Treatment of Adult-Type Citrullinemia with Administration of Citrate

YOSHIKIYAJIMA, TAKASHIHIRASAWA and TAKEYORI SAHEKI*

Clinic of Internal Medicine, Iwaki-Kyoritsu General Hospital, Iwaki 972, and *Department of Biochemistry, Tokai University School of Medicine, Isehara 259-11


A 48-year-old man who showed a regular diurnal fluctuation of blood ammonia level was diagnosed as adult-type citrullinemia with quantitative estimation of plasma amino acids and urea cycle enzymes in the liver. The restriction of daily protein intake less than 50 g and oral administration of lactulose had no effect on the blood ammonia level. The efficacy of four amino acid solutions which had been used in order to improve the hepatic encephalopathy was compared each other in this case. Glutamate-arginine mixture had the same effect as glutamate solution in lowering the blood ammonia level, and glutamate was thought to be essential. Also, oral administration of citrate showed a remarkable effect almost comparable to intravenous administration of glutamate. Analysis of plasma amino acids 3 hr after citrate administration demonstrated an increase in glutamate and a decrease in citrulline. These observations suggest that a metabolic pathway of citrate – α-ketoglutarate – glutamate – glutamine takes part in disposing free ammonia in the blood. – adult-type citrullinemia; diurnal fluctuation of blood ammonia; citrate therapy

Hepatocerebral disease with hyperammonemia and relapsing encephalopathy has been known as Inose-type in Japan (Inose 1950) and is now considered to be identical with portal-systemic encephalopathy described by Sherlock et al. (1954). But there were several cases which had no evidence of massive portal-systemic shunting as to cause relapsing encephalopathy (Takahashi et al. 1972; Koyama et al. 1973). Recently these cases have been shown to have an increased level of serum citrulline and some investigators proposed the abnormality of urea cycle as the etiology (Tsuji et al. 1976; Yamauchi et al. 1980). The congenital hyperammonemia resulting from the urea cycle abnormality is known among pediatricians (Hsia 1974) and there were some patients who survived to adulthood (Scott-Emuakpor et al. 1972; Fell et al. 1974). It remained unclear whether or not this entity is exactly the same as the adult type.

This paper deals with a patient with adult-type citrullinemia who had a first
episode of encephalopathy at the age of 48 years and was diagnosed so for the first time in his life. In this case, regular diurnal fluctuation of blood ammonia was seen and oral administration of citrate lowered the ammonia level and improved clinical features.

CASE REPORT

A 48-year-old Japanese man was admitted to our Hospital for the first time because of repetitive episodes characterized by disorientation and excitation. There was no history of liver or other diseases. He had been fond of peanut and fishes since childhood. His family had no history of liver disease, or his parents had no traceable consanguinity. His first episode occurred at forty-eight years of age. At midnight he was walking about in the room saying something that could not be comprehended and intended to leap out of a window at last. Hyperventilation and asterixis were seen simultaneously at that time. The next day when he admitted to our Hospital, the examination of blood ammonia revealed an abnormally high level of 186 µg/100 ml.

After admission he was free of neuropsychiatric signs and symptoms except that encephalopathic episodes occurred two to three times a month when blood ammonia levels were high. As his presumptive diagnosis was portal-systemic encephalopathy, restriction of daily protein intake less than 50 g and oral administration of lactulose were begun. For a month he showed no episodes and was discharged to be cared in the outpatient clinic. But two weeks later, he had an episode and readmitted to our Hospital.

At the time of second admission the patient was described to be slightly emaciated. He was in an alert mental state, but had no abnormal findings in neuropsychiatric examination. Physical examination revealed a spider nevus on the anterior chest and palmar erythema bilaterally. There were no hepatosplenomegaly, ascites, icterus, nor pretibial edema. Routine laboratory tests revealed: hemoglobin 10.6 g/100 ml, WBC 4500/mm³, platelets 9.4 × 10⁴/mm³, serum cholesterol 73 mg/100 ml, blood urea nitrogen 20 mg/100 ml, creatinine 1.4 mg/100 ml, SGOT 80 IU, SGPT 35 IU, alkaline phosphatase 16.3 King U, serum total bilirubin 2.5 mg/100 ml, direct bilirubin 0.7 mg/100 ml, ICG R₁₅ 15.4%, total serum protein 5.6 g/100 ml, albumin/globulin ratio 1.22, and serum electrolytes were within normal limits except Fe 168 µg/100 ml and Cu 171 µg/100 ml. The plasma ceruloplasmin level was as high as 128 mg/100 ml. Blood gas analysis showed slight metabolic acidosis, i.e., base excess of −5.2 mEq/liter.

Laparoscopic examination showed slightly undulating surface of the liver but no evidence of manifest nodularity. Histological examination revealed that lobular architecture was preserved and moderate fatty infiltration and slight fibrosis were present. Selective celiac arteriography showed no particular findings from arterial phase to venous phase nor did demonstrate portal-systemic collateral circulation. Electroencephalography at non-episodic interval revealed low frequency waves of high amplitude all over the head which denoted high grade organic
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changes of the cerebral cortex. But so-called triphasic waves were not recorded. EEG in an episode could not be taken as the patient was always in an exciting state at that time.

After admission there were episodes two or three times a month and arginine-glutamate mixture was effective to improve this encephalopathy. Characteristics of the encephalopathy were disorientation with excitation and hyperammonemia not provoking comatous state. Since the establishment of the efficacy of arginine-glutamate mixture in lowering the blood ammonia level, it was administered by drip infusion from 6:00 p.m. to 8:00 p.m. every day. During the following three months the patient had no episodes and showed gradual improvement in general condition. But hypoproteinemia was still present and he gained no weight. Since the establishment of the efficacy of citrate, it was administered every day.

SPECIAL STUDY FOR UREA CYCLE

Method

Blood ammonia was measured by the modified method of Okuda and Fujii (1966) (normal range 40–80 μg/100 ml). Quantitative estimation of plasma amino acids was done by an automatic amino acid analyzer (Hitachi Model KLA5). Urea cycle enzymes were determined by the method of Schimke (1962) with a material obtained by laparotomy. The enzyme protein of argininosuccinate synthetase (ASS) was quantitatively estimated by a single radial immunodiffusion method with an antibody to ASS of rat liver (Saheki et al. 1977).

Result

Diurnal fluctuation of the blood ammonia level. To prevent the hyperammonemia, protein intake was restricted to less than 50 g per day and the clinical course was observed. Symptoms of encephalopathy or asterixis occurred at night. Therefore, the blood ammonia was measured four times a day, i.e. 9:00 a.m., 2:00

![Fig. 1. Diurnal fluctuation of blood ammonia levels of three consecutive days. Daily protein intake was fixed at 50 g and no any medications were taken. The patient had no neuropsychiatric signs nor symptoms even at the highest level of the blood ammonia.](image-url)
p.m., 8:00 p.m., 11:00 p.m. during three consecutive days in order to elucidate the correlation between hyperammonemia and encephalopathy. It was revealed that the levels of blood ammonia depicted a gentle curve which had its peak between 8:00 p.m. and 11:00 p.m. (Fig. 1). After that period blood ammonia was measured only at 9:00 a.m. and 8:00 p.m. every day. The average of fasting levels of blood ammonia was 120 μg/100 ml at 9:00 a.m., and 430 μg/100 ml at 8:00 p.m. Encephalopathy was found at 870 μg/100 ml on the average (Fig. 2).

The effect of lactulose on the blood ammonia level. Daily protein intake was fixed at 50 g and lactulose was orally administered after every meal. The dosage
was increased from 30 ml to 40 ml per day and he had soft stools three to four times a day. But lactulose could not prevent the nocturnal rise of blood ammonia and encephalopathy appeared at the sixth day of lactulose medication. From that time administration of lactulose was ceased (Fig. 3).

The effect of glutamate and citrate on the blood ammonia level. Four solutions of amino acids which had been used for the improvement of hepatic encephalopathy were compared with each other in this patient. Each amino acid, 20 g, dissolved in 500 ml of physiological saline, was administered by drip infusion from 6:00 p.m. for 2 hr. The Blood ammonia level was measured just after the end of the infusion. The most potent one was the solitary use of glutamate which was almost equal to the mixture of arginine and glutamate. On the other hand, the least effective one was the solitary use of arginine. Citrate in the form of Na-salt was administered orally at 5:00 p.m. every other day. Blood ammonia measured at 8:00 p.m. revealed that the blood ammonia lowering effect of citrate was equivalent to the drip infusion of 20 g of glutamate (Fig. 4).

![Graph showing the effect of glutamate and citrate on blood ammonia level](image)

Fig. 4. The effect of glutamate and citrate on the blood ammonia level. Numbers in the bottom of the columns represent trials of the amino acid infusion. Vertical bars represent ±s.d.

Plasma amino acids. The serums were sampled at 9:00 a.m. and 8:00 p.m. to analyze amino acids and ammonia. Citrulline, ornithine, glutamate, asparate and methionine all had high values, while other amino acids were lower than normal values. Among them citrulline was remarkable in its rise at night. The data at 8:00 p.m. on July 27 showed an abnormally high level of citrulline under the influence of the mixture of arginine and glutamate administered immediately before the sampling. On September 14, 22.8 g of sodium citrate was administered orally at 5:00 p.m. and analysis of amino acids at 8:00 p.m. revealed an increase of glutamate and a decrease of citrulline (Table 1).

Urea cycle enzymes. Among five enzymes associated with urea cycle in the liver ASS was only one which had a decreased activity. Its activity was 15% of the
The quantity of the enzyme protein of ASS measured by an antibody to ASS of rat liver was 20% of the control. Michaelis constants (Km) for citrulline and aspartate were not increased (Table 2).

**DISCUSSION**

The concept of congenital defect of urea cycle is known among pediatricians, and the disease is classified into five types (Hsia 1974). Citrullinemia due to deficiency of ASS is a member of this entity and many cases have been reported. In most cases patients were infants and died within childhood but some of them survived to adulthood (Scott-Emuakpor et al. 1972). On the other hand, in the field of internal medicine and psychiatrics, citrullinemia with no signs nor symptoms until adulthood has been known (Tsuji et al. 1976; Yamauchi et al. 1980). In this paper such a case was called “adult type” and the patient is just an adult-type citrullinemia.

Analysis of urea cycle enzymes of the liver revealed the deficiency of ASS
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But it is a question why he had no clinical signs nor symptoms until 48 years old. It seems that in this case the partial deficiency of ASS activity was congenital and hyperammonemia with citrullinemia lasting since his birth was below the threshold level above which the encephalopathy could appear. But elevated blood ammonia even if under the threshold level was toxic to the liver and deprived the liver of its functional reserve gradually to the level which let the blood ammonia level rise above the threshold.

Generally in the case of the end stage of hepatic cirrhosis or fulminant hepatitis, a relatively low level of blood ammonia (100–200 µg/100 ml) could produce comatous state. Therefore other toxic substances besides ammonia have been thought to be essential in the pathogenesis of hepatic encephalopathy (Sherlock 1958). In this case, although the patient had a regular nocturnal rise of blood ammonia level to 400–500 µg/100 ml, he was usually free of neuropsychiatric episodes except occasional asterixis at most. Thus pure hyperammonemia caused by the defect of urea cycle seems to be well tolerated by the patient. This may also support the previous hypothesis that he had a hyperammonemia of subclinical level before the onset of the encephalopathy.

There are quantitative and qualitative abnormalities as to the cause of decreased enzyme activities. In general in the latter case, the amount of enzyme protein is within normal limit but the affinity of the enzyme to the substrate is

<table>
<thead>
<tr>
<th>Activity of urea cycle enzymes in liver (U/g liver)</th>
<th>Control mean±s.d. (range)</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamyl phosphate synthetase</td>
<td>4.06±1.99 (1.05–7.07)</td>
<td>3.13</td>
</tr>
<tr>
<td>Ornithine transcarbamylase</td>
<td>56.3±21.4 (35.5–81.9)</td>
<td>86.7</td>
</tr>
<tr>
<td>Argininosuccinate synthetase (ASS)</td>
<td>0.75±0.35 (0.24–1.39)</td>
<td>0.11</td>
</tr>
<tr>
<td>Argininosuccinase</td>
<td>1.8±0.7 (1.0–2.7)</td>
<td>2.2</td>
</tr>
<tr>
<td>Arginase</td>
<td>369±180 (236–800)</td>
<td>551</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzymological analysis</th>
<th>Control</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver ASS Activity</td>
<td>100%</td>
<td>ca. 15%</td>
</tr>
<tr>
<td>Amount of enzyme protein</td>
<td>100%</td>
<td>ca. 20%</td>
</tr>
<tr>
<td>Antigenicity against anti-rat ASS serum</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kinetics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Km for citrulline</td>
<td>4.8×10⁻⁵M</td>
<td>Not increased</td>
</tr>
<tr>
<td>Km for asparate</td>
<td>5.6×10⁻⁵M</td>
<td>Not increased</td>
</tr>
</tbody>
</table>
decreased. This type of enzyme abnormality with high Km values has been known among patients with congenital hyperammonemia (Tedesco 1967; Matsuda et al. 1971). In this case it was shown that Km values for both citrulline and aspartate were normal and the quantity of the enzyme protein was decreased.

There are many reports of adult-type citrullinemia with hyperammonemia but none of them referred to the diurnal fluctuation of blood ammonia. Takahashi et al. (1973) reported a case of adult-type citrullinemia in which the neuropsychiatric signs and symptoms used to aggravate at night, but he did not relate it to the ammonia level. In the present case the encephalopathic episodes had a marked trend to appear at night and this could be explained by the nocturnal rise of blood ammonia. In the field of congenital hyperammonemia, Batshaw et al. (1975) reported a case of thirteen years old female with carbamyl phosphate synthetase deficiency; the patient showed a rise of blood ammonia. Moser et al. (1967) reported two cases of argininosuccinic aciduria; the blood ammonia level of theirs was normal or only slightly elevated in the fasting state but marked elevations occurred postprandially. Hitherto the samplings of blood for the determination of ammonia have been done in the fasting state preferably at early in the morning. But, if the defective urea cycle is suspected as the pathogenesis, the blood samples should be taken postprandially, so much the better after the evening meals.

As to the treatment of hyperammonemia, protein restriction and intestinal sterilization with neomycin or lactulose have been proposed (Hsia 1974). In this case protein restriction to less than 50 g per day was not sufficient to prevent encephalopathic episodes and orally administered neomycin or lactulose never lowered the blood ammonia level. Morrow et al. (1967) also reported a case of four years old child with citrullinemia; orally administered neomycin was ineffective in this case. Levin et al. (1969) reported a case of congenital hyperammonemia with ornithine transcarbamylase deficiency; in this patient citrate, administered before the feed, was found to be effective in reducing the postprandial rise of blood ammonemia.

The mechanism of citrate in reducing ammonia rise has been though as follows: Citrate is converted to α-ketoglutarate through the citric acid cycle which now catches free ammonia in the blood to yield glutamate. Then glutamine synthesis takes place by incorporation of another ammonia with glutamate. Thus two molecules of ammonia are disposed in the process in which one molecule of citrate yields one molecule of glutamine. On this hypothesis, the use of citrate is more efficient than that of glutamate and is more desirable treatment since it can supplement the depletion of α-ketoglutarate caused by the hyperammonemia.

Citrate was effective in reducing the nocturnal rise of blood ammonia level and exerted the same effect as the drip infusion of glutamate on the present case. The analysis of plasma amino acid concentrations 3 hr after citrate ingestion demonstrated an increase in glutamate and a decrease in citrulline, which supported the previously mentioned hypothesis of citrate action through the citric acid cycle.
Acknowledgment

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References

linemia in adult caused by partial deficiency of liver argininosuccinate synthetase. 
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