学 位 論 文 の 要 旨

氏名　　小 林 弘 典

学 位 論 文 名　**ESI-MS/MS Study of Acylcarnitine Profiles in Urine From Patients With Organic Acidemias and Fatty Acid Oxidation Disorders**
発 表 雑 誌 名　**Journal of Chromatography. B**
（巻，初頁～終頁，年）　**855, 80-87, 2007**

著 者 名　**Hironori Kobayashi, Yuki Hasegawa, Mitsuru Endo, Jamiyan Purevsuren, Seiji Yamaguchi**

INTRODUCTION

Mass screening of newborns for inherited metabolic disorders using electrospray ionization tandem mass spectrometry (MS/MS) is in common use worldwide; disorders including amino acidemias, urea cycle disorders, organic acidemias (OAs) and fatty acid oxidation disorders (FAODs) can be detected. The procedure only requires sampling of a blood spot. However, it is sometimes difficult to make a definite diagnosis by MS/MS analysis alone, and a confirmatory test such as urinary organic acid analysis using GC/MS, enzyme determination, or molecular analysis may be required.

Urinary acylcarnitine analysis may also be useful in disease identification, but there have been only a few reports. In this study, we evaluated the utility of MS/MS analysis of urinary acylcarnitines in patients with OAs, FAODs, and carnitine deficit disorders.

MATERIALS AND METHODS

Urine samples were studied from 44 patients with OAs or FAODs. The cases included 16 with methylmalonic acidemia (MMA-emia); 5 with propionic acidemia (PPA-emia); 4 each with very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency and glutaric acidemia type 2 (GA2); 3 with glutaric aciduria type 1 (GA1); 2 each with 3-methylcrotonyl-CoA carboxylase (MCC) deficiency, 3-ketothiolase (BKT) deficiency and medium-chain acyl-CoA dehydrogenase (MCAD) deficiency; and one each with multiple carboxylase deficiency (MCD), 3-hydroxy-3-methylglutaryl-CoA lyase (HMGL) deficiency, short chain acyl-CoA dehydrogenase (SCAD) deficiency, tri-functional protein (TFP) deficiency, carnitine palmitoyltransferase 2 (CPT2) deficiency, and carnitine uptake defect (CUD). Three patients presenting with secondary carnitine deficiency were also included in the study.

Acylcarnitine analysis of blood spots and serum was performed using routine methods for MS/MS. In urine acylcarnitine analysis, 100 μl of urine was diluted with methanol 1:10 (v/v). After 30-min

incubation, aliquots were centrifuged for 5 min at 13,000 g; 10 μl of the supernatant was then transferred to a 96-well microplate and 100 μl of the methanol reference standard kit was added to each well. After drying under a gentle stream of nitrogen, 60 μl of 3N n-butanol-HCl was added and butylation was performed at 65°C for 15 min. After drying, the sample was reconstituted in 100 μl of 80% acetonitrile:water (4:1 v/v).

An API 3000 triple quadrupole tandem mass spectrometer in combination with a SIL-HTc autosampler was used, with a sample volume of 10 μl. Quantitative analysis was achieved by comparing the signal intensity of an analyte against the corresponding internal standard.

RESULTS AND DISCUSSION

1. Urinary acylcarnitines in MMA-emia and PPA-emia

MMA-emia and PPA-emia are the most common OAs, and are detectable through an elevation of C3, C3/C2 or C3/C16 in MS/MS using blood filter paper. However, diagnostic difficulty has been reported in some cases such as mild form of MMA-emia or PPA-emia. Urinary acylcarnitine analysis also missed such a mild case. It was suggested that some of these cases might be detectable with urinary acylcarnitine analysis under carnitine loading.

2. Urinary acylcarnitines in organic acid disorders other than MMA-emia and PPA-emia

In all cases tested, 4 of GA1, 2 each of MCC deficiency and KT deficiency, one each of MCD and HMGL deficiency, diagnostic markers of acylcarnitines for each disease were successfully detected in urine. It was reported that C5DC (glutarylcarnitine) or glutaric acid were not high in some cases of GA1, and that urinary excretion of C5DC is a good diagnostic marker even in such an ambiguous case. Urinary C5DC was high in all cases tested in our study, too, and it was supported that urinary acylcarnitine analysis could be an informative tool in diagnosis of GA1.

3. Urinary acylcarnitines in fatty acid oxidation disorders

Urinary acylcarnitines were evaluated in 14 cases of FAODs. In the patient with SCAD deficiency, elevation of C4 was found in blood. In urinary acylcarnitine profile, an elevation of C4 was also observed. In 2 cases of MCAD deficiency, marked elevation of C8 and mild elevation of C6, C10 and C10:1 in urine were observed. In cases of long-chain FAODs, 5 cases of VLCAD deficiency; 4 cases of GA2; one case each of TFP deficiency and CPT2 deficiency, specific abnormalities in urinary acylcarnitine profiles were not observed.

In FAODs, urinary acylcarnitine profiles in SCAD deficiency and MCAD deficiency were similar to blood acylcarnitine profiles. Additionally, it is unlikely that urinary acylcarnitine analysis is useful for cases of long-chain FAODs including VLCAD deficiency, TFP deficiency or CPT2 deficiency.

4. Blood and urine acylcarnitines in cases with blood carnitine deficit

Urinary acylcarnitines were investigated in four patients who showed a free carnitine deficit in blood. One case with congenital carnitine uptake defect showed decrease in all acylcarnitines and free carnitine in blood; however, the level of free carnitine in urine was consistently high. In another two cases, a reduced level of free carnitine in blood was seen, whereas an increased peak for C5 was observed. The urinary acylcarnitine profiles for these 2 cases showed a low level of free carnitine and an increased level of C5, as in the blood profiles. It was indicated that these two cases had received pivalate prodrug antibiotics, which caused an increase in C5, for long period. In the forth case, a reduced level of free acylcarnitines in blood was observed, whereas the C5-OH level was elevated. Urinary organic acid analysis indicated biotin deficit. The urinary acylcarnitine profile showed low free carnitine and increased C5-OH, but no elevation of C3. Combination of urinary acylcarnitine analysis, urinary organic analysis, and clinical history suggested that the patient had deficits in carnitine and biotin due to malnutrition. In these 4 cases with free carnitine deficit in blood, urinary acylcarnitine analysis was helpful for differential diagnosis.

In the current study, we examined the utility of urinary acylcarnitine analysis for diagnosis and evaluation of disorders detected in screening. The advantages of this approach are a short analysis time and high sensitivity, with only a very small amount of urine. Furthermore, comparison of metabolic profiles between blood and urine may be informative for confirmation of diagnosis and for understanding of pathophysiology.

It was confirmed that diagnostic acylcarnitine markers for each OA was enhanced with carnitine loading. Even in the mild form of MMA-emia, the levels of C3 and C3/C16 increased in urine after carnitine loading, despite the absence of abnormalities in blood. Therefore, in cases of OAs with ambiguous metabolic profiles urinary acylcarnitine analysis after carnitine loading may be helpful for confirmation of diagnosis, particularly.

CONCLUSION

Our results suggest that urinary acylcarnitine analysis is useful for evaluation of some OAs and FAODs except for long-chain FAODs, and for differential diagnosis of free carnitine deficit. In neonatal mass screening using MS/MS, urinary acylcarnitine analysis will be useful as a second tier test.