

ENZYME-REPLACEMENT THERAPY IN MUCOPOLYSACCHARIDOSIS I

EMIL D. KAKKIS, M.D., PH.D., JOSEPH MUENZER, M.D., PH.D., GEORGE E. TILLER, M.D., PH.D., LEWIS WABER, M.D., PH.D., JOHN BELMONT, M.D., PH.D., MERRY PASSAGE, M.S., BARBARA IZYKOWSKI, R.N., JEFFREY PHILLIPS, M.D., ROBIN DOROSHOW, M.D., IRV WALOT, M.D., RICHARD HOFT, M.D., AND ELIZABETH F. NEUFELD, PH.D.

ABSTRACT

Background Mucopolysaccharidosis I is a lysosomal storage disease caused by a deficiency of the enzyme α -L-iduronidase. We evaluated the effect of enzyme-replacement therapy with recombinant human α -L-iduronidase in patients with this disorder.

Methods We treated 10 patients with mucopolysaccharidosis I (age, 5 to 22 years) with recombinant human α -L-iduronidase at a dose of 125,000 U per kilogram of body weight given intravenously once weekly for 52 weeks. The patients were evaluated at base line and at 6, 12, 26, and 52 weeks by detailed clinical examinations, magnetic resonance imaging of the abdomen and brain, echocardiography, range-of-motion measurements, polysomnography, clinical laboratory evaluations, measurements of leukocyte α -L-iduronidase activity, and urinary glycosaminoglycan excretion.

Results Hepatosplenomegaly decreased significantly in all patients, and the size of the liver was normal for body weight and age in eight patients by 26 weeks. The rate of growth in height and weight had increased by a mean of 85 and 131 percent, respectively, at 52 weeks in the six prepubertal patients. The mean maximal range of motion of shoulder flexion and elbow extension increased significantly. The number of episodes of apnea and hypopnea during sleep decreased 61 percent. New York Heart Association functional class improved by one or two classes in all patients. Urinary glycosaminoglycan excretion decreased after three to four weeks of treatment; the mean reduction at 52 weeks was 63 percent of base-line values. Five patients had transient urticaria during infusions. Serum antibodies to α -L-iduronidase were detected in four patients.

Conclusions In patients with mucopolysaccharidosis I, treatment with recombinant human α -L-iduronidase reduces lysosomal storage in the liver and ameliorates some clinical manifestations of the disease. (N Engl J Med 2001;344:182-8.)

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MUCOPOLYSACCHARIDOSIS I is a lysosomal storage disease caused by a deficiency of α -L-iduronidase, an enzyme that cleaves the terminal α -L-iduronic acid residues in the glycosaminoglycans heparan sulfate and dermatan sulfate.^{1,2} The deficiency blocks the degradation of these glycosaminoglycans, which accumulate in lysosomes. Mucopolysaccharidosis I has a wide spectrum of clinical severity and has been subdivided into three syndromes: Hurler's syndrome (severe), Hurler-Scheie syndrome (inter-

mediate), and Scheie's syndrome (mild). Patients with Hurler's syndrome have many medical problems, including progressive developmental delay, corneal clouding, airway obstruction, cardiac disease, hepatosplenomegaly, and severe joint restriction, and most die by the age of 10 years.¹ Patients with Hurler-Scheie syndrome have many of the same medical problems, but the rate of progression is slower, they have little or no mental retardation, and they die in their teens or 20s. Patients with Scheie's syndrome have less extensive disease and a potentially normal life span.¹⁻³ The difference in severity is due primarily to the effect of various mutations, some of which permit residual enzyme activity.⁴

Bone marrow transplantation is an effective treatment for patients with Hurler's syndrome, especially if it is performed early in the course of the disease, before the onset of developmental decline.⁵⁻⁸ However, the substantial morbidity and mortality of the procedure and the need for matched donor marrow limit its usefulness.

Enzyme-replacement therapy is a potential treatment for lysosomal storage disease.⁹⁻¹¹ The first successful use of this therapy was in patients with Gaucher's disease, who were treated with mannose-terminated placental glucocerebrosidase.¹² Enzyme-replacement therapy has also been used for the treatment of patients or animals with other lysosomal storage diseases, such as Fabry's disease,¹³ Pompe's disease,^{14,15} and Maroteaux-Lamy syndrome.¹⁶

The cloning of complementary DNA encoding α -L-iduronidase^{17,18} led to the production of recombinant α -L-iduronidase.¹⁹ Studies in dogs with mucopolysaccharidosis I revealed that intravenously administered α -L-iduronidase was distributed throughout the body and that it reduced lysosomal storage in many tissues.^{20,21} On the basis of these data, we performed a 52-week study of α -L-iduronidase replacement in patients with mucopolysaccharidosis I.

From the Department of Pediatrics, Division of Medical Genetics, Harbor-UCLA Medical Center, Torrance, Calif. (E.D.K., M.P., B.L., J.P., R.D., I.W., R.H.); BioMarin Pharmaceutical, Novato, Calif. (E.D.K.); the University of North Carolina, Chapel Hill, Chapel Hill (J.M.); Vanderbilt University Medical Center, Nashville (G.E.T.); the University of Texas Southwestern Medical Center, Dallas (L.W.); Baylor College of Medicine, Houston (J.B.); and the Department of Biological Chemistry, UCLA School of Medicine, Los Angeles (E.F.N.). Address reprint requests to Dr. Kakkis at BioMarin Pharmaceutical, Inc., 371 Bel Marin Keys Blvd., Suite 210, Novato, CA 94949, or at ekakkis@biomarinpharm.com.

Other authors were Kian Ti Yu, M.D., Susie Okazaki, Dave Lewis, M.D., and Ralph Lachman, M.D. (Harbor-UCLA Medical Center, Torrance, Calif.); and Jerry N. Thompson, Ph.D. (University of Alabama at Birmingham, Birmingham).

METHODS

Study Subjects

We studied 10 patients who had mucopolysaccharidosis I (Table 1). Each patient had typical clinical manifestations of the disorder, and in each, the diagnosis was confirmed by the biochemical determination of α -L-iduronidase deficiency in leukocytes. Five patients were found to have one W402X or Q70X allele, but the other mutations have not yet been fully characterized (Hopwood JJ, et al.: personal communication).

Clinical Studies

Patients were premedicated with diphenhydramine (0.5 to 1.25 mg per kilogram of body weight), and recombinant human α -L-iduronidase (diluted in normal saline with 0.1 percent human serum albumin) was then administered intravenously once weekly over a three-hour period at a dose of 125,000 U per kilogram; the rate was 3000 U per kilogram during the first hour and 61,000 U per kilogram during each of the following two hours. The length of the infusion was increased to four to six hours in patients who had hypersensitivity reactions.

At base line and at 6, 12, 26, and 52 weeks, the patients underwent a physical examination including history-taking, magnetic resonance imaging of the abdomen and brain, and range-of-motion measurements. Echocardiography and electrocardiography were performed at base line and at 12, 26, and 52 weeks. Corneal photographs were obtained and a skeletal survey was performed at base line and at weeks 26 and 52. A skin biopsy was performed at base

line to establish cultures of fibroblasts for the determination of enzyme activity and genotyping.

Range of motion was measured with a goniometer, and the maximal patient-initiated range was recorded for each motion. Shoulder flexion was evaluated in terms of the movement of the patient's elbow anteriorly from the side of his or her body, and elbow and knee extension was evaluated in terms of the patient's ability to straighten each of these joints. Degrees of restriction represent the difference between the normal maximal range of motion for age and the measured value.

Polysomnography was performed at base line and at week 26 according to the guidelines of the American Thoracic Society. The number of episodes of apnea (defined as the cessation of airflow for at least 10 seconds) and hypopnea (defined as a decrease in oronasal airflow of at least 50 percent in association with desaturation of at least 2 percent or evidence of arousal), the number of minutes during sleep in which oxygen saturation fell below 89 percent, and the total sleeping time were recorded. From these data we calculated an apnea-hypopnea index by dividing the total number of episodes of apnea and hypopnea by the number of hours of sleep.

Biochemical studies included measurement of enzyme activity in leukocytes and brushings of buccal mucosa, urinary glycosaminoglycan excretion, and tests for serum antibodies to α -L-iduronidase (enzyme-linked immunosorbent assay and Western blotting). Studies to monitor toxicity included complete blood counts, chemistry panels, urinalysis, 24-hour creatinine clearance, and measurements of serum complement (C4, C3, and serum total complement activity [CH₁₀₀]) before and after the infusion. Organ volumes were determined by analysis of digital images obtained by

TABLE 1. CHARACTERISTICS OF THE PATIENTS.*

PATIENT NO.	SEX/AGE (YR)	CLASSIFICATION ACCORDING TO CLINICAL SEVERITY	RELATED CLINICAL PROBLEMS	RELATED SURGERY
1	M/17	Hurler-Scheie syndrome	Malaise and fatigue, joint stiffness, corneal clouding, deafness, airway obstruction, cardiac-valve disease, frequent infections, hepatosplenomegaly, inguinal hernia, digital contractures, growth deficiency	Tracheostomy
2	F/11	Hurler-Scheie syndrome	Malaise and fatigue, joint stiffness, corneal clouding, airway obstruction, cardiac-valve disease, frequent infections, hepatosplenomegaly, digital contractures, growth deficiency, scoliosis	Corneal transplantation (one eye)
3	M/9	Hurler-Scheie syndrome	Headaches, malaise and fatigue, joint stiffness, airway obstruction, frequent infections, hepatosplenomegaly, inguinal hernias, digital contractures, growth deficiency	Carpal-tunnel release, tonsillectomy and adenoidectomy
4	M/8	Hurler-Scheie syndrome	Headaches, malaise and fatigue, joint stiffness, airway obstruction, frequent infections, hepatosplenomegaly, umbilical hernia	Tonsillectomy and adenoidectomy
5	M/12	Hurler-Scheie syndrome	Headaches, malaise and fatigue, joint stiffness, corneal clouding, airway obstruction, cardiac-valve disease, frequent infections, hepatosplenomegaly, digital contractures, growth deficiency	
6	M/22	Hurler-Scheie syndrome	Malaise and fatigue, joint stiffness, corneal clouding, airway obstruction, cardiac-valve disease, frequent infections, hepatosplenomegaly, umbilical and inguinal hernias, growth deficiency	Mitral-valve replacement, carpal-tunnel release, CPAP (nightly)
7	F/17	Scheie's syndrome	Malaise and fatigue, joint stiffness, corneal clouding, cardiac-valve disease, hepatosplenomegaly, digital contractures	Aortic-valve replacement
8	F/5	Hurler's syndrome	Developmental delay, malaise and fatigue, corneal clouding, airway obstruction, cardiac-valve disease, frequent infections, hepatosplenomegaly, growth deficiency, gibbous deformity	Tonsillectomy and adenoidectomy
9	F/9	Hurler-Scheie syndrome	Developmental delay, malaise and fatigue, joint stiffness, corneal clouding, airway obstruction, cardiac-valve disease, frequent infections, hepatosplenomegaly, umbilical and inguinal hernias, digital contractures, growth deficiency	Tonsillectomy and adenoidectomy, ventriculoperitoneal shunt
10	M/14	Hurler-Scheie syndrome	Developmental delay, malaise and fatigue, joint stiffness, corneal clouding, airway obstruction, cardiac-valve disease, frequent infections, hepatosplenomegaly, inguinal hernias, scoliosis	Carpal-tunnel release, lumbar spinal fusion surgery

*Growth deficiency was defined as a value below the 5th percentile for height and weight. CPAP denotes continuous positive airway pressure.

magnetic resonance imaging with the use of Advantage Windows workstation software (General Electric). The organ volume was measured in milliliters and expressed as a percentage of body weight, assuming a density of 1 g per milliliter of tissue.

The protocol was approved by the human-subjects institutional review board at each institution. All patients or their parents or guardians gave written informed consent.

Biochemical Studies

Recombinant α -L-iduronidase was produced in Chinese-hamster-ovary cells with the use of bioreactors and standard column chromatography and was extensively analyzed for safety and purity. We measured the activity of α -L-iduronidase according to the method of Shull et al.²⁰ or with a new assay that involved more substrate and a higher temperature and whose results are reported in SI units. When the new assay is used, a dose of 125,000 U of α -L-iduronidase per kilogram is equivalent to 100 SI units per kilogram. Urinary glycosaminoglycan excretion was measured according to an adaptation of the method of Bjornsson.²² Enzyme-linked immunosorbent assays for antibodies to α -L-iduronidase involved a variation of the method of Shull et al.,²⁰ and Western blotting was performed according to a standard method. Uronic acids and *N*-sulfated urinary glycosaminoglycans were analyzed according to the orcinol, carbazole, and 3-methyl-2-benzothiazoline hydrazone methods and by electrophoretic separation.

Statistical Analysis

We used two-tailed *t*-tests to compare the mean values for liver and spleen volumes, urinary glycosaminoglycans, and range-of-motion data before and after treatment. We analyzed the rate of growth in height and weight by comparing the mean slopes of the best-fit lines for the one to two years before treatment for each patient with the best-fit lines for the one year of treatment. The mean change in the growth rate was analyzed by two-tailed *t*-tests. We used the Wilcoxon signed-rank test to analyze changes in New York Heart Association functional class.

RESULTS

All patients received weekly infusions of recombinant human α -L-iduronidase for 52 weeks. The mean activity of α -L-iduronidase in leukocytes was 0.04 U per milligram before treatment and 4.98 U per milligram, or 15 percent of normal, an average of seven days after an infusion of α -L-iduronidase (i.e., immediately before the next infusion). Enzyme activity was not detectable in brushings of buccal mucosa before treatment, but seven days after an infusion, it reached a level of 1 percent of normal.

Five patients (Patients 1, 4, 5, 6, and 7) had transient urticaria on the trunk, face, arms, and legs during an infusion given during week 4 or later, and in four patients (Patients 1, 5, 6, and 7) it recurred during subsequent infusions beginning at or after week 20. During these episodes, urticaria began midway through an infusion but resolved soon after the infusion was completed. In the four patients with recurrent urticaria, the episodes eventually became less frequent and less severe and finally stopped. In three patients (Patients 1, 5, and 6), the urticaria was accompanied by angioedema (thickening of the tongue and tightening of the throat) on a total of nine occasions and by moderate transient hypoxemia on three occasions. Patient 8 had one episode of facial swelling without urticaria during the infusion at week 47. These

symptoms usually resolved about one hour after the infusion was stopped. In patients with recurrent urticaria, the rate of enzyme infusion was decreased or the dose temporarily reduced, and they were given increased medications such as diphenhydramine before, and in some cases during, an infusion.

Four patients (Patients 2, 7, 8, and 9) had biochemical evidence of complement activation during infusions given at weeks 6 and 12, as evidenced by decreased serum total complement activity (i.e., decreased CH₁₀₀) and decreased C3 or C4 concentrations after infusions. Although these patients were usually asymptomatic, Patient 8 had fever, chills, and "fussy behavior" on three occasions. By week 26, there was no complement activation during infusion in any patient. In the four patients who had transient complement activation, serum α -L-iduronidase antibodies were detected by week 8, but the antibody levels subsequently declined or became undetectable. The antibodies did not immunoprecipitate native enzyme and did not inhibit enzyme activity *in vitro* (Anand VA, Kakkis ED: unpublished data), nor did they alter efficacy *in vivo* on the basis of urinary glycosaminoglycan excretion. In all 10 patients IgG antibodies developed to Chinese-hamster-ovary cell proteins that were present as a trace impurity in the enzyme preparation, but clinically important adverse events were not correlated with the presence or titer of these antibodies. There were no abnormalities in blood counts, serum chemical values, or urinalysis during treatment. Other mucopolysaccharidosis-related complications during treatment consisted of cervical subluxation requiring cervical fusion, mitral-valve replacement with coronary bypass grafting, repeated lumbar fusion, and a ventriculoperitoneal shunt, each in one patient.

Reduction in Lysosomal Storage

Liver volume decreased by 19 to 37 percent from base line in nine patients and by 5 percent in one patient at 52 weeks; the mean decrease was 25 percent ($P < 0.001$). By 26 weeks, the size of the liver was normal for body weight and age in eight patients (Fig. 1). In the two patients (Patients 6 and 9) who had the largest liver size relative to body weight at base line, the size of the liver was close to normal at 52 weeks (3.2 and 3.3 percent of body weight, respectively).

In eight patients the size of the spleen decreased by 13 to 42 percent from base line. The mean decrease was 20 percent among all 10 patients ($P < 0.001$).

Urinary glycosaminoglycan excretion declined rapidly after 3 to 4 weeks of treatment, and by 8 to 12 weeks it was 60 to 80 percent below the base-line values. At 52 weeks, the mean reduction was 63 percent (range, 53 to 74 percent; $P < 0.001$). The excess urinary glycosaminoglycan excretion (that above the upper limit of the normal value for age) was reduced by a mean of 80 percent in these patients. The results were confirmed by an assay of uronic acids and *N*-sul-

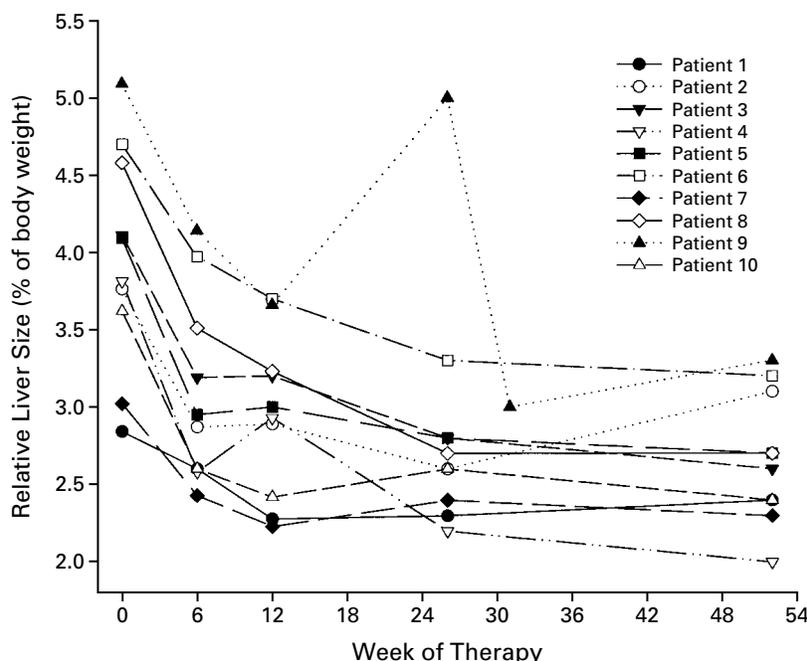


Figure 1. Changes in Liver Size in Patients with Mucopolysaccharidosis I during α -L-Iduronidase Therapy. Liver size was measured in terms of volume and expressed as the percentage of body weight, given a density of 1 g per milliliter of tissue. Patient 9 had an episode of hepatitis at 26 weeks that was believed to be due to a concomitantly taken medication and that resolved by week 30. This episode was thought to account for the transient increase in the size of her liver. The upper bounds of the 95 percent confidence interval of normal values (i.e., within the normal range for age, as adapted from the data of Stocker and Dehner²³) are 3.5 percent for boys 5 to 12 years of age, 3.2 percent for girls 5 to 12 years of age, 2.2 percent for boys 13 to 17 years of age, 2.7 percent for girls 13 to 17 years of age, 2.6 percent for men 18 years of age or older, and 2.9 percent for women 18 years of age or older.

fate (a test specific for heparan sulfate). Electrophoretic studies of urine revealed a significant reduction in the excretion of heparan sulfate and dermatan sulfate, but the excretion of dermatan sulfate was still greater than normal in all patients.

Clinical Studies

Increases in Height and Weight

The height increased by a mean of 6.0 cm (5 percent) in the six prepubertal patients, and their mean rate of growth in height increased from 2.80 cm per year to 5.17 cm per year during treatment ($P=0.01$) (Table 2). For all 10 patients, body weight increased by a mean of 3.2 kg (9 percent), and the mean increase was 4.2 kg (17 percent) among the 6 prepubertal patients. In these six patients, the mean rate of growth in weight increased from 1.66 kg per year before treatment to 3.83 kg per year during treatment ($P=0.04$) (Table 2).

Range of Motion

Restriction of shoulder flexion decreased during treatment in six of the eight patients in whom it was evaluated at base line. The mean decrease in joint re-

striction was 28 degrees in the right shoulder ($P<0.001$) and 26 degrees in the left shoulder ($P=0.002$) (Fig. 2A). Among all 10 patients, the degree of restriction of elbow extension decreased by a mean of 7.0 degrees in the right elbow ($P=0.03$) and 7.0 degrees in the left elbow ($P=0.007$) (Fig. 2B). The degree of restriction of knee extension decreased by a mean of 3.2 degrees on the right ($P=0.10$) and 3.0 degrees on the left ($P=0.09$) in the 10 patients (Fig. 2C).

Analysis in individual patients revealed that the joints with the greatest degree of restriction before treatment had the greatest improvement. For example, at base line, Patients 5, 9, and 10 could not flex their shoulders beyond 100 degrees, and the range of motion increased by 21 to 51 degrees after treatment. The improvements in the range of motion were accompanied by patient-reported increases in physical activities, such as being able to wash their hair, hold a hamburger normally, hang from monkey bars, and play sports better.

Airway Function

Seven of the 10 patients had apnea, and these 7 had a decrease in the number of episodes of apnea and hy-

TABLE 2. HEIGHT AND WEIGHT OF SIX PREPUBERTAL PATIENTS BEFORE ENZYME-REPLACEMENT THERAPY AND AFTER ONE YEAR OF THERAPY.

PATIENT No.	AGE (YR)	HEIGHT			WEIGHT		
		GROWTH RATE BEFORE TREATMENT	GROWTH RATE AT 52 WK	INCREASE	GROWTH RATE BEFORE TREATMENT	GROWTH RATE AT 52 WK	INCREASE
		cm/yr		%	kg/yr		%
2	11	3.76	5.67*	51	2.08	3.58*	72
3	9	2.97	5.75*	93	1.51	4.41*	192
4	8	4.22	5.71*	35	3.59	9.33*	160
5	12	3.51	8.63*	146	1.53	2.35	54
8	5	1.30	2.19	68	0.62	1.54	148
9	9	1.05	3.07	192	0.61	1.76	188
Mean	9	2.80	5.17*	85	1.66	3.83*	131
P value				0.01			0.04

*The normal growth rate is 5.0 cm per year for height and 3.0 kg per year for weight.

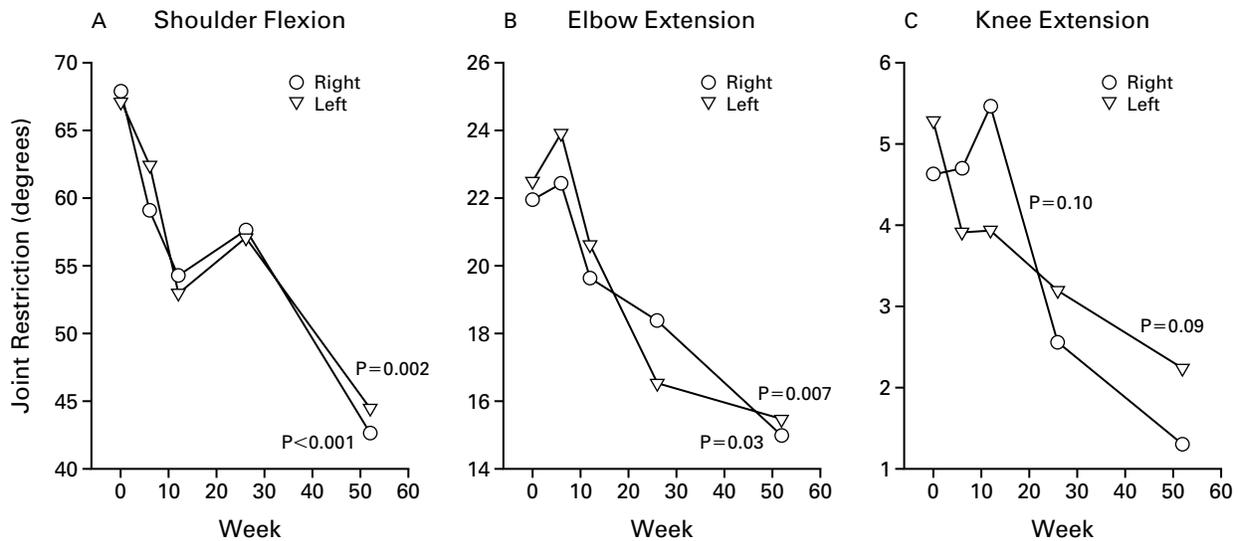


Figure 2. Mean Changes in the Restriction of Range of Motion of Shoulder Flexion (Panel A), Elbow Extension (Panel B), and Knee Extension (Panel C) in Patients with Mucopolysaccharidosis I during α -L-Iduronidase Therapy.

The mean degrees of restriction in the range of motion of right- and left-shoulder flexion are not shown for two patients, because shoulder flexion was not evaluated in these two patients at base line. The values represent the difference between the normal maximal range of motion for age and the measured value.

popnea during treatment, from 155 per night to 60 per night (a 61 percent decrease), with a change in the mean apnea-hypopnea index from 2.1 to 1.0 event per hour. Three of these seven (Patients 2, 6, and 9) had clinically important sleep apnea, and in all three this disorder improved during treatment. In Patient 2, the apnea-hypopnea index decreased from 4.5 events per hour at base line to 0.4 event per hour at 26 weeks, and the total length of time during sleep in which oxygen desaturation fell below 89 percent decreased

from 48 minutes to 1 minute per night. At base line, Patient 6 required continuous positive airway pressure at night because of severe desaturation (with continuous positive airway pressure, the oxygen saturation was below 89 percent during 61 of 368 minutes of sleep). After 52 weeks of treatment, oxygen saturation was less than 89 percent for only 8 of 332 minutes of sleep, and continuous positive airway pressure was not used. Patient 9 had an apnea-hypopnea index of 9.5 events per hour before treatment and 4.0 events

per hour after 26 weeks of treatment. In Patient 8, the initial apnea-hypopnea index of 0.1 event per hour increased to 3.1 events per hour at 26 weeks and to 9.3 events per hour at 52 weeks for reasons that were unclear. Eight of 10 patients or their families reported that their breathing had improved, and 5 of 7 reported quieter nighttime breathing, an improved quality of sleep, and decreased daytime somnolence.

Cardiac Function

The New York Heart Association functional classification was determined by serial interviews with the patients. All 10 patients reported an improvement by one or two classes (Fig. 3), but there was no objective data from echocardiographic studies to verify direct cardiac benefit. The improved functional scores may reflect improvements in aspects of the disease other than cardiac function. When base-line echocardiograms were compared with those obtained after 52 weeks of treatment, tricuspid regurgitation or pulmonary regurgitation was decreased in four patients, but mitral regurgitation worsened in two patients (Patients

2 and 7). At base line, Patient 6 had atrial flutter and clinical signs of cardiac failure, including dyspnea at rest and pitting edema. After 12 weeks of treatment, he had sinus rhythm with first-degree block and his dyspnea at rest and pitting edema had resolved.

Symptomatic Changes

Before treatment, all 10 patients reported a lack of endurance and limitations in their ability to perform daily activities, but exercise tolerance was not formally tested. During treatment, all patients had improved endurance and fewer limitations in their ability to perform daily activities, and after 26 weeks of treatment, many were able to walk farther, run, and play sports. Patients 3, 4, and 5 reported the resolution of severe, incapacitating headaches after 6 to 12 weeks of treatment.

Ophthalmic Changes

The extent of corneal clouding did not change in any of the eight patients with this problem. Several patients reported decreased photophobia or conjuncti-

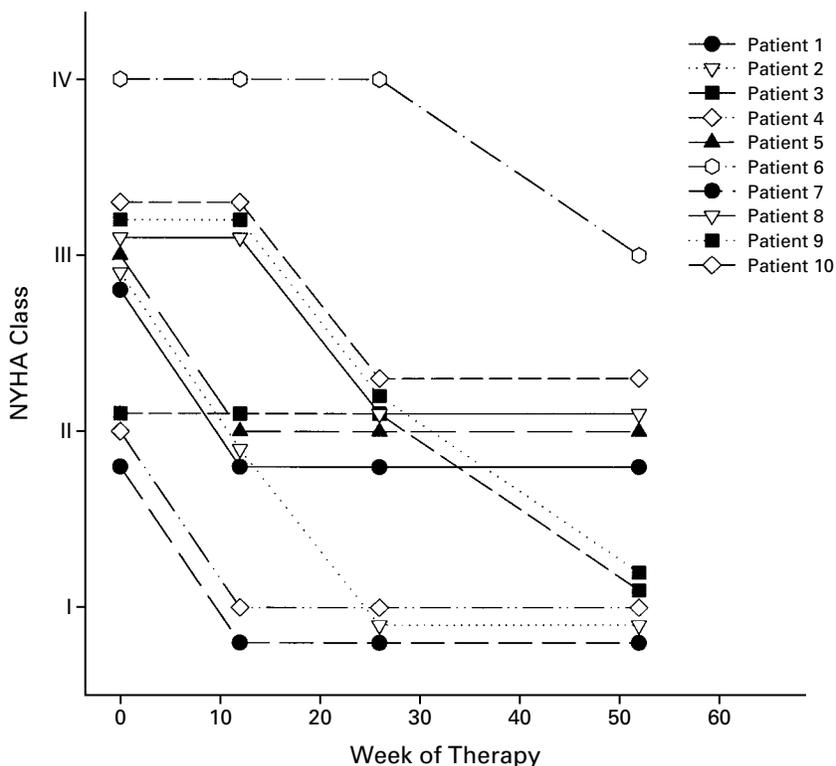


Figure 3. Changes in New York Heart Association (NYHA) Functional Class in Patients with Mucopolysaccharidosis I during α -L-Iduronidase Therapy.

The changes in scores were based on information obtained from serial interviews with the patients. New York Heart Association class I indicates no symptoms with ordinary activity; class II, symptoms with ordinary activity and a slight limitation of activity; class III, symptoms with less-than-ordinary activity and marked limitation of activity; and class IV, symptoms with any type of activity or at rest. The difference between pretreatment scores and scores at 52 weeks was significant ($P=0.002$).

val irritation. Visual acuity improved from 20/1000 to 20/200 (in one eye) in one patient and slightly in two others.

DISCUSSION

Our findings indicate that intravenous administration of recombinant human α -L-iduronidase results in clinical and biochemical improvement in patients with mucopolysaccharidosis I. The normalization of liver size and near-normalization of urinary glycosaminoglycan excretion are consistent with data from studies in dogs with mucopolysaccharidosis I, which demonstrated clearance of storage in the liver and decreased urinary glycosaminoglycan excretion in as little as two weeks.^{20,21}

This trial was not designed to assess the effect of enzyme-replacement therapy on the mental retardation of patients with Hurler's syndrome. Most of our patients did not have mental retardation, and studies of patients who were treated by bone marrow transplantation⁷ indicate that to be effective, therapy for mental retardation must be preventive.

Hypersensitivity reactions to the infusions of recombinant human α -L-iduronidase were less severe than those predicted on the basis of studies in dogs.^{20,21} Though important in some patients, recurrent urticaria was manageable with premedication and adjustments in the infusion rate. Antibodies specific to α -L-iduronidase were detected in four patients, usually in association with subclinical complement activation, and both the antibody levels and the extent of complement activation declined with time. Similar IgG-mediated immune responses have been noted in patients with Gaucher's disease who were treated with glucocerebrosidase,²⁴ although the events were more frequent in our patients. Patients with mucopolysaccharidosis I who are homozygous for mutations that result in the production of no protein (the so-called null genotype) may have a greater immune response than did our 10 patients, none of whom had a null genotype (Hopwood JJ, et al.: personal communication).

In conclusion, our findings demonstrate that recombinant human α -L-iduronidase can reduce lysosomal storage and ameliorate some aspects of clinical disease in patients with mucopolysaccharidosis I. Long-term studies of enzyme-replacement therapy will be required to assess the overall effects of this treatment on morbidity and mortality.

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