Successful treatment of a child with citrullinemia

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The amino acids formed by degradation of proteins ingested produce ammonia. The ammonia which is broken down and excreted as urea through a process known as the Kiebs-Hensleit cycle or the urea cycle (Rezvani, 1995). The urea cycle consists of five enzymes necessary for the synthesis of carbamyl phosphate, citrulline, argininosuccinate, arginine, and urea: carbamyl phosphate synthetase (CPS), ornithine transcarbamylase (OTC), argininosuccinate synthetase (AS), argininosuccinate lyase (AL), and arginase (ARG) (Lloyd, 1992). Congenital deficiencies of the enzymes involved in the urea cycle are diseases that are almost fatal without treatment, showing symptoms like vomiting, lethargy, dyspnea, and coma due to hyperammonemia coming from the accumulation of ammonia and metabolic precursors resulting from the deficiency of one of these enzymes (Batshaw and Brusilow, 1983). Among these, the disease manifested by the congenital deficiency of argininosuccinate synthetase (AS) which is associated with the formation of argininosuccinate in citrulline is called argininosuccinate synthetase deficiency or citrullinemia. There have been two reports on this so far in Korea; one in July 1987 by Kim et al. and the other by Park et al. in 1995. We are to report a case of successful treatment of a child with citrullinemia who was transferred to our hospital due to dyspnea, lethargy, feeding difficulties, convulsions and cyanosis together with some document studies related to this case.

Key words: Citrullinemia, Urea cycle disorder

CASE REPORT

A ten days old baby boy had been admitted to the NICU with the chief symptoms of dyspnea, lethargy, and convulsions. The patient was the second child of a 29 year-old mother, born at 40 weeks through a normal vaginal delivery. There was nothing special about the family history, except that the first born was a male who died 3 days postpartum after a coma without any explanation. The postpartum status of the child was good, started bottle-feeding and was discharged without any problems on day 3, but on day 4 after showing feeding difficulties, lethargy, dyspnea and a bout of convulsions the baby fell into a coma and was administered at some other hospital. The results of the lab taken then pointed out hyperammonemia. With the suspicion of an enzymatic disease of the urea cycle, amino acid levels in the blood and urine were tested and anticonvulsants were administered with mechanical ventilation therapy. Lactulose and neomycin were given as well for the treatment of the persisting hyperammonemia, but high levels of serum ammonia continued and therefore peritoneal dialysis was required. Consequently serum ammonia levels began to fall and the child regained consciousness to be transferred to our hospital ten days post-administration at other hospital.

When admitted the child was in a lethargic state, and vital signs were as follows: pulse rate 130 bpm, respiratory rate 40 per min, and body temperature 36.5°C. Body weight was 3,660 g. Pupil size and light reflexes were normal and the anterior fontanel was not swollen. There were neither any signs of anemia in the conjunctiva nor jaundice in the sclera. Breath sounds and heart sounds were both normal and although there appeared to be no abdominal swelling present, the liver was palpable by two fingerbreadths. The neurological exam was normal except for hypotonia of the muscles. Blood cell tests, electrolyte tests and ABGA tests taken on arrival were all within normal limits, but the serum ammonia was increased to a level over 285 μmol/dl and SGOT and SGPT were 37 IU/l and 77 IU/l respectively. In the amino acid analysis plasma citrulline was increased to 820.4 nmol/ml with arginine decreased to 44.4 nmol/ml, and the citrulline in urine was increased noticeably to a level of 10.1 mmol/g^{-Cr}. (Table 1)

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Table 1. Serial Amino Acid Analysis of plasma and urine

A. A.	Plasma (nmol/ml)				
	1 wk	3 mo.	6 mo.	1 yr.	Ref.
Arginine	44.4	149.0	96.3	121.5	45 - 130
Citrulline	820.4	2433.0	3280.2	2681.4	16 - 55
Glutamine	1772.7	355.0	275.6	116.5	390 - 650
Ornithine	22.8	144.0	248.9	46.5	27 - 80
A. A.	Urine (mmol/g^{-cr})				
	1 wk	3 mo.	6 mo.	1 yr.	Ref.
Citrulline	10.1	12.13	22.49	3.05	0.01 - 0.07
Glutamine	12.9	0.61	1.02	0.03	0.34 - 1.66
Ornithine	0.15	0.08	0.05	0.01	0 - 0.08

After being transferred to our hospital peritoneal dialysis and neomycin enemas were continued, and 250 mg/kg/day of sodium benzoate and the same amount of sodium phenylacetate were administered orally with 200 mg/kg/day of arginine as well by the sameroute, with restrictions in protein intake. On day 3, serum ammonia levels fell below 100 μmol/dl so peritoneal dialysis was stopped and a mixture of a regular formula, Pro-Phree™ , and Cyclinex™ was given through a nasogastric tube, gradually increasing the total protein level starting at 0.5g/kg/day. But on day 8 as the serum ammonia level increased to 285 μmol/dl protein was restricted again, and after starting parenteral nutrition serum ammonia levels on day 12 fell below 100 μmol/dl, hence protein intake was increased, and afterwards maintained a level below 100 μmol/dl, which brought a steady increase in the amounts of Pro-Phree and regular formula plus administrations of total protein up to 2.0 g/kg/day while ammonia levels were well maintained, and the infant was discharged on day 20.

After leaving the hospital, through education of the parents, sodium benzoate and arginine were continuously given in addition to the regular diet (Fig.1) and the succeeding lab tests of blood ammonia levels and amino acids were taken likewise (Table 1). At 7 and 14 months the child was readmitted to the hospital as blood ammonia levels had risen due to acute enteritis with incessant fevers, vomiting, and diarrhea, and after enemas of lactulose and neomycin, sodium benzoate and arginine infusion plus total parenteral nutrition normal levels of ammonia were recovered, making discharge possible. Now at 18 months, the child has achieved a normal growth with weight, height, and head circumference all over the 50th percentile, in addition to normal motor and mental development. Also instead of the Pro-Phree previously administered protein-free formula, a product of Maeil Research Institute, was given mixed with a regular formula, maintaining a total protein intake of 1.5 g/kg/day with 250 mg/kg/day of sodium benzoate and 400 mg/kg/day of arginine administered; since then, the child has been enjoying good health.

DISCUSSION

The first child with citrullinemia, born of a marriage between cousins was reported to be admitted at 9 months with a chief complaint of severe vomiting and mental retardation, and found to have high levels of citrulline in serum, CSF, and urine (McMurray, *et al.*, 1962). Also after Visakorpi (1962) reported of citrullinemia that same year tens of cases have been reported up till now, those mostly in Japan (Matsuda *et al.*, 1982).

Ammonia is made from amino acids absorbed in the body, but animals cannot tolerance even minimal amounts, whether intracellular or extracellular, and excretion is required; those that excrete ammonia in forms of urea as in humans are called 'ureotelic' (Kim, 1992). For ammonia to change into urea a series of reactions known as the Klebs-Hensleit cycle or the urea cycle is required, complete with all five of the enzymes participating in the reaction, and if even one is genetically deficient there is a defect in urea synthesis and ammonia is accumulated, causing toxemia. Citrulline is formed by the conversion of an ammonium ion and carbon dioxide to carbamoyl phosphate, using 2 ATP and the enzyme ornithine carbamoyl transferase, then transferred into the cytoplasm to be combined with aspartate which is another nitrogen source of urea to form argininosuccinate, and afterwards forming arginine to be finally converted into urea and releasing ornithine, only to be reuptaken into mitochondria and react with carbamoyl phosphate, thus starting the urea cycle once more. The frequency of congenital enzymatic diseases of the urea cycle is not clearly known but roughly estimated to be one in 30,000 births, of which most are dead before diagnosed (Conn and Banagale, 1986). Before the

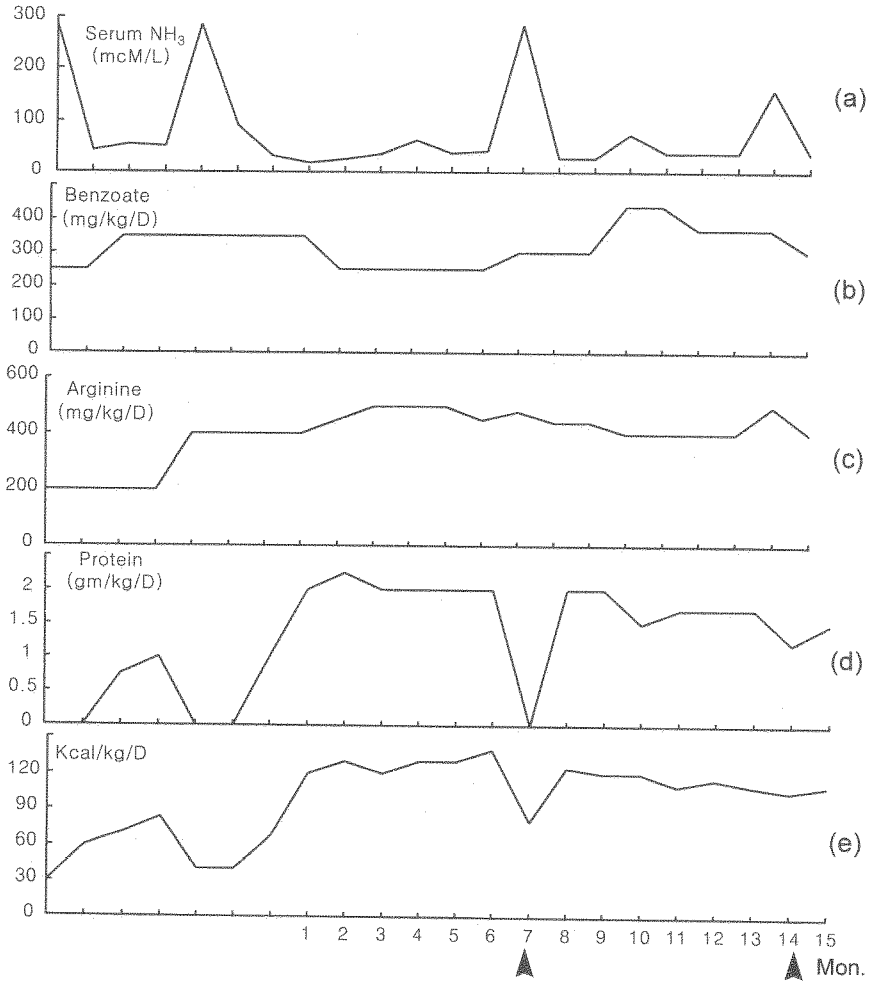

Fig. 1. Chronological progression to age 15 months of patient's serum NH_3 level (a), sodium benzoate dosage (b), arginine dorsage (c), protein intake (d), caloric intake (e). Arrowheads indicate acute febrile illnesses.

1980's deficiencies of OTC and AL were the most common of the five enzymes, making AS deficiency a much rarer disease (Koch, 1981). According to European study, out of 247 antenatally diagnosed urea cycle diseases, citrullinemia was the most common with 108, with AL and OTC deficiencies following in order (Kamoun *et al*, 1995).

Citrullinemia is inherited as an autosomal recessive and the argininosuccinate synthetase gene was found to be in chromosome 9 by using human-hamster cell hybrids (Critt *et al.*, 1977). Kobayashi *et al.* (1989) found it adequate to amplify (by PCR) and sequence mutant cDNA derived from the stable RNA located in the cutaneous fibroblasts of patients with citrullinemia. They discovered 9 mutants among 11 patients, in which three of them had deletions in exons 5, 6 or 7; the one in exon 6 was a point mutation. Among the other 6 mutants, 5 had a conversion of C:G to T:A at the CpG dinucleotides, which caused a loss of the restriction site of the enzyme Msp1 in 3 of them. They also found out that out of the 23 Japanese patients with typical citrullinemia 10 had deletions of exon 7, hence reporting less variation of mutation forms in comparison to the US (Kobayashi *et al.*, 1994). Out of the 20 mutations of ASmRNA which they discovered 14 single base mutations, deletion mutations of the exons 5, 6, 7, and 13 of mRNA, and an insertion mutation of the first 7 bases in exon 16 plus another of 37 bases between exons 15 and 16 (Kobayashi *et al.*, 1995).

In most cases over 50% die during this period due to severe symptoms starting from birth, and as they manifest as fretting, feeding difficulties, tachypnea and lethargy and progress to rigidity, convulsions, dyspnea, and coma suspected to be sepsis develop. When occurring in infancy they are often associated with retardation in psychomotor development and the severity and patterns vary. The hair of the child of this age is characteristically patchy and friable. Besides showing bizarre behavior nocturia, insomnia, nightmares, vomiting, diarrhea, tremors of the hands and feet, post-prandial delirium, lethargy, convulsions, delusions, illusions, and temporary coma, etc. Developmental retarda-

tion, both mentally and physically, are seen in some. The child tends to like beans, peas, or peanuts and dislike rice, vegetables, and sweets, which is thought to be due to arginine deficiency. In Japan there have been reports of adult-type citrullinemia, making the onset variable, ranging from school children to 48 year-old adults. In most cases they are citrullinemia type II with problems in quantities of AS. Citrullinemia is classified into three types according to AS. Type I citrullinemia shows abnormal AS activity in the liver, kidneys, and cultured fibroblasts (Saheki *et al.*, 1981), which Tedesco *et al.* (1967) found the changes in the Michaelis constant of the enzyme to be the cause. Type II citrullinemia shows low levels of AS in the liver but not in the kidneys or in cultured skin fibroblasts. Type III citrullinemia displays no enzymatic activity of AS due to lack of translation activity of AS mRNA (Kobayashi *et al.*, 1986, 1993).

The diagnosis of the child with defects in the urea cycle are made as follows. In neonates with symptoms previously described ammonia levels in the blood are increased and suspicion arises when the anion gap of the biochemical tests is normal and finally children with citrullinemia have increased levels of the precursor citrulline besides decreased levels of the product arginine in the blood, urine, and CSF (Saheki *et al.*, 1982). The metabolites of orotic acids and such from the cycle synthesizing pyrimidine from carbamoyl phosphate are increased, and levels of glutamate and glutamine which transport ammonia of the urea cycle are definitely increased (Matsuda *et al.*, 1982; Saheki *et al.*, 1982). As seen in this case citrulline levels in the blood and urine are markedly increased as such is the case with the blood levels of glutamine and alanine, with low arginine levels, hence although AS activation levels in the hepatocytes were not measured the diagnosis of citrullinemia can be made. According to amino acid tests taken 3 months after administering sodium benzoate and arginine, although high levels of citrulline were continuously maintained in the blood it in itself did not seem to have any neurotoxicity. In contrast, glutamine and arginine were normalized and responded well to therapy.

Hyperammonemia, which requires a differential diagnosis from citrullinemia, are divided into hereditary and non-hereditary causes. The former consists of lysinuric protein intolerance, defects in transporting metabolic materials of the urea cycle like the hyperammonemia-homocitrullinemia-hyperornithinemia syndrome, and the latter refers to neonatal transient hyperammonemia, Reye syndrome, defects in supplying arginine, and valproate therapy.

The ultimate goal in the therapy of a child with citrullemia is to prevent neurological damage due to hyperammonemia and to promote normal growth by supplying sufficient amounts of nutrients. When diagnosed prenatally initial diet and conservative therapy would suffice, but as seen in this case most children are in a fairly progressed state, which requires active therapy from the start. This includes lowering blood ammonia levels through peritoneal dialysis, hemodialysis and oral administraton of lactulose, and methods of stimulating nitrogen excretion pathways by using those rather than the urea cycle consist of the formation of hippuric acid by combining sodium benzoate with glycine and hence its excretion in urine (Gatley and Sherratt, 1977). Sodium phenylacetate or sodium phenylbutyrate is combined with glutamine in the liver (Ambrose *et al.*, 1933)(or glutamine and taurine in kidney (James *et al.*, 1972)) to form phenylacetylglutamine and excreted. Amounts of administration differ according to frequencies of feeding but usually 250-500 mg/kg/D three to four times a day or while feeding (Bachmann, 1984). These drugs especially need cyanocobalamine, folate, niacin, pantothenic to react, therefore Vitamin B should be administered 5 times more than the daily requirement (Zeman, 1991).

Diet therapy is also very important as conservative therapy besides the initial active therapy. The basic protocol of nutrient supplementation is to prevent catabolism of body protein by a well-fortified diet as well as hyperammonemia by specific amounts of dietary protein. The daily requirements are listed in Table 2 (Acosta and Yannicelli, 1993). In the case of the child previously mentioned protein and nutrients were initially supplemented by total parenteral nutrition while undergoing peritoneal dialysis, and then by mixing a general

Table 2. Recommended daily nutrient intake(range) for patients with urea cycle disorder

Age	Nutrient	
	Protein(g/kg)	Energy(kcal/Kg)
Infant		
0 to < 3 mo	2.20 - 1.25	150 - 125
3 to < 6 mo	2.00 - 1.15	140 - 120
6 to < 9 mo	1.80 - 1.05	130 - 115
9 to < 12 mo	1.60 - 0.90	120 - 110
Girls and Boys	(g/day)	(kcal/day)
1 to < 4 yr	8 - 12	945 - 1890
4 to < 7 yr	12 - 15	1365 - 2415
7 to < 11 yr	14 - 17	1730 - 3465
Women		
11 to < 15 yr	20 - 23	1575 - 3150
15 to < 19 yr	20 - 23	1260 - 3150
>= 19 yr	22 - 25	1785 - 2625
Men		
11 to < 15 yr	20 - 23	2100 - 3885
15 to < 19 yr	21 - 24	2200 - 4095
>= 19 yr	23 - 32	2625 - 3465

formula (12.6% protein), Pro-Phree, Cyclinex-1(7% essential amino acid) total protein amounts increased from 0.5 g/kg/D to 2.0g/kg/D, while the calories in total were set at 120 kcal/kg/D. At present protein-free formulas supplied by Maeil Research Institute are being used instead of Pro-Phree. In addition, arginine not only helps nitrogen excretion but must be administered as a conditional essential amino acid at levels of 400-700 mg/kg daily (Brusilow and Horwich, 1995).

But, as seen in this child since differential diagnoses of other abnormalities of the urea cycle cannot be made before the definite diagnosis a dose of 200 mg/kg must be given every day, and after the results of the amino acid tests have been obtained and the child has been diagnosed as citrullinemia administrations must be increased to 450 mg/kg/D.

Together with the therapy mentioned above continuous observation of the status of the child is also essential but first, there must be a follow-up to see whether growth and development is normal or not. At the same time nutrition status evaluated by blood tests must be tested quantitatively twice every week until amino acid concentrations in blood are stabilized, and once in every two to three months thereafter. When glutamine concentrations in particular increase it means that hyperammonemia is imminent, and in this case records of the diet for the last 3 days must be checked and tests referring to ammonia levels in the blood and infection must be executed. If protein administrations and calorie supplements in total are adequate and there is no possibility of infection, then protein intake must be decreased by 10%. During the acute phase ammonia levels in the blood must be frequently measured until normalized; when they are within normal range evaluation must be done once every week up till 6 months and after that once every month. If blood ammonia levels are above normal reduction of protein amounts or increase of amounts of sodium benzoate or sodium phenylacetate is required. Albumin levels in blood must be checked likewise once every 3 months and protein intake must be increased by 10% when low. If catabolism of body proteins is suspected 3-methylhistidine levels in urine should be measured and protein administration increased when over 25mmol/mol creatinine. Since glycine is necessary for normal synthesis of heme those who are infused with sodium benzoate should have their blood cells checked once every 3 months, and once every 6 months if not infused. When glycine seems to be deficient pyroglutamic acid levels in urine must been checked to see if they are over 325 μmol/mmol creatinine.

It is also our opinion that the establishment of a plan including management in terms of nutrition, evaluation of growth and development, treatment of acute illnesses starting with infection, and continous blood tests for the long-term management of the child is equally important as well as the initial active therapy through early diagnosis, with reference to the treatment of a child with citrullinemia.

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