Urocanic Aciduria: A Defect in the Urocanase Activity in the Liver of a Mentally Retarded

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YOSHIDA, T., TADA, K., HONDA, Y. and ARAKAWA, Ts. *Urocanic Aciduria: A Defect in the Urocanase Activity in the Liver of a Mentally Retarded.* Tohoku J. exp. Med., 1971, 104 (4), 305-312 — A new entity of inborn error of histidine metabolism was described. This was characterized by 1) urocanic aciduria, which was exaggerated after an oral dose of L-histidine, 2) a delayed clearance curve of serum histidine levels after an oral dose of L-histidine, 3) urinary excretion of no detectable amounts of formiminoglutamic acid after an oral load with L-histidine or after an intravenous injection of urocanate, 4) a marked decrease in the urocanase activity of the liver, and 5) mental retardation of probably early onset. ——— urocanic aciduria; urocanase; mental retardation; formiminoglutamic acid; histidine metabolism

As inborn errors of metabolism of histidine, histidinemia (Auerbach *et al.* 1962) and formiminotransferase deficiency (Arakawa *et al.* 1965) have been reported up to date. The present report deals with a new inborn error of the histidine metabolism, urocanic aciduria, which is characterized by mental retardation, urocanic aciduria and a defective activity of urocanase of the liver (cf. Fig. 1).

Fig. 1. Metabolic pathways of histidine and biochemical lesions in histidinemia (I), formiminotransferase deficiency syndrome (II) and urocanic aciduria (III).

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REPORT OF THE CASE

T.O., a 16-year-old boy. He was born to healthy and non-consanguineous parents after uneventful pregnancy and delivery, but weighing 2,600 g. His developmental history was not clear, but his physical development seemed to be slightly retarded: sitting up at the age of 11 months, walking alone at the age of 18 months. The patient was suffered from tuberculous meningitis at the age of 19 months, and treated at a local hospital for 10 months and recovered with a sequence of right sided hemiplegia and convulsions. His mother died of pulmonary tuberculosis when the patient was 9 years old.

Since 12 years of age, he has been admitted into an Institution for mentally retarded, Nishitaga, Sendai. On his admission, physical examination revealed a fairly well nourished boy with apathetic countenance. He could not walk alone nor speak any meaningful words. Body weight was 27.4 kg (normal, 33.0 kg), the head circumference being 52 cm (normal 52.8 cm). The lungs and heart were clear on auscultation. The liver and spleen were not palpable. Hemiplegia and contracture were noticed on the right side.

Laboratory findings: Routine urinalysis gave no abnormality. Serum electrolytes, lipids and protein were found to be normal. Peripheral blood picture was that: red cell count was 415×10^4 per mm^3 , hemoglobin 13.4 g per 100 ml, hematocrit 41%, and white cell count 9,250 per mm^3 . Liver function tests were negative for CCFT, 8.1 units for ZTT, 1.3 units for TTT, 7 units for GOT, 4 units for GPT, and 22.5 KA units for alkaline phosphatase.

Serum urea-nitrogen was 7.8 mg/100 ml, fasting blood glucose 82 mg/100 ml, uric acid 5.2 mg/100 ml, serum pyruvate 0.93 mg/100 ml, serum lactate 8.6 mg/100 ml and serum folic acid (L. casei) 3.8 m γ /ml.

Urinary spot test for metachromasia showed a negative result.

TABLE 1. Serum amino acid levels ($\mu\text{moles/l}$) of the patient (T.O.)

Amino acids	Patient (T.O.)	Normal range (Tada, 1964)
Threonine	49	48-134
Serine	66	216-699
Glutamine	320	
Proline	33	116-181
Glutamic acid	39	62-289
Glycine	149	164-400
Alanine	118	168-483
Valine	117	80-310
Methionine	14	8-25
Isoleucine	19	29-90
Leucine	42	73-164
Tyrosine	19	37-97
Phenylalanine	15	28-125
Ornithine	27	17-65
Lysine	146	50-148
Histidine	56	38-70
Arginine	16	Trace-89

TABLE 2. *Urinary amino acid levels (μ moles/mg of creatinine) of the patient (T.O.)*

Amino acids	Patient (T.O.)	Normal range*
Aspartic acid	0.04	Trace-0.10
Threonine	0.23	0.17-0.68
Serine	0.58	} 0.53-2.94
Glutamine	0.83	
Glutamic acid	0.17	0.10-0.40
Glycine	2.87	1.39-3.76
Alanine	0.51	0.51-1.42
α -Aminobutyric acid	0.05	Trace
Valine	0.25	Trace-0.28
Cystine	0.19	Trace
Methionine	0.06	Trace-0.10
Isoleucine	0.09	0.07-0.21
Leucine	0.07	0.09-0.37
Tyrosine	Trace	0.07-0.61
Phenylalanine	0.02	0.07-0.51
β AIB	0.59	0 -1.53
Ornithine	0.02	Trace-0.95
Lysine	0.72	0.07-0.56
Histidine	0.88	0.52-2.86
Arginine	0.02	Trace-0.10

* Urinary amino acid patterns of normal children, assayed by an automatic amino acid analyzer in our laboratory (Tada 1964).

The urinary excretion of xanthurenic acid after an oral load with tryptophan (100 mg per kg of body weight) was found to be 2.8μ moles/kg, being within normal limits.

Free amino acid patterns of the serum and urine were examined with an automatic amino acid analyzer, and were found to be in normal limits (cf. Tables 1 and 2).

Electroencephalography revealed a dysrhythmia. Pneumoencephalography showed an asymmetrical dilatation of cerebral ventricles with the Evans' ratio of 0.50 (cf. Fig. 2 a & b).

Histological examination of the liver biopsied showed no abnormality.

Chromosome analysis revealed no abnormality in both number and configuration.

Urinary excretion of uric acid was found to be within normal limits.

SPECIAL STUDIES

Serum histidine levels after an oral load with L-histidine: 100 mg of L-histidine monohydrochloride per kg of body weight were given orally and serum histidine levels were estimated by an automatic amino acid analyzer. The results showed that the serum histidine levels raised markedly and remained to be higher in the patient than in controls (cf. Fig. 3).

Urinary excretion of imidazole compounds and organic acids after an oral load with L-histidine: Urine specimens were collected for 24 hours after an oral dose

Fig. 2. Pneumoencephalograms of our patient, showing enlargement of the left lateral ventricle and calcified foci.

Fig. 3. Histidine tolerance curves in the patient and controls. Histidine monohydrochloride (100 mg per kg of body weight) was given orally.

of L-histidine monohydrochloride, 300 mg per kg of body weight, and examined an imidazole compounds (Auerbach *et al.* 1962, Humbel 1970) and organic acids (Whereat *et al.* 1968). Large amounts of urocanic acid and considerable amounts of both imidazole pyruvic acid and imidazole propionic acid were detected in the chromatograms of the urine specimens collected after the oral histidine load, which gave a positive reaction for the ferric chloride test.

Neither imidazole pyruvic acid nor imidazole propionic acid was detected by the chromatography in urine specimens from the patient before the oral histidine load and in urine samples from controls with or without the oral histidine dose.

Urocanic acid in the urine specimens after the oral histidine load was estimated semiquantitatively according to Auerbach's method (Auerbach *et al.* 1962) and it was found that average figure of urinary urocanic acid was 5.49 mmoles per day in the patient, while under 0.1 mmoles per day in controls (cf. Fig. 4).

Histidase activity of the skin: About 10 mg of stratum corneum were obtained from the finger tip skin and used for the assay of histidase by Zannoi and La Du's method (1963). The results showed that there was no difference of the histidase activity between the patient and controls (cf. Table 3).

Urocanase activity of the liver: Liver specimens, 40–50 mg in wet weight, were obtained by a needle biopsy from the patient and two controls without hepatic involvement. The urocanase activity was estimated according to Tabor and Mehler's method (1955) with a slight modification as follows: the reaction system

Fig. 4. Urinary excretion of urocanic acid (mmoles per day) after a histidine loading (0.3 g of histidine monohydrochloride per kg of body weight) in the patient and controls.

TABLE 3. *The histidase (histidine α -deaminase) activity in stratum corneum*

		μ Moles of urocanic acid formed, hr/g wet weight
Our patient (T.O.) 16 y. 0 m.		9.43
Controls		
1.	17 y. 2 m.	2.65
2.	14 y. 6 m.	2.57
3.	14 y. 0 m.	4.69
4.	8 y. 0 m.	5.75
5.	17 y. 9 m.	11.71

consisted of 1.5 ml of 0.04 M potassium phosphate buffer (pH 7.3) containing 20 mg of liver specimens, and 1 mM of urocanic acid. The incubation was done at 37°C for 30 min., and terminated by an addition of 1.5 ml of 5% perchloric acid. The mixture was well mixed, then centrifuged. The supernatant was used for an estimation of the optical density at 277 m μ . The precipitate was used for the protein estimation (Lowry *et al.* 1951).

The blank test was carried out in the same way except that urocanic acid was added after the completion of the incubation.

The urocanase activity was expressed in terms of μ moles of urocanic acid which were decomposed during the incubation for 30 minutes with the liver specimens.

The results showed that a marked decrease in the urocanase activity of the liver was found in the patient (cf. Table 4).

TABLE 4. *The urocanase activity in the liver from the patient and controls. The urocanase activity was expressed as μ moles of urocanic acid which were decomposed during the incubation for 30 min with liver specimens*

	μ Moles per g of wet weight	μ Moles per mg of protein
Our patient (T.O.) 16 y. 0 m.	0.51	0.0059
Controls		
1. 7 y. 2 m.	2.81	0.0427
2. 17 y. 7 m.	4.32	0.0658

Urinary excretion of formiminoglutamic acid (FIGLU) after an oral dose of histidine monohydrochloride or after an intravenous injection of urocanate: Formiminoglutamic acid (FIGLU) was estimated by Tabor and Wyngarden's method (1959) on urine specimens collected for 8 hours after an oral dose of L-histidine monohydrochloride, 0.33 g per kg of body weight. The results revealed that no detectable amounts of FIGLU was found in the patient urine (cf. Table 5).

TABLE 5. *Urinary excretion of FLGLU for 8 hours after an oral histidine load (0.33 g of L-histidine monohydrochloride per kg of body weight)*

	μ Moles per 8 hours
Our patient (T.O.) 16 y. 0 m.	0
Controls	
1. 19 y. 8 m.	26.5
2. 17 y. 11 m.	40.6
3. 19 y. 6 m.	32.9
4. 10 y. 1 m.	38.5

One gram of urocanic acid dihydrate was dissolved in 33 ml of distilled water and the solution was added with 1N NaOH solution to adjust the pH to 7.4. The mixture was autoclaved for 15 min., and used for an intravenous injection, in a dose of 33 mg of urocanate per kg of body weight.

The urine specimens were collected for 8 hours after the intravenous injection of the urocanate solution and assayed for FIGLU. The results showed that no detectable amount of FIGLU was found in the patient urine (cf. Table 6).

TABLE 6. *Urinary excretion of FLGLU for 8 hours after an intravenous injection of urocanate (33 mg per kg of body weight)*

	μ Moles for 8 hours
Our patient (T.O.) 16 y. 1 m.	0
Control 10 y. 5 m.	10.6

COMMENT

Our clinical and biochemical investigation upon a 16-year-old boy revealed a peculiar disorder which has not been reported in the literature up to date, which was characterized by 1) urocanic aciduria, which was exaggerated after an oral dose of L-histidine, 2) a delayed clearance curve of serum histidine levels after an oral dose of L-histidine, 3) urinary excretion of no detectable amounts of FIGLU after an oral dose of L-histidine or after an intravenous injection of urocanate, 4) a marked decrease in the urocanase activity of the liver, and 5) mental retardation of probably early onset.

Succinic acid was said to have an inhibitory effect upon urocanase (Hug *et al.* 1968), but urinary succinate excretion was found to be not increased in our patient.

Mental retardation of our patient seemed to be of early onset, but it was not excluded that the brain lesion due to tuberculous meningitis from which he had suffered at the age of 19 months might participate to some extent in mental retardation of our patient.

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