의학석사 학위논문

탠덤 질량분석기를 이용한 헌터 증후군과 연관된 iduronate-2-sulfatase 효소 활성도 직접 검사법 개발

Development of Direct Assay of Iduronate-2-Sulfatase for Mucopolysaccharidosis II (Hunter Syndrome) Using UPLC-Tandem Mass Spectrometry

2015 년 2 월

서울대학교 대학원

의학과 검사의학 전공

이 경 훈

탠덤 질량분석기를 이용한 헌터 증후군과 연관된 iduronate-2-sulfatase 효소 활성도 직접 검사법 개발

지도 교수 송 정 한

이 논문을 의학석사 학위논문으로 제출함

2015 년 2 월

서울대학교 대학원

의학과 검사의학 전공

이 경 훈

이경훈의 의학석사 학위논문을 인준함

2015 년 1 월

위 원 장 신 충호 (인)

부위원장 송 정 한 (인)

위 원 송 상 헌 (인)

Development of Direct Assay of Iduronate-2-Sulfatase for Mucopolysaccharidosis II (Hunter Syndrome) Using UPLC-Tandem Mass Spectrometry

by
Kyunghoon Lee, M.D.
(Directed by Junghan Song, M.D.)

A thesis submitted to the Department of Medicine in partial fulfillment of the requirements for the Degree of Master of Science in Medicine (Laboratory Medicine) at Seoul National University College of Medicine

February 2015

Approved by Thesis Committee:

Professor Choong Ho Shin **Chairman**
Professor Junghan Song **Vice chairman**
Professor Sanghoon Sng

ABSTRACT

Development of Direct Assay of Iduronate-2-Sulfatase for Mucopolysaccharidosis II (Hunter Syndrome) Using UPLC-Tandem Mass Spectrometry

Kyunghoon Lee

Department of Laboratory Medicine

The Graduate School

Seoul National University

Introduction: Mucopolysaccharidosis type II (MPS II), also known as Hunter syndrome, is caused by a deficiency in iduronate-2-sulfatase. MPS II is a chronic, progressive lysosomal storage disorder that affects multiple organ systems but is challenging to diagnose during the early stages. Here we developed and evaluated the performance of ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) with a commercially available substrate for the detection of MPS II.

Methods: 4-Methylumbelliferyl α-L-idopyranosiduronic acid 2-sulfate (IDS-S) was used as a substrate for IDS, and its enzymatic product, 4-

methylumbelliferyl α-L-idopyranosiduronic acid (IDS-P), was directly measured by UPLC-MS/MS. We determined the precision of our enzyme assay and the effects of sample amounts and incubation time on the results. Dried blood spots (DBSs) of 26 normal newborns and two patients with MPS II were analyzed.

Results: The intra- and inter-assay precisions were 7.9–10.5% and 4.8–10.2%, respectively. The amount of product obtained was proportional to the number of DBSs; however, a slight flattening was observed in the product versus DBS curve for more than one DBS. For our enzyme assay, the amount of product obtained increased linearly with the incubation period from 0 to 15 h. The enzyme activities measured in the DBSs were consistently lower in patients with MPS II than in normal newborns.

Conclusions: The performance of our enzyme assay was generally acceptable. In addition, it may be possible to simultaneously test multiple enzymes related to lysosomal storage diseases. To the best of our knowledge, this is the first report describing the use of MS/MS for the diagnosis of MPS II with a commercially available substrate. Our method provides a rapid, inexpensive, effective screening tool for the diagnosis of MPS II worldwide.

Keywords: Mucopolysaccharidosis, Hunter syndrome, Tandem mass spectrometry, Dried blood spot

Student number: 2013-21684

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

ABG	acid β-glucocerebrosidase
CE	collision energy
CV	coefficient of variation
CXP	collision cell exit potential
DBS	dried blood spot
DP	declustering potential
GAA	acid α-glucosidase
GAG	glycosaminoglycan
GLA	α-galactosidase
IDS	iduronate-2-sulfatase
IDS-S	4-methylumbelliferyl α-L-idopyranosiduronic acid 2-sulfate disodium salt
IDS-P	4-methylumbelliferyl α-L-idopyranosiduronic acid sodium salt
IDS-IS	4-methylumbelliferyl α-L-idopyranoside
IDU	α-L-iduronidase
IS	internal standard
LDS	lysosomal storage disorder
MPS	mucopolysaccharidoses
MPS II	mucopolysaccharidosis Type II
MRM	multiple reaction monitoring
MS/MS	tandem mass spectrometry
P	product
S	substrate
UPLC	ultra-performance liquid chromatography

INTRODUCTION

Lysosomes are intracellular organelles that contain numerous hydrolytic enzymes essential for the degradation and recycling of biological molecules, such as glycosaminoglycan, oligosaccharides, glycoproteins, and lipids. Lysosomal storage disorders (LSDs), including approximately 50 different diseases, have a combined incidence of 1 in every 1,500–7,000 births [1-2]. Mucopolysaccharidoses (MPSs) are a type of LSD caused by a deficiency in the enzyme(s) that break down glycosaminoglycans (GAGs), among which dermatan sulfate, heparin sulfate, keratin sulfate, and chondroitin sulfate are clinically significant. Multisystem cellular and organ dysfunctions are caused by accumulation of GAGs within lysosomes.

MPS Type II (MPS II), also known as Hunter syndrome, is caused by a deficiency of iduronate-2-sulfatase (IDS; EC 3.1.6.13), which catalyzes the hydrolysis of the 2-sulfate groups in dermatan and heparin sulfates [3]. MPS II is a chronic, progressive LSD affecting multiple organ systems and exhibits variable age of onset, presentation of symptoms, and rate of progression [3-6]. MPS II is an X-linked recessive disorder that mainly affects males; however, a few females have been diagnosed with MPS II because of skewed X-inactivation patterns or inheritance of another sex chromosome abnormality. A variety of mutations in the *IDS* gene, located on chromosome Xq27.3–q28, lead to a deficiency of IDS [7] and hence development of MPS II.

Since patients with MPS II are generally asymptomatic at birth, with initial symptoms appearing after 12 months of age, early diagnosis of MPS II is a challenging task. Although there are several methods for the diagnosis of

MPS II published in literature, routine newborn screening is not yet performed. In the 1970s, IDS activity was detected using radioactive substrates from heparin [8-10]. More recently, fluorometric methods have been developed; however, these multistep methods require long incubation times and excess purified α-iduronidase [11, 12]. Direct measurement of IDS activity using tandem mass spectrometer (MS/MS) has been reported in recent works [13, 14]. However, because of modification to the reaction step, these assays require use of a synthetic substrate produced directly in the researchers' laboratory [13, 14]; hence, this substrate is not commercially available.

Therefore, in this study, we developed an MS/MS-based direct assay of IDS activity for MPS II (Hunter syndrome) with commercially available synthetic substrates. Furthermore, we tested the potential of our new technique for clinical use in newborn screening programs.

MATERIALS AND METHODS

1. Materials

The substrate, product, and internal standard (IS) used to test for MPS II were as follows: 4-methylumbelliferyl α-L-idopyranosiduronic acid 2-sulfate disodium salt (IDS-S), 4-methylumbelliferyl α-L-idopyranosiduronic acid sodium salt (IDS-P), and 4-methylumbelliferyl α-L-idopyranoside (IDS-IS). These reagents were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). High-performance liquid chromatography (HPLC)-grade methanol and water (J.T. Baker, Phillipsburg, NJ, USA) were used. All other reagents, including acetonitrile, sodium acetate, lead(II) acetate, and formic acid, were of research grade or better and were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2. Enzyme assay procedures

For sample preparation, one 3.2-mm diameter punched–out dried blood spot (DBS) was incubated with 30 μL of 0.025 mM IDS-S and 10 mM lead(II) acetate in 0.1 M sodium acetate buffer (pH 5.0) for 15 h at 37°C. Next, 10 μL of 1 μg/mL IDS-IS in 80% methanol was added. The reaction was quenched with 200 μL acetonitrile, and the mixtures were centrifuged at 15,700 × *g* for 5 min. One hundred microliters of supernatant was transferred to each well of a 96-well microplate, and 10 μL of each sample was loaded into the autosampler of the ultra-performance liquid chromatograpy (UPLC) system.

3. UPLC-MS/MS conditions

Sample were analyzed on an LC-30A Nexera UPLC system (Shimadzu Co., Kyoto, Japan) with an AQUITY Waters CSH C18 column (50.0 × 2.1 mm, 1.7 μm; Waters, Watford, UK). The flow rate was 0.7 mL/min. The mobile phase was a gradient of a mixture of 0.05% formic acid (solvent A) and acetonitrile in 0.05% formic acid (solvent B). The gradient profile used was (A:B) 95:5 v/v at the start, followed by a linear gradient to 10:90 v/v until 3.5 min and returned to 95:5 v/v at 4.0 min. Total running time was 4.0 min. An AB Sciex API 6500 triple quadrupole tandem mass spectrometer (AB Sciex Pte., Ltd., Framingham, MA, USA) was operated using the following settings: declustering potential (DP), 71–121 V; Collision energy (CE), 23–41 V; and collision cell exit potential (CXP), 18–26 V, depending on the analytes (Table 1). Quantification was achieved by multiple reaction monitoring (MRM) in positive-ion mode. Peak area integration and data analysis were performed using Analyst 1.6 and MultiQuant 2.1 software (AB Sciex).

	DP(V)	CE(V)	CXP(V)	MRM transition (*m/z*)
IDS-S	71	41	18	477.0 → 220.9
IDS-P	86	33	24	352.9 → 177.0
IDS-IS	121	23	26	339.0 → 177.0

Table 1. Conditions for tandem mass spectrometry and multiple reaction monitoring (MRM) transitions of substrate, product, and internal standard

Abbreviations: DP, declustering potential; CE, collision energy; CXP, collision exit potential; MRM, multiple reaction monitoring; IDS-S, 4-methylumbelliferyl α-L-idopyranosiduronic acid 2-sulfate disodium salt; IDS-P, 4-methylumbelliferyl α-L-idopyranosiduronic acid sodium salt; IDS-IS, 4-methylumbelliferyl α-L-idopyranoside.

4. Determining of precision

Intra-assay precision (intra-assay coefficients of variation [CVs]) was determined by performing 10 replications for each sample at low, medium, and high concentrations. To evaluate the inter-assay CVs, enzyme activities of the same samples were measured on 3 consecutive days.

5. Effect of sample amount and incubation time

To evaluate whether the product obtained in the reaction was linearly related to the amount of enzyme used in the assay, we measured the enzyme activities for 0.5, 1, 1.5, and 2 DBSs. In addition, to assess the effects of incubation time, the enzyme reactions were monitored at 0, 5, 10, 15, and 20 h.

6. Evaluation of ion suppression

Ion suppression was evaluated by the post-column infusion model method [15]. The compound was introduced into the mass detector at a constant rate by a syringe pump connected to column effluent, and a constant electrospray ionization response was observed if no ionization interferences occurred when an extract from a biological specimen was injected into the UPLC portion of the instrument. As mentioned above, the infusion rate of the internal standard or product was kept constant through the syringe, and a blank specimen without internal standard, substrate, and product was simultaneously injected into the UPLC. MS/MS signals were then closely monitored.

7. DBSs of patients and normal individuals

To validate the capability of our system to detect MPS II in newborns, we analyzed three DBSs from two patients with MPS II. All patients were previously diagnosed as having MPS II on the basis of their clinical symptoms and findings of established molecular tests. Enzyme activities in the DBSs obtained from 26 newborns without any enzymatic abnormalities were measured as controls.

RESULTS

1. Materials and method development of UPLC-MS/MS

Substrates, products, and internal standards were fully separated using UPLC. We were able to reduce the chromatographic separation time to 3 min, allowing an injection cycle time of 4 min (Figure 1). No ion suppression was observed at the retention time for IDS-IS and IDS-P (Figure 2).

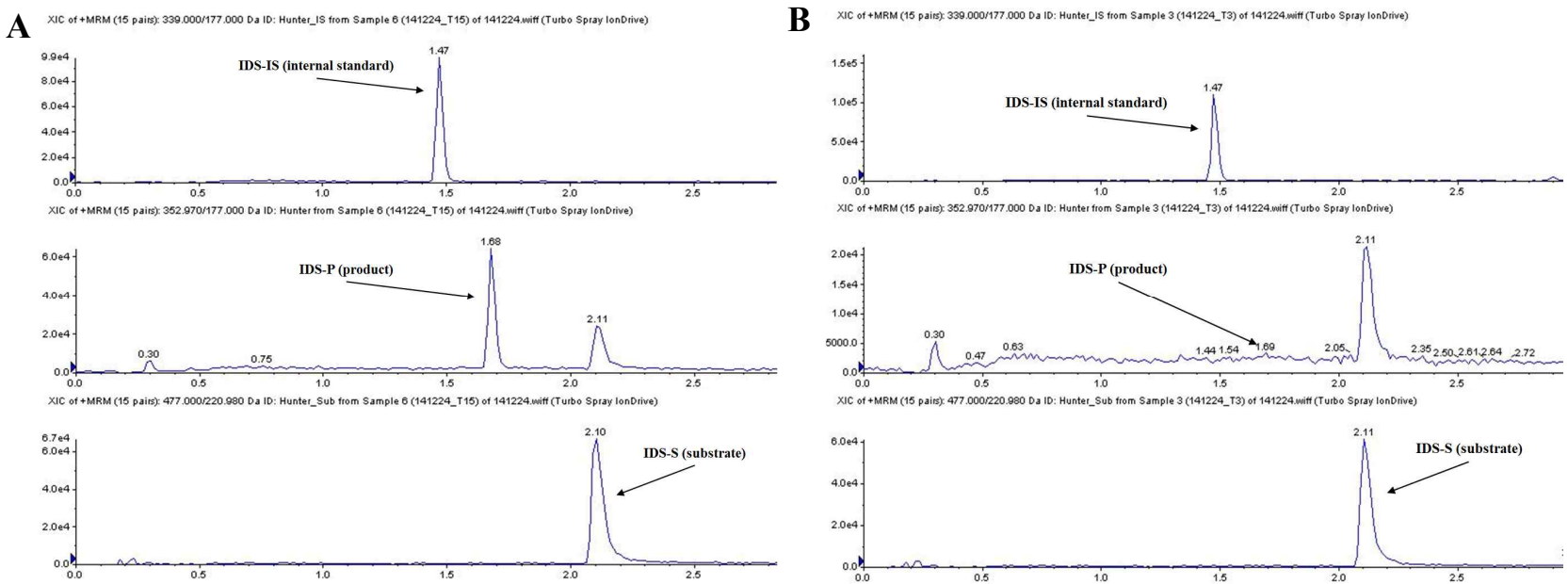

Figure 1. Representative UPLC-MS/MS MRM chromatograms from the IDS enzyme reaction mixture in samples from normal control (A) and patient with MPS II (B).

Abbreviations: IDS, iduronate-2-sulfatase; IDS-S, 4-methylumbelliferyl α-L-idopyranosiduronic acid 2-sulfate disodium salt; IDS-P, 4-methylumbelliferyl α-L-idopyranosiduronic acid sodium salt; IDS-IS, 4-methylumbelliferyl α-L-idopyranoside.

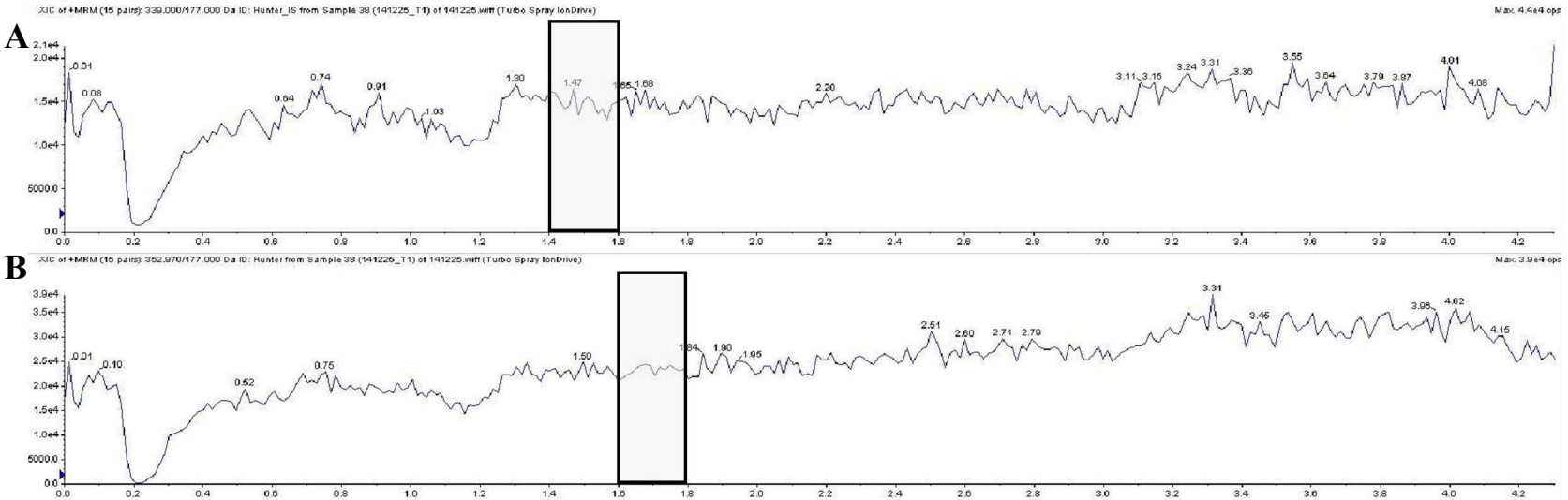

Figure 2. Evaluation of ion suppression. Internal standard (A) and product (B).

2. Precision

The intra-assay CVs were 8.5%, 7.9% and 10.5% at low, medium, and high concentrations, respectively. In addition, the inter-assay CVs were 6.6%, 4.8%, and 10.2% at low, medium, and high concentrations, respectively (Table 2).

Level	Low	Medium	High
Intra-assay CV(%)	8.5	7.9	10.5
Inter-assay CV* (%)	6.6	4.8	10.2

Table 2. Intra- and inter-assay precisions at low, medium, and high concentrations

* Enzyme activities of the same samples were measured on 3 consecutive days.

Abbreviation: CV, coefficient of variation

3. Effect of sample amount and incubation time

The amount of product obtained was proportional to the number of punched DBSs used in the assay. However, a slight flattening was observed in the product versus number of punched DBSs at more than one DBS (Figure 3).

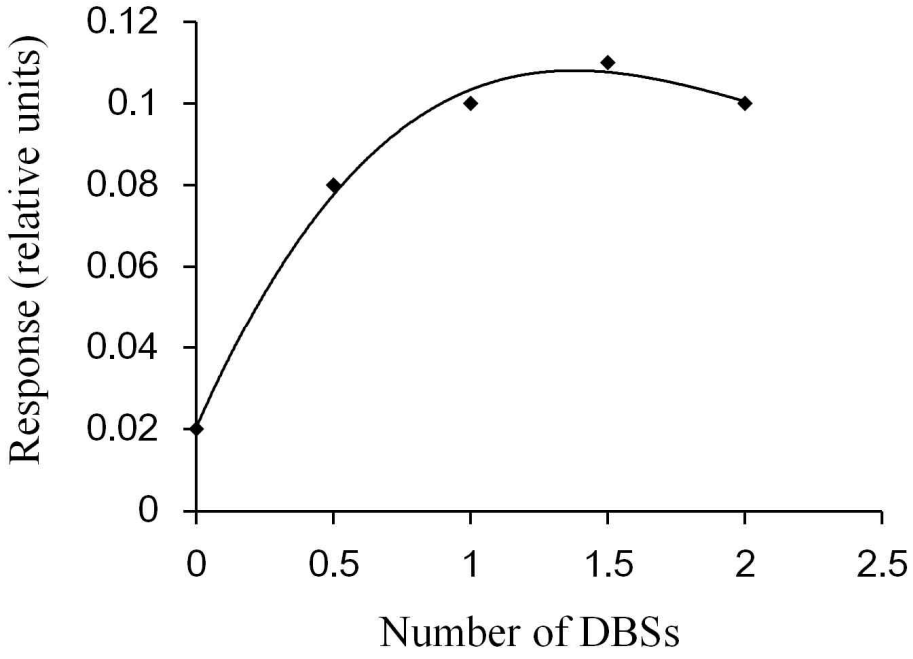

Figure 3. Effect of sample amount on enzyme activity. Representative graph for the IDS enzyme assay with respect to the number of DBSs. Each incubation time was 15 h.

Abbreviations: DBS, dried blood spot; IDS, iduronate-2-sulfatase

In our enzyme assay, the amount of product obtained increased linearly from 0 to 15 h. However, at more than 15 h of incubation, the curve for the amount of product versus incubation time plateaued. Therefore, we concluded that the 15-h incubation was optimal, and this incubation time was used for all subsequent enzyme assays (Figure 4).

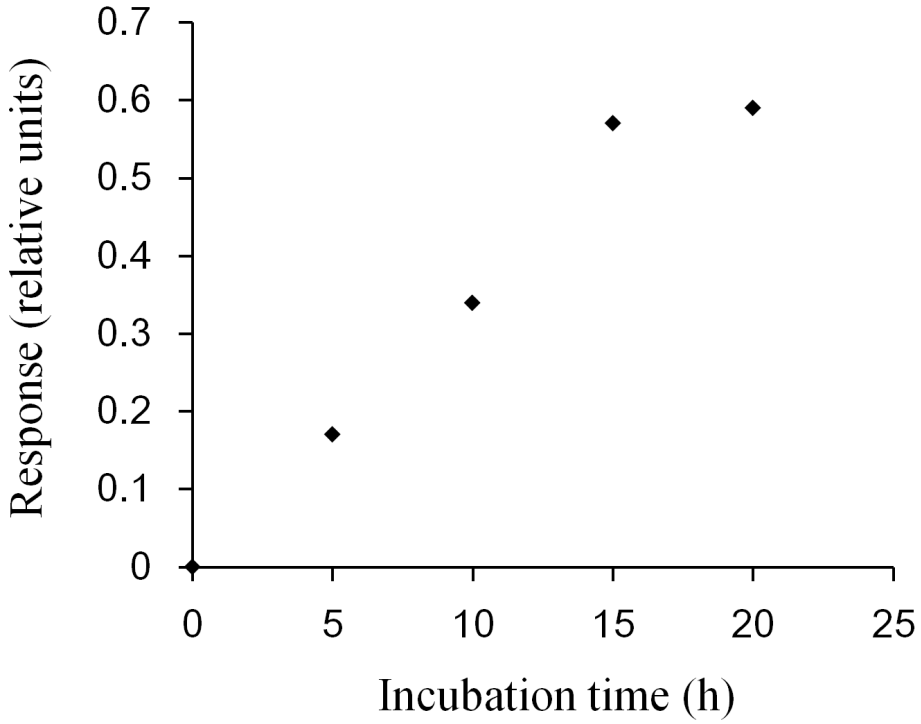

Figure 4. Effect of incubation time on enzyme activity. Representative graph for the time course of the IDS enzyme assays. This enzyme reaction was monitored for 20 h.

Abbreviations: IDS, iduronate-2-sulfatase

4. Enzyme activities in DBSs of patients and normal newborns

Finally, we analyzed IDS enzyme activities in DBSs of normal newborns and patients, as shown in figure 5. The enzyme activities in DBSs from patients with MPS II were markedly lower than those in the DBSs of normal newborns.

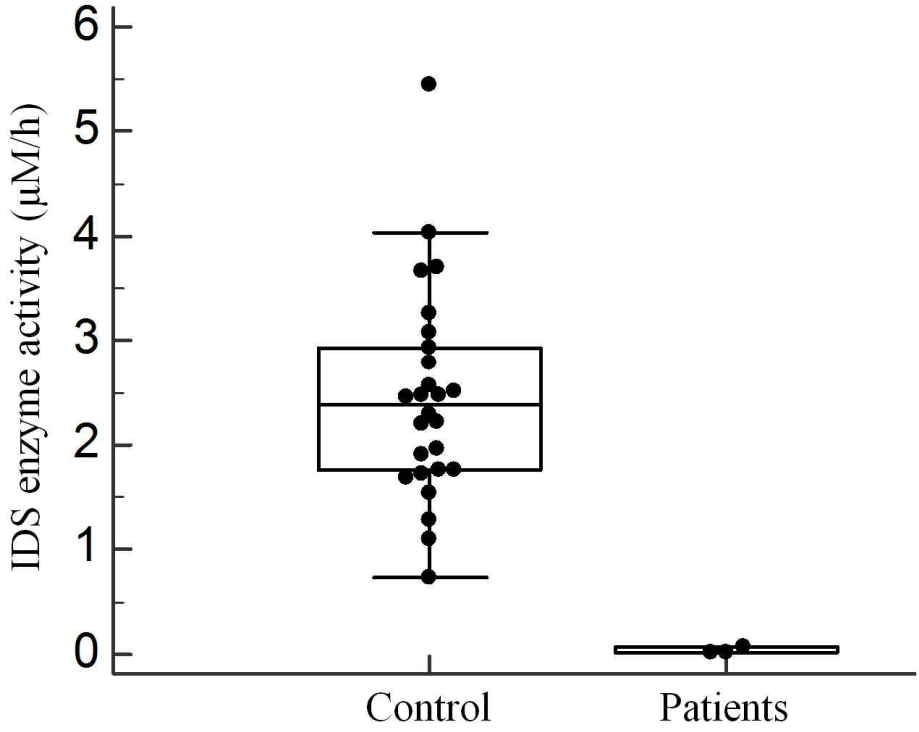

Figure 5. Comparison of enzyme activities in DBSs from healthy newborns (n = 26) and two patients with MPS II (two DBSs were obtained from one patient).

Abbreviations: IDS, iduronate-2-sulfatase; DBS, dried blood spot.

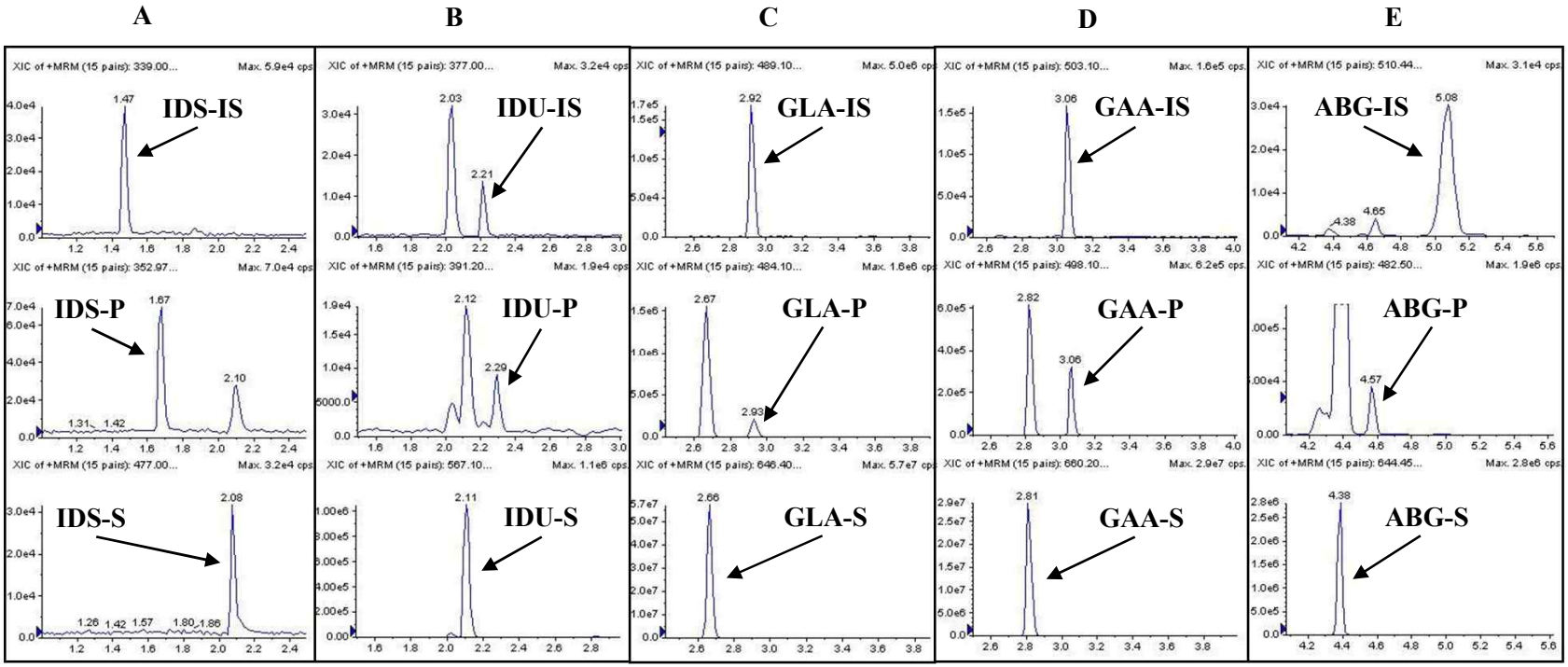

Figure 6. Representative UPLC-MS/MS MRM chromatograms from the mixture of IDS (A), IDU (B), GLA (C), GAA (D), and ABG (E) enzyme reactions in a sample with a normal newborn.

Abbreviations: IDS, iduronate-2-sulfatase; IDU, α-L-iduronidase; GLA, α-galactosidase; GAA, acid α-glucosidase; ABG, acid β-glucocerebrosidase; IS, internal standard; P, product; S, substrate.

DISCUSSION

To date, three methods, i.e., radiometric [8-10], fluorometric [11, 12], and LC-MS/MS assays [13, 14], have been used for IDS enzyme assays in the diagnosis of MPS II. However, these assays are associated with multiple challenges. For example, the initial radiometric assay was expensive, laborious, and radio-hazardous. Additionally, the multistep fluorometric method requires long incubation times and excess purified α-iduronidase. Finally, the substrates for the recently published LC-MS/MS assays are not commercially available. Therefore, we combined and modified the fluorometric and LC-MS/MS methods using 4-methylumbelliferyl α-L-idopyranosiduronic acid 2-sulfate as a substrate of IDS and detection of the enzymatic product, 4-methylumbelliferyl α-L-idopyranosiduronic acid, by UPLC-MS/MS. To the best of our knowledge, this is the first MS/MS-based enzyme activity assay for detection of MPS II with commercially available reagents. Moreover, our method is less expensive than other methods because it does not require additional enzymatic or synthetic steps. The performance of the assay in terms of precision, optimal incubation time, and required sample amount was generally acceptable.

Since the MS/MS techniques were developed for newborn screening of LSDs, several methods for direct multiplex MS/MS have been published [16-18]. However, despite the relatively common occurrence of MPS II, no enzyme assay for diagnosis of MPS II had been developed. For newborn screening for the detection of several LSDs, we performed multiplex MS/MS

tests for five LSDs, including Hunter, Hurler, Fabry, Pompe and Gaucher diseases, which were selected because they were the common targets of enzyme replacement therapy in Korea. All substrates, products, and internal standards were fully separated using UPLC. The chromatographic separation time was 6.6 min, allowing an injection cycle time of 7 min; this was slightly extended from our single analysis method in order to resolve targets with more difficult separation requirements (the total running time was just 4 min in our single method; Figure 6). Clear separation of five enzymatic products from their residual substrates is most important because soft ionization of residual substrates may result in product peaks and interference products when laborious pre-analytical LLE or SPE steps [16, 19] or turboflow online sample cleanup steps [20] are not used. As shown in Figure 6, we could achieve the clear separation of enzymatic products from their residual substrates without online or offline sample cleanup steps.

One limitation of this study was that only a small number of DBSs (26 DBSs from normal controls and three DBSs from two patients with MPS II) were collected. Therefore, from our results, we could not determine an appropriate cut-off level to diagnose MPS II. However, the enzyme activities in DBSs were clearly different between normal newborns and patients with MPS II. Because this is an X-linked recessive disease, the extent of IDS enzyme activity in heterozygotes must also be considered and assessed.

Furthermore, our study was a retrospective analysis focused on screening and diagnosis as we used DBSs that had been previously collected. Since the samples here were not sequential DBSs obtained from any patient

with MPS II, we were not able to compare enzyme activities between untreated and treated patients in order to assess our method for monitoring and prognosis. The IDS enzyme activity of one patient who has been treated for several years was lower than those in healthy controls and similar to those of untreated patients (data not shown). Thus, it is necessary to perform a prospective study using sequential DBSs from patients diagnosed using newborn screening methods to elucidate the relationship between IDS enzyme activity and enzyme replacement therapy in MPS II.

Finally, our study was a short-term study because patients with MPS II typically exhibit initial clinical features between the ages of 2 and 4 years. Therefore, to determine the clinical utility of our enzyme assay, an extended study will be required to compare IDS enzyme activities of newborns and those exhibiting initial symptoms.

In conclusion, we first evaluated the performance of direct IDS enzyme assay using UPLC-MS/MS with commercially available reagents and found the performance to be generally acceptable. On the basis of our experience in the field of biochemical diagnosis of MPS II (Hunter syndrome), we are going to optimize our assay precisely and apply our novel multiplex newborn screening method for the identification of several LSDs. Thus, we expect that our method will provide a rapid, inexpensive, effective screening tool for the diagnosis of MPS II worldwide. Furthermore, because our next study will be a long-term study, it will be able to provide valuable data on IDS enzyme activity levels in newborns with MPS II and the benefits of early treatment for MPS II in newborns without any symptoms.

REFERENCES

1. Wraith JE. Lysosomal disorders. Semin Neonatol 2002 Feb;7(1):75-83.
2. Staretz-Chacham O, Lang TC, LaMarca ME, Krasnewich D, Sidransky E. Lysosomal storage disorders in the newborn. Pediatrics. 2009 Apr;123(4):1191-207.
3. Parenti G, Wraith EJ, The mucopolysaccharidoses. In: Blau N, Duran M, Gibson KM, Dionisi-Vici C, eds. Physician's Guide to the Diagnosis, Treatment, and Follow-Up of Inherited Metabolic Diseases, 2014th ed. New York: Springer; 2014. p. 449-463.
4. Coutinho MF, Lacerda L, Alves S. Glycosaminoglycan storage disorders: a review. Biochem Res Int. Epub 2012; DOI: 10.1155/2012/471325.
5. Muenzer J, Beck M, Eng CM, Escolar ML, Giugliani R, Guffon NH, et al. Multidisciplinary management of Hunter syndrome. Pediatrics. Epub 2009 Nov 9; DOI: 10.1542/peds.2008-0999.
6. Martin R, Beck M, Eng C, Giugliani R, Harmatz P, Muñoz V, et al. Recognition and diagnosis of mucopolysaccharidosis II (Hunter syndrome). Pediatrics. Epub 2008 Feb; DOI: 10.1542/peds.2007-1350.
7. Froissart R, Da Silva IM, Maire I. Mucopolysaccharidosis type II: an update on mutation spectrum. Acta Paediatr Suppl. 2007 Apr;96(455):71-7.
8. Bach G, Eisenberg F Jr, Cantz M, Neufeld EF. The defect in the

Hunter syndrome: deficiency of sulfoiduronate sulfatase. Proc Natl Acad Sci U S A. 1973 Jul;70(7): 2134-8.

9. Hall CW, Liebaers I, Di Natale P, Neufeld EF. Enzymic diagnosis of the genetic mucopolysaccharide storage disorders. Methods Enzymol. 1978;50:439-56.

10. Hopwood JJ. α-L-Iduronidase, β-D-glucuronidase, and 2-sulfo-L-iduronate 2-sulfatase: preparation and characterization of radioactive substrates from heparin. 1979 Mar;69:203-16.

11. Voznyi YV, Keulemans JL, van Diggelen OP. A fluorimetric enzyme assay for the diagnosis of MPS II (Hunter disease). J Inherit Metab Dis. 2001 Nov;24(6):675-80.

12. Civallero G, Michelin K, de Mari J, Viapiana M, Burin M, Coelho JC, et al. Twelve different enzyme assays on dried-blood filter paper samples for detection of patients with selected inherited lysosomal storage diseases. Clin Chim Acta. 2006 Oct;372(1-2):98-102.

13. Wang D, Wood T, Sadilek M, Scott CR, Turecek F, Gelb MH. Tandem Mass Spectrometry for the Direct Assay of Enzymes in Dried Blood Spots: Application to Newborn Screening for Mucopolysaccharidosis II (Hunter Disease). Clin Chem. 2007 Jan;53(1):137-40.

14. Wolfe BJ, Blanchard S, Sadilek M, Scott CR, Turecek F, Gelb MH. Tandem Mass Spectrometry for the Direct Assay of Lysosomal Enzymes in Dried Blood Spots: Application to Screening Newborns for Mucopolysaccharidosis II (Hunter Syndrome). Anal Chem. 2011 Feb 1;83(3):1152-6.

15. Annesley TM. Ion suppression in mass spectrometry. Clin Chem. 2003 Jul;49(7):1041-4.

16. Han M, Jun SH, Song SH, Park KU, Kim JQ, Song J. Use of Tandem Mass Spectrometry for Newborn Screening of 6 Lysosomal Storage Disorders in a Korean Population. Korean J Lab Med. 2011 October; 31(4): 250–256.

17. Mechtler TP, Metz TF, Müller HG, Ostermann K, Ratschmann R, De Jesus VR, et al. Short-incubation mass spectrometry assay for lysosomal storage disorders in newborn and high-risk population screening. J Chromatogr B Analyt Technol Biomed Life Sci. 2012 Nov 1;908:9-17.

18. Gucciardi A, Legnini E, Di Gangi IM, Corbetta C, Tomanin R, Scarpa M, et al. A column-switching HPLC-MS/MS method for mucopoly saccharidosis type I analysis in a multiplex assay for the simultaneous newborn screening of six lysosomal storage disorders. Biomed Chromatogr. 2014 Aug;28(8):1131-9.

19. Li Y, Scott CR, Chamoles NA, Ghavami A, Pinto BM, Turecek F, et al. Direct multiplex assay of lysosomal enzymes in dried blood spots for newborn screeningomal storage disorders. Clin Chem. 2004 Oct;50(10):1723-4.

20. Metz TF, Mechtler TP, Orsini JJ, Martin M, Shushan B, Herman JL, et al. Simplified newborn screening protocol for lysosomal storage disorders. Clin Chem. 2011 Sep;59(9):1286-94.

국문 초록

서론: 탠덤 질량분석기를 이용하여 상업적으로 구입 가능한 시약들을 가지고 뮤코다당침착증 2 형 (헌터증후군)에 대한 진단에 대한 검사법의 수행능을 평가하였다.

방법: 탠덤 질량분석기 분석에서 iduronate-2-sulfatase 효소 검사의 조건과 과정을 개발하여 효소 검사의 정밀도 및 결과에 대한 dried blood spot(DBS) 양과 반응시간의 영향을 알아보았다. 26 명의 정상 신생아와 2 명의 헌터증후군 환자의 DBS 를 분석하였다.

결과: 검사내, 검사간 정밀도는 각각 7.9-10.5%, 4.8-10.2%를 나타내었다. 생성물의 양은 DBS 개수에 비례하였으나, 1개 이상의 DBS가 사용되었을 때에는 생성물과 DBS 개수 곡선이 편평해지는 것이 관찰되었다. 그리고 반응시간 15시간까지는 생성물의 양은 반응시간과 직선적으로 비례하여 증가하였다. 헌터증후군 환자의 DBS에서 측정한 효소활성은 일관되게 정상 신생아의 것보다 낮은 결과가 관측되었다.

결론: 우리가 개발한 효소활성 검사는 수용할 만한 수행능을

나타내었다. 그리고 우리는 개발한 검사가 리소좀 축적병의 원인이 되는 여러가지 효소 중에서 다섯가지 효소의 활성을 동시에 검사할 수 있는 가능성을 확인할 수 있었다. 본 논문은 상용화된 시약을 이용하여 탠덤 질량분석기에 의한 효소 활성 검사한 최초의 보고이다. 이 검사는 전세계 어디에서나 헌터증후군에 대하여 빠르고, 싸고, 효과적인 선별검사 방법으로 사용될 것이다.

주요어 : 뮤코다당축적증, 헌터증후군, 탠덤 질량분석기

학 번 : 2013-21684