arginase activity in tissue extracts. ND, Not determined

Tissue	Activity (nmol arginine hydrolyzed/mg protein in 30 min)		
	Normal	Patient M.O.	Patient M.U.
Kidney	500 ± 100	5,192	8,564
Liver	19,500 ± 600	334	532
Brain	50 ± 10	492	ND

Southern analysis

Southern blot analysis of genomic DNA, digested with a variety of restriction endonucleases and hybridized with a near full-length (1.400bp), ^{32}P-labeled liver arginase cDNA probe, revealed no evidence of a gross gene deletion or rearrangement in either patient (data not shown).

Northern analysis

Results of Northern blot analysis of poly-A$^+$-selected RNA from postmortem liver tissue of both patients, probed with the 1,400-bp AI cDNA, are shown in Fig. 1. Patient M.O. demonstrated AI mRNA of normal size and quantity, while the AI mRNA band was absent in patient M.U. Amounts of RNA loaded in each lane were equivalent as determined by reprobing of the blots with cDNAs for actin, cyclophyllin, and 28S ribosomal RNA (data not shown).

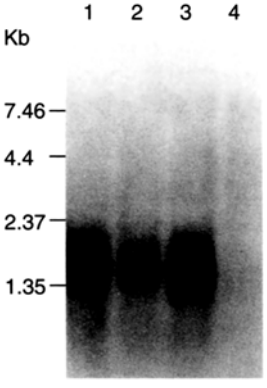

Fig. 1. Northern blot analysis of poly-A$^+$-selected liver RNA. Postmortem liver tissue of patient M.O. *(lane 3)*, probed with the 1,400-bp liver arginase cDNA, demonstrated AI mRNA of normal size and quantity as compared to that of control liver *(lanes 1, 2)*, while the AI mRNA band of patient M.U. *(lane 4)* was extremely faint, though apparently of normal size when visible. Amounts of total RNA loaded per lane were equivalent, except for control lane 2, which contained one-half the amount. This was confirmed by reprobing with cDNAs for actin, cyclophyllin, and 28S rRNA (not shown). Size markers are indicated in kilobases *(kb)* on left

Discussion

Prior to our better understanding of the basic biochemical defects, which has led to the formulation of more rational therapies, most patients with severe inborn errors died early of overwhelming metabolic imbalance. Now, with life expectancies for these patients increasing under attentive care, we are discovering some unusual and unexpected long-term sequelae in some of the disorders; examples include ovarian failure in females with galactosemia (Kaufman et al. 1981), destruction of the globus pallidus in methylmalonic acidemia (Heidenreich et al. 1988), central nervous system deterioration and visual impairment in cystinosis (Kaiser-Kupfer et al. 1986; Gahl and Kaiser-Kupfer 1987), and maternal phenylketonuria (Lenke and Levy 1980).

Our experience with the long-term natural history of liver arginase deficiency may serve to raise similar concerns in this disorder. Despite its well-known symptoms of mental retardation and muscle spasticity, early experience with hyperargininemic children had produced a general impression that this was the most benign of the urea cycle disorders, at least as regards its propensity for serious hyperammonemic crises (Cederbaum et al. 1979; Brusilow and Horwich 1989; Bernar et al. 1986). In the face of virtually absent liver arginase activity, these patients maintain persistent low-level ureagenesis and exhibit a relatively milder clinical course. For this reason, and because of the paucity of patients followed for an extended period, little attention has been paid to the long-term risks of hyperammonemia in this disorder. If anything, the general notion extant in other urea cycle and organic acid disorders that if the patients can be carried through the stresses of the newborn and infancy periods successfully, then their subsequent management will be much easier, has been accepted for arginase deficiency with even more certainty.

Belying this concept, the clinical course of the two patients described here points up the necessity for continued clinical vigilance in this disorder. Even well into adulthood, these patients are susceptible to a rapidly developing and fatal hyperammonemia brought on by the anorexia associated with common upper respiratory infections. In this setting, the rapidly ensuing sequelae of cerebral edema, seizures, pseudobulbar palsy and aspiration pneumonia may be virtually impossible to reverse even in the face of intravenous sodium benzoate and phenylacetate therapy and hemodialysis. The most prudent approach would seem to be prevention, in the form of early recognition of flulike illnesses and prompt institution of parenteral therapy.

Like most of the other hyperargininemic patients studied (Grody et al. 1992), neither of these individuals showed evidence of a gross arginase gene deletion on Southern blot analysis. Patient M.U., however, showed evidence of a markedly reduced-to-absent arginase mRNA on Northern blot analysis, indicating greatly reduced steady-state mRNA levels. Concordant with this finding, patient M.U. also showed no immunologically crossreacting arginase material on Western blot analysis (method described in Grody et al. 1989; data not shown).

Laboratory findings in these two patients also support our hypothesis that the persistent low-level ureagenesis seen in this disorder is due to the activity of an extra-hepatic isozyme of arginase found predominantly in the kidney. Residual AI activity was negligible, and in one patient AI mRNA was absent. Both patients' kidney arginase activities were markedly elevated above normal, of a magnitude similar to that of their terminal plasma arginine levels. This induction appeared to be restricted to the kidney and, to a lesser extent, the brain; AII activity in liver did not exceed the low level, comprising 1%–2% of total liver arginase activity in normals (Spector et al. 1983), typically found in this organ. Our earlier in vitro work had shown just such an apparent induction (up to 4- to 5-fold basal levels) in tissue culture, using human embryonic kidney cells (and rat hepatoma cells) whose growth media was supplemented with increasing levels (0.1–6.0 m*M*) of L-arginine (Grody et al. 1989), and now we have observed it in vivo in two patients. Such tissue-specific, substrate-driven induction of a biochemically related but genetically distinct isozyme may be unique in the setting of a human inborn error of metabolism, and raises the possibility of augmentation of AII levels, as opposed to direct replacement of AI, as a novel approach for eventual gene therapy of this disorder.

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