(4), macroglossia (3), umbilical hernia (2), carpal tunnel syndrome (2), heart failure (2), and left ventricular hypertrophy (1).

Urinary glycosaminoglycan (GAG)

All nine evaluable patients had elevated urinary GAG levels at baseline (mean 106.4 mg/g creatinine, approximately 8 times the upper limit of normal); one patient lacked an appropriate baseline value (Table 1). Following idursulfase treatment, urinary GAG levels decreased rapidly within the first three months of treatment and remained low for the remainder of the study (Fig. 2A). There was a statistically significant mean decrease in the urinary GAG level of −79.9 ± 2.2% from baseline to 12 months ($p = 0.004$). All nine evaluable patients showed a >70% decrease in urinary GAG levels and had normal values by the end of the study.

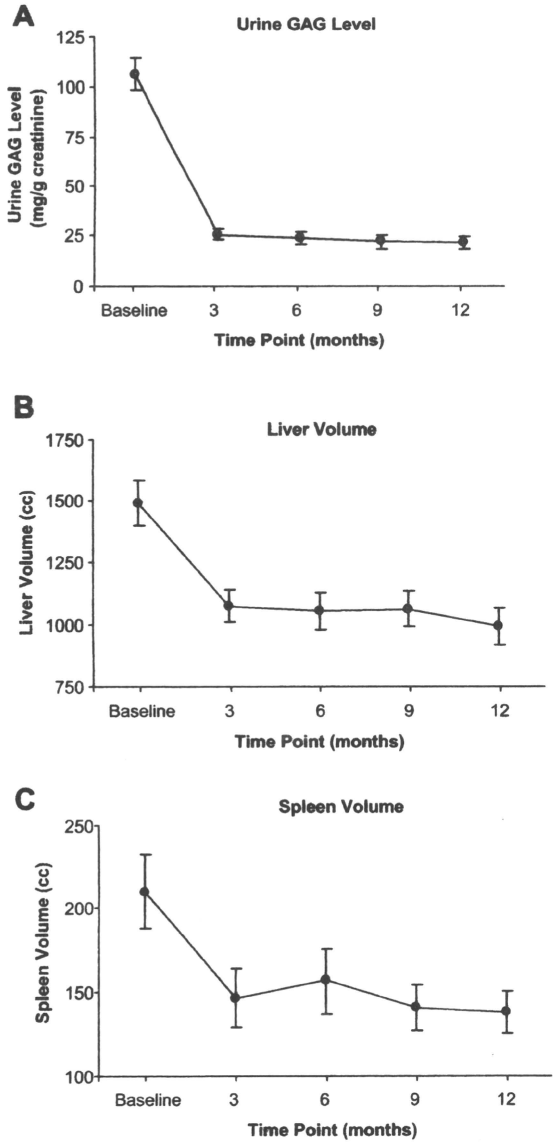

Fig. 2. The effects of idursulfase treatment on lysosomal storage over 12 months. (A) Urinary GAG level. (B) Liver volume. (C) Spleen volume. All changes are reported as mean ± SEM.

Liver and spleen volumes

At baseline, 9 (90%) patients had hepatomegaly (mean 1.3 MN, multiples of normal) and all 10 (100%) patients had splenomegaly (mean 2.4 MN) by CT. After 12 months of treatment, mean liver volume decreased by −33.2 ± 4.0% and mean spleen volume decreased by −31.0 ± 5.5% (Fig. 2B and C; Table 1), and both changes were statistically significant ($p = 0.002$). Most of the reductions occurred within the first three months of treatment. By the end of the study, all patients had liver volumes within the normal range and spleen volumes that were <2 MN, demonstrating efficient reduction of lysosomal GAG storage.

6-Minute Walk Test (6MWT)

At baseline, the mean 6MWT distance was 286.0 m for the seven patients who could perform the test (Table 1). All but one patient walked <399 m, the lower limit of normal for healthy adult men in the United States [24]. Three patients could not perform the 6MWT: one patient broke his leg just prior to the start of the study; one patient was wheelchair-bound secondary to shortness of breath and muscle weakness; and one patient was obese and could only walk a few steps with assistance. By the end of the study, the mean 6MWT distance had increased by 54.5 ± 27.0 m (Fig. 3A). This change represents a relative increase of 37.4%, and included one patient whose 6MWT distance increased by 131%. Four patients (57%) showed a clinically meaningful improvement of ⩾54 m [25], while the one patient with a normal 6MWT at baseline showed a decline (−71 m).

Percent predicted forced vital capacity (FVC)

Nine patients underwent spirometry at baseline and all showed a restrictive lung disease pattern: three were classified as having a severe defect (<50% predicted FVC) and five had a very severe defect (<34% predicted FVC) [26]. At baseline, mean percent predicted FVC was 39.9% (Table 1), and after 12 months it increased by 3.8 ± 2.8 percentage points (Fig. 3B). This improvement corresponds to a relative increase of 15.0% over baseline, which is considered clinically meaningful (⩾15% relative change) [25] and was achieved by four (44%) patients. Similarly, mean FVC increased by 16.3% over the baseline of 1.4 L. The mean forced expiratory volume in 1 s (FEV_1):FVC ratio remained unchanged at 0.70 during the study.

Cardiac

All patients had valve disease that remained stable during the study. The mean ejection fraction (EF) was normal at baseline and showed little change over 12 months (67.0–64.3%, change of −2.8 ± 2.5%) (Table 1). One patient with pre-existing cardiac failure showed gradual worsening during the study (EF 27–14%). At baseline, mean LVMI was slightly elevated at 139.9 g/m^2 (normal <131 g/m^2), and 50% (3/6) of evaluable patients had an elevated LVMI. After 12 months, mean LVMI decreased by −12.4%, with four patients showing a clinically meaningful improvement of >10% [27]. The patient with the largest LMVI at baseline showed a further increase (254.1–312.9 g/m^2).

Joint range of motion

Fig. 4 and Table 1 show the changes in joint range of motion observed during the study. At baseline, patients had significant joint contractures involving the shoulder (flexion, extension, and abduction), knee (flexion and extension), hip flexion and extension), and elbow (flexion and extension). Following 12 months of treatment, several joints showed increased range of motion, including mean

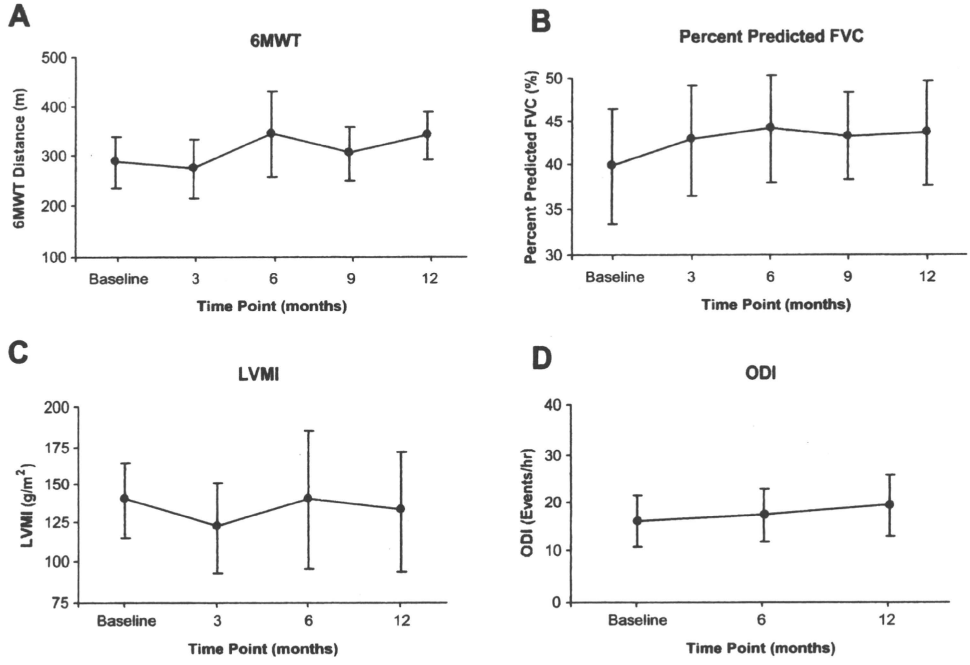

Fig. 3. The effects of idursulfase treatment on clinical endpoints over 12 months. (A) 6-Minute Walk Test. (B) % Predicted forced Vital Capacity. (C) Left Ventricular Mass Index. (D) Oxygen Desaturation Index. All changes are reported as mean ± SEM.

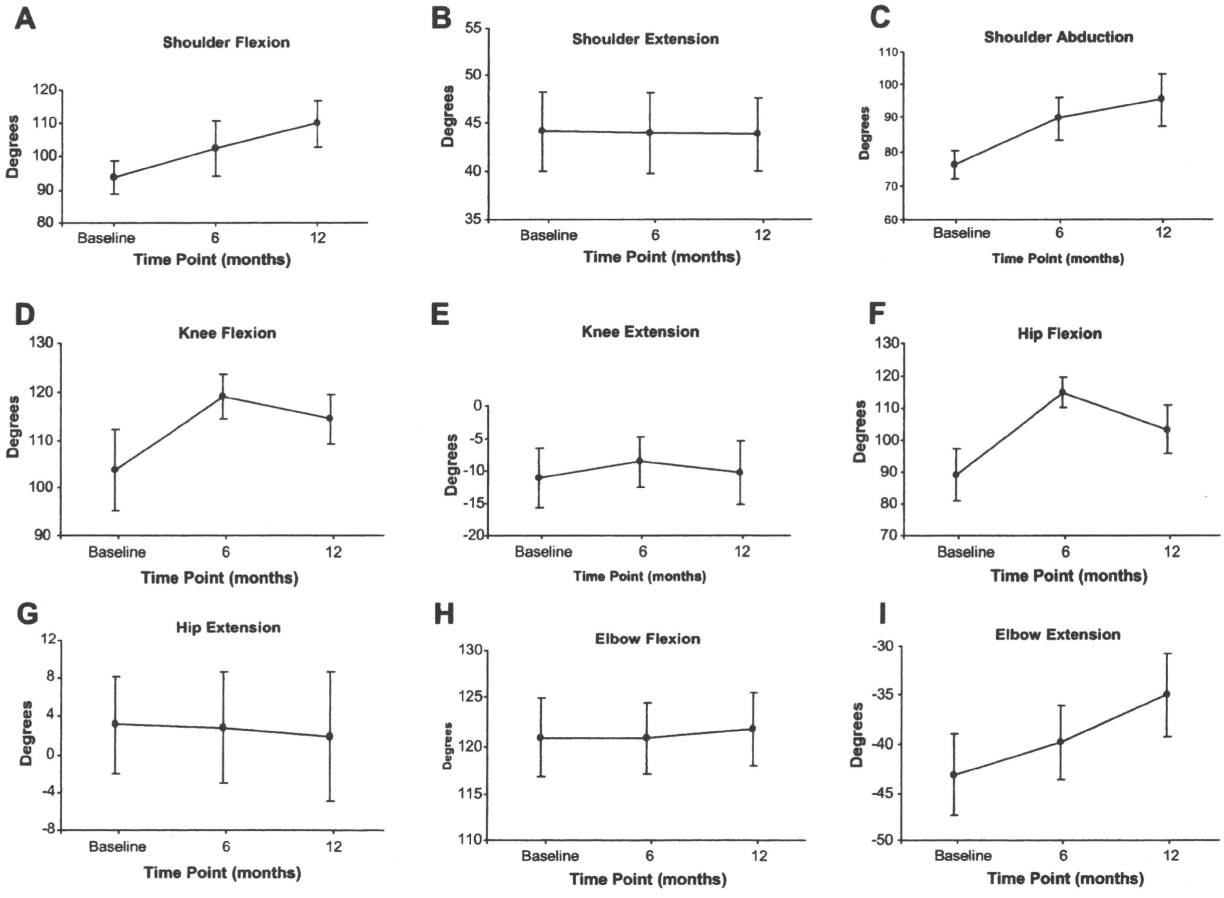

Fig. 4. The effects of idursulfase treatment on joint range of motion over 12 months. (A) Shoulder flexion. (B) Shoulder extension. (C) Shoulder abduction. (D) Knee flexion. (E) Knee extension. (F) Hip flexion. (G) Hip extension. (H) Elbow flexion. (I) Elbow extension. All changes are reported as mean ± SEM.

shoulder flexion (15.0 ± 7.3 degrees), shoulder abduction (19.0 ± 8.8 degrees), knee flexion (10.7 ± 10.3 degrees), hip flexion (14.2 ± 5.1 degrees; $p = 0.031$), and elbow extension (8.1 ± 3.4 degrees). However, most of the changes did not achieve statistical significance. Shoulder extension (−0.3 ± 4.1 degrees), elbow flexion (0.9 ± 2.5 degrees), knee extension (0.8 ± 2.5 degrees), and hip extension (−1.3 ± 1.8 degrees) showed little change during the study. Fig. 1 shows a 23 year-old study patient with severely limited shoulder range of motion (abduction and flexion), which improved following one year treatment with idursulfase.

Oxygen desaturation index (ODI)

At baseline, the mean oxygen desaturation index (ODI) was 18.5 events/h ($n = 9$), which is moderately abnormal [23]. Three patients had a normal ODI (<5 events/h), two had a mildly abnormal ODI (5–15 events/h), and four had a moderately to severely abnormal ODI (>15 events/h). During the study, the mean ODI increased by 3.9 ± 3.5 events/h, which was largely due to a single patient with an increase of 26.8 events/h. The other seven patients had stable ODI values (changes ⩽ 10 events/h).

Safety

Idursulfase was well-tolerated over the course of the study. Adverse events were mainly mild, unrelated, and attributable to expected symptoms of MPS II disease. Fifty percent (5/10) of patients experienced a total of 11 drug-related adverse events. Urticaria was the most frequent event (five events in two patients), followed by erythema (two events in the same patient). Similarly, 50% (5/10) of patients experienced infusion-related reactions (i.e. adverse events assessed as drug-related and occurring within 24 h of the infusion). The highest patient incidence involved skin reactions, i.e. urticaria and erythema (three patients each), while dyspnea, abdominal pain, and vasovagal syncope also were observed in one patient each. Except for one patient who experienced several episodes of urticaria between 9 and 12 months, the other four patients had infusion-related reactions only once or twice during the first three months of treatment. Management of infusion-related reactions included antihistamine therapy and temporary interruption of the infusion, and all events were followed by a successful patient recovery. There were no clinical laboratory abnormalities reported as related to idursulfase.

Two patients experienced serious adverse events, including one death, in the study. A 26 year-old male experienced an infusion-related reaction involving diffuse urticaria, flushing, and numbness of the tongue 1 h after initiation of the fifth infusion. The patient was pre-medicated with antihistamines without further events. A 42 year-old male had an infusion-related reaction reported by the investigator as vasovagal syncope, which consisted of hypotension, vomiting, weak pulse, and decreased consciousness and occurred 30 min into the first infusion. Subsequent infusions were preceded by corticosteroid pre-medication administration without further infusion-related reactions. The patient had a history of cardiac valve incompetence and cardiac failure requiring medications, including furosemide. Later in the study, he experienced an increase in leg edema secondary to worsening congestive heart failure. He was depressed and attempted suicide by drug overdose (not idursulfase). Upon arrival at the hospital, the patient went into cardiac arrest. Subsequent resuscitation measures were unsuccessful, and he died due to hypoxic encephalopathy, pneumonia and renal failure.

Antibodies

Anti-idursulfase IgG antibodies were detected in 60% (6/10) of patients, two of who became seronegative later in the study. No IgE antibodies were detected in patients who underwent testing for infusion-related reactions. The mean reductions in urinary GAG levels did not differ between patients who were seropositive at any time (−80.9% ± 3.8%; $n = 5$) and those who remained seronegative throughout the study (−78.6% ± 1.8%; $n = 4$). Although hypersensitive reactions or infusion-related adverse reactions tended to occur in the antibody-positive patients (four antibody-positive patients versus one antibody-negative patient), there was no correlation between the presence of antibodies and other adverse events. Furthermore, the frequency of hypersensitivity reactions did not correlate with antibody titer.

Discussion

The most remarkable difference between this and previous clinical studies of idursulfase [19,20] relates to the patient demographics and characteristics. The purpose of the JET study was to provide access to treatment for the most seriously ill MPS II patients while awaiting regulatory approval of idursulfase in Japan, which occurred in October 2007. Patients in the JET study had a mean age of 30.1 years, all were Japanese, and all were seriously ill (mean percent predicted FVC 39.9% and mean 6MWT distance 286.0 m). By comparison, MPS II patients in the Phase 1/2 and Phase 2/3 studies of idursulfase were younger (mean ages 13.9 years and 14.2 years), predominantly Caucasian (100% and 83%, respectively), and less severely affected (mean percent predicted FVC 55.1% and 55.4%; mean 6MWT distance 397 m and 395 m) [19,20]. Despite these patient differences, the JET study has shown that idursulfase is a safe and effective (Table 1) treatment for Japanese patients with MPS II and its risk–benefit profile is similar to that reported in previous studies.

In this study, idursulfase efficiently reduced GAG storage, as evidenced by the statistically significant reductions in urinary GAG levels ($p = 0.004$) and hepatosplenomegaly ($p = 0.002$) (Fig. 2; Table 1). These pharmacodynamic changes appeared to translate into clinical benefit, as evidenced by trends towards improvement in functional capacity (mean 54.5 m increase in 6MWT), respiratory function (mean 15.0% relative increase in percent predicted FVC), joint range of motion (mean increases ranging from 8.1–19.0 degrees for several joints), and LVMI (mean −12.4% decrease). Cardiac EF and valve disease remained mostly stable, although one patient with severe congestive heart failure showed progressive worsening and one patient with a greatly elevated LVMI showed a further increase. The mean ODI increased slightly by 3.9 events/h, but importantly 89% (8/9) of patients showed no clinically significant changes.

The safety profile of idursulfase in the JET study was similar to that of previous studies with no new or unexpected adverse events despite the older and more seriously ill patient population. Most adverse events were considered by investigators to be disease-related and unrelated to idursulfase. The most common drug-related adverse events were infusion-related reactions, occurring in 50% of patients. The most common infusion-related reactions were skin reactions consisting of urticaria and erythema. There were two related serious adverse events that occurred during the infusions—one involving urticaria, flushing, and numbness of the tongue, and the other involving vasovagal syncope. The one patient death was attributed to suicide from a drug overdose and was not related to idursulfase.

MPS II is a progressive and debilitating multisystem disease that is associated with a shortened lifespan, primarily from cardiorespiratory compromise [28]. Therefore, it is noteworthy that in this one-year study, cardiac and respiratory functions were improved or stable in most patients. Decreasing lung volumes are known to be associated with increased morbidity and mortality [26];

given the low percent predicted FVC values at baseline in study patients (mean 39.9%), a relative increase of 15% is of particular importance. The American Thoracic Society defines a >15% relative change in FVC occurring over a one-year period as being clinically meaningful [26]. Similarly, the 54.5 m mean increase in 6MWT distance also is considered to be a clinically meaningful improvement, based on a study of adult men with chronic obstructive pulmonary disease [25]. The 6MWT is a sub-maximal exercise test that is a composite assessment of cardiac, respiratory, and musculoskeletal function. Because all three of these organ systems are involved in the MPS disorders, walking tests have been widely used as primary efficacy endpoints in clinical trials of enzyme replacement therapy for other MPS disorders, including MPS I [29,30] and MPS VI [31].

We observed no evidence for an effect of race on immunogenicity or safety. IgG antibodies were detected in 60% (6/10) of patients treated with idursulfase, which is similar to the 49.6% rate seen in the Phase 2/3 study that enrolled predominantly Caucasian and other non-Asian patients [20]. In addition, the adverse event profile was similar in all respects; infusion-related reactions occurred in 50% of patients in the current study compared to 69% of patients receiving weekly idursulfase in the Phase 2/3 study [20].

Limitations of this study include its open-label treatment, lack of control group, and small sample size. Other aspects of the study design, however, including the treatment dose and regimen, study duration, and efficacy and safety assessments were identical or very similar to those used in the Phase 2/3 study [20]. A placebo effect in this study cannot be excluded, especially for effort-dependent assessments such as the 6MWT and active joint range of motion. Nevertheless, the magnitude of change in the 6MWT distance was similar to those observed in previous studies of idursulfase [19,20]. Determination of FVC by spirometry is less susceptible to a placebo effect given the requirement for test–retest reproducibility at each assessment [21]. This study enrolled only 10 patients, which may not have had sufficient power to detect a statistically significant clinical response even if clinical improvements were present. On the other hand, the biomarkers of lysosomal GAG clearance, i.e. liver and spleen volumes and urinary GAG level, did have sufficiently large effect sizes (change/standard deviation of change) to show statistically significant differences. Finally, the study involved only adult males, all of whom had a substantial pre-existing disease burden. This study shows that many disease features of seriously ill patients, including diminished cardiorespiratory function, restricted joint range of motion, and hepatosplenomegaly can improve with idursulfase treatment. An even better response is expected in young children prior to final organ maturation and the development of chronic tissue damage. In this regard, a study in MPS II patients $\leqslant$5 years of age is underway.

Conclusions

Idursulfase was generally well-tolerated and produced clinical improvements in adult Japanese patients with attenuated MPS II treated with the labeled dose, 0.5 mg/kg administered intravenously once weekly. Treatment with idursulfase also resulted in substantial reductions in hepatosplenomegaly and urinary GAG excretion, indicating efficient clearance of lysosomal GAG. The safety profile and immunogenicity of idursulfase appear to be similar between Japanese and previously studied Caucasian patients.

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References

[1] E.F. Neufeld, J. Muenzer, The mucopolysaccharidoses, in: C.R. Scriver (Ed.), The Metabolic and Molecular Bases of Inherited Disease, McGraw Hill, New York, 2001, pp. 3421–3452.
[2] R. Martin, M. Beck, C. Eng, R. Giugliani, P. Harmatz, V. Munoz, J. Muenzer, Recognition and diagnosis of mucopolysaccharidosis II (Hunter syndrome), Pediatrics 121 (2008) e377–386.
[3] J.E. Wraith, Enzyme replacement therapy with idursulfase in patients with mucopolysaccharidosis type II, Acta. Paediatr. Suppl. 97 (2008) 76–78.
[4] W. Lissens, S. Seneca, I. Liebaers, Molecular analysis in 23 Hunter disease families, J. Inherit. Metab. Dis. 20 (1997) 453–456.
[5] C.H. Kim, H.Z. Hwang, S.M. Song, K.H. Paik, E.K. Kwon, K.B. Moon, J.H. Yoon, C.K. Han, D.K. Jin, Mutational spectrum of the iduronate 2 sulfatase gene in 25 unrelated Korean Hunter syndrome patients: identification of 13 novel mutations, Hum. Mutat. 21 (2003) 449–450.
[6] K.M. Timms, M.L. Bondeson, M.A. Ansari-Lari, K. Lagerstedt, D.M. Muzny, S.P. Dugan-Rocha, D.L. Nelson, U. Pettersson, R.A. Gibbs, Molecular and phenotypic variation in patients with severe Hunter syndrome, Hum. Mol. Genet. 6 (1997) 479–486.
[7] E. Vafiadaki, A. Cooper, L.E. Heptinstall, C.E. Hatton, M. Thornley, J.E. Wraith, Mutation analysis in 57 unrelated patients with MPS II (Hunter's disease), Arch. Dis. Child. 79 (1998) 237–241.
[8] P. Li, A.B. Bellows, J.N. Thompson, Molecular basis of iduronate-2-sulphatase gene mutations in patients with mucopolysaccharidosis type II (Hunter syndrome), J. Med. Genet. 36 (1999) 21–27.
[9] P.J. Wilson, G.K. Suthers, D.F. Callen, E. Baker, P.V. Nelson, A. Cooper, J.E. Wraith, G.R. Sutherland, C.P. Morris, J.J. Hopwood, Frequent deletions at Xq28 indicate genetic heterogeneity in Hunter syndrome, Hum. Genet. 86 (1991) 505–508.
[10] Y. Yamada, S. Tomatsu, K. Sukegawa, Y. Suzuki, N. Kondo, J.J. Hopwood, T. Orii, Mucopolysaccharidosis type II (Hunter disease): 13 gene mutations in 52 Japanese patients and carrier detection in four families, Hum. Genet. 92 (1993) 110–114.
[11] T. Ochiai, Y. Suzuki, T. Kato, H. Shichino, M. Chin, H. Mugishima, T. Orii, Natural history of extensive Mongolian spots in Mucopolysaccharidosis type II (Hunter syndrome): a survey among 52 Japanese patients, J. Eur. Acad. Dermatol. Venereol. 21 (2007) 1082–1085.
[12] P.J. Meikle, J.J. Hopwood, A.E. Claque, W.F. Carey, Prevalence of lysosomal storage disorders, JAMA. 281 (1999) 249–254.
[13] B.J. Poorthuis, R.A. Wevers, W.J. Kleijer, J.E. Groener, J.G. de Jong, S. van Weely, K.E. Niezen-Koning, O.P. van Diggelen, The frequency of lysosomal storage diseases in The Netherlands, Hum. Genet. 105 (1999) 151–156.
[14] R. Pinto, C. Caseiro, M. Lemos, L. Lopes, A. Fontes, H. Ribeiro, E. Pinto, E. Silva, S. Rocha, A. Marcao, I. Ribeiro, L. Lacerda, G. Ribeiro, O. Amaral, M.C. Sa Miranda, Prevalence of lysosomal storage diseases in Portugal, Eur. J. Hum. Genet. 12 (2004) 87–92.
[15] F. Baehner, C. Schmiedeskamp, F. Krummenauer, E. Miebach, M. Bajbouj, C. Whybra, A. Kohlschutter, C. Kampmann, M. Beck, Cumulative incidence rates of the mucopolysaccharidoses in Germany, J. Inherit. Metab. Dis. 28 (2005) 1011–1017.
[16] A. Vellodi, E. Young, A. Cooper, V. Lidchi, B. Winchester, J.E. Wraith, Long-term follow-up following bone marrow transplantation for Hunter disease, J. Inherit. Metab. Dis. 22 (1999) 638–648.
[17] E.M. Kaye, Lysosomal storage diseases, Curr. Treat. Options Neurol. 3 (2001) 249–256.
[18] J. Muenzer, J.C. Lamsa, A. Garcia, J. Dacosta, J. Garcia, D.A. Treco, Enzyme replacement therapy in mucopolysaccharidosis type II (Hunter syndrome): a preliminary report, Acta. Paediatr. Suppl. 91 (2002) 98–99.
[19] J. Muenzer, M. Gucsavas-Calikoglu, S.E. McCandless, T.J. Schuetz, A. Kimura, A phase I/II clinical study of enzyme replacement therapy with idursulfase in mucopolysaccharidosis II (Hunter syndrome), Mol. Genet. Metab. 8 (2007) 329–337.
[20] J. Muenzer, J.E. Wraith, M. Beck, R. Giugliani, P. Harmatz, C.M. Eng, A. Vellodi, R. Martin, U. Ramaswami, M. Gucsavas-Calikoglu, S. Vijayaraghavan, S. Wendt, A.C. Puga, B. Ulbrich, M. Shinawi, M. Cleary, D. Piper, A.M. Conway, A. Kimura, A phase II/III clinical study of enzyme replacement therapy with idursulfase in mucopolysaccharidosis II (Hunter syndrome), Genet. Med. 8 (2006) 465–473.
[21] Standardization of spirometry. 1994 update. American Thoracic Society, Am. J. Respir. Crit. Care Med. 152 (1995) 1107–1136.
[22] ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, ATS statement: guidelines for the six-minute walk test, Am. J. Respir. Crit. Care Med. 166 (2002) 111–117.
[23] I. Fietze, K. Dingli, K. Diefenbach, N.J. Douglas, M. Glos, M. Tallafuss, W. Terhalle, C. Witt, Night-to-night variation of the oxygen desaturation index in sleep apnoea syndrome, Eur. Resp. J. 24 (2004) 987–993.
[24] P.L. Enright, D.L. Sherrill, Reference equations for the six-minute walk in healthy adults, Am. J. Respir. Crit. Care Med. 158 (1998) 1384–1387.
[25] D.A. Redelmeier, A.M. Bayoumi, R.S. Goldstein, G.H. Guyatt, Interpreting small differences in functional status: the Six Minute Walk test in chronic lung disease patients, Am. J. Respir. Crit. Care Med. 155 (1997) 1278–1282.

[26] Lung function testing: selection of reference values and interpretative strategies. American Thoracic Society. Am. J. Rev. Respir. Dis. 144 (1991) 1202–1218. .

[27] G. de Simone, M.L. Muiesan, A. Ganau, C. Longhini, P. Verdecchia, V. Palmieri, E. Agabiti-Rosei, G. Mancia, Reliability and limitations of echocardiographic measurement of left ventricular mass for risk stratification and follow-up in single patients: the RES trial, J. Hypertens. 17 (1999) 1955–1963.

[28] J.E. Wraith, M.Scarpa, M. Beck, O.A. Bodamer, L. De Meirleir, N. Guffon, A.M. Lund, G. Malm, A.T. Van der Ploeg, J. Zeman, Mucopolysaccharidosis type II (Hunter syndrome): a clinical review and recommendations for treatment in the era of enzyme replacement therapy, Eur. J. Pediatr. 167 (2008) 267–277 (Epub 2007 Nov 23).

[29] J.E. Wraith, L.A. Clarke, M. Beck, E.H. Kolodny, G.M. Pastores, J. Muenzer, D.M. Rapoport, K.I. Berger, S.J. Swiedler, E.D. Kakkis, T. Braakman, E. Chadbourne, K. Walton-Bowen, G.F. Cox, Enzyme replacement therapy for mucopolysaccharidosis I: a randomized, double-blinded, placebo-controlled, multinational study of recombinant human alpha-L-iduronidase (laronidase), J. Pediatr. 144 (2004) 581–588.

[30] L.A. Clarke, J.E. Wraith, M. Beck, E.H. Kolodny, G.M. Pastores, J. Muenzer, D.M. Rapoport, K.I. Berger, M. Sidman, E.D. Kakkis, G.F. Cox, Long-term efficacy and safety of laronidase in the treatment of mucopolysaccharidosis I, Pediatrics 123 (2009) 229–240.

[31] P. Harmatz, R. Giugliani, I. Schwartz, N. Guffon, E.L. Teles, M.C. Miranda, J.E. Wraith, M. Beck, L. Arash, M. Scarpa, Z.F. Yu, J. Wittes, K.I. Berger, M.S. Newman, A.M. Lowe, E. Kakkis, S.J. Swiedler; MPS VI Phase 3 Study Group, Enzyme replacement therapy for mucopolysaccharidosis VI: a phase 3, randomized, double-blind, placebo-controlled, multinational study of recombinant human *N*-acetylgalactosamine 4-sulfatase (recombinant human arylsulfatase B or rhASB) and follow-on, open-label extension study, J. Pediatr. 148 (2006) 533–539.

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CASE REPORT

Enzyme replacement therapy in a patient with Fabry disease and the development of IgE antibodies against agalsidase beta but not agalsidase alpha

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Abstract Fabry disease is an X-linked inherited lysosomal storage disorder caused by an inborn deficiency of the enzyme α-galactosidase A. Enzyme replacement therapy (ERT) with agalsidase alpha or beta isozymes is an effective treatment. Cross-reactivity of immunoglobulin G (IgG) antibodies with agalsidase alpha and beta has been reported, but no such reaction has been recorded for IgE antibodies. We present the case of a patient with Fabry disease who developed antiagalsidase beta IgE antibodies without cross-reactivity to agalsidase alpha. A 17-year-old boy with Fabry disease had suffered from severe atopic dermatitis since infancy, and he complained for several years of peripheral pain during the summer months and when exercising. Fabry disease was confirmed by family history and a positive enzyme test, and ERT was commenced. Following infusion of agalsidase beta (1.0 mg/kg), the patient complained of a high temperature in his hands and feet, and purulent eczema developed. The infusion dose was reduced to 0.2 mg/kg, but the hyperthermia did not change, although its duration decreased. After three infusions, eosinophilia developed (9.4%; 573 cells/μl blood) and remained unresolved after four infusions with agalsidase beta. Treatment with this enzyme was discontinued, and agalsidase alpha (0.2 mg/kg) started. This produced immediate resolution of the eosinophilia, which has been maintained during follow-up. In conclusion, this patient developed IgE antibodies against agalsidase beta, which demonstrated no cross-reactivity to agalsidase alpha. These findings emphasize the importance of analyzing IgE antibodies against both enzymes when patients exhibit severe infusion-related events.

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Introduction

Fabry disease is an X-linked inherited lysosomal storage disorder caused by a deficiency of the enzyme α-galactosidase A due to mutations in the *GLA* gene (Nagueh 2003; Schaefer et al 2005). It is a rare disease, with an incidence estimated to be between 1:40,000 and 1:117,000 worldwide (Mehta et al 2004), and there are no known ethnic predispositions (Zarate and Hopkin 2008). Alpha galactosidase deficiency results in accumulation of globotriaosylceramide and other neutral glycolipids, which eventually lead to cellular abnormalities, triggering inflammation and fibrosis in various organs, including the skin, kidney, nervous system, and heart (Mehta and Ginsberg 2005; Zarate and Hopkin 2008). Patients with the classic form suffer from acroparesthesias, hypohidrosis, angiokeratomas, corneal opacities, cerebrovascular ischemia/stroke, cardiac disorders, and renal dysfunction (Nagueh 2003). Life expectancy is reduced by approximately 20 years in untreated men and by about 15 years in untreated women (MacDermot et al 2001; Fabry 2002). Female mortality is usually due to cardiac failure, whereas renal failure is the most frequent cause of death in men. In recent years, organ transplantation and dialysis have increased the longevity of Fabry disease patients and, as a consequence, the preva-

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Table 1 Plasma α-galactosidase activity in the patient, the patient's mother, and a control individual

	α-galactosidase activity in plasma (nmol/ml per 2 h)
Patient	0.41
Patient's mother	5.48
Control	11.49

lence of cardiac and cerebrovascular events is increasing (Linhart et al 2007; Zarate and Hopkin 2008). Prior to 2001, treatment of Fabry disease was largely for symptom control. However, the introduction of enzyme replacement therapy (ERT) using one of two available enzyme preparations, agalsidase alpha or beta, was an important milestone in managing this rare condition (Lidove et al 2007). These authors undertook a systematic review of the two enzymes and identified 11 trials in Fabry disease. Both agalsidase alpha and beta produced significant clinical benefits on the heart, kidneys, nervous system, and quality of life. Based upon data presented to the European Agency for the Evaluation of Medicinal Products, Lidove and colleagues (2007) summarized the principal characteristics of the two enzymes:

- Agalsidase alpha: produced from a human fibroblast cell line that has undergone human glycosylation; frequency of immunoglobulin G (IgG) antibodies approximately 55%, with no IgE response; about 10% of patients develop an infusion reaction; usual dosage is 0.2 mg/kg every 2 weeks infused over 40 min.
- Agalsidase beta: produced from Chinese hamster ovary cells that have undergone nonhuman glycosylation; frequency of IgG antibodies approximately 80%, and an IgE response has been documented; about 50% of patients develop an infusion reaction; usual dosage is 1 mg/kg every 2 weeks infused over 2–4 h.

To date, more than 2,000 Fabry disease patients have been treated with either agalsidase alpha or beta (Hoffman 2009). Both enzymes induced an IgG response in Fabry patients, but antibody formation did not influence clinical efficacy or outcomes (Eng et al 2001). Cross-reactivity of IgG antibodies against agalsidase alpha and beta has been observed in Fabry patients (Linthorst et al 2004). In contrast, an IgE antibody response to ERT has only been reported for patients treated with agalsidase beta (Banikazemi et al. 2007; Bodensteiner et al 2008), not in those receiving agalsidase alpha (Schiffmann et al 2006; Pastores et al 2007; Tesmoingt et al. 2009). Consequently, no cross-reactivity of IgE antibodies between agalsidase beta and alpha has been reported to date.

In this article, we present the case of a teenager with Fabry disease who required ERT and in whom we initiated therapy with agalsidase beta. He immediately developed IgE antibodies and a number of adverse effects to agalsidase beta, and we subsequently managed this individual by first modifying the dosage of agalsidase beta and finally by converting to agalsidase alpha. Herein, we report the clinical and antibody responses documented at each stage of his treatment.

Clinical presentation

The 17-year-old boy had experienced severe atopic dermatitis since childhood, and had been suffering peripheral pain during the summer months and also when exercising since his teenage years. Fabry disease was diagnosed as a result

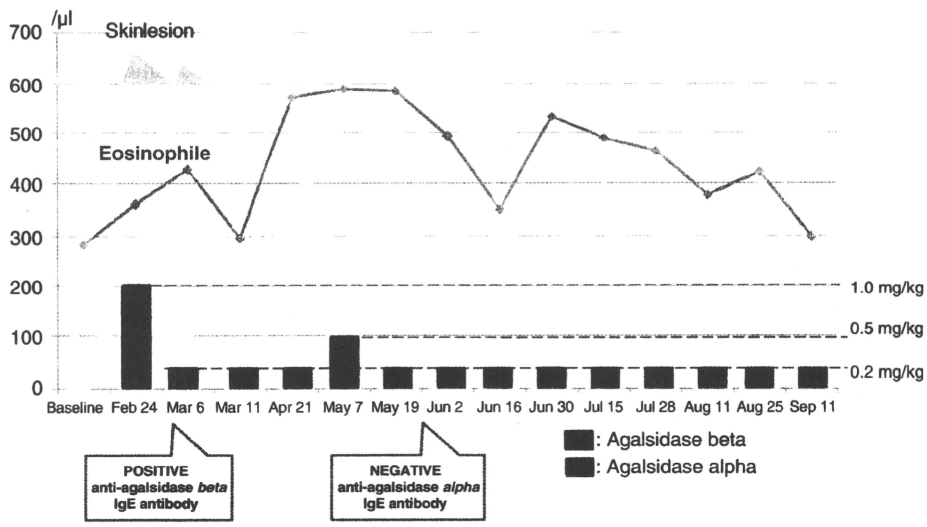

Fig. 1 Clinical course of enzyme replacement therapy

of a family history and following enzymatic analysis. The patient's grandmother and uncle both died from heart failure when they were middle aged (50–60 years old). The uncle had received ERT for the 2 years prior to his death. The patient's mother has suffered from peripheral pain since childhood and with cardiomyopathy and left ventricular hypertrophy since her 40s. She had been receiving ERT for 1 year. The patient's plasma α-galactosidase activity was 0.41 nmol/ml per 2 h compared with 5.48 nmol/ml per 2 h for his mother and 11.49 nmol/ml per 2 h in a control individual (Table 1). The patient exhibited positive serological allergen-specific IgE antibody test [radioallergosorbent test (RAST)] results to eggs and milk. Based on clinical presentation and family history, the patient was approved for treatment with ERT, and agalsidase beta was chosen. Routine follow-up included a full clinical assessment, immunological evaluation (determination of IgG and IgE antibody titers), hematological analysis, biochemical examination of liver and kidney function, urinalysis, investigation of possible adverse effects, and histology as deemed appropriate by the attending physician. Prior to commencing ERT, the patient had normal IgE antibody levels, and eosinophilia was not present.

Following the first infusion of agalsidase beta (1.0 mg/kg), the patient was positive for antiagalsidase beta IgE antibodies (Fig. 1). ERT-induced hyperthermia occurred in his hands and feet and persisted overnight. Two days following administration, purulent eczema developed at the areas of high temperature (Figs. 2a, b). The dose of agalsidase beta was reduced to 0.2 mg/kg for the second infusion (Fig. 1). The patient reported that the high temperature in his hands and feet was a concern but that it was less severe. At this point, he remained positive for the IgE antibody against agalsidase beta. ERT was changed to agalsidase alpha[1] infusion at 0.2 mg/kg for the third infusion, and hyperthermia was attenuated. When agalsidase beta was reintroduced for the fourth infusion at 0.2 mg/kg, the patient did not report any symptoms. However, eosinophilia appeared at 9.4% (573 cells /μl blood) (Fig. 1). Therefore, the dose of agalsidase beta was increased to 0.5 mg/kg for the fifth treatment. The patient

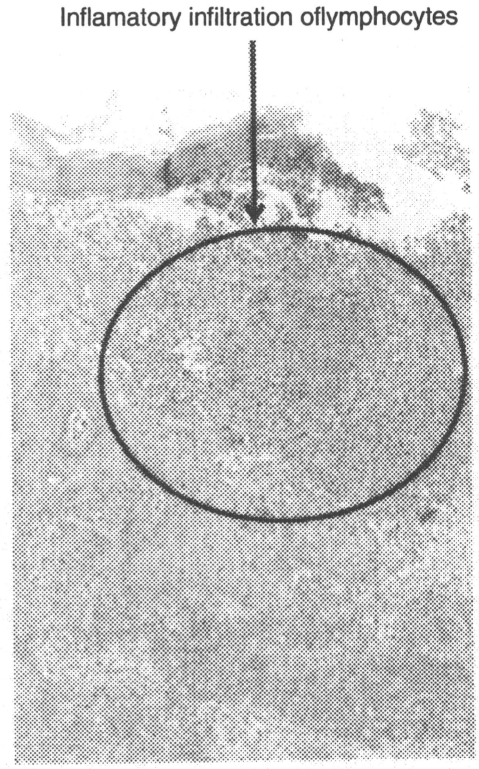

Fig. 3 Inflammatory infiltration of lymphocytes following infusion of agalsidase beta (0.5 mg/kg)

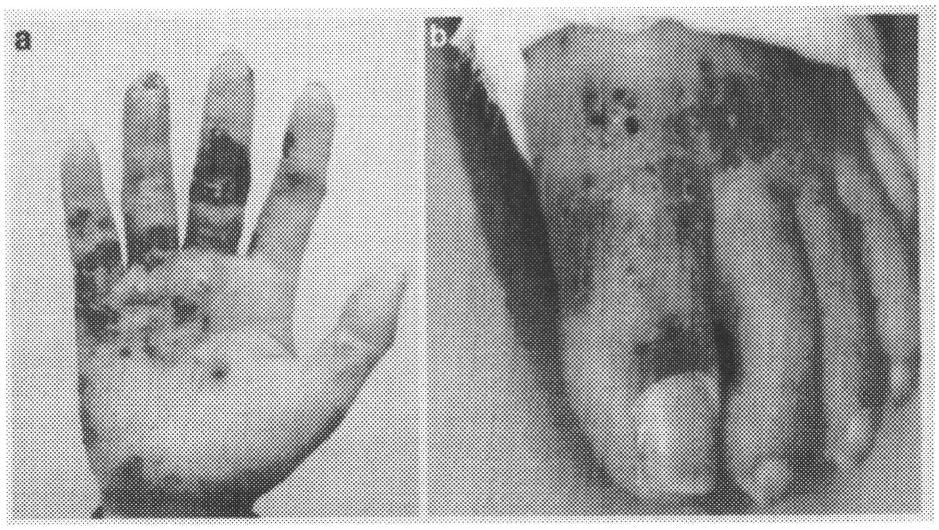

Fig. 2 Eczema on the hands (**a**) and feet (**b**) following infusion of agalsidase beta (1.0 mg/kg)

did not complain of hyperthermia, but eosinophilia continued, as confirmed by histological examination of biopsied skin that showed inflammatory infiltration of lymphocytes (Fig. 3).

The decision was made to discontinue agalsidase beta and replace it with agalsidase alpha 0.2 mg/kg for the sixth infusion. The patient was negative for the IgE antibody against agalsidase alpha at this point. The change in ERT resulted in resolution of the eosinophilia, which was reduced to normal levels, and this improvement was maintained during follow-up to the last observation point (11 September 2009) (Fig. 1). At this time, the patient was negative for both alpha and beta isoforms of the IgG antibody.

Discussion

Cross-reactivity of IgG antibodies against agalsidase alpha and beta is a common finding in many patients with Fabry disease (Linthorst et al 2004). However, a similar cross-reactivity of IgE antibodies between the two enzymes has not been documented. Our results demonstrate that in this 17-year-old boy, IgE antibodies developed that reacted specifically with agalsidase beta and resulted in eosinophilia and adverse clinical symptoms such as purulent eczema on the feet and hands. An IgE antibody response to agalsidase beta has been reported previously (Banikazemi et al 2007; Bodensteiner et al 2008). The latter group found that agalsidase beta could be successfully reinstated following the IgE response and long-term therapy maintained. Our results augment previous data, as in this patient, initial treatment and rechallenge with agalsidase beta clearly provoked an IgE antibody response, whereas treatment and rechallenge with agalsidase alpha produced no such effect. Generally speaking adverse infusion-related events are uncommon, and agalsidase beta is well tolerated (Keating and Simpson 2007; Ramaswami et al 2007); however, it is important to determine the IgE antibody response to both agalsidase alpha and beta in patients with Fabry disease. Further investigation is also required to fully elucidate the cross-reactivity potential of the IgE-mediated antibody response in order to optimize treatment for the individual patient.

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References

Banikazemi M, Bultas J, Waldek S et al (2007) Agalsidase-beta therapy for advanced Fabry disease: a randomized trial. Ann Intern Med 146:77–86

Bodensteiner D, Scott CR, Sims KB et al (2008) Successful reinstitution of agalsidase beta therapy in Fabry disease patients with previous IgE-antibody or skin-test reactivity to the recombinant enzyme. Genet Med 10:353–358

Eng CM, Guffon N, Wilcox WR et al (2001) Safety and efficacy of recombinant human alpha-galactosidase A–replacement therapy in Fabry's disease. N Engl J Med 345:9–16

Fabry H (2002) Angiokeratoma corporis diffusum- Fabry disease: historical review from the original description to the introduction of enzyme replacement therapy. Acta Paediatr Suppl 91(439):3–5

Hoffman B (2009) Fabry disease: recent advances in pathology, diagnosis, treatment and monitoring. Orphanet J Rare Dis 4:21

Keating GM, Simpson D (2007) Agalsidase Beta: a review of its use in the management of Fabry disease. Drugs 67:435–455

Lidove O, Joly D, Barbey F et al (2007) Clinical results of enzyme replacement therapy in Fabry disease: a comprehensive review of the literature. Int J Clin Pract 61:293–302

Linhart A, Kampmann C, Zamorano JL et al (2007) Cardiac manifestations of Anderson-Fabry disease: results from the international Fabry outcome survey. Eur Heart J 28:1228–1235

Linthorst GE, Hollak CE, Donker-Koopman WE, Strijland A, Aerts JM (2004) Enzyme therapy for Fabry disease: neutralizing antibodies toward agalsidase alpha and beta. Kidney Int 66:1589–1595

MacDermot KD, Holmes A, Miners AH (2001) Anderson-Fabry disease: clinical manifestations and impact of disease in a cohort of 98 hemizygous males. J Med Genet 38:750–760

Mehta A, Ginsberg L (2005) Natural history of the cerebrovascular complications of Fabry disease. Acta Paediatr 94(Suppl):9–10

Mehta A, Ricci R, Widmer U et al (2004) Fabry disease defined: baseline clinical manifestations of 366 patients in the Fabry Outcome Survey. Eur J Clin Invest 34:236–242

Moore DF, Kaneski CR, Askari H, Schiffmann R (2007) The cerebral vasculopathy of Fabry disease. J Neurol Sci 257:258–263

Nagueh SF (2003) Fabry disease. Heart 89:819–820

Pastores GM, Boyd E, Crandall K et al (2007) Safety and pharmacokinetics of agalsidase alfa in patients with Fabry disease and end-stage renal disease. Nephrol Dial Transplant 22:1920–1925

Ramaswami U, Wendt S, Pintos-Morell G (2007) Enzyme replacement therapy with agalsidase alfa in children with Fabry disease. Acta Paediatr 96:122–127

Schaefer E, Mehta A, Gal A (2005) Genotype and phenotype in Fabry disease: analysis of the Fabry Outcome survey. Acta Paediatr Suppl 94(447):87–92

Schiffmann R, Ries M, Timmons M et al (2006) Long-term therapy with agalsidase alfa for Fabry disease: safety and effects on renal function in a home infusión setting. Nephrol Dial Transplant 21:345–354

Tesmoingt C, Lidove O, Reberga A et al (2009) Enzyme therapy in Fabry disease: severe adverse events associated with anti-agalsidase cross-reactive IgG antibodies. Br J Clin Pharmacol 68:765–769

Zarate YA, Hopkin RJ (2008) Fabry's disease. Lancet 372:1427–1435

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RESEARCH REPORT

Validation of keratan sulfate level in mucopolysaccharidosis type IVA by liquid chromatography–tandem mass spectrometry

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Abstract Mucopolysaccharidosis type IVA (MPS IVA, Morquio A disease), a progressive lysosomal storage disease, causes skeletal chondrodysplasia through excessive storage of keratan sulfate (KS). KS is synthesized mainly in cartilage and released to the circulation. The excess storage of KS disrupts cartilage, consequently releasing more KS into circulation, which is a critical biomarker for MPS IVA. Thus, assessment of KS level provides a potential screening strategy and determines clinical course and efficacy of therapies. We have recently developed a tandem mass spectrometry liquid chromatography [LC/MS/MS] method to assay KS levels in blood. Forty-nine blood specimens from patients with MPS IVA [severe (n=33), attenuated (n=11) and undefined (n=5)] were analyzed for comparison of blood KS concentration with that of healthy subjects and for correlation with clinical severity. Plasma samples were digested by keratanase II to obtain disaccharides of KS. Digested samples were assayed by LC/MS/MS. We found that blood KS levels (0.4–26 μg/ml) in MPS IVA patients were significantly higher than those in age-matched controls (0.67–4.6 μg/ml; P<0.0001). It was found that blood KS level varied with age and clinical severity in the patients. Blood KS levels in MPS IVA peaked between 2 years and 5 years of age (mean 11.4 μg/ml). Blood KS levels in severe MPS IVA (mean 7.3 μg/ml) were higher than in the attenuated form (mean 2.1 μg/ml) (P=0.012). We also found elevated blood KS levels in other types of MPS. These findings indicate that the new KS assay for blood is suitable for early diagnosis and longitudinal assessment of disease severity in MPS IVA.

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Abbreviations

KS	keratan sulfate
MPS IVA	mucopolysaccharidosis type IVA
LSD	lysosomal storage disease
MPS	mucopolysaccharidoses
GAGs	glycosaminoglycans
GALNS	*N*-acetylgalactosamine-6-sulfate sulfatase
QC	quality control
CV	coefficient of variation
DMB	dimethylmethylene blue
LC/MS/MS	liquid chromatography tandem mass spectrometry
ERT	enzyme replacement therapy
ML	mucolipidoses
ELISA	enzyme-linked immunosorbent assay
HPLC	High-performance liquid chromatography

Introduction

Mucopolysaccharidoses (MPS) are a family of heritable disorders caused by deficiency of lysosomal enzymes required for degradation of glycosaminoglycans (GAGs) (Neufeld and Muenzer 2001). Each known MPS type involves deficiency of a specific lysosomal enzyme required for the stepwise degradation of specific GAG(s).

Mucopolysaccharidosis type IVA (MPS IVA, Morquio A disease) is an autosomal recessive disease caused by the deficiency of *N*-acetylgalactosamine-6-sulfate sulfatase (GALNS). The enzyme and its deficiency were discovered and identified by the use of oligosaccharide substrates prepared from chondroitin 6-sulfate (C6S) containing *N*-acetylgalactosamine (GalNAc) 6-sulfate. Thus, the enzyme was originally named GalNAc-6-sulfate sulfatase, abbreviated to GALNS (Dorfman et al. 1976). Subsequently, it was shown that the enzyme also removed the 6-sulfated galactose residues of keratan sulfate (KS) (Glössl and Kresse 1982). GALNS is required to degrade KS and C6S. Clinically, the severe form is characterized by short trunk dwarfism, kyphoscoliosis, coxa valga, odontoid hypoplasia, abnormal gait, joint mobility problems, restriction of chest wall movement and a life span of 20–30 years. Patients with the attenuated form can have a near normal quality of life, with mild involvement of the skeleton (Northover et al. 1996; Montaño et al. 2007, 2008). Although there is no approved treatment available, enzyme replacement therapy and bone marrow transplantation are under investigation on MPS IVA human patients and mouse models.

The pathogenesis of the bone dysplasia in MPS IVA is largely unknown, but it is speculated that the accumulation of KS could be toxic to osteoblasts (Fang-Kircher et al. 1997). A cost effective and sensitive method to measure a biomarker for the disease would be useful for making an early diagnosis, determining disease severity and systematically monitoring patients' responses to treatment regimens.

One potential disease marker for MPS IVA, total GAG in the urine, can be measured spectrometrically using dimethylmethylene blue (DMB) (Whitley et al. 1989) or alcian blue (Bjornsson 1993). However, these methods are not applicable to blood without prior protease treatment, as protein in the specimen interferes with the binding of the dye to the GAG. In addition, the dye itself is also prone to decompose, leading to a high background. For total GAG levels in urine, the range in approximately 20% of patients with MPS IVA overlapped the normal range (Tomatsu et al. 2004). Therefore it is difficult to distinguish the patients from healthy controls on the basis of the level of urine GAG excretion.

Another potential biomarker of this disease is the blood KS level. KS is synthesized mainly in cartilage and is one of the substrates accumulated in MPS IVA disease. The excessive storage of KS is known to cause severe skeletal dysplasia in patients with Morquio A syndrome. The measurement of KS in blood in these patients would provide information to help one to assess the longitudinal prognosis and efficacy of therapies, as well as early diagnosis. There are established procedures for measuring KS. The cetylpyridinium chloride method followed by thin-layer chromatography has been used semiquantitatively for the analysis of urine samples from patients with MPS IVA (Fujimoto and Horwitz 1983; Beck et al. 1986). Monoclonal antibody assay [enzyme-linked immunosorbent assay-inhibition assay (ELISA)-Inhibition] is also available for KS measurement (Thonar et al. 1985). However, those methods involve multiple laborious steps. We have recently reported a sandwich ELISA method for detection of KS in biological fluids. The results demonstrated that KS levels in the blood and urine of patients with MPS IVA are significantly elevated in comparison with those of healthy controls of equivalent ages (Tomatsu et al. 2004). The sandwich ELISA method provides the total amount of KS quantitatively but does not provide the level of sulfation and composition of KS. High-performance liquid chromatography (HPLC) is another sensitive and accurate method to measure each specific GAG, although it is still laborious and does not allow us to assay large numbers of samples simultaneously (Tomatsu et al. 2005; Kinoshita and Sugahara 1999; Toyoda et al. 1998; Yamada et al. 2000). We have recently developed a sensitive, specific and reproducible KS assay system by liquid chromatography tandem mass spectrometry (LC/MS/MS) (Oguma et al. 2007). We have also shown the importance of blood KS measurement to assess the efficacy of treatment during enzyme replacement therapy (ERT) on an MPS IVA mouse model (Tomatsu et al. 2008a), resulting in substantial reduction of KS level.

Moreover, we have recently demonstrated that KS level in blood is elevated in each type of MPS and mucolipidoses (ML) examined by sandwich ELISA, in contrast to the conventional understanding (Tomatsu et al. 2005).

In this study we evaluated KS levels in the blood of patients with MPS IVA by using a recently developed LC/MS/MS method, and we showed its feasibility as a method to evaluate the clinical course of MPS IVA. In addition, we assessed the elevation of KS levels in other types of MPS and ML.

Materials and methods

Subjects Blood (plasma) samples were obtained from 49 patients with MPS IVA (33 severe, 11 attenuated and five undefined) after informed consent had been obtained from each patient. For all samples, the ages of the patients were identified. Blood samples were also obtained from 125 healthy controls. The diagnosis of MPS IVA was made on the basis of a reduced enzyme activity (GALNS) of ≤5% the normal level in plasma, leukocytes or fibroblasts. We classified clinical severity according to patients' heights, as described previously (Montaño et al. 2008; Tomatsu et al. 2004). We obtained 101 blood (plasma) samples from patients with MPS and ML, except for those with MPS IV, ranging between 0 and 39 years of age (MPS I, n=31; MPS II, n=28; MPS III, n=19; MPS VI, n=6; MPS VII, n=6; ML, n=11).

KS assay Briefly, LC/MS/MS was used for the analysis of the disaccharides produced from KS. An API 4000 mass spectrometer equipped with a turbo-ion spray was used (Applied Biosystems, Foster City, CA, USA) (Oguma et al. 2007). KS in human plasma was digested to disaccharides by keratanase II (Seikagaku Corporation, Tokyo, Japan). The disaccharides were analyzed by LC/MS/MS using multiple reaction monitoring in negative ion mode. Separation by LC was performed on a Hypercarb column [2.0 mm internal diameter (i.d.)×150 mm, 5 μm) with gradient elution by acetonitrile–0.01 M ammonium bicarbonate (pH 10). The flow rate of the mobile phase was 0.2 ml/min. After digestion of the blood, KS was digested with keratanase II, and the disaccharide compositions of Galβ1→4GlcNAc(6S) and Galβ1(6S)→4GlcNAc(6S) were recognized. The C-6 position of the GlcNAc residue or both the Gal and GlcNAc residues was sulfated. When GALNS enzyme is deficient, the 6-sulfated galactose residues of KS are not removed in patients with MPS IVA; therefore, an accumulation of Galβ1(6S)→4GlcNAc (6S) disaccharides was expected. Blood samples with KS concentrations were assayed in duplicate using the appropriate dilution.

Data analysis The data obtained were analyzed to determine whether the levels of KS varied significantly with respect to age and clinical phenotype of MPS IVA patients. The variability of KS levels in the blood of both MPS IVA patients and controls was plotted according to age. For comparison between patient and control samples, Student's t-test, the Mann–Whitney U test or Welch's t-test was applied, depending on the distribution of the values. All data were analyzed with StatView statistical software (StatView J 4.5; Abacus Concepts, Inc.).

Results

Assay validation The intra- and inter-day precision and recoveries of Galβ1→4GlcNAc(6S) or Galβ1(6S)→4GlcNAc(6S) were previously obtained for human plasma and serum (Oguma et al. 2007). The results indicated that the method here had satisfactory precision and recovery without any effect by plasma proteins.

Blood (plasma) KS concentrations The KS values for the blood (plasma) samples from 49 MPS IVA subjects (average age 14.3 years; range 2–65 years), 125 control subjects (average age 12.1 years; range 0–62 years), and 101 non-type IVA MPS and ML are reported in Tables 1, 2 and 3 and Figs. 1 and 2.

Blood KS concentrations were found to vary with age. For all ages, the average blood KS concentrations in healthy controls and patients with MPS IVA were 2.3 μg/ml and 5.7 μg/ml, respectively (P<0.001). In healthy control newborn infants, blood KS concentration was under 2.0 μg/ml, and it rose, reaching a peak between the ages of 0 and 2 years (mean 2.9 μg/ml for control), and the concentrations stayed relatively constant till the individual reached 15 years of age. After 15 years, blood KS concentrations decreased to1.5 μg/ml and stabilized thereafter. When control subjects and MPS IVA patients in each age range (2–5 years, 5–10 years, 10–15 years, and over 15 years) were compared, the blood concentrations of KS were different between groups (mean 2.6 μg/ml vs 11.4 μg/ml, P<0.001; 2.2 μg/ml vs 6.4 μg/ml, P=0.055; 2.9 μg/ml vs 3.2 μg/ml, P=0.81; 1.5 μg/ml vs 2.1 μg/ml, P=0.020) (Tables 1 and 2, Fig. 1). Thus, at younger ages, there was more difference in KS levels between MPS IVA patients and the age-matched controls. KS level in patients with MPS IVA was reduced and had nearly normalized at over 10 years old. Blood KS levels in severe MPS IVA (mean 7.3 μg/ml) were higher than in the attenuated form (mean 2.1 μg/ml) (P=0.012) (Table 2). This finding suggests that KS level could be associated with clinical severity (KS is metabolized), although it still remains noteworthy that the patients with the attenuated form had a

Table 1 Blood KS evaluated by LC/MS/MS in healthy controls (SD, standard deviation)

Age group	Number	Data	Age (years)	Gal GlcNAc (6S) (μg/ml)	Gal(6S) GlcNAc (6S) (μg/ml)	Total (μg/ml)	Gal GlcNAc (6S) (%)	Gal(6S) GlcNAc (6S) (%)
$\chi \leq 2$	50	Average	0.5	2.2	0.4	2.6	85.7	14.2
		SD	0.5	0.7	0.2	0.9	4.5	4.6
$2 < \chi \leq 5$	25	Average	3.3	2.1	0.5	2.6	82.5	17.5
		SD	1.0	0.7	0.2	0.8	3.7	3.7
$5 < \chi \leq 10$	8	Average	7.5	1.8	0.4	2.2	82.6	17.3
		SD	1.7	0.6	0.2	0.8	4.7	5.0
$10 < \chi \leq 15$	7	Average	12.4	2.4	0.5	2.9	82.2	17.6
		SD	1.5	0.7	0.1	0.8	2.1	2.2
$15 < \chi$	37	Average	34.4	1.2	0.3	1.5	76.4	23.6
		SD	13.1	0.5	0.1	0.6	4.0	3.8
All ages	125	Average	12.1	2.0	0.4	2.3	86.7	18.3
		SD	16.3	1.0	0.2	0.9	28.5	6.1

lower mean KS because of their older age compared with patients with the severe phenotype.

The level of plasma KS was also compared between each type of MPS and ML and the age-matched controls (Tables 1 and 3 and Fig. 2). Plasma KS levels in MPS I showed that 4 out of 31 (12.9%) patients had above the mean+2 standard deviations (SD) of the age-matched controls (Fig. 2). Patients with MPS II had the highest mean KS in their blood among all types of MPS and ML patients except for patients with MPS IV (mean 6.0 μg/ml). Twenty-two out of 28 (78.6%) had plasma KS values above the mean + 2SD of age-matched controls (Fig. 1). Plasma KS values in 5 of 19 (26.3%) MPS III patients, 3 of 6 (50%) MPS VI patients, 1 of 6 MPS VII patients, and 2 of 11 (18.2%) ML patients were above the mean + 2SD of the age-matched controls (Fig. 2).

Composition in KS The compositional ratio of Galβ1(6S)→4GlcNAc(6S) to Galβ→4GlcNAc(6S) in KS derived from the blood samples of MPS IVA patients was compared, since it was expected to be reflected by a deficiency of GALNS enzyme, which digests at the C-6 position of sulfated galactose. For the healthy control newborn infants, the ratio of Galβ1(6S)→4GlcNAc(6S) was 10.6%, and it rose between the ages of 0 and 2 years (mean 16.3%) and the concentrations stayed relatively constant until they reached 15 years. After 15 years, the compositional ratio of Galβ1(6S)→4GlcNAc(6S) increased

Table 2 Blood KS evaluated by LC/MS/MS in MPS IVA patients (SD, standard deviation)

Data	Number	Data	Age (years)	Gal GlcNAc (6S) (μg/ml)	Gal(6S) GlcNAc (6S) (μg/ml)	Total (μg/ml)	Gal GlcNAc (6S) (%)	Gal(6S) GlcNAc (6S) (%)
$2 < \chi \leq 5$	12	Average	2.7	9.2	2.2	11.4	80.3	19.7
		SD	0.9	5.4	1.4	6.8	3.8	3.8
$5 < \chi \leq 10$	13	Average	7.8	5.2	1.2	6.4	78.7	21.3
		SD	1.3	4.7	1.0	5.7	7.7	7.7
$10 < \chi \leq 15$	9	Average	12.8	2.5	0.7	3.2	71.5	28.5
		SD	1.8	2.0	0.2	2.2	14.4	14.4
$15 < \chi$	15	Average	30.0	1.7	0.5	2.1	76.9	23.1
		SD	16.0	1.1	0.3	1.3	7.2	7.2
All ages	49	Average	14.3	4.6	1.1	5.7	77.3	22.7
		SD	14.1	4.7	1.1	5.8	8.8	8.8
Severe	33	Average	12.8	5.9	1.4	7.3	79.6	20.4
		SD	13.1	5.5	1.4	6.8	6.9	6.9
Attenuated	11	Average	19.5	1.6	0.5	2.1	71.9	28.1
		SD	19.3	1.8	0.3	2.0	13.1	13.1

Table 3 Blood KS evaluated by LC/MS/MS in other types of MPS and ML patients (SD, standard deviation)

Type	Number	Data	Age (years)	Gal GlcNAc (6S) (μg/ml)	Gal(6S) GlcNAc (6S) (μg/ml)	Total (μg/ml)	Gal GlcNAc (6S) (%)	Gal(6S) GlcNAc (6S) (%)
MPS I	31	Average	8.3	2.9	0.7	3.7	77.1	22.9
		SD	9.2	2.5	0.3	2.7	6.6	6.5
MPS II	28	Average	13.7	5.0	1.1	6.0	81.4	18.6
		SD	9.3	3.5	0.6	4.1	3.1	2.8
MPS III	19	Average	4.6	3.4	0.8	4.2	81.4	18.7
		SD	2.8	2.2	0.5	2.6	3.2	2.9
MPS VI	6	Average	2.7	4.5	1.0	5.5	81.9	18.7
		SD	2.2	3.1	0.7	3.7	4.1	4.0
MPS VII	6	Average	11.3	2.5	0.4	2.9	81.8	18.0
		SD	13.1	2.5	0.4	2.8	5.5	6.4
ML	11	Average	1.0	3.5	0.7	4.1	84.9	15.9
		SD	2.1	3.0	0.7	3.2	4.4	3.0

to 23.6% and stabilized thereafter. In all ages, the ratio of Galβ1(6S)→4GlcNAc(6S) was significantly higher in patients with MPS IVA than in healthy controls (mean 22.7% vs 18%, $P<0.001$) (Tables 1 and 2, Fig. 2), suggesting that KS at the C-6 position of galactose is more sulfated in patients. When control subjects and MPS IVA patients in each age range (2–5 years, 5–10 years, 10–15 years, and over 15 years) were compared, their ratios of Galβ1(6S)→4GlcNAc(6S) to Galβ→4GlcNAc(6S) were as follows: mean 17.5% vs 19.7%; 17.3% vs 21.3%; 17.6% vs 28.5%; 23.6% vs 23.1%, respectively (Tables 1 and 2, Fig. 1).

Discussion

The accumulation of undegraded KS leads to damage of cartilage cells, causing systemic skeletal chondrodysplasia in patients with MPS IVA. KS, which contributes over 25% of the cartilage GAGs in adults, is one of the most important components in bone. When cartilage proteoglycans, such as KS, are not degraded properly, they are stored mainly in chondrocytes, where KS is synthesized. Pathohistological examinations of the bone and cartilage cells are useful for the diagnosis of MPS IVA; however, it is not

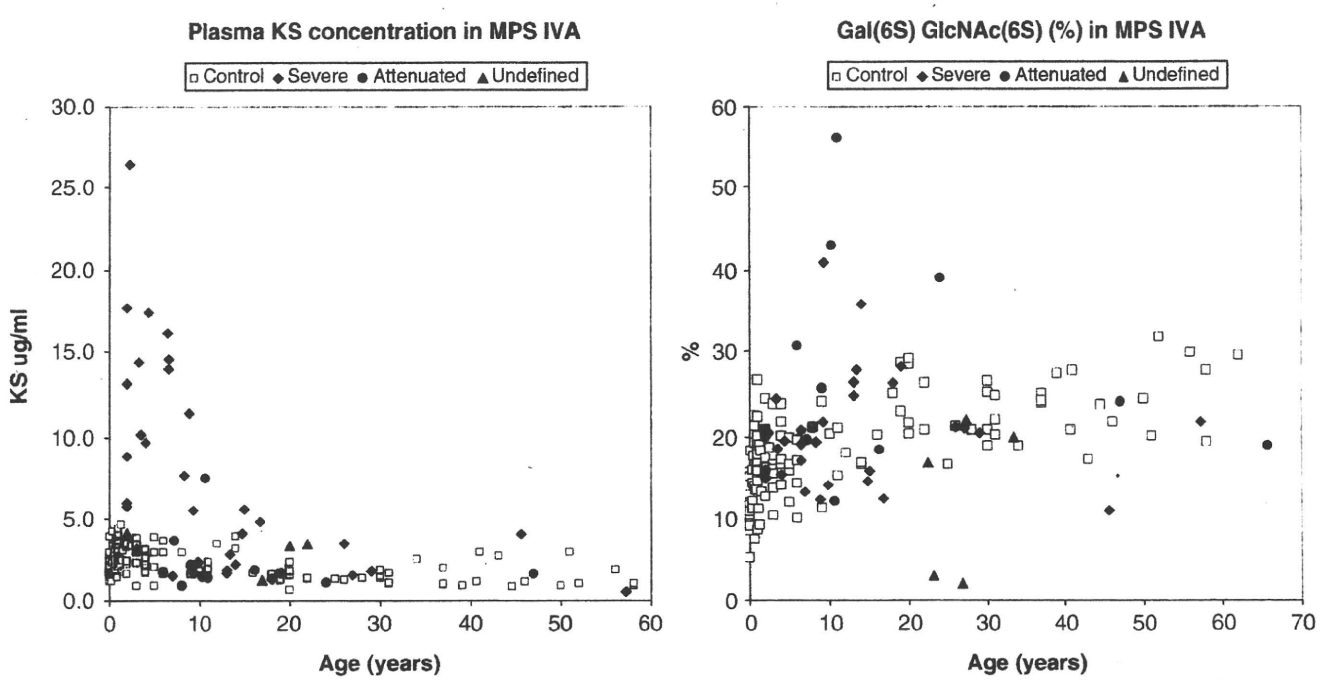

Fig. 1 Concentrations of blood KS in MPS IVA patients and healthy individuals. Results from 49 specimens from individuals with MPS IVA (severe, 33; attenuated, 11; undefined, 5) and 125 from healthy individuals are plotted with respect to age. Left panel blood, KS of patients with MPS IVA and healthy individuals. Right panel, ratio of Galβ(6S)→4GlcNAc(6S) in KS compositions (%)

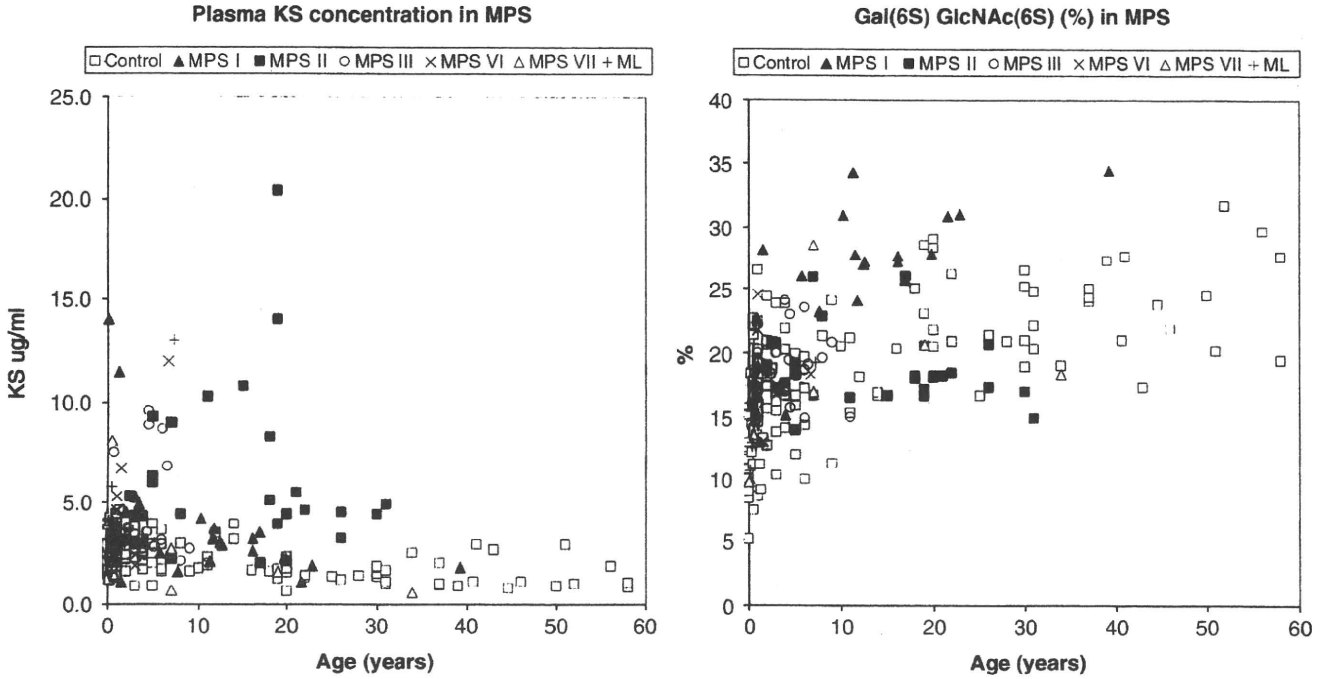

Fig. 2 Concentrations of blood KS in patients with MPS types I, II, III, VI, and VII and ML and healthy individuals. Results from 101 specimens from individuals with MPS and ML (MPS I, 31; MPS II, 28; MPS III, 19; MPS VI, 6; MPS VII, 6; ML, 11) and 125 from healthy individuals are plotted with respect to age. Left panel, blood KS of patients with MPS and ML and healthy individuals. Right panel, ratio of Galβ(6S)→4GlcNAc(6S) in blood KS compositions (%) in patients with MPS and ML and healthy individuals

practicable to obtain biopsy samples from MPS IVA patients. Alternatively, molecular analysis and standard enzyme assay are used as diagnostic techniques for MPS IVA. Measurements of KS concentrations in blood samples should also provide critical information about the clinical status and prognosis of MPS IVA patients, efficacy of therapies and early diagnosis as a biomarker.

The monoclonal antibody used for sandwich ELISA in previous studies (Seikagaku, Tokyo, Japan) (Tomatsu et al. 2004, 2005) is known to be specific for Galβ1(6S)→4GlcNAc(6S); both galactose and *N*-acetyl-glucosamine have to be sulfated (Caterson et al. 1983; Mehmet et al. 1986). Therefore, this analytical method does not provide complete quantification and qualification of total KS. Concentrations of KS-derived disaccharides and their compositions in human samples are measured by the present LC/MS/MS method. Keratanase II, used for the digestion of KS, recognizes both Galβ1→4GlcNAc(6S) and Galβ1(6S)→4GlcNAc(6S), in which *N*-acetyl-glucosamine is sulfated. Thus, the current LC/MS/MS method measures the KS molecules with disaccharides of Galβ1→4GlcNAc(6S) and Galβ1(6S)→4GlcNAc(6S). Although unmeasured KS could be still present, the best choice is to use keratanase II-digested samples, since the enzyme covers the broadest range of KS saccharides, leading to 10–100 times higher KS concentration than sandwich ELISA method.

Our results of KS compositions are of great interest. The compositional ratio of Galβ1(6S)→4GlcNAc(6S) to Galβ→4GlcNAc(6S) in KS derived from blood samples of patients with MPS IVA was higher than that of the age-matched healthy controls, except for the group over 15 years old, reflected by a deficiency of the GALNS enzyme, which digests at the C-6 position of sulfated galactose. However, there was no difference in the ratio of Galβ1(6S)→4GlcNAc(6S) to Galβ→4GlcNAc(6S) in the group over 15 years old between healthy controls and MPS IVA patients. The compositional ratio of Galβ1(6S)→4GlcNAc(6S) increased even in the healthy controls. KS sulfation may increase with the patient's age, although the physiological significance of its increase remains unknown. In this study we could not clearly correlate between clinical severity and Galβ1(6S)→4GlcNAc(6S) ratio; therefore, further longitudinal studies of each individual MPS IVA patient with a different phenotype are needed.

Unaffected healthy young children would be expected to have a high cartilage turnover, resulting in higher KS level. Age-dependent changes in KS turnover (Tomatsu et al. 2005; Thonar et al. 1988) showed that blood KS level rose progressively during the first 5 years of life, remained elevated until 10–12 years of age, and then declined after teenage, until it stabilized at the age of 15 years, although longitudinal data from the individual children are required to confirm this age dependency. Elongation of the long

bones during growth occurs through a process of endochondral ossification in which new cartilage is continuously laid down before it is degraded and replaced by bone. The decreased level of KS after teenage in healthy children is consistent with the fact that the growth rate begins to decline during this period.

In the initial progressive stage between ages 0 and 5 years, the mean blood KS concentration in patients with MPS IVA was the highest. After 10 years of age, the KS level in most MPS IVA patients declined to near normal or normal levels. Blood KS levels in MPS IVA could be reflected by two factors: (1) maturation-related changes as observed in unaffected healthy children, (2) the severity of progressive chondrodysplasia. MPS IVA patients under 10 years old had markedly elevated blood KS levels, indicating that the progression of cartilage destruction in most patients is rapid in young age and that KS is released from cartilage and leached into the circulation. Patients with high levels of KS in their blood at a young age likely have the severe form of MPS IVA, where the cartilage is overloaded with undegraded KS. One may speculate that the blood concentrations in patients with MPS IVA reflect the amount of stored KS in cartilage tissues. When the growth plate has closed or torn, the synthesis of KS in cartilage will decline. In fact, the destruction of cartilage in most patients could be completed by teenage, resulting in little release of KS from the cartilage to the circulation and almost no place for the synthesis of KS.

We have reported that a newborn infant affected by MPS IVA showed lower-spine radiographs with anterior beaking of the lumbar vertebrae as well as minor anomalies on the phalanges that suggested skeletal dysplasia (Ohashi et al. 2009). Subsequent radiographs showed progression of the anterior beaking, progressive kyphosis, platyspondyly and irregularities of the vertebral bodies, which are signs characteristic of severe MPS IVA disease. Thus, one can speculate that KS accumulation in bone has already started before birth and that the KS level could already be elevated at the newborn stage. To understand this phenomenon of normalization of blood KS concentration in MPS IVA patients and to predict when elevation of KS levels will start, one needs to determine the blood KS levels sequentially at different ages for the same individuals, longitudinally.

There are well-known relationships between types of MPS and specific GAG(s) that accumulate (Neufeld and Muenzer 2001). Elevation of KS level in blood or urine was considered to be specific for MPS IV. However, we have recently demonstrated that patients with MPS and ML, other than those with MPS IV, had elevated blood KS levels in addition to the GAGs originating from the respective enzyme defect (Tomatsu et al. 2005). In this study, we also confirmed that some of the patients affected by other types of MPS and ML had elevated blood KS levels. The mechanism for the secondary elevation of KS remains unclear, since the current theory on the pathway of KS metabolism cannot explain this phenomenon.

Most patients with MPS and ML have severe bone dysplasia, as in MPS IV. Therefore, elevated levels of KS in the blood of other MPS and ML patients could be related to underlying bone disease, especially of cartilage tissues. It is noted that the mean age of patients in this study was younger in the order ML, MPS VI, MPS I, and MPS II. Generally, progressive bone disease is observed in ML, MPS VI, and MPS I at younger ages than that for MPS II. Therefore, elevation of KS in ML, MPS VI, and MPS I was observed in younger patients, while the elevation in MPS II was observed even in teenaged or older patients. It is also noteworthy that most patients with MPS and ML had a compositional ratio of Galβ1(6S)→4GlcNAc(6S) to Galβ→4GlcNAc(6S) in KS similar to that seen in the age-matched healthy controls, suggesting that the interaction between KS and GALNS in removing the sulfate in the C-6 position in galactose is not inhibited. Since KS is synthesized mainly in cartilage, the successful reduction of KS could provide more specificity for the bone pathology of MPS disease.

Assessment of urinary KS by LC/MS/MS was out of the scope of this study. However, preliminary study of urine samples from patients with MPS IVA has shown that KS levels were markedly elevated. The KS levels in both MPS IVA patients and healthy controls were age dependent. In the controls, the KS levels were highest in newborn infants, as observed from total urinary GAGs. While the degree of decline in the urinary KS concentration proportional to age was equivalent to that of blood KS concentration, urinary KS level in patients with MPS IVA remained higher than those of the controls, even after 15 years of age (unpublished data).

In summary, the determination of blood KS concentrations by LC/MS/MS should provide a useful tool to assess clinical status in patients with MPS IVA and to measure response to treatments such as enzyme replacement therapy, bone marrow transplantation, and gene therapy as observed in MPS IVA mice treated by enzyme replacement therapy (Tomatsu et al. 2008b).

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References

Beck M, Glossl J, Grubisic A, Spranger J (1986) Heterogeneity of Morquio disease. Clin Genet 29:325–331

Bjornsson S (1993) Simultaneous preparation and quantitation of proteoglycans by precipitation with alcian blue. Anal Biochem 210:282–291

Caterson B, Christner JE, Baker JR (1983) Identification of a monoclonal antibody that specifically recognizes corneal and skeletal keratan sulfate. Monoclonal antibodies to cartilage proteoglycan. J Biol Chem 258:8848–8854

Dorfman A, Arbogast B, Matalon R (1976) The enzymic defects in Morquio and Maroteaux-Lamy syndrome. Adv Exp Med Biol 68:261–276

Fang-Kircher SG, Herkner K, Windhager R, Lubec G (1997) The effects of acid glycosaminoglycans on neonatal calvarian cultures—a role of keratan sulfate in Morquio syndrome? Life Sci 61:771–775

Fujimoto A, Horwitz AL (1983) Biochemical defect of non-keratan-sulfate-excreting Morquio syndrome. Am J Med Genet 15:265–273

Glössl J, Kresse H (1982) Impaired degradation of keratan sulphate by Morquio A fibroblasts. Biochem J 203:335–338

Kinoshita A, Sugahara K (1999) Microanalysis of glycosaminoglycan-derived oligosaccharides labeled with a fluorophore 2-aminobenzamide by high-performance liquid chromatography: application to disaccharide composition analysis and exosequencing of oligosaccharides. Anal Biochem 269:367–378

Mehmet H, Scudder P, Tang PW, Hounsell EF, Caterson B, Feizi T (1986) The antigenic determinants recognized by three monoclonal antibodies to keratan sulphate involve sulphated hepta- or larger oligosaccharides of the poly (N-acetyllactosamine) series. Eur J Biochem 157:385–391

Montaño AM, Tomatsu S, Gottesman GS, Smith M, Orii T (2007) International Morquio A registry: clinical manifestation and natural course of Morquio A disease. J Inherit Metab Dis 30:165–174

Montaño AM, Tomatsu S, Brusius A, Smith M, Orii T (2008) Growth charts for patients affected with Morquio A disease. Am J Med Genet A 15;146:1286–1295

Neufeld EF, Muenzer J (2001) The mucopolysaccharidoses. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 8th edn. McGraw-Hill, New York, pp 3421–3452

Northover H, Cowie RA, Wraith JE (1996) Mucopolysaccharidosis type IVA (Morquio syndrome): a clinical review. J Inherit Metab Dis 19:357–365

Oguma T, Tomatsu S, Okazaki O (2007) Analytical method for determination of disaccharides derived from keratan sulfates in human serum and plasma by high-performance liquid chromatography/turbo-ionspray ionization tandem mass spectrometry. Biomed Chromatogr 21:356–362

Ohashi A, Montaño AM, Colón JE, Oguma T, Luisiri A, Tomatsu T (2009) Sacral dimple: Incidental findings from newborn evaluation. Acta Paediatr 98:768–769, 910–912

Thonar EJ, Lenz ME, Klintworth GK, Caterson B, Pachman LM, Glickman P, Katz R, Huff J, Kuettner KE (1985) Quantification of keratan sulfate in blood as a marker of cartilage catabolism. Arthritis Rheum 28:1367–1376

Thonar EJ, Pachman LM, Lenz ME, Hayford J, Lynch P, Kuettner KE (1988) Age related changes in the concentration of serum keratan sulphate in children. J Clin Chem Clin Biochem 2:57–63

Tomatsu S, Okamura K, Taketani T, Orii KO, Nishioka T, Gutierrez MA, Velez-Castrillon S, Fachel AA, Grubb JH, Cooper A, Thornley M, Wraith E, Barrera LA, Giugliani R, Schwartz IV, Frenking GS, Beck M, Kircher SG, Paschke E, Yamaguchi S, Ullrich K, Isogai K, Suzuki Y, Orii T, Kondo N, Creer M, Noguchi A (2004) Development and testing of new screening method for keratan sulfate in mucopolysaccharidosis IVA. Pediatr Res 55:592–597

Tomatsu S, Okamura K, Taketani T, Orii KO, Nishioka T, Gutierrez MA, Velez-Castrillon S, Fachel AA, Grubb JH, Cooper A, Thornley M, Wraith E, Barrera LA, Giugliani R, Schwartz IV, Frenking GS, Beck M, Kircher SG, Paschke E, Yamaguchi S, Ullrich KK, Isogai K, Suzuki Y, Orii T, Kondo N, Creer M, Noguchi A (2005) Keratan sulfate levels in mucopolysaccharidoses and mucolipidoses. J Inherit Metab Dis 28:187–202

Tomatsu S, Montaño AM, Ohashi A, Oikawa H, Oguma T, Dung VC, Nishioka T, Orii T, Sly WS (2008) Enzyme replacement therapy in a murine model of Morquio A syndrome. Hum Mol Genet 17:815–824

Tomatsu S, Montaño AM, Ohashi A, Oikawa H, Oguma T, Dung VC, Nishioka T, Orii T, Sly WS (2008) Enzyme replacement therapy in a murine model of Morquio A syndrome. Hum Mol Genet 17:815–824

Toyoda H, Demachi Y, Komoriya S, Furuya N, Toida T, Imanari T (1998) Characterization and determination of human urinary keratan sulfate. Chem Pharm Bull 46:97–101

Whitley CB, Ridnour MD, Draper KA, Dutton CM, Neglia JP (1989) Diagnostic test for mucopolysaccharidosis I. Direct method for quantifying excessive urinary glycosaminoglycan excretion. Clin Chem 35:374–379

Yamada H, Miyauchi S, Morita M, Yoshida Y, Yoshihara Y, Kikuchi T, Washimi O, Washimi Y, Terada N, Seki T, Fujikawa K (2000) Content and sulfation pattern of keratan sulfate in hip osteoarthritis using high performance liquid chromatography. J Rheumatol 27:1721–1724

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ORIGINAL ARTICLE

Dermatan sulfate and heparan sulfate as a biomarker for mucopolysaccharidosis I

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Abstract Mucopolysaccharidosis I (MPS I) is an autosomal recessive disorder caused by deficiency of α-L-iduronidase leading to accumulation of its catabolic substrates, dermatan sulfate (DS) and heparan sulfate (HS), in lysosomes. This results in progressive multiorgan dysfunction and death in early childhood. The recent success of enzyme replacement therapy (ERT) for MPS I highlights the need for biomarkers that reflect response to such therapy. To determine which biochemical markers are better, we determined serum and urine DS and HS levels by liquid chromatography tandem mass spectrometry in ERT-treated MPS I patients. The group included one Hurler, 11 Hurler/Scheie, and two Scheie patients. Seven patients were treated from week 1, whereas the other seven were treated from week 26. Serum and urine DS (ΔDi-4S/6S) and HS (ΔDiHS-0S, ΔDiHS-NS) were measured at baseline, week 26, and week 72. Serum ΔDi-4S/6S, ΔDiHS-0S, and ΔDiHS-NS levels decreased by 72%, 56%, and 56%, respectively, from baseline at week 72. Urinary glycosaminoglycan level decreased by 61.2%, whereas urine ΔDi-4S/6S, ΔDiHS-0S, and ΔDiHS-NS decreased by 66.8%, 71.8%, and 71%, respectively. Regardless of age and clinical severity, all patients showed marked decrease of DS and HS in blood and urine samples. We also evaluated serum DS and HS from dried blood-spot samples of three MPS I newborn patients, showing marked elevation of DS and HS levels compared with those in control newborns. In conclusion, blood and urine levels of DS and HS provide an intrinsic monitoring and screening tool for MPS I patients.

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Abbreviations

DS	Dermatan sulfate
HS	Heparan sulfate
LSD	Lysosomal storage disease
MPS	Mucopolysaccharidoses
GAG	Glycosaminoglycan
DMB	Dimethylmethylene blue
ΔDiHS	Unsaturated disaccharide unit from heparan sulfate
ΔDiHS-0S (ΔHexUAα1–4GlcNAc)	2-acetamido-2-deoxy-4-O-(4-deoxy-a-L-threo-hex-4-enopyranosyluronic acid) -D-glucose
ΔDiHS-NS [ΔHexUAα1-4GlcN(2-N-sulfate)]	2-deoxy-2-sulfamino-4-O-(4-deoxy-a-L-threo-hex-4-enopyranosyluronic acid)-D-glucose
ΔDi-4S/6S [ΔHexUAα1–4GlcNAc(4/6-*O*-sulfate)]	2-acetamido-2-deoxy-4-O-(4-deoxy-a-L-threohex-4-enopyranosyluronic acid)-4/6-O-sulfo-D-glucose
HPLC	High-performance liquid chromatography
LC/MS/MS	Liquid chromatography tandem mass spectrometry
NBS	Newborn screening
HCII-T	Complex heparin cofactor II–thrombin

Introduction

Mucopolysaccharidosis I (MPS I) is an autosomal recessive disorder caused by deficiency of a lysosomal enzyme α-L-iduronidase (IDUA; EC 3.2.1.76), leading to accumulation of its catabolic substrates dermatan sulfate (DS) and heparan sulfate (HS) in tissues. MPS I patients exhibit a wide spectrum of clinical phenotypes: from the most severe phenotype patients, known as Hurler syndrome, to the most attenuated form, Scheie syndrome. Hurler patients suffer from progressive multiorgan dysfunction in the first year of life and develop cognitive delay, heart valvular disease, respiratory abnormality, dysostosis multiplex, rigidity of joints, hepatosplenomegaly and corneal clouding, which lead to death in early childhood. Scheie patients have no direct central nervous system (CNS) compromise, but have mild visceral organ involvement and can have a normal life span. To understand clinical heterogeneity, residual activity, specific metabolic pathway, and genotype/phenotype correlation have been well studied (Litjens et al. 1996; Froissart et al. 2002; Terlato and Cox 2003; Fuller et al. 2005). This has been clearly illustrated in MPS I where sensitive methods to detect residual IDUA activity and oligosaccharide determination in fibroblasts provide discrimination between patients with and without CNS involvement (Fuller et al. 2005).

Allogeneic hematopoietic stem cell transplantation and enzyme replacement therapy (ERT) have been performed for MPS I patients. These therapies altered the progression of this disorder with substantial success (Wraith et al. 2004; Church et al. 2007). Measurement of urinary glycosaminoglycan (GAG) excretion has been widely used as a biomarker for MPS I and other types of MPS (Byers et al. 1998; Gallegos-Arreola et al. 2000; Mabe et al. 2004). Urinary GAG levels help initial diagnosis and could provide to be a measure for responsiveness to treatment. However, GAG excretion is unlikely to reflect clinical severity and specific clinical signs and symptoms, and GAG cannot easily be measured in blood. Therefore, additional biomarkers correlating clinical severity and response to therapies would be useful for patient management. Several biomarkers for MPS have been investigated and proposed. For example, serum levels of a GAG-regulated serpin–protease complex heparin cofactor II–thrombin (HCII-T) was elevated in MPS I patients, or serum levels of HCII-T showed responsiveness to treatment, although values in many patients did not drop to the control range during treatment (Randall et al. 2006, 2008). Moreover, dipeptidyl peptidase IV (DPP-IV) activity measured by mass spectrometry (MS) decreased in MPS I patients undergoing ERT, indicating that it could be a useful biomarker for monitoring treatment efficacy in MPS disease. As DPP-IV has an important regulatory role in metabolism, it is possible that its elevation could cause some of the secondary pathology seen in MPS (Beesley et al. 2009). Thus, several new potential biomarkers secondarily elevated in MPS I have been evaluated.

Meanwhile, a few studies have been performed investigating the level of DS and HS as a biomarker for MPS. The ratio of urine DS to chondroitin sulfate (CS) was measured by the size and density of spots seen from two-dimensional electrophoresis of extracted GAGs. This semiquantitative technique showed decreased ratio of DS/CS during the course of hemopoietic stem cell transplant for MPS I patients (Church et al. 2007). Accumulation of a disaccharide fragment [hexosamine-N-sulfate(α-1,4) hexuronic acid; HNS-UA] marker of HS storage was also assessed within multiple organs of a naturally occurring mouse model of MPS IIIA (Sanfilippo A disease) by mass spectrometry (King et al. 2006). Our previous study by the sandwich enzyme-linked immuno-

sorbent assay (ELISA) method demonstrated elevation of blood and urine HS, suggesting their usefulness as biomarkers of disease in MPS I patients, although the ELISA method to measure blood DS levels has not thus far been established.

Overall, despite the fact that DS and HS are primary storage materials in most MPS, there is limited understanding in affected patients with regard to when these specific GAGs are elevated in circulation, how they are correlated with the clinical signs and symptoms, and whether these metabolites can be useful as biomarkers to monitor treatment effectiveness. Recently, we developed a liquid chromatography tandem mass spectrometry (LC/MS/MS) method to analyze a specific GAG, such as DS, HS, and keratan sulfate (KS), and their compositions in normal control samples (Oguma et al. 2007a, b; Tomatsu et al. in press). The aims of this study were to: (1) evaluate reductions by LC/MS/MS of DS and HS levels in urine and serum samples of MPS I patients during ERT as a biomarker, and (2) evaluate DS and HS values in MPS I newborns in comparison with normal newborns. We found that DS and HS are potentially useful biomarkers for monitoring the efficacy of treatment in MPS I and also that it is applicable to the newborn screening (NBS) of the same.

Materials and methods

Participants

Serum and urine samples were obtained from 14 MPS I patients (cases 1–14) on ERT (one Hurler, 11 Hurler/Scheie, two Scheie): case 1 (female) 7.7 years, Hurler/Scheie; case 2 (male) 19.8 years, Hurler/Scheie; case 3 (male) 16.9 years, Scheie; case 4 (female) 22.8 years, Hurler/Scheie; case 5 (male) 11.6 years, Hurler/Scheie; case 6 (male) 12.4 years, Hurler/Scheie; case 7 (male) 10.3 years, Hurler/Scheie; case 8 (female)- 21.5 years, Hurler/Scheie; case 9 (female) 11.8 years, Hurler/Scheie; case 10 (male) 39.3 years, Scheie; case 11 (male) 11.3 years, Hurler; case 12 (female) 11.6 years, Hurler/Scheie; case 13 (male) 5.8 years, Hurler; case 14 (male) 16.2 years, Hurler/Scheie. The starting age for monitoring ranged between 5.8–39.3 years. Seven patients were treated by weekly infusions with 0.58 mg/kg dose from week 1, whereas another seven (placebo group) treated beginning week 26. Serum and urine samples were collected at baseline (pretreatment), week 26, and week 72 prior to enzyme infusion. We analyzed 50 control serum samples in comparison and obtained DBS samples from three MPS I newborns and 50 normal control newborns.

Liquid chromatography tandem mass spectrometry (LC/MS/MS) method

Disaccharides from DS, HS, and KS from human serum and urine were prepared and measured, as described previously (Oguma et al. 2007a, b). The samples on DBS were eluted by double-distilled water (ddH_2O). Briefly, each sample of serum (or urine or eluted DBS) was digested by chondroitinase B, heparitinase, and keratanase II mixture solution at 37°C for 3 h. After filtration, the digested sample was applied to LC/MS/MS; API-4000 mass spectrometer equipped with a turbo ion spray (Applied Biosystems, Foster City, CA, USA). Analysis of disaccharides was performed by LC/MS/MS using multiple reactions monitoring in negative ion mode. Separation of LC was performed on a Hypercarb (2.0 mm i.d. ×150 mm, 5 μm) with a gradient elution. When DS was digested with chondroitinase B, 2-acetamido-2-deoxy-4-O-(4-deoxy-α-L-threohex-4-enopyranosyluronic acid)-4/6-O-sulfo-D-glucose (ΔDi-4S/6S) in DS was recognized. When HS was digested with heparitinase, 2-acetamido-2-deoxy-4-O-(4-deoxy-α-L-threo-hex-4-enopyranosyluronic acid) -D-glucose (ΔDiHS-0S) and 2-deoxy-2-sulfamino-4-O-(4-deoxy-α-L-threo-hex-4-enopyranosyluronic acid)-D-glucose (ΔDiHS-NS) in HS were recognized. After digestion of blood, KS was digested with keratanase II, and disaccharide compositions of (6S) and Galß1(6S)→4GlcNAc(6S) were recognized. Samples with DS, HS, and KS concentrations were assayed in duplicate using the appropriate dilution.

Sandwich ELISA method

While we validate the DS and HS levels provided by LC/MS/MS, the KS and HS ELISA assays were performed in comparison, as previously described (Seikagaku Co, Tokyo, Japan) (Tomatsu et al. 2004, 2005a, b). Absorbance was measured at 450 nm with the microplate reader. KS and HS concentrations were read by applying the absorbances of each sample to the calibration curve. We assayed serum and urine samples from 14 MPS I patients.

Measurement of urinary GAGs

Urinary GAGs were measured in comparison with blood and urine DS and HS values using dimethyl-methylene blue (DMB), as described previously (Whitley et al. 1989). Creatinine was measured by mixing 10 μl of a tenfold-diluted urine sample with 50 μl saturated picric acid (Sigma-Aldrich, St. Louis, MO, USA) and 50 μl 0.2 M sodium hydroxide (NaOH). Absorbance at 490 nm