ELSEVIER

Journal of Chromatography B, 000 (2001) 000–000

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Screening for inborn errors of metabolism using gas chromatography–mass spectrometry

Keming Xu[a,*], Liwen Wang[a], Hong Cai[a], Ting Zhang[a], Chunhua Zhang[b], Isamu Matsumoto[b]

[a]*Capital Institute of Pediatrics, No. 2 Ya-bao Lu, Beijing, 100020, PR China*
[b]*Matsumoto Institute of Life Science (MILS), Japan*

Abstract

Screening of newborns for inborn errors of metabolism (IEM) in China is both a challenging and undeveloped area for gynecologists and pediatricians. Since 1999, the Capital Institute of Pediatrics has been studied as regards screening for IEM using advanced gas chromatography–mass spectrometry (GC–MS) method in collaboration with the Matsumoto Institute of Life Science (MILS), Japan, and has successfully diagnosed 51 cases of IEM in a total of 393 patients. Galactosemia, phenylketonuria and methylmalonic acidemia were the most frequent disorders among 51 cases of IEM. Treatment by suitable drugs and/or diet therapy was very effective in the most cases.

Keywords: Inborn error of metabolism

1. Introduction

Inborn errors of metabolism (IEM) are a genetic disease, caused primarily by alteration of a specific gene which results in deficiency of a specific enzyme activity and leads to disturbance of biochemical reactions in human cells and organs [1–3]. If these diseases are not diagnosed and treated promptly prior to onset and/or soon after onset, these patients either die in the newborn period, or are left with some neurological deficits such as mental-motor retardation. The clinical manifestations of IEM are variable in most of the diseases. Therefore, it is very difficult to make a correct diagnosis from clinical symptoms alone, and a number of biochemical tests are required. One of the latest modern biochemical techniques is GC–MS analysis for IEM. This method was applied to diagnose IEM in 1966 by Tanaka et al. [4], and elegantly improved by Matsumoto et al. and Kuhara et al. over the last few years [5–7]. Matsumoto's method allows screening of inborn errors not only of amino acids and organic acids, but also of carbohydrate and nucleic acids and their metabolites. In February 1999, the Capital Institute of Pediatrics began a pioneering collaborative study with the Matsumoto Institute of Life Science (MILS), Japan, to screen for IEM using a chemical diagnostic method in high-risk children with mental-motor retardation of unknown causes and/or infants suspected of having some IEM. Of the patients subjected to GC–MS analysis, 51 cases of IEM have been found in the last 17 months.

*Corresponding author. Tel.: +86-10-6512-8462; fax: +86-10-6512-8367.

E-mail address: xdcui@public.intercom.com.cn (K. Xu).

0378-4347/01/$ – see front matter
PII: S0378-4347(01)00042-1

The results will be discussed in this paper.

2. Clinical data

A total of 393 patients from the Capital Institute of Pediatrics neurological department and from another hospital in Beijing were referred for GC–MS analysis. There were 226 males and 127 females with ages ranging from 3 months to 14 years.

3. Experimental method

3.1. Urine filter paper set

As reported before [8], a urine filter paper set consists of filter paper for urine collection and a drying bag (called a ''set bag'') that allows wet filter paper to dry during mailing. The filter paper is Toyo Roshi No. 2, 2×3 cm in area, and can contain sufficient urine for GC–MS analysis. The filter paper part is covered with non-woven film to prevent tearing. The drying bag is equipped with a sealing device and is filled with desiccant that can over 90% dry filter paper containing 3 ml urine within 8 h. To collect urine, the filter paper (covered with non-woven film) should be soaked in a urine sample, drained well, placed in the set bag, and then sealed. The urine filter paper was sent to MILS by air-mail from our Institute.

3.2. Sample treatment

Sample pretreatment and GC–MS conditions were basically as described [8,9]. The filter paper was taken from the filter paper set, and the non-woven film was removed. The filter paper was folded once, and was placed in the outer tube of a 2.5-ml disposal syringe that was set on the spit tube. Then 1.5 ml of distilled water was slowly poured on top of the filter paper, left for 5 min, and centrifuged at 2000 rpm for 5 min to obtain urinary elute. Next, 0.1 ml of elute was treated in the same way as fresh urine. That is, the elute (0.1 ml) was incubated with 20 μl urease solution (containing 15 μl of enzyme) at 37°C for 15 min. Then 20 μg *N*-heptadecanoate (Tokyo Kasei) was added as an internal standard. After removing precipitate by centrifugation, it was evaporated to dryness, and the residue was trimethysilylated with 100 μl of *N,O*-bistrimethylsilyltrifluoroacetamide (BSTFA) and 10 μl of trimethychlorosilane (TMCS) at 90°C for 40 min. Finally 1 μl of the derived extract was injected into GC–MS.

3.3. GC–MS analysis

All analyses were done on a Shimadzu GC–MS QP-5050A using helium as carrier gas with velocity of 24 ml/min to an ultra alloy-5 silica capillary column (30 m×0.25 mm I.D., 0.25-μm film thickness, Frontier Laboratories, KK). The temperature was raised from 60 to 325°C at 17°C/min. Each 1-μl sample was automatically injected in 20:1 split mode and mass spectrum was scanned by electron impact (EI) mode every 0.25 s in low resolution mode from m/z 50 to m/z 650.

3.4. Data analysis

Peaks in the TIC chromatogram that showed the profile of urinary metabolites were identified from each mass spectrum. Abnormally excreted amounts of marker compounds for 101 metabolic diseases were checked for screening of inborn errors of metabolism.

4. Results

4.1. Results of screening

Abnormal urine profiles were found in 51 cases among 393 patients tested (Table 1). The chemical diagnoses included 11 cases of galactosemia (21.5%), six cases of phenylketonuria (PKU, 11.8%), six cases of methylmalonic aciduria (MMA, 11.8%), three cases of Fanconi syndrome (5.9%), three cases of glucosuria (5.9%), two cases each of glutaric aciduria, lysinuria and homoserinuria, one each of fructose-1,6-diphosphatase deficiency (FDPD), pyroglutamic aciduria, long-chain fatty acetyl CoA dehydrogenase deficiency (LCAD) and neuroblastoma (2%, respectively), and 12 cases of ketonuria.

Table 1
Major clinical manifestation in 51 patients

Diagnosis	*n*	Mental-motor retardation	Seizure	Hypo- or hyper-tonic	Hair yellow	Special smell
Galactosemia	11	8	2	5	4	3
PKU	6	6	6	3	4	6
MMA	6	6	1	5	4	0
Lysinuria	4	4	2	3	2	1
Homoserinuria	2	1	1	1	1	0
FDPD	1	1	1	1	1	1
Pyroglutamic aciduria	1	1	1	0	0	0
LCAD	1	1	1	1	0	0
Glutaric aciduria	1	1	0	1	0	0
Fanconi syndrome	3	3	0	1	0	0
Simple glycosuria	1	0	0	0	0	0
Diabetes mellitus	2	0	0	0	0	0
Ketouria	12	9	4	7	6	5

Two of the six patients with MMA were treated with a special diet and vitamin B_{12}, three cases will be treated by the same method and the parents abandoned one case. Of 11 patients with galactosemia, four cases have been treated in our hospital with a galactose-free diet, two cases have been treated in their local hospital with a galactose-free diet, and two cases were lost to follow-up. The clinical manifestations and biochemical analyses of urine were normal in three cases with temporary galactosemia: they were completely cured. Accompanying diseases were infant hepatitis, underdeveloped liver function and pneumonia. Six cases with PKU were sent to another hospital in Beijing, where a special PKU diet was provided (Table 2).

As described above, seven cases (two cases of MMA, four cases of galactosemia and one case of PDFD) have been treated in our hospital. After treatment, mental-motor development was markedly improved, and the biochemical analysis of urine was normal in six cases: the case with MMA, in spite of significant improvements in clinical manifestation, showed abnormal urinary metabolism. Three of the 51 cases are described below.

Table 2
Rate of discovery of clinical manifestation after treatment

Clinical manifestation	Cases	Percent
Mental-motor retardation	41	80.4
Seizure	19	37.3
Hypo/hypertonia	28	54.9
Yellow hair	22	43.1
Special urine smell	16	31.4
Recurrent vomiting	4	7.8

4.1.1. Case 1

C.K. was a 10-month-old boy with mental-motor retardation, hypotonia, yellow hair. He was unable to sit. He appeared dull, and did not react to his environment or to his mother. Tendon reflexes were weak. CT test suggested maldevelopment of the brain. GC–MS analysis revealed galactosemia (Fig. 1) [9]. After 1 month of treatment with a galactose-free diet, he could sit, became animated, and responded to voices. After 1 year, he started walking independently, and urine GC–MS analysis was normal.

4.1.2. Case 2

L.X. was a 5-month-old boy, hospitalized because he was unable to hold his head up and did not recognize his mother. His head was small with head circumstance of 40 cm. His hair was yellow, he did not react to his surroundings, and he exhibited hypotonia in his upper extremities and hyperreflexia. GC–MS analysis revealed MMA (Fig. 2) [10,11]. After 2 weeks of vitamin B_{12} therapy and a special diet, he appeared alert, smiled, responded to his parents, and could hold up his head. After 2 months

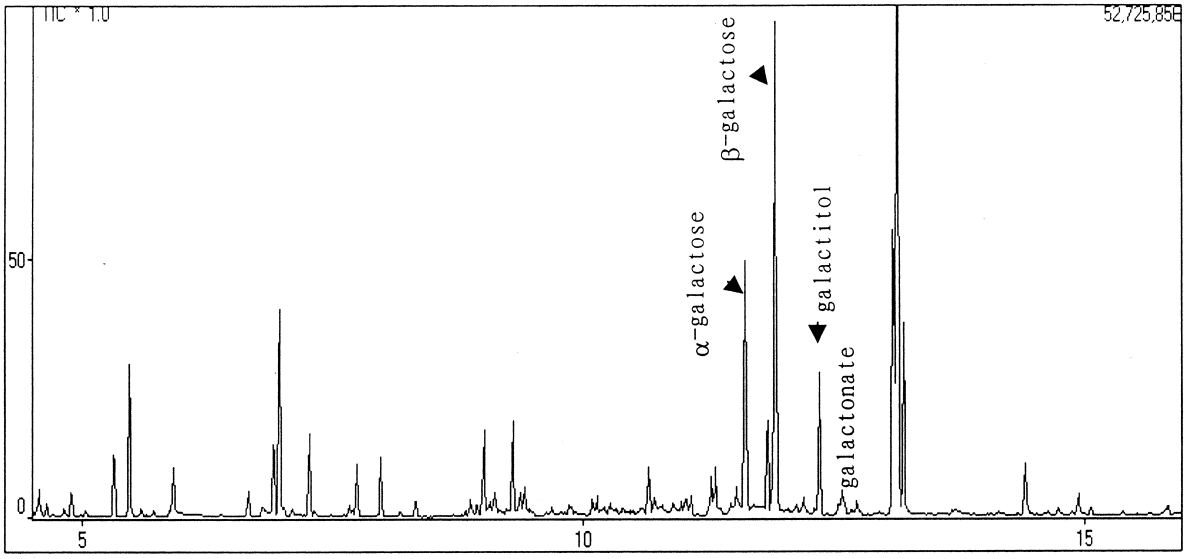

Fig. 1. TIC chromatogram of urinary organic compounds of a patient with galactosemia.

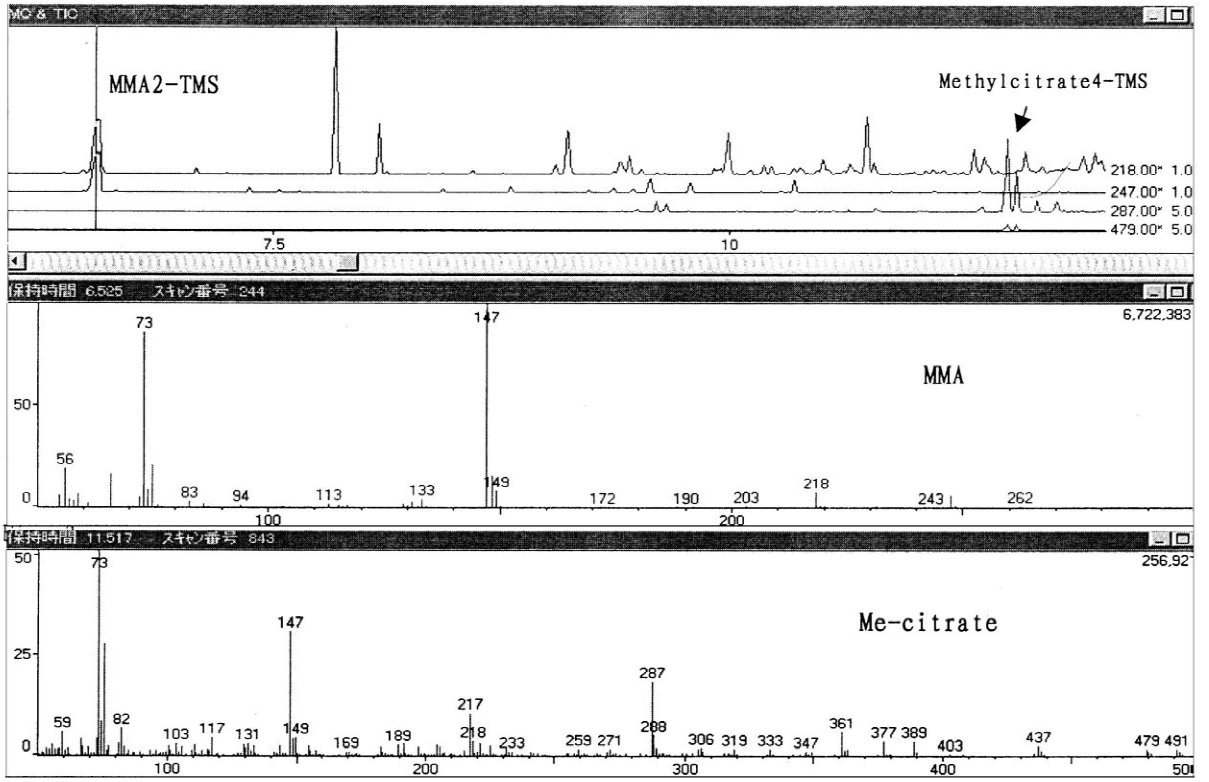

Fig. 2. Mass chromatogram and mass spectra of methylmalonate (MMA) and methylcitrate (Me-citrate) in the urine of a patient with MMA.

of therapy he responded to his name and could follow moving objects with his eyes. The hypotonia and hyperreflexia disappeared. After 9 months he could imitate his parents' actions, and expressed pleasure or cried when praised or criticized. GC–MS urine analysis was normal.

4.1.3. Case 3

H.X. was a 5-month-old male hospitalized for hypotonia. He had mental-motor retardation. His expression was dull, he could not follow objects and did not react to his environment. He could only hold his head up for 1 min. CT scan showed maldevelopment of the brain. GC–MS analysis revealed FDPD (Fig. 3) [12]. After 5 days of restricting fructose and sucrose in his diet, the patient became alert, often smiled and after 6 months was very interested in his surroundings, could grab things actively and recognized his parents. The hypotonia disappeared and GC–MS urine analysis was normal.

5. Discussion

IEM is usually manifested in the postnatal period or early infancy. If diagnosed at the right time, most patients develop normally, except for some types of IEM. The clinical manifestations in most types of IEM are not specific, so a special laboratory test should be conducted in order to make the correct diagnosis. By using GC–MS, it was possible to diagnose 11 types of IEM as described in this paper. Of 51 cases of IEM exhibiting abnormal urinary profiles, 31 cases could be effectively treated with a

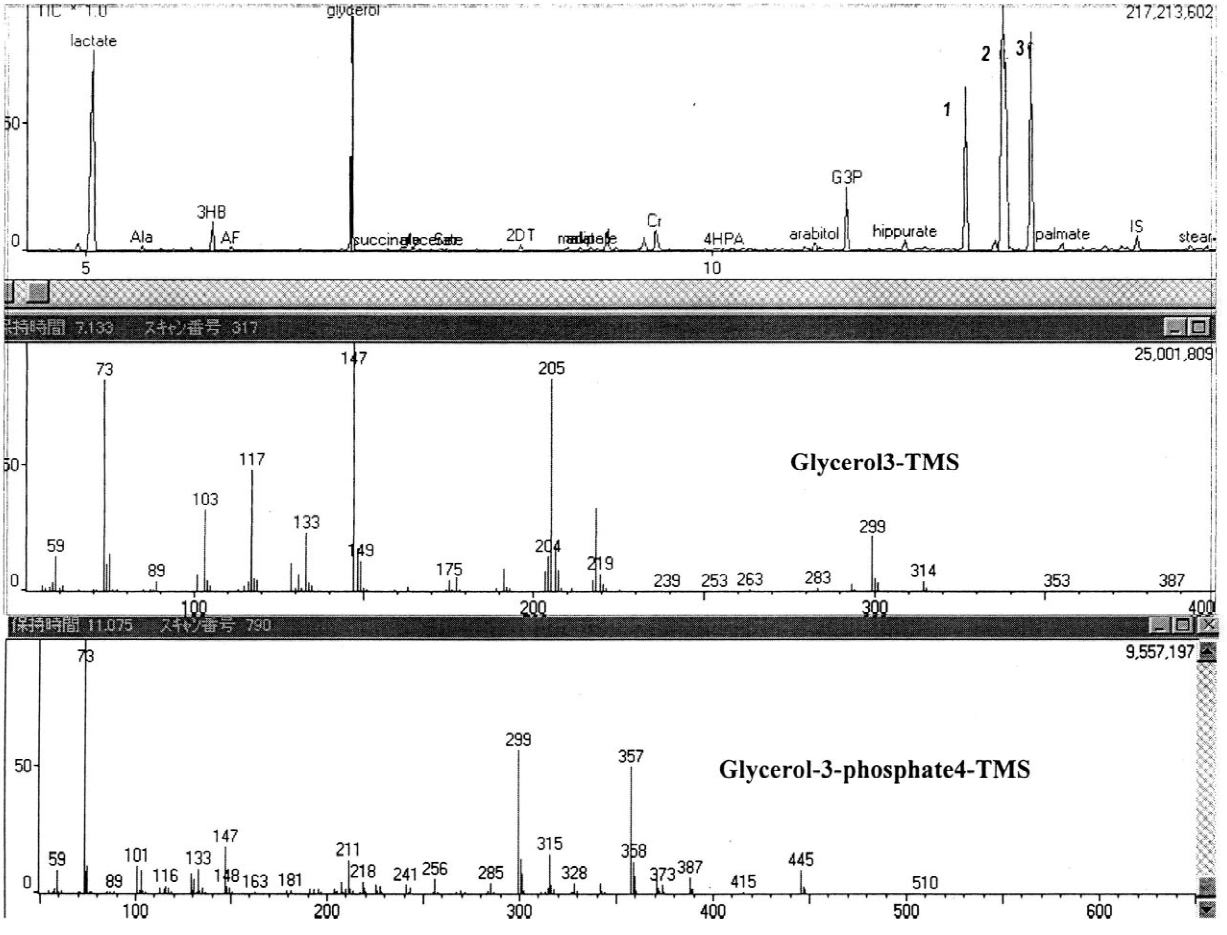

Fig. 3. TIC chromatogram and mass spectra of glycerol and glycerol-3-phosphate (G3P) in urine of a patient with FDPD.

special diet and drug therapy, showing good progress. If these patients had been diagnosed earlier during the postnatal period, mental-motor retardation would not have occurred, the patient would not have been critically ill even after disease onset, and many patients could have progressed normally.

In China, IEM has not been properly recognized due to the lack of modern laboratory facilities. The chemical diagnostic method of GC–MS for detecting IEM is accurate, sensitive and specific, which is valuable for screening for IEM. The screening of IEM should be conducted as early as possible. It is also very important to increase the number of target diseases.

The authors hope to encourage the use of this method in China.

References

[1] I. Matsumoto, T. Kuhara, in: D.M. Desiderio (Ed.), Mass Spectrometry, Plenum, New York, 1993, p. 259.
[2] I. Matsumoto, T. Kuhara, Mass Spectrom. Rev. 6 (1987) 77.
[3] S.I. Goodman, S.P. Markey (Eds.), Diagnosis of Organic Acidemias By Gas Chromatography–Mass Spectrometry, Alan R. Liss, New York, 1981, p. 1.
[4] K. Tanaka, M.A. Budd, M.L. Efron, K.I. Isselbacher, Proc. Natl. Acad. Sci. USA 56 (1966) 236.
[5] I. Matsumoto, S. Sakamoto, T. Kuhara, M. Sudo, M. Yoshino, Practical Chemical Diagnosis, Soft Science, Tokyo, 1995.
[6] I. Matsumoto, T. Kuhara (Eds.), Advances in Chemical Diagnosis and Treatment of Metabolic Disorders, Vol. 2, Kanazawa Medical University Press, Kanazawa, 1994, p. 143.
[7] T. Kuhara, T. Shinka, Y. Inoue et al., Proc. Jpn. Soc. Biomed. Mass Spectrom. 22 (1997) 119.
[8] C. Zhang, K. Xu, P.D. Usha, Y. Wang, I. Matsumoto, J. Chromatogr. B 764 (2000) 41.
[9] I. Matsumoto, T. Kuhara, Mass Spectrom. Rev. 15 (1996) 43.
[10] R.A. Chalmers, A.M. Lawson, in: Organic Acids in Man, Chapman and Hall, London, 1982, p. 211.
[11] C. Zhang, M. Matsumoto, Y. Inoue, C. Ning, T. Shinka, T. Furumoto, T. Kuhara, I. Matsumoto, J. Kanazawa Med. Univ. 20 (1995) 272.
[12] M. Iga, M. Kimura, T. Ohura, Y. Kitakawa, S. Yamaguchi, J. Chromatogr. B 764 (2000) 75.