A Phase 1 Trial of Recombinant Human Acid Sphingomyelinase (rhASM) Enzyme Replacement Therapy in Adults with ASM Deficiency (ASMD)

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Introduction

Acid sphingomyelinase deficiency (ASMD) is an autosomal recessive, lysosomal storage disorder that results when sphingomyelin is unable to be normally catabolized to ceramide and phosphorylcholine. Consequently, sphingomyelin accumulates within cells primarily of the reticuloendothelial system, leading to hepatosplenomegaly, anemia, thrombocytopenia, and interstitial lung disease. Growth retardation and an atherogenic lipid profile also are common findings. Patients who have little to no residual ASM activity exhibit the most severe symptoms with onset in infancy, failure to thrive, neurodegeneration, and death by age 3 (Niemann-Pick disease type A, NPD A). Patients with higher amounts of residual ASM activity have variable ages of onset, heterogeneous presentations and somatic symptoms, little to no neurologic involvement, and generally survive into adulthood (NPD B). Currently, there is no treatment for patients with ASMD.

Enzyme replacement therapy (ERT) has been successfully used to treat several lysosomal storage disorders, including Gaucher disease, Mucopolysaccharidosis types I, II, and VI, Fabry disease, and Pompe disease. Recombinant human lysosomal enzymes are administered intravenously and taken up into cells by receptor-mediated endocytosis for subsequent targeting to lysosomes. Proof of principle for the treatment of ASMD was demonstrated by Dr. Edward Schuchman's laboratory (Mount Sinai Medical Center) in an ASM knockout mouse (ASMKO) model where intravenous injections of recombinant human ASM (rhASM) efficiently reduced sphingomyelin levels in liver and spleen, and to a lesser extent in lung (Miranda, et al., FASEB 2000;14:1988). However, sphingomyelin levels were not reduced in brain because of the inability of rhASM to cross the blood-brain barrier.

Additional studies confirmed that biweekly doses of rhASM reduced sphingomyelin levels in ASMKO mice in a dose-dependent manner (0.3-3 mg/kg). The no observed adverse effect levels (NOAEL) for single and repeat dosing were determined to be 0.3 and 3 mg/kg, respectively in ASMKO mice. Subsequent attempts to deplete sphingomyelin levels in lung with higher doses of rhASM led to unexpected toxicity. At doses ≥10 mg/kg, ASMKO mice but not normal animals experienced liver inflammation, adrenal hemorrhage, cardiovascular shock and death in the setting of very elevated cytokine levels, suggesting cytokine release syndrome. The toxicity and cytokine elevations seen with high doses of rhASM could be ameliorated or prevented by prior treatment of ASMKO mice with several lower doses of rhASM, suggesting that the rate and amount of sphingomyelin degradation plays a key role.

We now report the first-in-human study of rhASM conducted in adults with ASMD.

Methods and Study Design

Design

This was a single-center, single-dose, dose escalation Phase 1 trial conducted at Mount Sinai Medical Center in New York City, USA from December 2006 to April 2009. The primary trial objectives were to evaluate the safety and pharmacokinetics of single doses of rhASM in adults with non-neuronopathic ASMD (Niemann-Pick B). Single doses of 0.03, 0.1, 0.3, 0.6 and 1.0 mg/kg rhASM were infused sequentially by dose cohort. The original trial design called for a minimum of 15 patients (5 cohorts of 3 patients each). Due to difficulties with patient enrollment, the protocol was amended such that the first 2 cohorts enrolled 3 patients each and the last 3 cohorts were to enroll 2 patients each. An independent data monitoring committee oversaw the conduct of the trial and all study procedures were approved by the IRB.

Patients

To be eligible for the study, patients had to be 18-65 years of age and have deficient ASM enzyme activity, a spleen volume ≥2x normal, AST and ALT ≤250 IU/L, bilirubin ≤3.6 mg/dL, INR ≤1.5, DLco >30% predicted, and platelets ≥60,000/μL. Patients were excluded if they had cirrhosis (by liver biopsy), significant cardiac disease, total splenectomy, or were taking medications or herbal supplements that were potentially hepatotoxic, promoted bleeding, or inhibited rhASM.

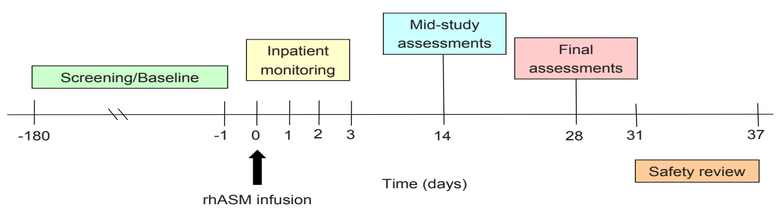
Visits

Once screening was completed and eligibility was confirmed, patients were admitted to the cardiac care unit (CCU) overnight for baseline telemetry and infused the following morning with rhASM. Patients were monitored for 72 hrs post-dose while on telemetry (24 hrs in the CCU and 48 hrs in the General Clinical Research Center). Patients returned for an overnight visit on Day 14 and an outpatient visit on Day 28.

Assessments

- Physical exam – Days 0, 1, 2, 14, and 28
- Chemistries, hematology, and urinalysis - preinfusion, then 24, 48, 72 hrs; Days 14, 28
- Liver function tests - preinfusion, then q12 hrs through 72 hrs, Days 14, 28
- Aldosterone, cortisol, delta-4-androstenedione - preinfusion, then q12 hrs through 72 hrs
- ACTH stimulation test – Screening, Day 14
- Telemetry – continuous through 72 hrs
- ECG, echocardiogram - preinfusion, end of infusion, and postinfusion at 1, 2, 6, 12, and 24 hrs, Day 14
- Cardiac biomarkers (BNP, cardiac troponin-I, CPK-MB) - preinfusion, then 2, 6, 12, 24 hrs
- Sphingomyelin, ceramide levels in plasma – preinfusion, then 24, 48, 72 hrs; Days 14 and 28
- Pulmonary function testing and CXR – Screening, Day 14
- Liver and spleen volumes by MRI - Screening, Day 14
- Liver and skin biopsies – Screening, Day 14
- Biomarkers – predose, Days 14, 28
- Anti-rhASM IgG testing – preinfusion, Day 28
- Pharmacokinetics – within 30 min preinfusion, 15 min after start of infusion, end of infusion, and at following time points post-infusion: 15, 30, 45 min; 1, 2, 3, 4, 6, 8, 12, 18, 24, 48, 72 hrs
- Cytokines (IL-1a, IL-1b, IL-6, G-CSF, GM-CSF, MIP-1a, TNF-a) – pharmacokinetic timepoints (above) plus Day 14

Phase 1 Study Patient Flow



Demography and Baseline Characteristics

Cohort Number	Dose (mg/kg)	Patient ID	Age at Infusion (yr)	Gender	Age at Symptom Onset (yr)	Age at Diagnosis (yr)	Spleen Volume (X norm)	ASM Activity (% norm)
1	0.03	10202	23	F	2.2	2.2	12.5	14
		10401	21	M	0.5	8.1	15.1	25
		10503	19	M	5.9	5.9	16.1	15
2	0.1	10304	45	F	38.7	38.7	4.8	14
		10605	26	F	2.9	3.3	12.3	17
		10906	41	F	9.6	9.6	8.7	29
3	0.3	10807	24	M	1.0	3.0	9.5	18
		11509	54	F	53.3	53.3	6.1	24
4	0.6	12010	18	M	Unknown	13.9	8.7	12
		12313	18	M	1.4	3.0	10.1	6
5	1.0	12112	46	M	3.5	3.5	14.5	6

A total of 13 patients were enrolled and 11 patients infused with rhASM. The mean age of infused patients was 30.8 yrs, all were Caucasian (non-Hispanic/non-Latino), and mean spleen volume was 10.8 multiples of normal. Patient 10807 had a partial splenectomy; the remaining patients had intact spleens at study entry.

Results

Treatment Emergent Adverse Events Considered Related (Possibly, Probably, or Definitely) to Treatment

Dose (mg/kg)	Patient No.	Adverse Event	Start Day[a]	Severity	Action Taken
0.3	11509	Fever	2	Moderate	None
		Myalgia	2	Moderate	None
		Nausea	2	Moderate	None
		Acute phase reaction	2	Moderate	None
0.6	12010	Leg pain	1	Moderate	None
		Abdominal pain	2	Mild	None
		Hip pain	2	Moderate	None
		Acute phase reaction	3	Moderate	None
0.6	12313	Acute phase reaction	2	Moderate	None
		Elevated bilirubin	2	Moderate	None
		Lymphocytic infiltrate/ hepatocellular degeneration (liver biopsy)	14	Moderate	None
1.0	12112	Nausea/vomiting	1	Moderate	Medication
		Elevated bilirubin	3	Severe	None
		Fever	2	Moderate	Medication
		Fatigue	2	Moderate	None
		Acute phase reaction	2	Moderate	None
		Urobilinogen in urine	3	Moderate	None
		Scleral icterus	3	Moderate	None
		Increased fibrin D-dimer	29	Moderate	None

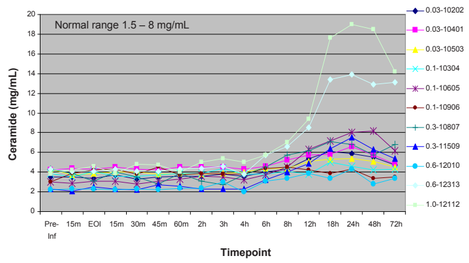
[a]Start day relative to infusion

Summary of Related Adverse Avents

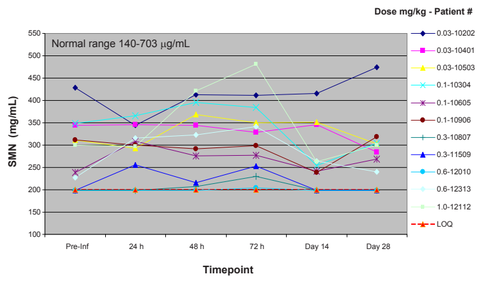
- There were no significant cardiovascular changes by telemetry, ECG, echocardiogram, or biomarkers (BNP, cardiac troponin-I, CPK-MB) and no measurable cytokine elevations or hormonal abnormalities.
- Four of six patients receiving ≥0.3 mg/kg rhASM experienced a total of 19 clinical and laboratory adverse events assessed as drug-related. The intensity of adverse events ranged from mild to severe, but most were moderate and did not require any intervention. They included fever (n=2), pain [myalgia; abdominal, leg, and hip pain] (n=2), nausea (n=2), scleral icterus & urine urobilinogen (n=1), fatigue (n=1), vomiting (n=1), lymphocytic infiltrate/ hepatocellular degeneration on liver biopsy (n=1), acute phase reaction (n=4), elevated bilirubin (n=2), and increased fibrin D-dimer (n=1).
- Onset of clinical symptoms began 12 hrs post-infusion and resolved by 72 hrs, except for hip pain in one patient that began after 72 hrs.
- Day 14 liver biopsy in one patient (0.6 mg/kg, #12313) showed two new foci of lymphocytic infiltrates: one was tiny (0.1 mm diameter) and the other was moderate (0.5 mm diameter) and associated with hepatocellular degeneration.
- The study was stopped when the first patient in cohort 5 (1 mg/kg, #12112) experienced a dose-limiting toxicity of hyperbilirubinemia (peak 4.7 mg/dL).

Substrate and Product

Plasma ceramide levels showed a dose-dependent rise by 6 hrs and peaked at 18-72 hrs



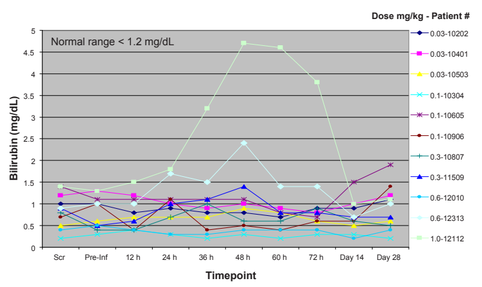
Plasma sphingomyelin levels were normal at baseline and showed no consistent trend over time



Liver

Total bilirubin showed a dose-related rise by 24 hrs and peaked at 48-60 hrs. The highest total bilirubin was 4.7 mg/dL in patient #12112 who received the highest dose (1 mg/kg). There were proportional increases in direct and indirect bilirubin. No increases were seen in ALT, AST, or alkaline phosphatase. There was a mild increase in GGT through 72 hrs (not shown) in two patients who received the highest (1 mg/kg, #12112) and lowest (0.03 mg/kg, #10503) doses. There was no indication of hemolysis as hemoglobin and hematocrit were stable.

Bilirubin - Total



Acute Phase Response (Inflammation)

CRP showed a transient dose-related rise by 24 hrs, peaked at 48-72 hrs, and returned to normal by Day 14. Other acute phase reactants also showed increases (% neutrophils, fibrinogen, ferritin, PT and PTT) and decreases (iron, albumin). There was no trend in platelet count or the level of fibrin-split products (not shown). Plasma levels of various cytokines (IL-1a, IL-1b, IL-6, G-CSF, GM-CSF, MIP-1a, TNF-a) were consistently below quantifiable levels. The laboratory acute phase reactants correlated with the clinical symptoms of nausea, headache and myalgias in some patients.

CRP

Fibrinogen

% Neutrophils

Ferritin

Preliminary Safety Observations

- Dose-related clinical and lab adverse events began at 0.3 mg/kg
- Two major safety laboratory observations
  1. **Hyperbilirubinemia**
     - Proportionate rise in direct and indirect bilirubin
     - No consistent markers of liver damage (AST, ALT, AP). Two patients had mildly ↑GGT at 48-72 hrs, and one patient had two new liver foci of lymphocytic infiltrates, one of which was associated with hepatocellular degeneration.
  2. **Acute phase response (Inflammation)**
     - ↑ CRP, % neutrophils, ferritin, fibrinogen, PT, PTT; ↓iron, albumin
     - The clinical symptoms of fever, nausea, vomiting, and pain are most likely related to the acute phase response
- No evidence of cytokine release syndrome, cardiovascular changes, or hormonal abnormalities

Conclusions

- This trial describes the first experience with enzyme replacement therapy in adult patients with ASMD. At bioactive doses in humans, rhASM did not cause significant cytokine elevations or cardiovascular changes.
- The major safety observations were dose-related hyperbilirubinemia and acute phase response. Both adverse events are likely related to the breakdown of sphingomyelin into ceramide and phosphorylcholine, but the exact molecular mechanisms are not fully understood.
- Several safety biomarkers were identified, including bilirubin, ceramide, and CRP.
- Based on the hyperbilirubinemia findings, the maximum tolerated starting dose of rhASM was 0.6 mg/kg. Within-patient dose escalation may be an option for higher repeat doses of rhASM.