Dexamethasone-Mediated Up-Regulation of the Mannose Receptor Improves the Delivery of Recombinant Glucocerebrosidase to Gaucher Macrophages

Yunxiang Zhu, Xuemei Li, Edward H. Schuchman, Robert J. Desnick, and Seng H. Cheng

Genzyme Corporation, Framingham, Massachusetts (Y.Z., X.L., S.H.C.); and Mount Sinai School of Medicine, New York, New York (E.H.S., R.J.D.)

Received September 17, 2003; accepted November 6, 2003

ABSTRACT

Enzyme replacement therapy for Gaucher disease uses a recombinant glucocerebrosidase (Cerezyme) whose oligosaccharide chains have been remodeled to expose the core mannose residues. This modification promotes the uptake of the hydrolase by Gaucher-affected macrophages via mannose receptor-mediated endocytosis. However, studies revealed that amounts of the infused enzyme were also delivered to other mannose receptor-bearing cells such as the liver sinusoidal endothelial cells. To maximize the delivery of Cerezyme to macrophages, agents that increased the cell surface levels of the mannose receptor specifically on macrophages were examined. Treatment with dexamethasone improved the in vitro uptake of Cerezyme by a macrophage but not by liver sinusoidal endothelial or hepatocyte cell lines. The enhanced uptake by the macrophages was due to an increase in surface mannose receptors because the activity could be blocked by the addition of mannans. Pretreatment of rats with the glucocorticoid also preferentially enhanced the delivery of Cerezyme to the Kupffer cells and splenic macrophages. This effect of dexamethasone also applied to substrate-laden macrophages isolated from Niemann-Pick A mice. Together, these data suggest that pretreatment with dexamethasone could specifically enhance the presentation of mannose receptors on Gaucher macrophages with resultant improvement in delivery of the enzyme to the affected cells.

Gaucher disease is an inherited lysosomal storage disorder that is caused by a deficiency of the lysosomal hydrolase glucocerebrosidase (Beutler and Grabowski, 2001). This deficiency results in the progressive accumulation of undegraded glucosylceramide primarily in the macrophages of the liver, spleen, lung, and bone marrow and in the most severely affected subjects, the central nervous system. Consequently, affected individuals demonstrate hepatosplenomegaly, hematological abnormalities, bone lesions, and pulmonary disease and in severe instances, neurological disease. Type 1 Gaucher disease, the most prevalent, is nonneuropathic, whereas types 2 and 3 (chronic) are characterized by neurological disease, an earlier age of onset, and significantly more severe systemic clinical symptoms. This spectrum of disease severity correlates inversely with the level of residual glucocerebrosidase activity, with the type 2 and 3 variants generally exhibiting lower levels of activity than type 1.

Presently, type 1 Gaucher disease is treated using a recombinant glucocerebrosidase (Cerezyme) that has been remodeled by sequential digestion of the oligosaccharide side chains to expose the core mannose residues (Furbish et al., 1981; Murray, 1987; Brady et al., 1994). This modification enhances the recognition and uptake of the enzyme by the mannose receptor on macrophages, the primary cells affected in Gaucher disease. Additionally, by removing the galactose residues, it also served to minimize the interaction of the enzyme with other carbohydrate receptors such as the asialo-glycoprotein receptor present on hepatocytes (Furbish et al., 1981; Lodish, 1991). However, because mannose receptors are also resident on other cell types such as the liver sinusoidal endothelial cells (Magnusson and Berg, 1989; Stang et al., 1990), a significant proportion of the infused mannose-terminated glucocerebrosidase is also internalized by these nonaffected cells. Biodistribution studies in mice showed that although remodeling significantly improved the delivery of Cerezyme to the macrophages, approximately 80 to 90% of the enzyme was still internalized by the parenchymal and endothelial cells (Bijsterbosch et al., 1996; Xu et al., 1996; Friedman et al., 1999). This uptake of Cerezyme by the unaffected parenchymal and endothelial cells may underlie the need for frequent maintenance infusions of large doses of Cerezyme.
enzyme for therapeutic effect (Beck et al., 1997; Beutler, 1997; Weinreb et al., 2002). Although increasing the dose of Cerezyme may compensate for the relative inefficiency in delivering Cerezyme to the affected macrophages, higher doses could saturate the receptors available on the cell surface. Indeed, mannose receptor-mediated uptake of Cerezyme has been shown to be saturable when administered at doses greater than 50 U/kg body weight, at least when tested in mice (Mistry et al., 1996; Friedman et al., 1999).

To enhance the delivery of Cerezyme to the target macrophage cells and thereby the efficacy of enzyme replacement therapy, we sought to determine the utility of increasing the abundance of mannose receptors specifically on the surface of these cells. Several agents are reportedly capable of modulating the density of the mannose receptor on macrophages, some of which have been approved for human clinical use (Mokoena and Gordon, 1985; Shepherd et al., 1985, 1994; Schreiber et al., 1990; Harris et al., 1992; Stein et al., 1992; Montaner et al., 1999; Piemonti et al., 1999). In this report, we examined the ability of one of these agents, the glucocorticoid steroid dexamethasone, to increase the expression of the mannose receptor specifically on macrophages. We showed that pretreatment with dexamethasone resulted in a greater uptake of Cerezyme by macrophages both in vitro and in vivo. Consequently, it may be possible to enhance the efficacy of enzyme replacement therapy for Gaucher disease or reduce the dose needed for treatment through the use of this strategy.

Materials and Methods

**Reagents and Chemicals.** Dexamethasone for the in vitro studies was purchased from Calbiochem (San Diego, CA), whereas that for the in vivo studies (water-soluble dexamethasone sodium phosphate) was from American Reagent Laboratories Inc. (Shirley, NY). Bovine serum albumin, yeast mannan, and other common chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. The macrophage-specific monoclonal antibody CD11b and 0.5-μm red fluoresbrite microbeads were from Serotech (Raleigh, NC) and Polysciences (Warrington, PA), respectively. Preclinical grade unmodified and remodeled recombinant glucocerebrosidase (Cerezyme) was from Genzyme Corporation (Boston, MA). All cell culture reagents were from Invitrogen (Carlsbad, CA). The anti-von Willebrand factor antibody was purchased from Cell Systems, Inc. (Kirkland, WA).

**Cell Culture Conditions.** The rat alveolar macrophage cell line NR8383 (CRL-2192) obtained from the American Type Tissue Culture Collection (Manassas, VA) was cultured in Kaighn’s modified F12K nutrient mixture supplemented with 2 mM L-glutamine, 15% (v/v) heat-inactivated fetal bovine serum (FBS), penicillin, and streptomycin. Hep3B cells were grown in minimal essential medium with Earle’s salts, 10% FBS, nonessential amino acids, sodium pyruvate, penicillin, and streptomycin. Human liver sinusoidal endothelial cells (LSEC) were obtained from Applied Cell Biology Research Institute (Kirkland, WA) through Cell Systems, Inc. LSECs were cultured in CS-C media containing 10% FBS. Culture dishes or flasks were routinely pretreated with attachment factor solution before seeding with the cells.

**Cerezyme Uptake in Vitro.** Macrophages, hepatocytes, and LSECs were grown under the conditions described above and were treated with dexamethasone or other drugs as indicated in the figure legends. When treatment was for greater than 2 days, fresh drug was added to the culture every 24 h. For the Cerezyme uptake studies using the macrophage cell line NR8383, the cells were first harvested from the culture flasks using phosphate-buffered saline (PBS). After washing with Kaghan’s media, the cells were resuspended in uptake media (Kaghan’s media containing 4 mg/ml bovine serum albumin and 25 mM Hepes, pH 6.8) to a final concentration of 10⁶ cells/ml. One million of the cell line was distributed and fixed in 1.5-ml microcentrifuge tubes in duplicate. Where appropriate, yeast mannan (dissolved in deionized water) was added to a final concentration of 2 mg/ml to inhibit mannose receptor-mediated uptake of the enzyme. After the addition of Cerezyme (1 U/ml), the cells were incubated at 37°C for 2 h with periodic mixing every 15 min. The cells were then pelleted by centrifugation at 1000g for 3 min in a microcentrifuge, washed twice with 1 ml of ice-cold PBS containing 1 mg/ml of yeast mannan, and twice more with PBS alone. Cell pellets were dissolved in 1 ml of 50 mM potassium phosphate, 0.25% Triton X-100, pH 6.5 (KP buffer), containing complete protease inhibitors (Roche Diagnostics, Indianapolis, IN). For the Cerezyme uptake studies with Hep3B cells and LSECs, the procedure used was similar to that described above for macrophages except that these studies were performed with adherent cells. The uptake media used with these cells was DMEM containing 4 mg/ml BSA and 25 mM Hepes, pH 6.8. After incubation with Cerezyme, the wells were washed twice with PBS containing 1 mg/ml yeast mannan and twice more with PBS and then lysed in 1 ml of KP buffer as for the macrophages. Lysates were assayed for glucocerebrosidase activity using the artificial fluorogenic substrate 4-methylumbelliferyl-β-D-glucopyranoside as described previously (Marshall et al., 2002). Protein levels were quantified using the MicroBCA protein assay kit (Pierce Chemical, Rockford, IL).

**Cerezyme Uptake in Vivo.** Animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services, NIH Publication No. 86-23). To assess the effect of dexamethasone on Cerezyme uptake by Kupffer cells in vivo, Fisher rats (female, ~130 g) were divided into four groups (n = 3 animals/group). Two groups of rats received vehicle (PBS) without dexamethasone. The other two groups were administered water-soluble dexamethasone sodium phosphate intraperitoneally at a dose of either 10 μg/rat or 100 μg/rat. For the treated groups, four doses of dexamethasone were given over two consecutive days, one dose in the early morning and one dose in the late afternoon of each day. On the 3rd day, 2.5 U of Cerezyme (~18 U/kg) was administered intravenously (via the tail vein as a bolus injection) to one group of rats that had been mock-treated with PBS and two groups of rats that had been pretreated with dexamethasone. Two hours after the injections, the rats were euthanized and their livers were dissected and fixed in 0.01% glutaraldehyde (Electron Microscopy Sciences, Fort Washington, PA) and 0.1% glutaraldehyde (Electron Microscopy Sciences) in PBS for 1 h at room temperature. The livers were then placed into an ice-cold 30% sucrose solution and incubated overnight at 4°C. The tissues were embedded in tissue-freezing OCT compound (Electron Microscopy Sciences), and 5-μm sections were prepared. Liver sections were stained for macrophages using the antibody CD11b (Serotech, Raleigh, NC) and for human glucocerebrosidase using the antibody IB5 and then visualized by immunofluorescence microscopy. A goat anti-mouse IgG conjugated to Alexa 568 and a goat anti-rat IgG conjugated to Alexa488 were used to visualize the macrophages and enzyme, respectively.

Quantitation of the effects of dexamethasone on Cerezyme uptake in vivo was performed with splenic macrophages isolated using the procedure by Gessani et al. (2000). Fisher rats (female, ~130 g) were treated with dexamethasone and Cerezyme as described above. After treatment, the spleens were harvested and then cut into small pieces. The spleen cells were released by gently squeezing the pieces between two frosted cover slides. The released cells were washed with 15 ml of RPMI 1640 medium containing 10% heat-inactivated FBS, and the red blood cells were lysed using red blood cell lysis buffer (Sigma-Aldrich). The isolated splenocytes were seeded onto 150-mm tissue culture dishes and incubated at 37°C for 1 h to allow the splenic macrophages to attach. After removing the unattached
cells, the attached cells (predominantly macrophages) were washed five times with PBS and then lysed in KP buffer. The lysates were then assayed for glucocerebrosidase activity and protein levels as described above. The purity of the isolated macrophages was confirmed by staining with the macrophage-specific antibody CD11b. The phagocytic activity of the isolated macrophages was also assessed by incubating them with 0.5-μm red Fluoresbeads (Poly-sciences) for 1 h at 37°C. The cells were pelleted, washed three times with PBS, treated with 4% paraformaldehyde, and stained with a rabbit anti-bovine CI-MPR antibody followed by a goat anti-rabbit IgG antibody conjugated to Alexa 546 (Molecular Probes, Eugene, OR). Confocal microscopy was used to determine whether the microbeads were on the cell surface or in the interior of the cells.

The total amount of glucocerebrosidase present in the spleens was also measured by homogenizing the tissues in KP buffer (1 ml KP buffer/100 mg wet tissue). After centrifugation at 14,000g for 15 min, the supernatants were collected and assayed for glucocerebrosidase activity using the artificial fluorogenic substrate as described above. Statistical analysis was performed using one-way analysis of variance followed by Newman-Keuls multiple comparison test.

**Uptake Studies Using Alveolar Macrophages from Niemann-Pick A Mice.** Lung alveolar macrophages were isolated according to the protocol of Brain and Frank (1968) from 4-month-old acid sphingomyelinase knockout mice (Dhami et al., 2001) that had been pretreated with dexamethasone for three consecutive days. Alveolar macrophages isolated from nontreated Niemann-Pick A mice were used as controls. The isolated macrophages were seeded onto 12-well tissue culture plates and incubated at 37°C for 1 h to allow for attachment. Cerezyme uptake studies and enzyme assays were then performed as described for the liver cells.

## Results

**Effect of Dexamethasone on in Vitro Uptake of Recombinant Glucocerebrosidase.** In an attempt to further improve the delivery of Cerezyme to macrophages, the target cells affected in Gaucher disease, we evaluated the relative utility of agents capable of up-regulating the mannose receptor on these cells. Figure 1 shows the results obtained with one such agent, dexamethasone, on Cerezyme uptake by alveolar macrophage (NR8383), hepatocyte (Hep3B), and LSEC cells. The cells were pretreated with dexamethasone for two consecutive days after which the extent of Cerezyme uptake by the different cells was assayed and compared with that of untreated controls. Incubation of the untreated macrophages and LSEC cells with Cerezyme for 2 h showed that these cells were capable of internalizing the enzyme (Fig. 1, A and B). Uptake of Cerezyme by the macrophages could be completely inhibited, and that by LSECs partially inhibited by the addition of yeast mannan. This suggests that uptake of the modified enzyme by the macrophages was mediated primarily through the mannose receptor, whereas that by LSECs involved the mannose but also other receptors. Three other members of the mannose receptor family [the M-type phospholipase A(2) receptor, DEC-205 and Endo180] have been identified and their binding to sugars and expression in various cell types characterized (East and Isacke, 2002). Endo 180 has been shown to be expressed on various cell types, including fibroblasts, endothelial cells, and macrophages, and binding to mannos is not as avid as binding to N-acetylglucosamine (Sheikh et al., 2000). Therefore, receptors on LSECs that bind Cerezyme via these other receptors (Sato and Beutler, 1993) may account for the population of internalized enzyme that was resistant to inhibition by mannan.

Macrophages that had been pretreated with dexamethasone exhibited an approximately 3-fold greater uptake of Cerezyme compared with the untreated controls (Fig. 1A). Although we did not quantify the mannose receptors on macrophages after dexamethasone treatment, the enhanced uptake of Cerezyme by the dexamethasone-treated macrophages was most likely the result of increased surface expression of the mannose receptor because the uptake could be completely inhibited by the addition of yeast mannan. Moreover, the ability of dexamethasone to enhance the expression of mannose receptors on macrophages has been reported previously by Shepherd et al. (1985). Associated with this increase in the presentation of the mannose recept-
tor on the cell surface of dexamethasone-treated macrophages was an enhancement in the uptake of lysosomal enzymes. This increased uptake of enzymes may explain the observed increase in the levels of endogenous glucocerebrosidase activity (~130% of untreated control) after dexamethasone treatment (compare first and fourth column in Fig. 1A). In addition, the stimulatory effect of dexamethasone on Cerezyme uptake by the macrophages was time-dependent. A minimum of 2 days of pretreatment was necessary before any increase in uptake of the enzyme was observed (data not shown). This timing correlates well with that reported for the de novo transcription and translation of the mannose receptor by macrophages after treatment by dexamethasone (Cowan et al., 1992). On the contrary, this increase in Cerezyme uptake was not observed in the dexamethasone-treated LSECs, indicating that dexamethasone had differential effects on the two different cell types (Fig. 1B). The hepatocyte cell line internalized Cerezyme poorly irrespective of whether it was pretreated with dexamethasone (Fig. 1C). This observation is consistent with previous findings that hepatocytes do not express the mannose receptor. Hence, pretreatment with dexamethasone had the effect of selectively increasing the uptake of Cerezyme by the macrophage but not by the LSEC or hepatocyte cells in vitro.

**Pretreating Rats with Dexamethasone Enhanced the Uptake of Cerezyme by Kupffer Cells.** Having demonstrated that dexamethasone could differentially affect the uptake of Cerezyme by macrophages, hepatocytes and endothelial cells in vitro, we next examined its effect in vivo. Rats were administered 2.5 U of Cerezyme (~18 U/kg), an amount determined previously to result only in minimal or no immunostaining of liver sections using the anti-human-specific glucocerebrosidase antibody 1B5. The liver thin sections were also stained with an anti-macrophage antibody (CD11b) to visualize the Kupffer cells. As shown in Fig. 2, liver sections from rats pretreated with dexamethasone consistently generated greater staining of Cerezyme in Kupffer cells than in the untreated animals. Moreover, a greater number of Kupffer cells stained positive for Cerezyme when a higher dose of dexamethasone was used. These in vivo data support the observations in vitro that pretreatment with dexamethasone can result in enhanced uptake of Cerezyme by the Kupffer cells.

**Pretreatment with Dexamethasone Also Increased the Uptake of Cerezyme by Splenic Macrophages in Vivo.** To provide a more quantitative assessment of the effect of dexamethasone in vivo, splenic macrophages were purified from rats treated with the drug, and the extent of Cerezyme uptake was determined. The spleen, another organ that is profoundly affected in Gaucher disease, was selected for these studies because of the greater ease with which the macrophages could be isolated and purified from this tissue. Splenic macrophages were isolated and enriched using the method of Gessani et al. (2000) that is based on the preferential ability of macrophages to attach to culture plates. Immunofluorescence studies using the microbead-specific antibody CD11b confirmed that this method routinely generated preparations that were greater than 90% macrophages (data not shown). This contrasts with the less than 10% labeling in the total splenocyte fraction, which is consistent with the reported percentage of macrophages in spleen. Most of the isolated macrophages also retained their phagocytic activity as indicated by their ability to internalize fluorescent 0.5-μm microbeads (data not shown).

Rats that had been pretreated with either dexamethasone or vehicle were injected with Cerezyme and their splenic macrophages then purified as indicated above and assayed for glucocerebrosidase activity. As shown in Fig. 3, macrophages isolated from rats that had been pretreated with dexamethasone and administered 18 U/kg Cerezyme had much higher levels (approximately 2-fold after subtracting the endogenous activity level) of activity than the corresponding vehicle-treated animals. These studies were repeated in three independent experiments with similar results for a total of 17 rats.

The effect of dexamethasone in animals administered dif-

![Fig. 2. Dexamethasone pretreatment increased the uptake of Cerezyme by liver Kupffer cells in vivo.Liver thin-sections from control and dexamethasone-treated and Cerezyme injected (18 U/kg) rats were coimmunostained with anti-macrophage (CD11b) and anti-human glucocerebrosidase (1B5) antibodies. The liver Kupffer cells are shown in green and the location of human glucocerebrosidase (Cerezyme) is shown in red. Arrows indicate Kupffer cells that were costained with the marker for Cerezyme. Increasing the dose of dexamethasone (from 10 to 100 μg/rat) increased the number of Kupffer cells that stained positive for human glucocerebrosidase. Slides shown are representative of results obtained from two independent experiments with three rats used per group in each study.](image1)

![Fig. 3. Dexamethasone pretreatment increased the uptake of Cerezyme by splenic macrophages in vivo. Splenic macrophages were isolated from control and dexamethasone-pretreated rats injected with or without Cerezyme. Purified macrophages (approximately 90% pure) were lysed and assayed for glucocerebrosidase activity. Control rats and rats pretreated with dexamethasone were administered either 18 or 72 U/kg Cerezyme. The data in the 18 U/kg groups were from three studies with a total of 17 rats (p < 0.05). The data in the 72 U/kg groups were from two studies with a total of 10 rats (p < 0.001). Values were normalized to endogenous glucocerebrosidase activity, which was set at 100%. The shaded columns represent animals that were treated with dexamethasone. Error bars represent standard deviation. The results indicated that dexamethasone pretreatment increased Cerezyme uptake at all the doses tested.](image2)
different doses of Cerezyme was also examined (Fig. 3). Administration of 72 U/kg Cerezyme, a dose approximating that used to treat Gaucher patients and shown previously to saturate the mannose receptors in humans (Mistry et al., 1996), generated results similar to those observed with 18 U/kg. In all cases, pretreatment with dexamethasone resulted in an approximately 2–3-fold greater uptake of Cerezyme by the rat splenocyte macrophages compared with their respective vehicle-treated counterparts. When adjusted for dose, the dexamethasone-mediated increase in Cerezyme uptake was estimated to be 3–4 fold. For example, the extent of Cerezyme internalized by splenic macrophages of animals pretreated with dexamethasone and 18 U/kg enzyme was similar to that of animals administered vehicle and 72 U/kg Cerezyme (Fig. 3). Analysis of the total spleen homogenates also revealed higher glucocerebrosidase activities in the animals that had been pretreated with dexamethasone (data not shown). However, the magnitude of the increase was lower than in the purified macrophages. This may be expected if the macrophage population within the total splenic homogenate was primarily responsible for internalizing the infused Cerezyme. Together, these results suggest that dexamethasone-mediated up-regulation of the mannose receptors (Mokoena and Gordon, 1985; Shepherd et al., 1985, 1994) improved the delivery of Cerezyme to macrophages in vivo, the cells that are primarily affected in Gaucher disease.

Dexamethasone Pretreatment Also Increased the Uptake of Unmodified Recombinant Glucocerebrosidase by Splenic Macrophages. The effect of dexamethasone on the uptake of unmodified recombinant glucocerebrosidase was also examined. Unlike Cerezyme, which has been specifically remodeled to expose the core mannose residues on its oligosaccharide side chains, unmodified recombinant glucocerebrosidase contains only a few exposed mannose residues by virtue of a single high-mannose oligosaccharide side chain (Friedman et al., 1999). Interestingly, despite having a lower abundance of exposed mannose residues, the uptake of the unmodified enzyme by splenic macrophages in vivo was also enhanced to a similar extent as the remodeled Cerezyme by dexamethasone pretreatment (Fig. 4). Hence, the effect of dexamethasone would seem to be applicable to both unmodified and carbohydrate-remodeled recombinant glucocerebrosidase.

Substrate-Laden Alveolar Macrophages Are Also Responsive to Dexamethasone Treatment. Because Gaucher macrophages, unlike the normal macrophages used in the above-mentioned studies, are laden with accumulated glucosylceramide, the effect of dexamethasone on substrate-burdened macrophages was also examined. In the absence of a viable animal model of Gaucher disease that accumulated extensive amounts of glucosylceramide in the macrophages, we elected to investigate the effects of dexamethasone on macrophages from the Niemann-Pick A mouse. The Niemann-Pick A mouse lacks acid sphingomyelinase activity and shows extensive lysosomal accumulation of sphingomyelin in the reticuloendothelial system of liver, spleen, bone marrow, and lung (Horinouchi et al., 1995; Dhami et al., 2001). Although the substrate that accumulates in the macrophages of Gaucher disease and Niemann-Pick A disease is different, the similarity in the pathology suggests that the macrophages from the Niemann-Pick A mouse may be an appropriate surrogate for those from Gaucher disease. The alveolar macrophages isolated from the Niemann-Pick A mouse reportedly also exhibit altered functions, suggesting that the diseased macrophages may respond differently to dexamethasone (Dhami et al., 2001). To test this, alveolar macrophages were lavaged from the lungs of Niemann-Pick A mice that were either pretreated with dexamethasone or vehicle for 3 days. The harvested alveolar macrophages were then plated onto culture plates, and their ability to internalize Cerezyme was assayed. Despite having extensive lysosomal storage, the alveolar macrophages that had been pretreated with dexamethasone showed significantly higher levels (approximately 3-fold) of Cerezyme than the vehicle-treated controls (Fig. 5). The uptake of Cerezyme was inhibited by mannan, indicating that internalization was primarily mediated via the mannose receptor. Hence, lysosomal storage of sphingomyelin in the alveolar macrophages did not affect the ability of dexamethasone to up-regulate the mannose receptors in vivo. This observation supports the possible

---

**Fig. 4.** Dexamethasone pretreatment increased the uptake of unmodified glucocerebrosidase by spleen macrophages in vivo. Splenic macrophages were isolated from control and dexamethasone-pretreated rats injected with or without unmodified glucocerebrosidase. Purified macrophages (approximately 90% pure) were lysed and assayed for glucocerebrosidase activity. Values were normalized to endogenous glucocerebrosidase activity, which was set at 100%. *p < 0.001 between the dexamethasone-treated and nontreated groups administered 7 U/kg Cerezyme (total of 11 rats in two separate experiments). Error bars represent standard deviation.

**Fig. 5.** Lipid-laden alveolar macrophages remained responsive to dexamethasone treatment. Acid sphingomyelinase knockout mice were pretreated with or without dexamethasone for 3 days after which the lung alveolar macrophages were isolated and allowed to attach to a 12-well dish as described under Materials and Methods. Cerezyme was then added to the cells and the extent of enzyme uptake determined. Values were normalized to endogenous glucocerebrosidase activity, which was set at 100%. Error bars represent standard deviation. In some studies, mannan was included to saturate the mannose receptor. The shaded columns represent animals that were treated with dexamethasone.
use of dexamethasone to enhance the delivery of Cerezyme to Gaucher-affected macrophages.

Discussion

Treatment with Dexamethasone Specifically Enhanced the Uptake of Remodeled Recombinant Glucocerebrosidase by Macrophages in Vitro and in Vivo.

The target cells for treatment by enzyme replacement therapy in Gaucher type I disease are the tissue macrophages of the liver, spleen, lung, and bone marrow. The finding of the mannose receptor on macrophage cells led to the development of a macrophage-targeted glucocerebrosidase (Cerezyme) through remodeling of the enzyme's carbohydrate moieties (Furbish et al., 1981; Brady et al., 1994). This modification proved to be essential for efficient delivery of the infused recombinant enzyme to the macrophages through mannose receptor-mediated endocytosis. However, biodistribution studies indicated that although more of the remodeled enzyme was delivered to the macrophages compared with the unmodified enzyme, the liver sinusoidal endothelial cells, which also bear the mannose receptor, internalized a significant amount of the infused enzyme (Bijsterbosch et al., 1996; Xu et al., 1996; Friedman et al., 1999). To further improve the delivery of the enzyme to the macrophages, approaches that specifically up-regulated the mannose receptor on this cell type were evaluated. It was hypothesized that increasing the presentation of mannose receptors on the macrophages would lead to greater receptor-mediated uptake of the enzyme and thereby the therapeutic efficacy of Cerezyme.

The levels of the mannose receptor on macrophages have been shown to be susceptible to modulation by a variety of inflammatory and anti-inflammatory agents. Addition of the cytokines interleukin-10 and interferon-γ to macrophages results in down-regulation of the receptor, whereas treatment with dexamethasone, interleukin-4, and prostaglandin E up-regulates its expression and in some instances, its activity as well (Shepherd et al., 1985; Cowan et al., 1992; Stein et al., 1992; Schreiber et al., 1993; Montaner et al., 1999). In the case of the clinically approved glucocorticoid dexamethasone, it has been reported that its primary effect is to induce the synthesis of receptor protein through an increase in the level of mannose receptor mRNA (Shepherd et al., 1985; Cowan et al., 1992). Here, we demonstrated that treatment in vitro with dexamethasone resulted in an increase in mannose receptors specifically on a macrophage cell line but not liver sinusoidal endothelial or hepatocyte cell lines as evidenced by a greater uptake of Cerezyme. The enhanced uptake was consistent with being strictly mannose receptor-dependent because it could be completely inhibited by yeast mannan. This differential effect of dexamethasone on uptake by the different cell types would seem to be recapitulated in the spleens and livers of animals treated with Cerezyme. Immunofluorescence studies using a human glucocerebrosidase-specific antibody indicated that a greater amount of the infused Cerezyme was internalized by the Kupffer cells in dexamethasone-pretreated animals. A similar increase in Cerezyme uptake by splenic macrophages was also detected with dexamethasone pretreatment. Dexamethasone-treated rats had greater amounts of enzyme delivered to their splenic macrophages than did nontreated animals, despite the use a 4-fold higher dose of enzyme in the latter group. When adjusted for dose, this represented an approximately 3-fold increase in Cerezyme uptake by the dexamethasone-treated splenic macrophages. The extent of the increase in uptake is consistent with that reported for the synthesis of mannose receptor after dexamethasone treatment (Shepherd et al., 1985; Cowan et al., 1992). This effect of dexamethasone was also realized in lipid-laden macrophages as maybe encountered in Gaucher-affected tissues. Sphingomyelin-filled alveolar macrophages isolated from a mouse model of Niemann-Pick A disease that had been pretreated with dexamethasone also exhibited a higher propensity to internalize Cerezyme compared with untreated controls. This indicates that the mannose receptors on alveolar macrophages were also up-regulated. Hence, it is likely that this strategy should also allow for improved delivery of Cerezyme to Gaucher-affected cells. By extension, it should also apply to the improved delivery of acid sphingomyelinase to Niemann-Pick A-affected macrophages.

1,25-Dihydroxyvitamin D3 has also been reported to up-regulate the mannose receptor on macrophages in vitro (Clohisy et al., 1987). However, attempts to use this agent in combination with Cerezyme in the clinic revealed no apparent increase in efficacy (Grabowski et al., 1998). Because no details of this trial have been reported thus far, the reason for the discrepancy in our animal studies with the human study using this strategy is difficult to reconcile. However, in light of this observation, we should be cautious about extrapolating the possible application of this strategy to humans.

Potential Benefits of Pretreatment with Dexamethasone on Enzyme Replacement Therapy for Gaucher Disease. Although enzyme replacement therapy for Gaucher type I disease has been shown to be effective, there remains debate as to the optimal dose and frequency of administration of the enzyme (Altarescu et al., 2000; Beutler, 2000). Studies to evaluate various treatment regimens, including the use of a high dose with a low frequency of reinfusion or a low dose with a high frequency of reinfusion have been equivocal due to the broad spectrum of disease severity among patients (Beutler, 2000). A regimen that is commonly used to attain a clinical response in patients involves the use of a high dose (60 U/kg) and low frequency of reinfusion (biweekly). Because infusion time (for 60 U/kg) can be approximately 2 h, interventions that support a reduction in dose or infusion interval are desirable. The demonstration here that pretreatment with dexamethasone could affect a greater uptake of Cerezyme by tissue macrophages might allow for a reduction in dose and therefore the time for infusion of the enzyme.

Another aspect of enzyme replacement therapy for Gaucher disease is the relatively long duration of treatment (several months) necessary to completely reverse the disease manifestations (Brady and Barton, 1994; Weinreb et al., 2002). A possible explanation may be that the amount of enzyme delivered to the target macrophages, despite the use of 60 U/kg, is suboptimal and that complete elimination of the accumulated substrate can occur only after several repeated infusions. Although increasing the dose may aid in rectifying this problem, higher doses of Cerezyme may also saturate the mannose receptors and thereby limit the benefit that could be provided to the affected macrophages. In contrast, the strategy to increase the surface density of the mannose receptor specifically on the affected macrophages
using dexamethasone should promote a greater uptake of the infused enzyme and thereby enhance the efficacy of enzyme replacement therapy. This increase in effectiveness could translate to a more rapid response time and to a reduction in dose and perhaps even decrease the frequency of infusions.

Yet another potential benefit associated with the use of dexamethasone in Gaucher disease is its anti-inflammatory activity. The total weight of accumulated glucocerebroside in the livers of affected Gaucher patients accounts for less than 2% of the observed hepatomegaly (Cox, 2001). It has been proposed that the organomegaly is more likely the result of chronic inflammation induced by the lipid-laden macrophages. This possibility has been substantiated in a murine model of Gaucher disease (Mizukami et al., 2002), which displayed significant systemic inflammation despite exhibiting only a minimal increase in accumulation of glucocerebroside. If this line of reasoning is correct, the anti-inflammatory activity of dexamethasone may provide a unique benefit to the treatment of Gaucher disease beyond improving the delivery of enzyme to the macrophages. The anti-inflammatory activity of dexamethasone may also negate any potential down-regulation of the mannose receptor by interferon-γ (Harris et al., 1992; Shepherd et al., 1994). The dual benefits of dexamethasone in improving the targeting of Cerezyme to macrophages and reducing inflammation may outweigh the potential side effects associated with the use of the glucocorticoid. As such, its inclusion represents a possible strategy to improve the treatment of Gaucher disease. However, safety considerations associated with repeated chronic use of dexamethasone in Gaucher patients will need to be carefully evaluated. Perhaps other dexamethasone-like compounds that are capable of facilitating the up-regulation of mannose receptors on macrophages, but without the associated toxicity, may be more appropriate for consideration.

Acknowledgments

We thank the Comparative Medicine Group for assistance with some of the animal studies. We also thank the Gaucher and Gene Transfer Research groups for informative discussions and Ronald Scheule and Canwen Jiang for help in proofreading the manuscript.

References


Address correspondence to: Dr. Seng H. Cheng, Genzyme Corporation, 31 New York Ave., Framingham, MA 01701-9322. E-mail: seng.cheng@genzyme.com