Studies on α -Ketoglutaric Aciduria in Type I Glycogenosis

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KODAMA, H., OKADA, S., INUI, K., YUTAKA, T. and YABUUCHI, H. *Studies on α -Ketoglutaric Aciduria in Type I Glycogenosis*. Tohoku J. exp. Med., 1980, 131 (4), 347-353 — Urinary excretion of the organic acids in patients with type I and III glycogenosis was investigated. In all patients with type I glycogenosis, urinary α -ketoglutarate concentration was about 10 times the normal value. α -Ketoglutaric aciduria was not improved by the acute or prolonged administration of a large dose of cofactors for pyruvate- and α -ketoglutarate dehydrogenase complex. On the other hand, the level of α -ketoglutarate in the urine from type I patients decreased in conjunction with the decrease of plasma lactate and pyruvate concentration after repeated oral glucose loading. Oral citrate loading brought an increased excretion of α -ketoglutarate in type I glycogenosis. It is possible that α -ketoglutarate dehydrogenase is the rate-limiting step in tricarboxylic acid cycle and in patients with glycogenosis type I, the excessive excretion of α -ketoglutarate may be caused by the limited activity of α -ketoglutarate dehydrogenase with excessive substrate. ——— glycogenosis; α -ketoglutaric aciduria; α -ketoglutarate dehydrogenase; citrate loading test

Type I glycogenosis, inherited glucose-6-phosphatase deficiency, is characterized by a number of abnormal biochemical findings including hypoglycemia, hyperlactic acidemia, hyperuricemia and hypertriglyceridemia (Howell et al. 1962; Cornblath and Schwartz 1976; Howell 1978). Study on tricarboxylic acid cycle, which is directly associated with glycogenolysis, is of particular interest (Dosman et al. 1974; Sadeghi-Nejad et al. 1974; Fernandes and Blom 1976). Chalmers and associates (1978) described abnormal urinary excretion of α -ketoglutarate in two patients with type I glycogenosis. But the reason for the excessive excretion of α -ketoglutarate remains uncertain. We examined urinary organic acids in patients with hepatic glycogenosis in various conditions. Our results may suggest that the excessive excretion of α -ketoglutarate in type I glycogenosis is caused at least in part by the overflow of pyruvate.

MATERIALS AND METHODS

Subjects

Seven cases of type I glycogenosis, 4 boys and 3 girls, aged from 2 to 14, and 2

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cases of type III glycogenosis, 10 and 12 year-old boys, were examined. Diagnosis was made on the basis of clinical and laboratory findings (Fernandes et al. 1974). Nine age-matched healthy children, 5 boys and 4 girls, were examined for obtaining the control values of the urinary excretion of organic acids. All tests were performed with informed consent.

Identification and quantitative analysis of urinary organic acids

Filtered fresh urine (0.33 ml with isovaleric acid 67 μ g as internal standard) was applied to a column (3 $\times$ 1000 mm SA-10A anion-exchange resin, Mitsubishi-Kasei, Tokyo, Japan) and eluted with 0.2 N HCl. Fraction which contained main organic acid was freeze-dried and trimethylsilylated with BSTFA (Pierce Co., Rockford Ill., U.S.A.). The individual organic acids were identified using GC-MS 9000 mass spectrometer (Shimadzu, Kyoto, Japan) with the conventional method. Each organic acid was detected by carboxylic acid analyser (Seishin Pharmaceutical Co., Tokyo, Japan), employed with liquid chromatography and a specific detection method for carboxylic acids (Nakajima and Ozawa 1976). Uric acid was not detectable by the analyser. Because lactate and 3-hydroxybutyrate were poorly separated, their measurement was specifically carried out by the enzymatic methods (Williamson et al. 1962; Haworth et al. 1976). Urinary content of organic acids was expressed as μ g/mg creatinine. Creatinine was measured by the Folin-Wu method (Bonsnes and Tauskey 1945).

Repeated oral glucose loading test

Studies were performed on 4 patients with type I glycogenosis. Glucose (1.75 g/kg) was orally given three time every 2 hr. Blood and urine samples were taken as indicated in Fig. 2.

Administration of cofactors of pyruvate- and α -ketoglutarate dehydrogenase complex

After an overnight fasting, 75 mg of lipoate, 150 mg of thiamine, 20 mg of nicotinic acid and 300 mg of pantothenic acid in 100 ml of isotonic saline were infused for 2 hr in 4 patients with type I glycogenosis. Blood and urine samples were examined before and 4 hr after the infusion.

For the prolonged administration of cofactors, thiamine (300 mg/day) and lipoate (100 mg/day) were orally given to the patients with type I glycogenosis for one month. Blood and urine samples were examined once a week.

Oral citrate loading test

After an overnight fasting, citrate (150 mg/kg) was administered per os to control subjects and 3 patients with type I glycogenosis in hypoglycemic and normoglycemic conditions. Urine samples were collected every hour until 3 hr after the loading.

RESULTS

Contents of urinary organic acids

Fig. 1 shows carboxylic acid analyser chromatograms of typical normal urine (upper panel) and of type I glycogenosis (lower panel). As shown in Table 1, excretion of excessive amount of lactate and α -ketoglutarate was observed in all urine samples from type I glycogenosis. These findings were consistently found in the various conditions of the patient. On the other hand, 3-hydroxybutyrate was increased in the urine from type III glycogenosis patients.

Fig. 1. Chromatogram of carboxylic acids present in urine from a healthy control (upper) and a patient with type I glycogenosis (lower). 1, amino acids; 2, glycolate+3-hydroxybutyrate+lactate; 3, pyruvate; 4, oxaloacetate+malate; 5, propionate; 6, citrate; 7, succinate; 8, isocitrate; 9, α -ketoglutarate; 10, adipate; 11, isovalerate (internal standard).

TABLE 1. Organic acid concentration in the urine from patients with glycogenosis and normal subjects

	Type I glycogenosis (n=7)	Type III glycogenosis (n=2)	Normal subjects (n=9)
Lactate	55 $\pm$ 36	14	29 $\pm$ 15
3-OH-butyrate	56 $\pm$ 20	628	33 $\pm$ 27
Pyruvate	32 $\pm$ 14	7	27 $\pm$ 23
Citrate	99 $\pm$ 95	65	294 $\pm$ 181
Succinate	14 $\pm$ 12	trace	5 $\pm$ 4
Isocitrate	477 $\pm$ 179	294	296 $\pm$ 132
α -Ketoglutarate	642 $\pm$ 292	28	54 $\pm$ 32
Adipate	13 $\pm$ 13	20	12 $\pm$ 9

Values are expressed as $\mu\text{g}/\text{mg}$ creatinine (mean $\pm$ s.d.).

Repeated oral glucose loading test

During oral glucose loading to the patients with type I glycogenosis plasma, glucose level was maintained within the normal limit and plasma lactate and pyruvate gradually decreased towards normal level (Fig. 2a). Urinary lactate and α -ketoglutarate were also normalized (Fig. 2b), while other urinary organic acids were unchanged.

Fig. 2. (a): Glucose (●-●), lactate (○-○) and, pyruvate (Δ-Δ) in plasma. (b): α -Ketoglutarate concentration in urine (●-●) during repeated oral glucose loading test in four patients with type I glycogenosis. Each bar expresses mean value $\pm$ S.D.

Fig. 3. Urinary α -ketoglutarate concentration after oral citrate loading (150 mg/kg) in patients with type I glycogenosis during hypoglycemic state (●-●) and normoglycemic state (○-○). In control subjects (shaded area) no increment of urinary α -ketoglutarate was observed.

Loading test of cofactors of pyruvate dehydrogenase complex

By the acute or prolonged administration of lipoic acid, thiamine, nicotinic acid and pantothenic acid to 4 patients with type I glycogenosis, glucose, lactate and

pyruvate levels in the plasma and lactate and α -ketoglutarate levels in the urine were not improved.

Oral citrate loading test

By oral citrate loading urinary citrate excretion was increased and reached the maximum in 1 hr. Urinary excretion of α -ketoglutarate in normal subjects was steady, whereas it was clearly increased in type I glycogenosis with the maximum increment of 788 $\mu\text{g}/\text{mg}$ creatinine at fasting hypoglycemic state and 160 $\mu\text{g}/\text{mg}$ creatinine at normoglycemic state after glucose load. Other organic acids were not changed. (Fig. 3).

DISCUSSION

A carboxylic acid analyser we employed was a simple and efficient tool for the quantitative analysis of carboxylic acids in biological samples although it had a certain limit as stated in the method section. The normal urinary organic acid pattern we obtained was in good agreement with the data analyzed by means of gas liquid chromatography (Björkman et al. 1976; Harrington et al. 1977). Increased amount of urinary 3-hydroxybutyrate was found in type III glycogenosis. This was reported by Fernandes and Pikaar (1972), and Binkiewicz and Senior (1973), and considered to be related to the existence of severe fasting ketosis in type III glycogenosis.

The occurrence of large amounts of α -ketoglutarate in urine of 2 cases of type I glycogenosis was observed by Chalmers et al. (1978) using conventional gas liquid chromatography. Our results obtained with the different method from theirs, carboxylic acid analyser, also support their findings. The reason for α -ketoglutaric aciduria has not been well understood. Chalmers et al. (1978) hypothesized that the lack of cofactors for α -ketoglutarate dehydrogenase might lead to the accumulation of α -ketoglutarate since the same cofactors are utilized for the metabolism of accumulated pyruvate. There are some reports that several diseases with lactic acidosis were improved by the cofactors of dehydrogenases. Clayton et al. (1967) and Hommes et al. (1968) reported that the clinical and biochemical improvements were observed in the patients with Leigh syndrome with large amount of lipoate. In other inborn errors of organic acid metabolism such as methylmalonic aciduria, thiamine responsive megaloblastic anemia, pyridoxine dependent homocystinuria and cystathioninuria, large doses of the cofactors involved in the reaction have resulted in improvement (Bejsovec et al. 1967; Barber and Spaeth 1969; Rogers et al. 1969). Thus, it is assumed that large doses of enzyme cofactors are effective if it is deficient. However, we found that the acute or prolonged administration of large amount of dehydrogenase cofactors to type I glycogenosis patients revealed no effects on the levels of lactate, pyruvate and α -ketoglutarate in blood and urine. Therefore, the occurrence of α -ketoglutaric aciduria associated with type I glycogenosis is not explained by the deficiency of cofactors.

As lactate and pyruvate in plasma were gradually decreased towards normal with oral administration of glucose to the patients with type I glycogenosis, urinary excretion of α -ketoglutarate was decreased (Fig. 2). This may indicate the parallel relationship between lactate (and pyruvate) and α -ketoglutarate. As can be seen in Fig. 3, the oral administration of citrate produced an increased urinary excretion of α -ketoglutarate in type I glycogenosis in 1 or 2 hr even at normoglycemic state. Since plasma lactate levels in patients with type I glycogenosis (30–40 mg/100 ml) was still above the normal limit when citrate was loaded at normoglycemic state, it is likely that the abnormal accumulation of lactate in the plasma results in the urinary excretion of α -ketoglutarate. Accordingly, the metabolic capacity or threshold of α -ketoglutarate dehydrogenase seems very limited.

We consider that the oral citrate loading test is very safe and easy way to evaluate the flux through the tricarboxylic acid (TCA) cycle without direct addition of pyruvate or lactate. This observation possibly indicates that α -ketoglutarate tends to accumulate among TCA components, and suggests that α -ketoglutarate dehydrogenase is the rate limiting step in TCA cycle. Alternatively, it may also be probable that α -ketoglutarate was produced, at least in part, by increased activity of transaminases in the condition which favors gluconeogenesis. This hypothesis is supported by the fact that urinary α -ketoglutarate excretion increased when blood sugar level is low and blood pyruvate level is high (Fig. 3). However, the direct analysis of liver enzymes which participate in gluconeogenesis and TCA cycle needs to be studied to clarify the basic mechanism of α -ketoglutaric aciduria.

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