

Infusion of recombinant human acid sphingomyelinase into Niemann-Pick disease mice leads to visceral, but not neurological, correction of the pathophysiology

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ABSTRACT An inherited deficiency of acid sphingomyelinase (ASM) activity results in the Type A and B forms of Niemann-Pick disease (NPD). Using the ASM-deficient mouse model (ASMKO) of NPD, we evaluated the efficacy of enzyme replacement therapy (ERT) for the treatment of this disorder. Recombinant human ASM (rhASM) was purified from the media of overexpressing Chinese Hamster ovary cells and i.v. injected into 16 five-month-old ASMKO mice at doses of 0.3, 1, 3, or 10 mg/kg every other day for 14 days (7 injections). On day 16, the animals were killed and the tissues were analyzed for their sphingomyelin (SPM) content. Notably, the SPM levels were markedly reduced in the hearts, livers, and spleens of these animals, and to a lesser degree in the lungs. Little or no substrate depletion was found in the kidneys or brains. Based on these results, three additional 5-month-old ASMKO animals were injected every other day with 5 mg/kg for 8 days (4 injections) and killed on day 10 for histological analysis. Consistent with the biochemical results, marked histological improvements were observed in the livers, spleens, and lungs, indicating a reversal of the disease pathology. A group of 10 ASMKO mice were then i.v. injected once a week with 1 mg/kg rhASM for 15 wk, starting at 3 wk of age. Although anti-rhASM antibodies were produced in these mice, the antibodies were not neutralizing and no adverse effects were observed from this treatment. Weight gain and rota-rod performance were slightly improved in the treated animals as compared with ASMKO control animals, but significant neurological deficits were still observed and their life span was not extended by ERT. In contrast with these CNS results, striking histological and biochemical improvements were found in the reticuloendothelial system organs (livers, spleens, and lungs). These studies indicate that ERT should be an effective therapeutic approach for Type B NPD, but is unlikely to prevent the severe neurodegeneration associated with Type A NPD.—Miranda, S. R. P., He, X., Simonaro, C. M., Gatt, S., Dagan, A., Desnick,

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TYPES A AND B Niemann-Pick disease (NPD) are two clinically distinct forms of an inherited lysosomal storage disorder resulting from the deficient activity of acid sphingomyelinase (ASM; sphingomyelin phosphodiesterase; E.C. 3.1.2.14) (1, 2). Type A NPD presents with failure to thrive, hepatosplenomegaly, and progressive neurodegeneration that generally leads to death by three years of age. In contrast, patients with Type B NPD develop hepatosplenomegaly and/or pulmonary insufficiency, but usually have little neurological involvement and survive into adolescence or adulthood (3).

ASM is the enzyme required to hydrolyze sphingomyelin (SPM) into ceramide and phosphocholine. In most normal tissues, SPM constitutes from 5 to 20% of the total cellular phospholipid, but in NPD patients these levels may be elevated up to over 70%. It is presumed that both forms of NPD are caused by the accumulation of SPM in various cell types (e.g., macrophages, neurons), leading to impairment of their normal cellular function. However, the precise nature of the phenotypic variation in NPD is not understood.

The rationale underlying enzyme replacement therapy (ERT) for the treatment of lysosomal storage disorders derives from early cell culture studies showing that cells from patients affected with these disorders could be metabolically 'corrected' when they

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were cultured together with either normal cells or conditioned media obtained from normal cells (4). Subsequently, it was found that the 'cross-correcting factors' present in normal cell media were lysosomal enzymes that were being released from the cells at low levels (5). Early attempts at ERT in human patients with lysosomal disorders were unsuccessful (6–11), primarily because of an inadequate understanding of receptor-mediated endocytosis and insufficient sources of highly purified enzymes. These limitations have now been largely overcome, and ERT has proved clinically successful for at least one lysosomal storage disorder: Type 1 Gaucher disease (12–15).

ERT for NPD became possible after the cloning of the human ASM cDNA and gene (16–18), the construction of mouse models deficient in ASM activity (19, 20), and the creation of a Chinese hamster ovary (CHO) cell line overexpressing recombinant human ASM (rhASM) (21). The ASM-deficient (ASMKO) mouse models have clinical, biochemical, and pathological findings similar to those seen in human NPD patients, making them valuable tools for the investigation of disease pathogenesis and evaluation of therapeutic approaches.

Relevant to the success of ERT for NPD, it has recently been shown that ASM is released from normal cells at high levels (22, 23). Intriguingly, this secreted form of ASM (also known as sSMase) required zinc cations to become activated, explaining why it had not been previously detected by standard enzyme assay systems, which generally were carried out in the presence of EDTA. In stark contrast to the secreted enzyme, the intracellular enzyme is highly active at low pH in the absence of exogenous zinc or other divalent cations. The secreted enzyme can be taken up by NPD cells via a receptor-mediated processes that delivers it to the lysosomes; within lysosomes, the internalized enzyme comes in contact with zinc and is activated.

As part of our ongoing efforts to develop an effective treatment for Types A and B NPD, the present study evaluated the efficacy of ERT using the ASMKO mouse model and compared these results to those obtained previously from bone marrow transplantation (BMT) (24, 25) and hematopoietic stem cell gene therapy (HSCGT) (S. R. P. Miranda et al. unpublished results). The data presented document the efficacy of ERT for the treatment of non-neurological Type B NPD and highlight its limitations for the treatment of neurological Type A NPD.

MATERIALS AND METHODS

Experimental animals

The ASMKO mouse model was created by gene targeting as described previously (19). Affected mice have no detectable

ASM activity, but develop normally until ~8 wk of age, when ataxia and mild tremors become noticeable. The disease then follows a neurodegenerative course that leads to death between 6 and 8 months of age. Characteristic lipid-laden foam cells (NPD cells) are found in most major organs, associated with elevated SPM levels. Homozygous (–/–) mice are distinguished from normal (+/+) or heterozygous (+/–) animals using a polymerase chain reaction assay (19). The animals were maintained on a 12 h light/dark cycle with water *ad libitum* and Purina rodent chow 5001.

Production and purification of recombinant human ASM

The eukaryotic expression vector p91023 was used to stably overexpress active human ASM in a CHO cell line (21). Briefly, this overexpression system relied on coamplification of the human ASM cDNA and the adjacent dihydrofolate reductase gene in the presence of methotrexate (26, 27). Stable amplification of the human ASM cDNA led to overexpression and secretion of rhASM. A clonal CHO cell population overexpressing rhASM was obtained by methotrexate amplification, adapted to suspension cultures, and maintained in a CELLMAX 100 bioreactor system (21). The CELLMAX 100 system enabled collection of ~1 l of culture media/week for enzyme purification. The purification method resulted in the isolation of rhASM in an essentially homogeneous form (21).

Enzyme administration

Three groups of test animals were injected with purified rhASM. Initially, doses of 0.3, 1, 3, and 10 mg rhASM/kg of mouse body weight were administered intravenously (i.v.) (50 μ l injection volume) into adult (5 month) ASMKO mice for 2 wk (4 mice each per dose). Mice received injections every other day during the 2 wk period (7 injections) and were killed 48 h after the last injection to assess their tissue SPM content (described below). Based on these results, three additional 5-month-old ASMKO animals were then injected i.v. with 5 mg/kg every other day for 8 days (4 injections) and killed for histological analysis on day 10 (see below). For long-term analysis, 1 mg/kg of rhASM was administered i.v. once a week for 15 wk into 10 ASMKO animals, starting when the animals were 3 wk old. Five animals each were used for biochemical or histological analysis. Immediately before each injection the enzyme was filtered using a 0.2 μ m filter.

ELISA and immunoprecipitation assays

To detect the presence of antibodies against injected rhASM, enzyme-linked immunoassays (ELISA) were performed on plasma from treated and untreated mice. Purified rhASM (1 μ g) was incubated for 18 h in 96-well microtiter plates. The wells were washed 3 \times with washing buffer 1 (WB1: 10 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% Tween 20) and then blocked for 1 h with 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) at room temperature. Dilutions of treated and untreated mouse plasma in WB1 (100 μ l of 10⁻², 10⁻³, or 10⁻⁴ dilutions) were added to the wells and incubated at 37°C for 3 h. The wells were then washed 3 \times with WB1, followed by the addition of 100 μ l of peroxidase-conjugated goat anti-mouse IgG (Sigma, St. Louis, Mo.) (1:500 dilution in WB1) and a 2 h incubation at room temperature. The wells were again washed 3 \times with WB1, then twice more with washing buffer 2 (10 mM Tris-HCl pH 7.5, 150 mM NaCl); 100 μ l of peroxidase substrate ('ready-to-use' peroxidase substrate for ELISA-ABTS solution; Boehringer Mannheim, Mannheim, Germany) was added to the wells and

incubated at room temperature for 30 min. An automated ELISA plate reader was used to determine the absorbance at 405 nm.

Immunoprecipitation assays were used to assess whether the antibodies present in the treated mice were inactivating the injected enzyme. A solution of 500 U (~1 µg) of rhASM, 1 ml of PBS, 10 µl of mouse plasma, and 1 mg/ml BSA was incubated for 18 h at 4°C on a rotating wheel; 100 µl of protein G-Sepharose diluted 1:1 in PBS was then added to the solution and incubated for 4 h at 4°C. After centrifugation at 14000 rpm for 2 min, supernatants and pellets were assayed for ASM activity.

ASM activity assay

SPM covalently linked to the fluorescent probe BODIPY (BODIPY dodecanoyl sphingosyl phosphocholine; BOD12-SPM) was synthesized as described previously for lissamine rhodamine SPM (LR12-SPM) (28), except that BODIPY dodecanoic acid (Molecular Probes Inc., Eugene, Oreg.) was condensed with sphingosyl phosphocholine. Cleavage of BOD12-SPM by ASM releases fluorescent ceramide, which can be quantified after separation by TLC or organic extraction. WBCs or tissues from the treated or control mice were obtained as described previously (25) and homogenized in 0.2% Triton X-100 on ice using three 10 s bursts of a Potter-Elvehjem tissue homogenizer (Thomas Scientific, Swedesboro, N.J.). Total protein was determined by the method of Stein et al. (29). The standard 15 µl ASM assay mixture consisted of 10 µl of sample (homogenized cells or tissues) and 2 nmol of BOD12-SPM suspended in 0.1 M sodium acetate buffer, pH 5.2, containing 0.6% Triton X-100 and either 5 mM EDTA (for detection of the non-zinc-dependent ASM activity) or 0.1 mM ZnCl₂ (for detection of the zinc-stimulated ASM activity) (23). After incubating the assay mixture at 37°C (up to 3 h), the samples were loaded onto TLC plates (LK6 D Silica gel 60, Whatman, Clifton, N.J.) and resolved using chloroform/methanol (95:5 v/v). After resolution, the band containing the fluorescently labeled ceramide was scraped from the TLC plates, extracted in chloroform/methanol/water (1:2:1 v/v) for 15 min at 55°C, and quantified in a spectrofluorometer (fluorescence spectrophotometer 204-A; Perkin Elmer, Norwalk, Conn.). The excitation and emission settings were 505 and 530 nm, respectively.

Sphingomyelin analysis

SPM levels were determined from the phosphate content in each sample (30). Tissues (brain, liver, lung, spleen, kidney, and heart) were homogenized in chloroform/methanol (1:2 v/v) for lipid extraction. Alkaline phospholipid hydrolysis was performed on the samples in 0.4 N KOH/90% methanol for 2 h at 55°C. To avoid interference from plasmalogen and other phospholipids in the brain extracts, acidic hydrolysis was carried out on these samples using 0.6 M HCl in ethanol for 30 min at 37°C prior to the alkaline hydrolysis. After hydrolysis, the samples were incubated with perchloric acid (70%) for 40 min at 180°C, followed by treatment with 0.5% ammonium molybdate and Fiske & Subbarow reducer (4 mg/ml; Sigma) for 10 min at 100°C. Absorbance at 830 nm was measured in a spectrophotometer (Model 1201, Milton Roy Spectronic Inc. Rochester, N.Y.).

Histopathological studies

Mice were anesthetized with ketamine (Sigma; 0.5 g/kg of body weight) and subjected to cardiac perfusion. An incision

was made in the right atrium to allow blood to flow out, and a cannula was introduced through the left ventricle into the aorta, delivering 50 ml of warm 0.9% NaCl solution. Tissue samples for light microscopy were fixed in buffered 10% formalin, paraffin-embedded, sectioned, and stained with hematoxylin and eosin.

Behavioral studies

Motor coordination tests were conducted using a rota-rod treadmill for mice (7650 Accelerating model, Ugo Basile Biological Research Apparatus, Italy) (31). In this apparatus, a motor sets the rotor in motion via the gear belt at a selected speed. When the mouse falls off its cylinder section, the plate below trips and the corresponding counter is disconnected, thereby recording the animal's endurance time in seconds. By subjecting animals to an accelerating drum, screening results are less scattered (32). The machine was set to an initial speed of 32 rpm and the acceleration was increased 1× every 25–30 s. Treated mice were analyzed at ~4 months of age (after the 10th injection) along with ASMKO and normal control animals. Their scores were registered in 2 consecutive days, carefully keeping the conditions in each test as similar as possible. Three tests were performed each day, with a rest time of 1 h between trials. A maximum time limit of 360 s/test was established.

RESULTS

Short-term ERT

Data regarding the plasma clearance and tissue distribution of rhASM after a single injection (1 mg/kg) into ASMKO mice has previously been reported, as has the uptake of rhASM by cultured NPD cells (21). To further evaluate the effects of ERT in ASMKO mice, a total of 16 five-month-old ASMKO mice were injected with 0.3, 1, 3, or 10 mg/kg of rhASM every other day for 14 days (7 injections, 4 animals per dose). The animals were killed 48 h after the last injection and their tissues were analyzed for SPM content. The mice used in these studies had clinical and pathological disease at the onset of ERT, including ataxia, tremors, elevated SPM levels in their organs, and foam cell infiltrates. Thus, these studies were designed to evaluate whether accumulated substrate could be depleted in diseased mice after short-term ERT. **Figure 1** shows the reduction in SPM content in different tissues of the treated animals. Note that a substantial SPM reduction occurred in the livers, spleens, and hearts, whereas only a partial reduction was observed in the lungs at the highest enzyme dose (10 mg/kg). Essentially no alterations in the SPM contents were observed in the kidneys and brains. Next, a group of three additional 5-month-old ASMKO mice received i.v. injections of rhASM (5 mg/kg) every other day for 8 days (four injections), and then were killed for histological analysis on day 10. Note the widespread infiltration of lipid-filled foam cells (indicated by the white open

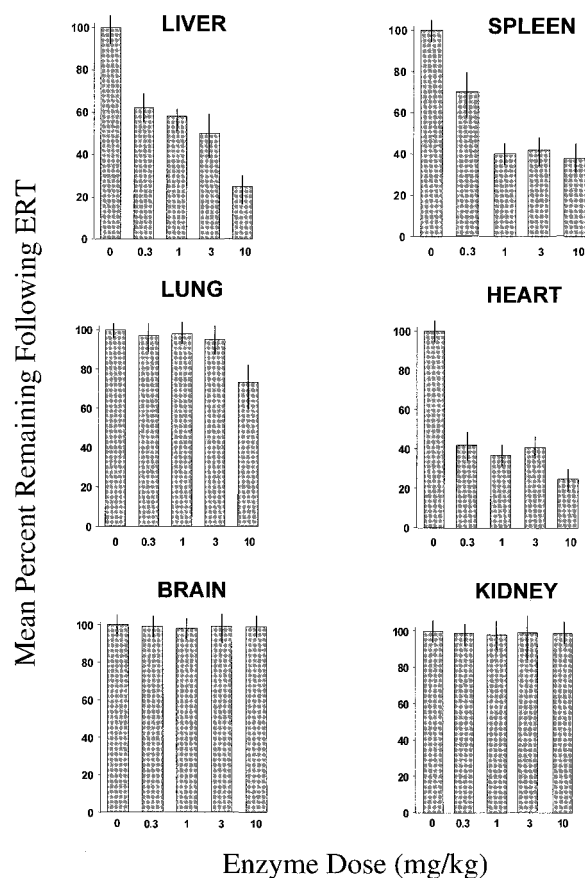


Figure 1. SPM content in tissues after short-term ERT. The tissue SPM contents were first determined in four untreated 5-month-old ASMKO animals. Four groups of 5-month-old ASMKO mice were then injected every other day with various doses of rhASM (four animals each dose) for 14 days (seven injections) and their SPM contents were determined on day 16. The mean values obtained after ERT (including mock injected, 0 mg/kg) were divided by the mean values of the untreated ASMKO control animals in each tissue to obtain the mean percent remaining after ERT. The standard errors of the mean are indicated by the vertical lines. Note that the greatest response was seen in the heart, followed by the spleen and liver. Only a partial response was noted in the lung at the highest enzyme dose (10 mg/kg).

spaces) throughout the livers and spleens of 5-month-old ASMKO animals and the almost complete occlusion of the airway spaces in the lungs (Fig. 2). Also note the marked improvement in the livers, spleens, and lungs of the animals treated by short-term ERT.

Long-term ERT

Based on the encouraging results of short-term ERT in older ASMKO animals (see above), a long-term ERT (15 wk) protocol was then carried out on 10 ASMKO mice using an intermediate dose of enzyme (1 mg/kg) injected once per week. In contrast to the short-term protocols, these recipients were 3 wk of age and presymptomatic at the time ERT was initiated.

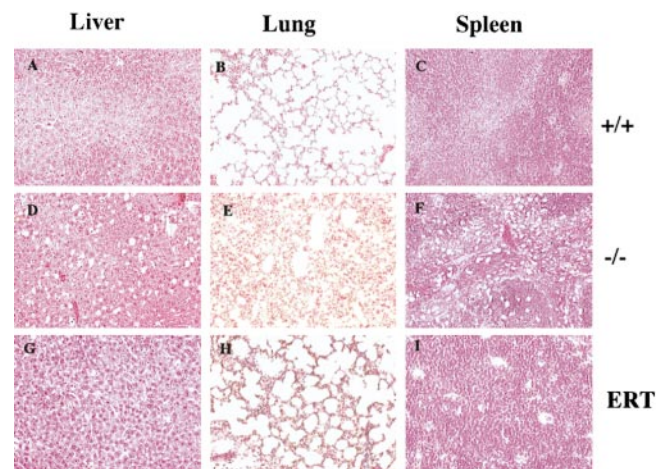


Figure 2. Histological analysis of tissues after short-term ERT. Three 5-month-old ASMKO animals were injected every other day with 5 mg/kg of rhASM for 8 days (four injections). Preparation of tissues for histological analysis was then carried out on day 10 as described in Materials and Methods. Magnification = 200 \times .

Figure 3 depicts the SPM results obtained from the long-term protocol. The data demonstrate that ERT prevented significant SPM accumulation in all organs analyzed, with the exception of the brain. **Figure 4** shows representative histological analysis of tissue sections from several organs obtained from treated ASMKO and control mice. Note that by 5 months of age, untreated ASMKO mice exhibited massive lipid storage as compared to normal animals. Numerous foam cells interspersed throughout the

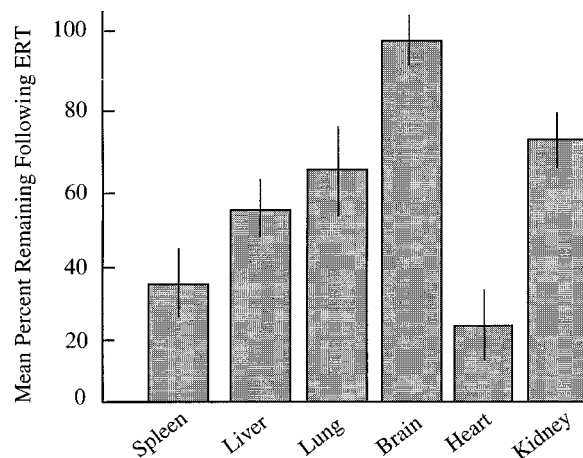


Figure 3. SPM content in tissues after long-term ERT. Ten ASMKO animals were injected once a week with 1 mg/kg of rhASM beginning at 3 wk of age for 15 wk. Five of the animals were killed to determine the SPM content of their tissues. For comparison, the tissue SPM contents also were determined in 5 untreated 5-month-old ASMKO mice. The mean values obtained after ERT were divided by the mean values of the untreated ASMKO control animals in each tissue to obtain the mean percent remaining after ERT. The standard errors of the means are indicated by the vertical lines. Note that similar to the short-term ERT (Fig. 1), the greatest response was seen in the heart, followed by the spleen, liver, and lung.

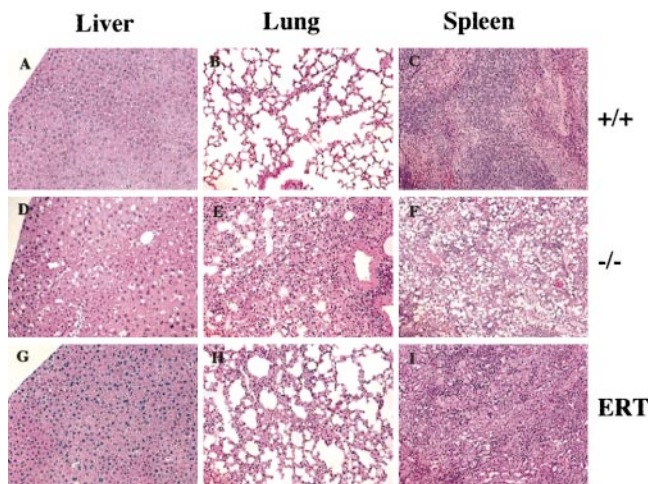


Figure 4. Histological analysis of tissues after long-term ERT. Ten ASMKO animals were injected once a week with 1 mg/kg of rhASM beginning at 3 wk of age for 15 wk. Five of the animals were then killed for histological analysis as described in Materials and Methods. Magnification = 200 \times .

liver and spleen sections were observed along with extensive infiltration of alveoli and septa in the lungs, occluding the airways. Notably, tissues from the treated ASMKO animals had an almost normal histological appearance.

Analysis of brain sections from the treated ASMKO mice revealed massive Purkinje cell drop-out equivalent to that observed in untreated ASMKO mice (not shown), suggesting that the injected rhASM did not cross the blood–brain barrier to a significant degree. Consistent with these results, all of the treated mice developed severe ataxia, exhibited behavioral abnormalities (see below), and died at about the same age as untreated ASMKO mice (average age of death \sim 6 months, not shown).

Adverse reactions and immune responses

Most of the ASMKO mice treated with rhASM showed no adverse reactions. However, 3 of the 10 animals in the long-term study died immediately after the injections. The cause of death was likely related to restraining, anesthesia, or the injection procedure itself. No anti-anaphylactic drugs were administered before the injections and no acute immune reactions were observed. Serum samples were collected every other week from the 7 remaining mice beginning 2 wk after the first enzyme injection. ELISA analyses were carried out to detect the presence of anti-rhASM antibodies and immunoprecipitation was used to determine whether such antibodies inactivated the enzyme activity. All of the treated animals had measurable levels of anti-hASM antibodies by the 5th injection (**Fig. 5**), but these antibodies did not inactivate the enzyme as determined by immunoprecipitation (not shown).

Behavioral assessment

Rota-rod performance was used to assess the motor behavior of treated mice in comparison with normal and affected control animals. **Figure 6** summarizes the average scores from six rota-rod trials performed on two consecutive days at \sim 4 months of age (i.e., after the 10th enzyme injection). Note that the treated animals had average scores, which were not significantly different from the ASMKO control animals, consistent with the lack of histological or biochemical improvement in the central nervous system (CNS).

DISCUSSION

In recent years significant efforts have been devoted to the evaluation of ERT for lysosomal storage diseases. Toward this end, several lysosomal hydrolases have been overexpressed in CHO cells (e.g., ref 33–35), leading to their large-scale purification. A

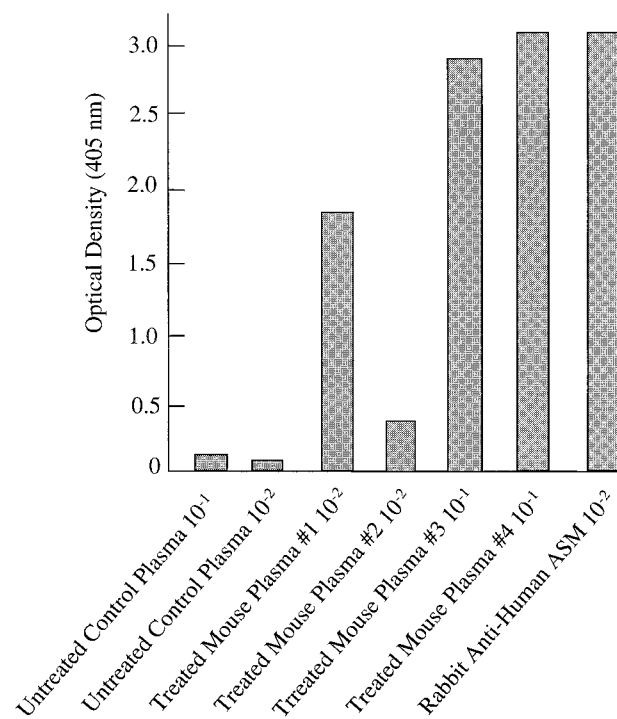


Figure 5. Injection of rhASM into ASMKO mice results in antibody production. An ELISA was developed to detect the presence of anti-rhASM antibodies in mouse plasma (see Materials and Methods for details) and used to assess whether mice subjected to long-term ERT were producing such antibodies. The results are shown for four treated mice analyzed after the 5th injection of rhASM (1 mg/kg, once per week). For comparison, ELISA results are shown using untreated ASMKO control mouse plasma (negative control) or a rabbit serum known to contain anti-human ASM antibodies (positive control). For the positive control, a peroxidase-conjugated goat anti-rabbit IgG was used instead of the standard goat anti-mouse IgG.

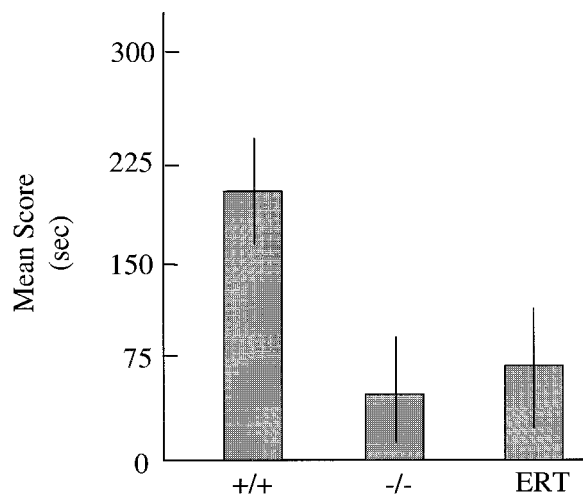


Figure 6. Behavioral assessment in mice subjected to ERT. Rota-rod scores from individual mice subjected to the long-term ERT protocol were averaged and plotted. Five animals from each group (+/+; -/-, ERT) were tested. The data points show the mean values along with standard error of the mean obtained after the 10th injection (1 mg/kg, once per week).

similar strategy was used to overexpress and purify rhASM (21). The physical and kinetic properties of the purified rhASM were nearly identical to those of enzyme purified from human urine, providing a readily available source of active enzyme for the evaluation of ERT in the ASMKO mice.

Our initial ERT studies revealed that the $T_{1/2}$ of rhASM after a single i.v. injection into ASMKO mice was ~2 min and that >90% of the injected enzyme was taken up by the liver (21). These studies also showed that the majority of the internalized enzyme was delivered to lysosomes and that ~30–50% of the enzyme uptake by cells was dependent on the mannose-6-phosphate receptor system.

The present study extends these findings and reports biochemical, histological, and clinical analysis of ERT in the ASMKO mice. The major organ sites of pathology in NPD are the liver, spleen and lung, as well as the brain in Type A NPD. Thus, our analysis was focused on these four organs. Initially, a short-term protocol was carried out in which 5-month-old, symptomatic ASMKO mice were injected with varying doses (ranging from 0.1 to 10 mg/kg) of rhASM every other day for 14 days. These studies revealed that the SPM levels in the livers and spleens (as well as the hearts) of the treated animals were markedly reduced as compared to age-matched ASMKO control animals, suggesting that the disease phenotype may be reversible. The SPM levels in the lungs were only modestly reduced at the highest enzyme levels (10 mg/kg), perhaps due to the reduced vascularization of this tissue as compared to the other three. No substrate reduction was observed in brains (or kidneys) from these short-term studies. Histological analysis of 5-month-old ASMKO mice

treated with only four injections (5 mg/kg) of rhASM confirmed these biochemical results and revealed that the extent of histological reversal in the lungs was even greater than that expected from the biochemical studies.

We then treated a group of 3 wk old, presymptomatic ASMKO animals with weekly rhASM injections of 1 mg/kg for 15 wk. As expected, anti-hASM antibodies were detected in the plasma of treated mice by the 5th injection, but immunoprecipitation analysis demonstrated that these antibodies were non-neutralizing. SPM levels determined at the end of the 15 wk treatment period revealed that the group receiving ERT had markedly lower substrate levels than untreated, age-matched ASMKO control animals. This was most evident in the hearts, spleens, and livers, but was also observed in the lungs and to a very limited degree in the kidneys. The only tissue for which there was no evidence of substrate reduction was the brain, consistent with the fact that by the end of the treatment schedule (i.e., when the animals were ~18 wk of age), all of the ASMKO mice receiving ERT had severe ataxia and neurological disease. Histological analysis again corroborated the biochemical results and showed a marked improvement in the reticuloendothelial organs of the ERT treatment group as opposed to untreated ASMKO controls.

It is of interest to compare these ERT results to previous results from our group using the ASMKO mice to evaluate two other therapeutic approaches, BMT and HSCGT (24, 25; S. R. P. Miranda et al., unpublished results). The most noticeable difference between BMT or HSCGT vs. ERT is in the effects on CNS disease. ERT led to essentially no improvements in CNS disease, whereas BMT and HSCGT slowed the progression of the CNS disease but did not prevent it. These latter results were obtained even though very few hematopoietically derived cells crossed the blood–brain barrier and no increases in ASM activity were detected in the treated brain homogenates (24, 25). Thus, the CNS disease in ASMKO mice is responsive to very low levels of ASM activity and the lack of a similar response by ERT is likely due to the fact that essentially no rhASM was able to cross the blood–brain barrier after i.v. injection. Other than the CNS effects, the therapeutic response of the ASMKO mice to ERT were similar to BMT or HSCGT, although these latter approaches seemed to be modestly more effective in treating the lung pathology. Responses in the livers and spleens were similar. One of the most notable results obtained from the ERT study was that as few as four injections of rhASM into older ASMKO mice could significantly reverse substrate accumulation in ASMKO mice. Since the BMT or HSCGT protocols were only carried out on presymptomatic

animals, these results could not be directly compared.

It is also of interest to compare our ERT results to those obtained in animal models of other lysosomal storage diseases. For example, in the murine model of mucopolysaccharidosis (MPS) type VII, ERT initiated early in life led to marked improvements in visceral organ glycosaminoglycan storage and partial improvements in learning, memory, and hearing deficits (36). ERT in the ASMKO mice also led to marked improvements in visceral organ storage, but no detectable neurological changes. It must be recognized, however, that the tests used to assess neurological changes in the two studies (e.g., cognitive in the MPS VII mice vs. motor coordination in the ASMKO mice) were different, perhaps explaining the differing results. In addition, the ERT treatment in the MPS VII mice was initiated slightly earlier than in the ASMKO mice, perhaps before complete closure of the blood-brain barrier. In avian and feline models of Pompe's disease and MPS type VI, respectively (37, 38), ERT initiated at various ages also led to variable amounts of visceral organ pathological changes and clinical improvement, again suggesting that ERT is an effective way to treat many of these disorders. Similar studies have been carried out in a dog model of MPS type I (39) and several other lysosomal disease models. Although a systematic assessment of age dependency has not been carried out in most cases, in general, the results clearly indicate that ERT should be beneficial for these disorders and that the earlier therapy is initiated, the better the clinical response.

In summary, the data presented here indicate that ERT is likely to be a useful therapeutic approach for non-neurological, Type B NPD patients and that even in older patients in which the SPM levels are markedly elevated, there may be some therapeutic response. The major organ sites of pathology in Type B NPD are the liver, spleen, and lung, three sites that showed a strong response to ERT in the mouse model. For Type A NPD, ERT is unlikely to evoke any therapeutic response in the CNS, limiting its usefulness for the treatment of this disorder. FJ

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