ESTABLISHING GAS CHROMATOGRAPHY - MASS SPECTROMETRY TO DIAGNOSE ORGANIC ACIDEMIAS IN THAILAND

Vorasuk Shotelersuk[1], Sumarlee Srivuthana[1], Suttipong Wacharasindhu[1], Valairat Dhamcharee[2], Somchit Jaruratanasirikul[3], Suthipong Pangkanon[4], Verachai Kaewpaluek[5] and Suphab Aroonparkmongkol[1]

[1]Unit of Endocrinology, Genetics and Metabolism, Department of Pediatrics; [2]Department of Anatomy, Chulalongkorn University and Hospital, Bangkok 10330, Thailand; [3]Department of Pediatrics, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand; [4]Queen Sirikit National Institute of Child Health, Bangkok, Thailand; [5]Unit of Toxicology, Department of Forensics Medicine, Chulalongkorn University and Hospital, Bangkok 10330, Thailand

Abstract. Disorders of organic acid metabolism are a group of disorders which has long been ignored by majority of Thai physicians. Part of this is due to lack of laboratories in Thailand to verify the diagnosis of the disorders. We have recently developed a technique to qualitatively analyze organic acids utilizing Gas Chromatography - Mass Spectrometry (GC-MS). Eight patients in four families were successfully identified as having organic acidemias (OA) by this method. Two families had methylmalonic acidemia, one had propionic acidemia, and the other had 3-methylcrotonyl CoA carboxylase deficiency. To our knowledge, this is the first laboratory in Thailand being able to use GC-MS to diagnose OA. Availability of a laboratory in Thailand and affordability of the test are expected to result in earlier diagnosis and identification of more cases of OA in Southeast Asian countries. Consequently, prompt and proper treatment can be anticipated which should lead to better prognosis for patients with this group of disorder.

INTRODUCTION

Patients presented with lethargy, hypotonia, hypertonia, tachypnea, seizures, ataxia, vomiting, failure to thrive, delayed development, and hepatomegaly may have organic acid disorders. Abnormal clinical chemistries such as cytopenia, metabolic acidosis, hyperammonemia, hypoglycemia, lactic acidemia, ketosis may also suggest abnormalities of organic acid metabolism (Goodman, 1996; Clarke, 1996). However, this group of disorders has long been ignored by many of the Thai physicians. Part of which may be due to unavailability of laboratories in Thailand to verify the diagnosis of the disorders.

The qualitative analysis of organic acids by gas chromatography - mass spectrometry (GC-MS) has well established as an important method for the diagnosis of disorders of organic acid metabolism since early 1980s (Sweetman, 1991). Here we reported accomplishment of utilizing GC-MS to identify organic acids and making diagnoses of patients with methylmalonic acidemia, propionic acidemia, and 3-methylcrotonyl CoA carboxylase deficiency. This will expedite the diagnosis of OA in Thai and other Southeast Asian patients. Therefore, prompt treatment and better prognosis can be anticipated.

MATERIALS AND METHODS

Urine organic acid analysis using GC-MS

Three drops of 6N HCl were added to 1 ml of urine or of 80 mg/100 ml control substrates (Table 1). NaCl was added until saturated. Then, 1 ml of ethylacetate, as a solvent to extract OA, was added. After the solution was mixed and centrifuged at 3,000 rpm for 3 minutes, the upper layer was transferred to a new tube and evaporated with nitrogen gas from N-evaporator till dry. We repeated extraction of organic acids two more times, each with 1 ml of ethylacetate. When it dried, BSTFA-TMCS [(N,O-bis (trimethylsilyl) trifluoroacetamide-trimethylchlorosilane) (Supelco, PA, USA)] 100 µl was added, then mixed, and heated at 90°C

Table 1
Standards used and their retention times.

No	Standards	Retention times (min)
1.	Methylmalonic acid	9.50
2.	Adipic acid	19.93
3.	Succinyl acetone	23.31, 24.55, 25.44
4.	Orotic acid	28.57
5.	Sebacic acid	33.22
6.	Undecanedioic acid	36.61

in a water bath for 10 minutes. The sample was then injected into the GC (HP 5890 series II PLUS) using Helium as carrier gas with the flow rate of 0.5 ml/minutes. The column used was HP-Ultra2, 25 m x 0.2 mm x 0.33 μm. The injection condition was "split (20:1), inlet at 250°C". The oven temperatures were 100°C for 1 minute, then increased with the rate of 3°C per minute to 250°C and sustained for 1 more minute. The substances were detected by mass selective detector (Hewett Packard 5972 series) at 280°C and were identified by a library kindly provided by Dr George Thomas of the Kennedy Kreiger, USA and Dr Tina Cowan at the University of Maryland, USA.

Patients

Family 1: Patient 1 was born at 37 weeks of gestation. The pregnancy, labor and delivery was unremarkable. His parents were second cousins (see pedigree in Fig 1). The patient's older brother (patient 2) died of hypoglycemia and severe metabolic acidosis at age 5 months. Patient 1 suffered from persistent pulmonary hypertension and pneumonia requiring ventilatory support for the first 16 days of life. Because his older brother was suspected of having an organic acidemia, the patient was given carnitine 300 mg/kg BW/day since the first week of life. At age 3 weeks, his general condition improved and he was discharged from the neonatal intensive care unit. The carnitine was discontinued and he was fed on regular formula. At age 2 months, he developed lethargy. Physical examination revealed mild dehydration, jaundice, and tachypnea. Laboratory data demonstrated pancytopenia with hemoglobin 9.52 g/dl, hematocrit 28.3%, white blood cell count 1,090 cells/mm^3, and platelet 19,300 /mm^3. Urine pH was 6 and urine ketone 2+. He did not have hypoglycemia. Serum sodium was 133 mEq/l, potassium 4.4 mEq/l, chloride 94 mEq/l, bicarbonate 17 mEq/l and the anion gap of 22 mEq/l. BUN was 6 mg/dl, Cr 0.5 mg/dl, total bilirubin 6.46 mg/dl, direct bilirubin 5.71 mg/dl, alkaline phosphatase 220, SGOT 69 U/l, SGPT 95 U/l, ionized calcium 1.33 mmol/l (normal range 1.15-1.8 mmol/l), magnesium 2.0 mg/dl (normal range 1.6-2.6 mg/dl), and ammonia 492 μg/dl (normal range: 25-94 μg/dl). Urine ferric chloride test and DNPH tests were negative. Urine p-nitroaniline test was positive.

Family 2: An 8-month-old boy (patient 3) presented with fever, vomiting and lethargy. He was born to a G3P2 27-year-old mother and a 32-year-old unrelated father. The pregnancy and delivery were unremarkable. He had an older brother (patient 4) who died at age 2 years because of severe acidosis. Another older sister had been normal. On physical examination, he was tachypneic, lethargic, and moderately dehydrated. Blood cell counts were within normal limits. Serum sodium was 134 mEq/l, potassium 2.5 mEq/l, chloride 98 mEq/l, bicarbonate 11 mEq/l and the anion gap 25 mEq/l. BUN was 2 mg/dl and Cr 0.6 mg/dl. Liver function tests were unremarkable. Ammonia level was 350 μg/dl (normal range: 25-94 μg/dl). Urine ferric chloride test and DNPH tests were negative. Urine p-nitroaniline test was positive.

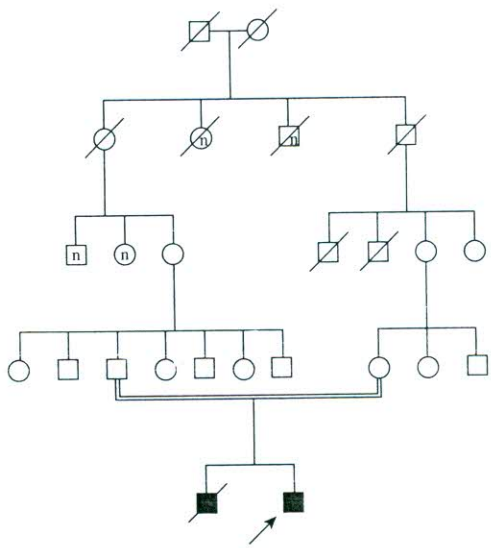

Fig 1–Pedigree of family 1.

Family 3: A one-month-old boy (patient 5) presented with lethargy for 3 days before admission. His parents were second cousins. His older brother (patient 6) died at age 3 months from severe metabolic acidosis without a definite diagnosis. However, the mother recognized the similar manifestations in her 2 children. Upon admission, his body weight was 2,700 g (his birth weight was 3,200 g). Physical examination revealed moderate dehydration and hepatomegaly with palpable liver 2 cm below his right costal margin. Laboratory data showed severe metabolic acidosis with initial bicarbonate of 6 mEq/l. The sodium was 132 mEq/l, potassium 4.8 mEq/l, and chloride 100 mEq/l. The blood sugar was 78 mg/dl, BUN 18 mg/dl, and Cr 0.8 mg/dl. The ammonia level was 600 μg/dl. Urine examination showed pH of 5.5, specific gravity 1.026, ketone 2+ and negative for protein and sugar.