Clinica Chimica Acta 454 (2016) 20–27

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Triplex tandem mass spectrometry assays for the screening of 3 lysosomal storage disorders in a Korean population

Sung Eun Cho [a], Joung Ryull Kwak [a], Hyojung Lee [a], Dong Hee Seo [a], Junghan Song [b,c,*]

[a] LabGenomics Clinical Laboratories, Seongnam, Republic of Korea
[b] Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea
[c] Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, Republic of Korea

ARTICLE INFO

Article history:
Received 9 September 2015
Received in revised form 14 December 2015
Accepted 15 December 2015
Available online 18 December 2015

Keywords:
Triplex tandem mass spectrometry assays
Lysosomal storage disorders
Korean population

ABSTRACT

Background: We evaluated the performance of triplex tandem mass spectrometry (MS/MS) assays using dried blood spots (DBSs) to screen for 3 lysosomal storage disorders (LSDs): Pompe, Fabry, and Gaucher diseases.
Methods: An Acquity UPLC CSH C18 column (Waters) and a TQD triple quadrupole mass spectrometer (Waters) in the multiple reaction monitoring (MRM) mode were used to evaluate the precision, carryover, and ion suppression of 3 enzyme assays (acid alpha glucosidase, acid α-galactosidase, and acid β-glucocerebrosidase) and were used to screen 1606 anonymous newborn DBSs.
Results: Intra- and inter-run CVs were between 0 and 14.1% and, between 0 and 18.9%. The carryover was 0%, −0.14%, and 0.39% for the 3 enzyme activities, respectively. No ion suppression was observed. The data from the 1606 anonymous newborn DBSs exhibited approximately bell-shaped distributions of 3 enzymatic activities (the median values were 16.46 μmol/h/l, 7.25 μmol/h/l, and 25.63 μmol/h/l, respectively).
Conclusions: The performance of the triplex tandem mass spectrometry assays for the screening of 3 lysosomal storage disorders in a Korean population using DBSs was acceptable.

1. Introduction

A lysosome is an intracellular organelle containing numerous acid hydrolases that degrade biological molecules such as proteins, glycoproteins, proteoglycans, lipids, and other complex macromolecules. Lysosomal storage disorders (LSDs) are caused by loss-of-function mutations in the genes encoding for lysosomal hydrolases; these mutations lead to the accumulation of intermediate metabolic products [1,2]. More than 50 LSDs with an estimated clinical prevalence of 1/7000 to 1/9000 in European populations, have been described [3–5]. Although several cases of LSDs in the Korean population have been reported, the incidence and prevalence of LSDs in Korea is unknown [2].

Pompe disease, Fabry disease and Gaucher disease are LSDs caused by deficiencies in acid α-glucosidase (GAA, EC 3.2.1.20), acid α-galactosidase (GLA, EC 3.2.1.22), and acid β-glucocerebrosidase (GBA, EC 3.2.1.45), respectively [6]. Enzyme replacement therapy (ERT) has become available [7], which makes the early diagnosis of these diseases more important. Even though, in case of Gaucher disease, ERT is available only for type 1 (nonneuropathic), which is the most common variant of Gaucher disease [8].

Li et al. [6] developed a novel approach to simultaneously determine GAA, GLA, and GBA activities in dried blood spots (DBSs) using tandem mass spectrometry (MS/MS).

Subsequently, several studies were performed to develop and modify the methods for sample extraction and, the composition of the assay cocktails, and to adapt the tandem mass spectrometry conditions to each respective laboratory [2,9,10]. Gelb MH et al. reviewed 3 screening methods for LSDs: (a) direct assay of enzymatic activities using tandem mass spectrometry or fluorometry, (b) immunocapture-based measurement of lysosomal enzyme abundance, (c) measurement of biomarkers, and concluded that tandem mass spectrometry better differentiates between nonaffected vs affected individuals than fluorometry techniques [11].

In this study, we determined the most appropriate method to screen for 3 LSDs, Pompe, Fabry, and Gaucher diseases, in a large reference laboratory in Korea using triplex tandem mass spectrometry assays to assess each enzyme activity in a DBS simultaneously. We evaluated the performance of this method, and we measured the above 3 enzyme activities in 1606 anonymous newborn DBSs to evaluate newborn reference ranges for the possible screening of Korean newborns for LSDs.

Abbreviations: GAA, acid α-glucosidase (Pompe disease); GLA, acid α-galactosidase (Fabry disease); GBA, acid β-glucocerebrosidase (Gaucher disease); CDC, Centers for Disease Control and Prevention; MRM, multiple reaction monitoring; S, substrate.

* Corresponding author at: Department of Laboratory Medicine, Seoul National University Bundang Hospital, 173-82 Gumi-ro, Bundang-gu, Seongnam 463-707, Republic of Korea.
E-mail address: songjhcp@snu.ac.kr (J. Song).

http://dx.doi.org/10.1016/j.cca.2015.12.019
0009-8981/

2. Material and methods

2.1. Materials and reagents

The substrate–internal standard mixture (S–IS; S&IS Analyte Specific Reagent Powder for GAA, GLA, and GBA) and the product–internal standard mixture (P–IS; 1:1 mol/mol, powder) were provided by Genzyme Pharmaceuticals trough the Centers for Disease Control and Prevention. The substrates (S) and internal standards (IS) are described below. GAA-S is (7-benzoylamino-heptyl)-[2-{4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)-phenylcabamoyl}-ethyl]-carbamic acid *tert*-butyl ester ($C_{34}H_{49}N_3O_{10}$) (molecular weight 659.8 Da). GAA-IS is (7-d5-benzoylamino-heptyl)-[2-(4-hydroxy-phenylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester ($C_{28}H_{34}N_5O_5D_5$) (molecular weight 502.7 Da). GLA-S is (6-benzoylamino-hexyl)-[2-{4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)-phenylcarbamoyl}-ethyl]-carbamic acid *tert*-butyl ester ($C_{33}H_{47}N_3O_{10}$) (molecular weight 645.7 Da). GLA-IS is (6-d5-benzoylamino-hexyl)-[2-(4-hydroxy-phenylcarbamoyl)-carbamic acid *tert*-butyl ester ($C_{27}H_{32}N_5O_5D_5$) (molecular weight 488.6 Da). GBA-S is D-glucosyl-β1-1′-*N*-dodecanoyl-D-*erythro*-sphingosine [C12-Glucocerebroside ($C_{36}H_{69}NO_8$)] (molecular weight 643.5 Da). GBA-IS is *N*-myristoyl-D-*erythro*-sphingosine [C14 ceramide ($C_{32}H_{63}NO_3$)] (molecular weight 509.5 Da).

CDC DBS samples at 4 different levels: base, low, medium, and high of GAA, GLA and GBA enzyme activities were provided by the Newborn Screening Quality Assurance Program of the CDC.

Four different levels were obtained: (1) unprocessed cord blood (high QC); (2) 50% cord blood in a leukocyte-reduced base pool (medium QC); (3) 5% cord blood in a leukocyte-reduced base pool (low QC), and (4) a leukocyte-reduced base pool (base QC). The CDC-reported activity values, i.e., the mean activities (range), are described in Table 1.

We purchased acetonitrile, methanol, and HPLC-grade water from J.T.Baker. Analytical reagent-grade formic acid was purchased from Wako Pure Chemical Industries. Citric acid trisodium salt (Sigma), monobasic sodium phosphate (Sigma), CHAPS hydrate (Sigma), acarbose (Sigma), anhydrous sodium acetate (Sigma), sodium taurocholate (Sigma), *N*-acetyl-D-galactosamine (GalNAc) (Sigma), were used to assemble the assay cocktail and were purchased from Sigma Aldrich. Glacial acetic acid (Duksan: UN2789) was purchased from Duksan Reagents. We purchased conical-bottomed 96-well plates from Corning, Inc. We used a Thermo shaker (Thermo shaker MB100-4A) and an Eppendorf centrifuge (Eppendorf centrifuge 5810 R; Eppendorf).

2.2. Preparation of the cocktail buffer and assay cocktail (S–IS cocktail)

All of the procedures, including those for preparing the assay buffer and the assay cocktail and, for conducting the enzyme assay were performed after modifying the methods developed by Zhang et al. [9] and Han M. et al. [2].

To prepare the GAA buffer solution, we added 10.20 g of monobasic sodium phosphate to HPLC-grade water. We added sodium citrate tribasic dihydrate to the monobasic sodium phosphate solution. We adjusted the pH to 4.0 using hydrochloric acid, resulting in a 250-ml citrate phosphate buffer with a concentration of 0.34 mol/l phosphate and 0.17 mol/l citrate. We used a 100-g/l solution of 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) in water as a detergent solution. We used 0.8 mmol/l of acarbose in water as an inhibitor solution.

To prepare the GAA assay cocktail, 1.8 ml of a 100-g/l solution of CHAPS, 15.9 ml of a 0.3-mol/l citrate-phosphate buffer, and 0.3 ml of a 0.8-mmol/l solution of acarbose in water were added to a vial containing GAA-S/IS.

To prepare the GLA buffer solution, we added 1.48 g of sodium acetate trihydrate to HPLC-grade water. We added 1.46 ml of pure glacial acetic acid to the sodium acetate trihydrate solution. We adjusted the pH to 4.6 using acetic acid, resulting in 250 ml of a 0.174 mol/l sodium acetate buffer. We used a 120-g/l solution of sodium taurocholate in water as a detergent solution. We used 1 mol/l of *N*-acetyl-D-galactosamine in water as an inhibitor solution.

The GLA assay cocktail was prepared by adding 0.45 ml of a 120-g/l solution of sodium taurocholate in water, 14.67 ml of a 0.174-mol/l sodium acetate buffer, and 2.88 ml of a 1- mol/l solution of *N*-acetyl-D-galactosamine in water to a vial containing GLA-S/IS.

To prepare the GBA buffer solution, we added 21.45 g of monobasic sodium phosphate to HPLC-grade water. We added sodium citrate tribasic dihydrate to the monobasic sodium phosphate solution. We adjusted the pH to 5.1 using HCl, resulting in 250 ml of a citrate phosphate buffer with 0.175 mol/l phosphate and 0.358 mol/l citrate. We used a 120-g/l solution of sodium taurocholate in water as a detergent solution.

To prepare the GBA assay cocktail, 2.4 ml of a 120-g/l solution of sodium taurocholate, and — 15.6 ml of a 0.62-mol/l citrate phosphate buffer were added to a vial containing GBA-S/IS.

2.3. Sample preparation

We punched 3.2 mm Whatman 903 DBSs (containing approximately 3.2 μl of dried blood) into 96-well polypropylene/conical bottom plates (Corning) using a puncher. The first and last well of each plate contained blank filter paper. We added 70 μl of a 20-mmol/l sodium phosphate elution buffer (pH 7.1) and sealed the plates with film. We centrifuged the samples for 1 min at 2000 ×*g* in an Eppendorf centrifuge (Eppendorf). The samples were extracted at 37 °C and 875 RPM with shaking for 1 h in a Thermo orbital shaker (Thermo).

We transferred 10 μl of the extract to a new conical-bottomed, 96-well plate that contained 15 μl of the enzyme assay cocktails for GAA, GLA, and GBA in each well. The plate was sealed and incubated at 37 °C and 235 RPM with shaking for 17 h in the Thermo orbital shaker.

After incubation, we centrifuged the plate for 1 min at 2000 ×*g*, and collected the 3 reacted samples into one well of a new plate. We added 150 μl 100% acetonitrile to quench the enzymatic reactions and

Table 1
LSD QC specimen certification set 8 analytical information (μmol/l/h).

Analytes	Lot numbers	Mean values X (95% confidence limits)	Lot numbers	Mean values X (95% confidence limits)	Lot numbers	Mean values X (95% confidence limits)	Lot numbers	Mean values X (95% confidence limits)
GAA	Base pool 381	0.09 (0.00–0.41)	Low 382	0.82 (0.47–1.17)	Medium 383	8.63 (7.10–10.17)	High 384	16.58 (13.83–19.32)
GLA	Base pool 381	0.45 (0.03–0.86)	Low 382	0.75 (0.45–1.05)	Medium 383	4.19 (3.19–5.19)	High 384	9.42 (7.48–11.36)
GBA	Base pool 381	0.35 (0.00–0.72)	Low 382	0.80 (0.39–1.21)	Medium 383	5.55 (4.47–6.63)	High 384	11.42 (8.90–13.94)

Abbreviations: LSD, lysosomal storage disorder; QC, quality control; GAA, acid α-glucosidase (Pompe disease); GLA, acid α-galactosidase (Fabry disease); GBA, acid β-glucocerebrosidase (Gaucher disease).