Enzyme Replacement Therapy in a Patient with Hyperargininemia

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MIZUTANI, N., HAYAKAWA, C., MAEHARA, M. and WATANABE, K. Enzyme Replacement Therapy in a Patient with Hyperargininemia. Tohoku J. exp. Med., 1987, 151 (3), 301-307 — In a patient with hyperargininemia, enzyme replacement therapy such as whole blood exchange transfusion or erythrocyte transfusion was performed, and its effect was confirmed in vitro as well as in vivo. The patient has been treated with the restriction of protein intake, oral administration of an essential amino acid mixture, and sodium benzoate or phenylacetic acid. With these treatments, his plasma ammonia levels were controlled. On the other hand, plasma and CSF concentrations of arginine were not so well controlled. With whole blood exchange transfusion and erythrocyte transfusion, plasma arginine concentrations and plasma ammonia levels were controlled. These effects have continued for about 3 months. Furthermore, the effect of exchanged erythrocytes on the blood arginine levels was also confirmed by in vitro experimentation that mixes arginine with erythrocytes in the medium RPMI 1640. Arginine concentration of the medium did not decrease when arginine was mixed with erythrocytes of the patient, but when arginine was mixed with erythrocytes of mother or normal controls, it decreased rapidly. In calculation, 10^7 of erythrocytes of the mother or normal controls was presumed to metabolize 4-5 nmole of arginine per day. From these results, the erythrocyte exchange transfusion is considered to be effective for the control of clinical and biochemical abnormalities in this disorder. ——— blood exchange transfusion; enzyme replacement therapy; erythrocyte transfusion; hyperargininemia

Hyperargininemia is a rare disorder of the urea cycle due to the deficiency of arginase which catalizes the conversion of arginine to ornithine and urea. The clinical features of this disorder are different from the other enzymopathies of the urea cycle. Especially, marked degree of spasticity of four extremities is characteristic. Up to data, there have been some attempts to treat the patient with this disorder. Restriction of protein intake (Terheggen et al. 1975; Qureshi et al. 1981; Cederbaum et al. 1982), oral administration of an essential amino acid mixture (Snyderman et al. 1979; Cederbaum et al. 1982), sodium benzoate and

Received November 12, 1986; accepted for publication February 7, 1987.
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phenylacetic acid (Batshaw et al. 1981), and exchange transfusion or gene replace-
ment therapy (Terheggen et al. 1975) have been tried, but the effects of most of
these trials were insufficient. In this paper, we report the biochemical effect of
blood exchange transfusion and erythrocyte transfusion in vivo as well as in vitro.

**CASE REPORT**

The details of the clinical findings of this patient have been reported (Mizutani et al.
1983).

The patient is a 5-year-7-month-old boy, the first of two children of healthy consan-
guineous parents. Physical examination at birth was completely normal. Mental and
physical development for the first 3 years was almost normal. He could walk unassisted at
13 months of age, but presumably walked on tip toe. Furthermore, there was an aversion to
protein-rich foods.

At 4 years and 2 months of age, he was found to have abnormal liver function tests, and
at 4 years and 7 months of age, he was found to have hyperammonemia.

On admission, his neurological examination revealed severe spasticity of the lower
extremities, hyperactive deep tendon reflexes, positive ankle and patellar clonus, and
Babinski’ reflexes.

Laboratory examination revealed abnormal liver function tests (GOT: 562 IU/liter,
GPT: 930 IU/liter). Plasma urea levels were low (2–8 mg/100 ml), and plasma ammonia
levels were markedly elevated (208–475 μg/100 ml). Urinary excretion of urea nitrogen
and uric acid were extremely low. Urinary excretion of hippuric and orotic acid markedly
increased.

Amino acid analysis revealed markedly elevated plasma and CSF concentration and
increased urinary excretion of arginine. Based on the complete deficiency of erythrocyte
arginase activity, the diagnosis of hyperargininemia was confirmed. Erythrocyte arginase
activities of his parents, younger brother, uncle and grandfather were less than a half of the
normal controls, and they were considered to be heterozygotes of hyperargininemia (Table
1).

Some trials of treatment such as restriction of protein intake (1.0–1.5 g/kg/day), oral
administration of an essential amino acid mixture, sodium benzoate (250 mg/kg/day) and
phenylacetic acid (100 mg/kg/day) were performed. With these treatments, his plasma
ammonia levels were controlled, and plasma and CSF concentrations of arginine showed a
slight decrease, but remained far above the normal range. Clinically, spasticity of the lower

<table>
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<tr>
<th>Table 1. The arginase activity of erythrocytes in his family</th>
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<tr>
<td>Erythrocyte arginase activity (μmole urea/hr/g hemoglobin)</td>
</tr>
<tr>
<td>Patient. 0</td>
</tr>
<tr>
<td>Younger brother 303</td>
</tr>
<tr>
<td>Mother 363</td>
</tr>
<tr>
<td>Father 304</td>
</tr>
<tr>
<td>Uncle 526</td>
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<tr>
<td>Grand father 594</td>
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<tr>
<td>Controls (n = 7) 971.1±93.0*</td>
</tr>
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Means ± s.d.
and upper extremities gradually increased, and mental deterioration appeared. When marked hyperammonemia and hyperargininemia appeared, we performed blood exchange transfusion one time in July 1983, and erythrocyte transfusion five times in 1984–1986.

**METHODS**

Amino acids in plasma, urine and CSF were determined with an automatic amino acid analyzer Hitachi 034. Plasma ammonia levels were measured by an enzymatic methods (Mondzac et al. 1965) or using a commercial kit “Amitest” (Tada et al. 1979).

Erythrocyte arginase activity was determined by a minor modification of the method of Shih et al. (1972).

Exchange transfusion of 200 ml/kg of fresh blood was performed.

Erythrocyte exchange transfusion was performed according to the following method. Erythrocytes of the patient were replaced with the aid of Hemonetics Pex blood cell separator. Normal packed erythrocytes were substituted for the erythrocytes of the patient, and mixed with the patient’s plasma through the cell separator, then returned to the patient. Erythrocyte replacement was performed at a rate of 10–25 ml/min, and total replaced volume of erythrocyte were 1170 ml. Erythrocyte arginase activity, plasma arginine and ornithine concentrations, plasma ammonia levels and plasma urea were measured during and after the exchange transfusion of fresh blood or erythrocytes.

Furthermore, to confirm the effect of exchanged erythrocytes on the reduction of arginine, we mixed erythrocyte and arginine in the medium RPMI 1640. The concentration of arginine in the medium was 900–1000 nmole/ml, and erythrocyte counts of the patient, mother and normal controls in the medium were 100–150 × 10⁴/mm³, respectively. Mixed medium was rest in the CO₂ incubator for 3 days, and amino acids of the medium were analyzed everyday.

**RESULTS**

Plasma arginine concentrations of the patient reduced gradually during the exchange transfusion of fresh blood and erythrocytes only, and normalized within 2 days. On the other hand, plasma concentrations of ornithine and urea rose corresponding to the decrease of plasma arginine concentrations. Plasma ammonia levels reduced within normal limit in 2 days (Figs. 1 and 2).

Erythrocyte arginase activity has been normalized when exchange transfusion of about 1000 ml of fresh blood and transfusion of about 400 ml of erythrocytes were performed, and at the end of exchange transfusion, the arginase activity of erythrocytes was 2 times the normal range (Figs. 1 and 2).

Exchanged erythrocyte arginase activity was normal for about one month, but thereafter, the activity decreased gradually, and 3 months after the exchange transfusion, erythrocyte arginase activity completely disappeared. Corresponding to the decrease of erythrocyte arginase activity, plasma concentrations of arginine and ammonia elevated gradually (Figs. 1 and 2). On the other hand, CSF concentrations of arginine unchanged in spite of exchange transfusion of blood or erythrocyte (Fig. 1).

Up to date, the patient has been treated with low-protein diet, oral administration of an essential amino acid mixture and sodium benzoate (250 mg/kg/day).
His ammonia levels were elevated sometimes to 200-350 µg/100 ml, and plasma concentrations of arginine ranged from 200 to 400 nmole/ml (Fig. 3).

In vitro experimentation, arginine concentrations of the medium did not diminish when arginine was mixed with erythrocytes of the patient, and ornithine concentrations of the medium were also unchanged. On the other hand, when arginine was mixed with erythrocytes of the mother or normal controls, arginine
concentrations of the medium decreased, and ornithine concentrations of the medium increased in accordance with the decrease of arginine concentrations (Fig. 4).

In calculation, it was presumed that arginase of 10⁷ erythrocytes of the mother or normal controls can metabolize 4-5 n mole of arginine per day.
DISCUSSION

Hyperargininemia is a rare disorder of the urea cycle. Clinical symptoms of this disorder such as severe spasticity of the four extremities, mental and physical deterioration are different from the other enzymopathies of the urea cycle. These symptoms are not explained by hyperammonemia only. It is suspected that the other factors such as elevated plasma arginine and its metabolites may underlie the occurrence of the neurological symptoms. Therefore, the treatment of the patient with hyperargininemia must aim to control hyperargininemia as well as hyperammonemia.

Our patient was treated with low-protein diet, oral administration of an essential amino acid mixture, and sodium benzoate or phenylacetic acid, the stimulator of the alternative pathways of the urea cycle. With these treatments, hyperammonemia was controlled, but plasma and CSF concentrations of arginine were not well controlled.

Recently, Sakiyama et al. (1984) reported that blood exchange transfusion and erythrocyte transfusion to a patient with hyperargininemia were effective for biochemical and clinical findings. We confirmed the effect of blood exchange transfusion and erythrocyte transfusion not only in vivo but also in vitro. It is considered that erythrocyte and liver arginase are kinetically the same (Berüter et al. 1978). The complete absence of erythrocyte arginase activity suggests the complete absence of liver arginase activity. It is inconceivable that exchanged erythrocyte arginase was transmitted into the liver cells, and affected the urea cycle. Furthermore, erythrocyte arginase activity is less than 2% of the total activity. Our results of in vitro experiments indicate that exchanged erythrocyte arginase acted on arginine directly, which was transferred into erythrocytes and metabolized arginine to ornithine and urea. Apart from the fact that exchanged erythrocyte arginase can decrease plasma arginine levels, it is doubtful that exchanged erythrocyte arginase affects arginine in the cells. This doubt is supported by the fact that CSF concentrations of arginine were unchanged in spite of blood exchange transfusion or erythrocyte transfusion. The details of these mechanism remain unexplained, because we could not measure arginine concentrations in the tissues or cells. On the other hand, it is true that plasma arginine is metabolized in erythrocytes which has no urea cycle. So, erythrocyte transfusion is considered to be effective for the purpose of decreasing not only hyperammonemia but also hyperargininemia.

It is very difficult to treat the patient with hyperargininemia. Some trials of treatment have been insufficient. But our experience of exchange transfusion and erythrocyte transfusion, and the results of our in vitro experiments suggest that erythrocyte exchange is effective to decrease plasma arginine concentrations and plasma ammonia levels. Repeated erythrocyte exchange transfusion in the early stage of the disease for example every 3 months, may be effective for the control
of clinical and biochemical findings.

We have been planning more effective enzyme replacement therapy, and performing the purification of the erythrocyte arginase by gel filtration method according to Berüter et al. (1978) using DEAE-sephadex chromatography and Sephacryl S-300.

Acknowledgments

This study was partly supported by Grant No. 84–11 from National Center for Nervous, Mental and Muscular Disorders (NCNMMD) of the Ministry of Health and Welfare, Japan.

References