

A study on α -ketoadipic aciduria by gas chromatographic-mass spectrometry

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INTRODUCTION

α -ketoadipate (α -KA), an intermediate in the catabolism of L-lysine, hydroxylysine, and L-tryptophan, undergoes oxidative decarboxylation to form glutaryl-CoA and then dehydrogenates to form crotonyl-CoA, the latter undergoes further degradation and enters in TCA cycle, as shown in Figure 1. α -ketoadipic aciduria (Mckusick 245130) is a rare inborn error in the metabolism of α -KA to glutaryl-CoA and is characterized by the increased excretion of α -KA, α -amino adipate (α -AA) and α -hydroxyadipate (α -HAA). Since Przyrembel *et al* first described it in 1975^[1], only 13 cases of α -ketoadipic aciduria have been reported over the past 25 years, including 7 symptomatic, and 6 asymptomatic even in the symptomatic siblings with α -ketoadipic aciduria^[1-10]. The clinical manifestations of this metabolic disorder showed heterogeneity. However, no follow-up study on either symptomatic or asymptomatic case has been available so far. We followed up two cases of α -ketoadipic aciduria clinically and metabolically using organic solvent extraction, new urease-pretreatment and gas chromatography-mass spectrometry (GC/MS).

MATERIALS AND METHODS

Subjects

Two male children were studied. Pregnancy and

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delivery were uneventful. Their clinical data were collected after diagnosis of α -ketoadipic aciduria.

Reagents

Creatinine-d₃ (methyl-d₃) was purchased from Nippon Sanso Ltd., Tokyo, Japan, and 2-amino adipate, 2-oxoadipate, 2, 2-dimethylsuccinate (DMS) and urease type C₃ were obtained from Sigma Chemical Co. St. Louis, MO, USA. All chemicals were of analytical grade.

Quantitation of α -KA, α -HAA, α -AA and glutarate

Twenty nmol of DMS was used as the analyte of internal standard for preparing standard curves of α -KA and glutarate, 50nmol of DMS was also chosen as the analyte of internal standard for making standard curves of α -AA and α -HAA. Comparing with peak area of internal standard, quantitation of urinary α -KA, α -HAA, α -AA and glutarate was performed using standard curves.

Sample preparation

Urine samples from two cases were collected at different detecting time. All samples were frozen at -20°C until analysis. Creatinine was determined and urine volumes equivalent to 1 μ mol creatinine were prepared prior to GC/MS analysis by organic solvent extraction^[11] or urease-pretreatment^[12]

Measurement

Samples were analyzed using GC/MS-computer systems of QP-5000 (Shimadzu, Japan) and HP-6890 (Hewlett Packard, USA) as well as the new diagnostic method described previously^[12,14,15]. The concentrations of these compounds were normalized to urinary creatinine and expressed as mmol per mol creatinine.

Loading tests of tryptophan and lysine (each was 100mg/kg of body weight) were made in Case 1 at the age of 1 year and 4 months.

RESULTS

The two cases were followed up clinically for a period of 15 years (8 months-15 years in Case 1) and 5 years (1 day - 5 years in Case 2), respectively. Case 1 was slightly delayed in growth after birth and CT on his head showed mild cortical atrophic change at the age of 9 months. At 1 year and 4 months, the analysis of first urine sample

revealed high levels of α -KA and α -AA using GC/MS. Loading test was performed. The values of α -KA, α -HAA, glutarate and α -AA reached 16-fold, 4-fold, 9-fold and 4.5-fold, respectively after lysine was taken orally. The concentrations of these four compounds increased 7-fold, 2-fold, 3-fold and 4-fold, respectively after tryptophan was also administered orally. Treatment was carried out with a low-lysine diet (70mg/kg daily) and a low-tryptophan diet (17mg/kg daily) after diagnosis. His CT was within normal range and his mild cortical atrophic change disappeared at the age of 4 years. The dietary treatment was discontinued due to normal development. His growth is normal at present. Case 2 developed cyanosis, clonic seizures and hypoglycemia 1 day after birth, then grew normally without low protein restriction.

Excretion of abnormal urinary metabolites profile compatible with α -ketoaciduria in two cases was continuously observed using organic solvent extraction, urease-pretreatment and GC/MS techniques (Table 1). Total ion current (TIC) chromatogram of TMS derivatives of organic acid in urine from Case 1 is shown in Figure 2. Ten

major compounds were identified. Peak 4, 6 and 7 represent glutarate, α -HAA and α -KA, respectively. Figure 3 shows the TIC of TMS derivatives of metabolites in urine from the same patient using urease-pretreatment and 9 major compounds were confirmed. Peak 2, 4, and 5 demonstrated glutarate, α -HAA and α -AA, respectively. Abnormal metabolites profile of α -KA, α -HAA, α -AA and glutarate were also detected in the urine of Case 2 with those techniques. Compared to the case with α -ketoaciduria, three abnormal peaks, including α -KA, α -HAA and glutarate after organic solvent extraction and α -AA, α -HAA and glutarate using urease-pretreatment, did not appear in the urine from healthy age-matched control at same retention time. The value of α -AA is much higher than that of α -KA, and glutarate was also detected and found increased in the urine of two cases. The concentrations of α -KA, α -HAA and glutarate ranged between 9-49 mmol/mol creatinine, 12-55 mmol/mol creatinine and 9-216 mmol/mol creatinine, respectively. The amounts of α -AA were 92-450 mmol/mol creatinine in analysis of urinary amino acid (Table 1).

Table 1 Urinary concentrations of metabolites

Cases	Detecting age	α -KA	α -AA	α -HAA	Glutarate	Total value of metabolites
1	1.4yrs	33(ND)	223(2-25)	28(ND)	29(0.04)	313
	4.2yrs	33(ND)	266(0-51)	17(ND)	24(0.04)	340
	15yrs	33(ND)	92(0-4)	21(ND)	53(ND)	199
2	13d	31(ND)	200(1-11)	12(ND)	9(0.03)	252
	29d	49(ND)	450(5-197)	17(ND)	54(0.03)	570
	5yrs	9(ND)	92(0-4)	55(ND)	216(ND)	372
Average		31.3	220.5	25	64.2	

Values are expressed as mmol/mol creatinine. ():control

α -AA: α -aminoacidipate, α -KA: α -ketoacidipate, α -HAA: α -hydroxyacidipate, ND: not detected; d: days, yrs: years

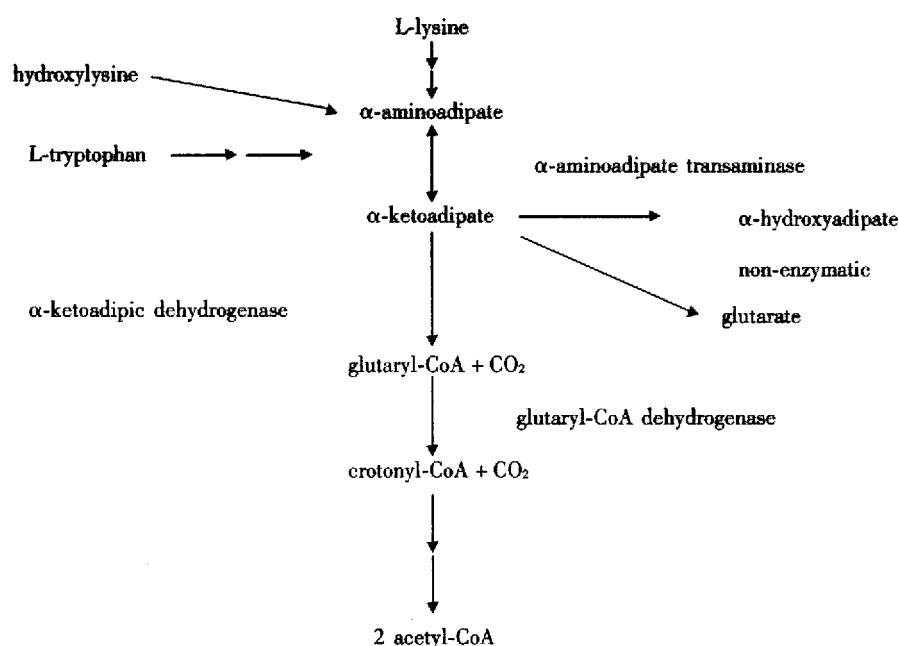


Figure 1 α -metabolic pathways of L-lysine, hydroxy-L-lysine and L-tryptophan leading to α -KA.

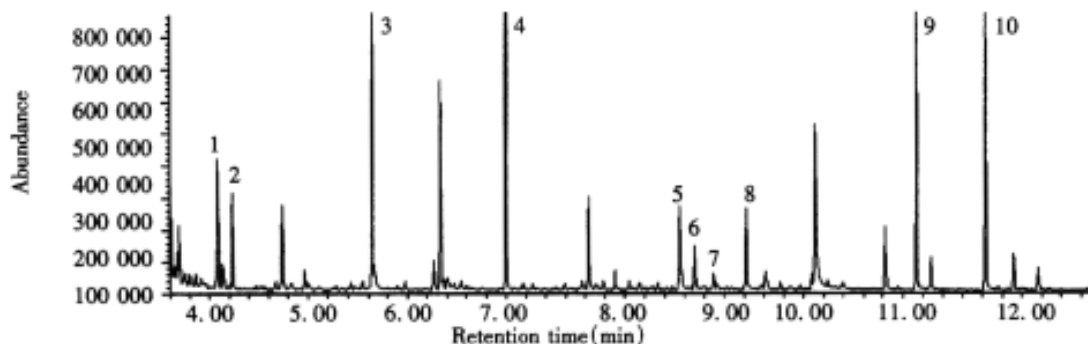


Figure 2 Total ion current (TIC) chromatogram of TMS derivatives of organic acids in urine from a patient with α -ketoadipic aciduria (case 1). Peak identities are: 1, lactate-2TMS; 2, glycolate-2TMS; 3, urea-2TMS; 4, glutarate-2TMS; 5, 4-hydroxyphenylacetate-2TMS; 6, α -hydroxyadipate-2TMS; 7, α -ketoadipate-3TMS; 8, cis-aconitate-3TMS; 9, 3-hydroxymyristate-2TMS (IS); 10, heptadecanoate-TMS (IS). IS: added as an internal standard.

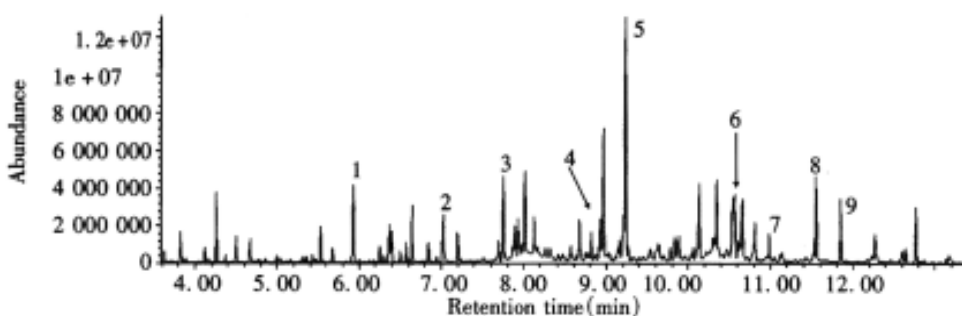


Figure 3 TIC chromatogram of TMS derivatives of metabolites in urine from a patient with α -ketoadipic aciduria (case 1). Peak identities are: 1, phosphate-3TMS; 2, glutarate-2TMS; 3, erythritol-4TMS; 4, α -hydroxyadipate-2TMS; 5, α -aminoadipate 3TMS; 6, histidine-3TMS; 7, gluconate-6TMS; 8, urate-4TMS; 9, heptadecanoate-TMS (IS). IS: added as an internal standard.

DISCUSSION

In analyzing organic acid, GC/MS has been proven by several institutions to be the most efficient method for chemical diagnosis of inborn error of metabolism.

In 1991, Shoemaker *et al* reported that urinary organic acids, amino acids and sugar could be analyzed simultaneously by GC/MS after excessive urea in the urine was degraded with urease and removed. This method, however, takes several hours, needs skillful technicians, and is not so practical. Therefore, Shoemaker's procedure was simplified for use in multiple sample analysis^[12]. As a result, rapid practical and simultaneous analysis of amino acids and organic acids became possible. Further improved-procedure was adopted, which is a stable isotope dilution method using not only d_3 -creatinine but also stable-isotope-labeled amino acids as internal standards. It only takes 1 hour for pretreatment of one sample and is a highly comprehensive diagnostic tool for a wide range of metabolic disorders^[13]. However, for detecting α -KA, the approach of organic solvent extraction is more sensitive than that of urease-pretreatment in this study.

α -ketoadipic aciduria was first described by Przyrembel *et al* in 1975 since then 7 cases have been reported^[1-3,6-9] using GC/MS or other analytical methods, and some subjects with α -ketoadipic aciduria were found by re-examination^[3]. In addition to symptomatic cases, 6

asymptomatic cases have also been reported^[2-5,10]. Up to now no follow-up study on this case with or without symptoms has been described by employing organic solvent extraction, urease-pretreatment and GC/MS techniques.

Almost all probands were identified when prominent spots of α -AA were noted on amino acid chromatography of urine, with subsequent investigations demonstrating α -aminoadipic acidemia and increased urine concentrations of α -KA and α -HAA. Different clinical symptoms were also described according to 7 affected individuals. The major manifestations include psychomotor retardation (5 cases)^[1,3,6-8], men talretardation (3 cases)^[1-3], hypotonia (3 cases)^[1,3,7] and seizures (2 cases)^[6,8]. The data indicate that the individuals with α -ketoadipic aciduria presented with nonspecific symptoms, but the central nervous system may be especially vulnerable. In this study, two cases showed symptoms at onset, including an 8-month-old boy with growth retardation (Case 1) and a boy with seizures (Case 2) as described above symptoms. In the two cases, the values of α -KA, α -AA, α -HAA and glutarate were always high at the different detecting time using present method. Meanwhile, loading tests of lysine and tryptophan were performed at 1 year and 4 months in Case 1. The concentrations of α -KA, α -AA, α -HAA and glutarate increased after lysine or tryptophan was taken orally. So the diagnosis of α -ketoadipic aciduria was confirmed. Three abnormal peaks were

still identified in recent detection as α -KA, α -HAA and glutarate after organic solvent extraction, and the three abnormal peaks of α -AA, α -HAA and glutarate also appeared by urease-pretreatment compared with healthy age-matched control.

In normal condition, α -KA and α -HAA could not be detected and only a trace of α -AA and glutarate exist in the urine, and when the patients are in the interim period, organic acid and amino acid in serum and urine are normal^[7]. In this study, abnormal excretion of these compounds was found and the value of α -AA was much higher than that of α -KA in the urine of two cases, but we still considered and diagnosed them as α -ketoadipic aciduria. Based on this α -amino adipic aciduria is caused by the deficiency of mitochondrial α -amino adipic acid aminotransferase, which leads to elevated urinary excretion of α -AA without α -KA.

A small amount of glutarate was also detected in urine in the two cases, but the level was much lower than that found in glutaric aciduria type I, and 3-hydroxyglutarate was not detected, so it is almost certainly because of spontaneous decarboxylation of α -KA or artifact^[6,7].

The accumulated metabolites suggest a block in α -ketoadipic acid dehydrogenase, and intact mutant fibroblasts are almost totally unable to oxidize α -amino[1-¹⁴C] adipate and α -keto[1-¹⁴C] adipate to ¹⁴CO₂, but a defect in α -ketoadipic acid dehydrogenase has not been demonstrated directly. If α -ketoadipic and α -ketoglutaric acid dehydrogenase are indeed the same, it is not clear a defect can produce so mild a phenotype. This may indicate that the two enzymes are different. Vallat *et al* recently reported that significant increment of α -AA occurred in the plasma and urine of 8 vigabatrin (VGB) treated children suggesting that VGB strongly inhibit α -amino adipic acid transaminase, α -ketoadipic acid dehydrogenase, or glutaryl-CoA dehydrogenase^[16,17]. However, more knowledge about underlying the mechanism is required.

Although protein restriction was reported to improve clinical symptoms like Case 1 and Case 2 in which no seizures occurred without treatment. Our results and results previously reported^[3,8], suggest that the clinical course of α -ketoadipic aciduria is incongruity and the condition is not apparently deleterious. Some authors investigated normal siblings of the patients, who excreted excessive amounts of α -KA and α -AA, and thought mental retardation may result from other causes^[2,3]. Others researchers consider that the analysis of mass spectrometry for this disorder would not be useful because α -ketoadipic aciduria is a nondeleterious inherited metabolic defect. But up to now, the relationship between the biochemical abnormality and clinical manifestations in α -ketoadipic aciduria

is still unclear.

Inheritance as an autosomal recessive trait is inferred from the pedigrees. There is no evidence so far that heterozygous carriers can be distinguished from control subjects. The incidence is not known. For these reasons, it is necessary to follow up this case with α -ketoadipic aciduria using GC/MS in order to clarify the mechanism of the clinical heterogeneity of this defect.

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