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Distribution of acid sphingomyelinase in rodent and non-human primate brain after intracerebroventricular infusion

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ABSTRACT

One treatment approach for lysosomal storage diseases (LSDs) is the systemic infusion of recombinant enzyme. Although this enzyme replacement is therapeutic for the viscera, many LSDs have central nervous system (CNS) components that are not adequately treated by systemic enzyme infusion. Direct intracerebroventricular (ICV) infusion of a high concentration of recombinant human acid sphingomyelinase (rhASM) into the CNS over a prolonged time frame (hours) has shown therapeutic efficacy in a mouse model of Niemann–Pick A (NP/A) disease. To evaluate whether such an approach would translate to a larger brain, rhASM was infused into the lateral ventricles of both rats and Rhesus macaques, and the resulting distribution of enzyme characterized qualitatively and quantitatively. In both species, ICV infusion of rhASM resulted in parenchymal distribution of enzyme at levels that were therapeutic in the NP/A mouse model. Enzyme distribution was global in nature and exhibited a relatively steep gradient from the cerebrospinal fluid compartment to the inner parenchyma. Additional optimization of an ICV delivery approach may provide a therapeutic option for LSDs with neurologic involvement.

Introduction

Lysosomal storage disorders are a family of about 50 diseases, most of which are caused by mutations in genes encoding hydrolytic enzymes. The reduced or deficient activity of these hydrolases results in the pathologic accumulation of substrate within lysosomes, leading to organ dysfunction and premature death (Neufeld, 1991). With the advent of recombinant enzyme replacement therapy (ERT) there are now treatments for some of the more common LSDs, such as Gaucher (Weinreb et al., 2002), Fabry (Desnick and Banikazemi, 2006), Pompe (van der Ploeg and Reuser, 2008), Hurler/Scheie (Wraith et al., 2005), and Hunter (Zareba, 2007). ERT by infusion into the systemic circulation effectively reduces storage in the affected visceral tissues, but not in tissues within the central nervous system due to the inability of these large molecular weight proteins to cross the blood–brain barrier. Bone marrow replacement has been used successfully to reduce the neuropathic manifestations in some Hurler patients (Whitley et al., 1993), but this approach has been less beneficial in patients with neuronopathic Gaucher and Niemann–Pick A (NP/A; acid sphingomyelinase deficiency) diseases (Malatack et al., 2003).

As a localized treatment strategy, direct administration of recombinant enzyme into the brain parenchyma has shown some promise. For example, glucocerebrosidase has been administered directly into the brain parenchyma by convection-enhanced delivery in rats (Zirzow et al., 1999) and in a Gaucher patient (Lonser et al., 2007). Imaging data after infusing contrast agents into non-human primates (NHPs) and the human patient indicated a distribution of enzyme localized to an area surrounding the site of administration (Lonser et al., 2007), suggesting that infusion into multiple parenchymal sites may be required to treat the entire brain. This may be an impractical treatment strategy in diseases with widespread pathology like NP/A, where abnormal substrate accumulation can be found in a variety of cell types in both the cerebrum and cerebellum (Schuchman and Desnick, 2001).

The cerebrospinal fluid (CSF) compartment has been used as a conduit to achieve more global CNS distribution of enzyme. For example, direct injections of sulfamidase into the cisterna magna reduced pathologic substrate in the brains of mucopolysaccharidosis

Abbreviations: BSA, bovine serum albumin; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; ERT, enzyme replacement therapy; Gd, gadoteridol; GDNF, glial cell line-derived neurotrophic factor; HRP, horseradish peroxidase; ICP, intracranial pressure; ICV, intracerebroventricular; IHC, immunohistochemistry; IV, intravenous; LSD, lysosomal storage disease; MPS, mucopolysaccharidosis; MRI, magnetic resonance imaging; NP/A, Niemann–Pick A; NHP, non-human primate; rhASM, recombinant human acid sphingomyelinase; RT, room temperature.

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(MPS) IIIA mice (Hemsley et al., 2007; Hemsley et al., 2008), and within select regions of Huntaway dogs (Hemsley et al., 2009). Studies in MPS I animal models have also shown success with an intrathecal approach (Dickson et al., 2007). In principle, intracerebroventricular (ICV) delivery could effect more widespread distribution of enzyme by taking advantage of the natural CSF flow from the ventricles. Widespread reduction of intracellular storage pathology has been demonstrated in rodent models after ICV administration of recombinant enzymes, and has resulted in improved motor function in acid sphingomyelinase (ASM) knockout mice (Dodge et al., 2009), increased survival in Gaucher mice (Cabrera-Salazar et al., 2010), and efficacy in additional LSD models (Chang et al., 2008; Lee et al., 2007).

In larger brains, ICV administration of high molecular weight molecules has given variable results in terms of distribution, probably due to the many parameters involved, such as (i) the nature of the brain (size, anatomical structure, gray/white matter distribution, CSF flow rate, etc.), (ii) the nature of the molecule (size, conformation, isoelectric point, receptor distribution, etc.), (iii) the details of the delivery (volume infused, infusion rate and time, etc.), and (iv) the methods used to evaluate distribution (radioactivity, ELISA, immunohistochemistry, etc.). For example, in the rat, bolus ICV injections of radiolabeled 5 kDa inulin led to distribution over the entire brain by 4 h (Proescholdt et al., 2000), but a short (<1 min) infusion of radiolabeled 7.4 kDa IGF-1 resulted in only limited (<1.25 mm) distribution into the brain parenchyma (Nagaraja et al., 2005). In primates, ICV infusions of glial cell line-derived neurotrophic factor (GDNF) led to behavioral/motor improvements in MPTP-induced hemiparkinsonian NHPs, where the target was the nigrostriatal pathway, and especially the putamen (Gash et al., 1996; Grondin et al., 2002; Zhang et al., 1997), but in clinical translation failed to improve the parkinsonian condition of patients (Nutt et al., 2003). An autopsy of one trial patient (3 weeks after his last dose of GDNF) showed no GDNF immunoreactivity at the cerebral infusion site or elsewhere, in contrast to the findings in NHPs (receiving daily doses of GDNF), where GDNF immunoreactivity was found not only at the infusion site but also within the caudate nucleus, septum, and overlying cortex (Kordower et al., 1999). These autopsy results have been taken to signify that the GDNF ICV infusion did not reach the intended target(s) in this group of patients.

Given this variability of results and our earlier successful use of an ICV delivery route in a mouse model of NP/A disease, in which rhASM was infused over a 6 h period (Dodge et al., 2009), we asked whether a similar ICV infusion strategy would lead to significant distribution of rhASM into the parenchyma of rats and NHPs. Our results indicate that, for both rats and NHPs, in all brain regions in contact with CSF this approach leads to protein distribution into the parenchyma at levels that were therapeutic in the NP/A knockout mouse. The distribution of rhASM shows a gradient of concentration, with high levels in parenchyma adjacent to pial and ependymal surfaces, and declining tissue levels at greater distances from ventricular and subarachnoid CSF spaces. With further refinement of delivery parameters, e.g. infusion time and rate, this approach may represent a plausible option for the treatment of neuronopathic LSDs.

Materials and methods

Recombinant human acid sphingomyelinase

Recombinant human acid sphingomyelinase was manufactured at Genzyme Corporation (Framingham, MA) and formulated at a final concentration of 20 mg/ml in artificial cerebrospinal fluid (aCSF; 148 mM NaCl, 3 mM KCl, 1.4 mM $CaCl_2$ dihydrate, 0.8 mM $MgCl_2$ hexahydrate, 0.8 mM NaH_2PO_4 heptahydrate, 0.16 mM Na_2HPO_4 monohydrate, pH 7.0).

Rat studies

Female Sprague–Dawley rats (CD IGS; Charles River Laboratories, Waltham, MA) 8–10 weeks old were housed individually with free access to food and water in an AAALAC accredited facility. All studies were reviewed and approved by the institutional animal care and use committee. Test articles were administrated by tail vein injection in 80 μl, or by ICV infusion (see below). At the end of each study, rats were euthanized with 2 ml of sodium pentobarbital (Euthasol; Virbac, Fort Worth, TX). For animals being perfused, PBS was perfused into the left cardiac ventricle at 83 ml/min for ~2 min. Blood was collected by retro-orbital eye bleed into BD gold microtainer tubes (VWR, Bridgeport, NJ). Serum was separated by centrifugation at 10,000×g for 10 min and stored at −80 °C. Brains were removed and sectioned coronally into either 3- or 6-mm blocks (see below) using a brain matrix (VWR Scientific, West Chester PA) while submerged in 20 mg/ml bovine serum albumin (BSA) in phosphate-buffered saline pH 7.2 (PBS; Gibco/Invitrogen, Grand Island, NY) to minimize contamination by CSF-borne rhASM. For immunohistochemical (IHC) analysis, brains were cut into 3 mm blocks and placed in 4% paraformaldehyde (PFA). For orientation purposes brains were notched on the side infused. Brains to be analyzed biochemically were cut into 6-mm blocks and placed between two glass slides before being submerged in 2-methyl butane cooled in dry ice. The slides were wrapped in aluminum foil and stored at −80 °C.

ICV infusion of rhASM into rats

Stereotactic surgery was performed 7 days prior to infusion to implant cannulae into the brains and to allow time for tissue to heal around the cannula. Rats were anesthetized with 3% isoflurane and placed in a stereotactic frame for placement of an indwelling guide cannula (Plastics One, Roanoke, VA) to a position 1 mm dorsal to the right lateral ventricle (A–P: −1.25 mm from bregma, M–L: −1.4 mm from bregma, D–V: −3.2 mm from dura, incisor bar: 0.0 mm; (Paxinos and Watson, 1998)). Guide cannulae were permanently affixed to the skull with anchor screws and dental acrylic (CMA Microdialysis Inc., North Chelmsford, MA). A dummy cannula (Plastics One) was inserted into the guide cannula to maintain cannula patency prior to insertion of the infusion probe. One hour before and 24 h after surgery rats were given ketoprofen (5 mg/kg; SC) for analgesia.

On the day of enzyme infusion, the dummy cannula was removed and replaced with an infusion probe (Plastics One) that extended 1 mm ventral to the tip of the guide cannula. Infusion probes were connected to a swivel (Instech Laboratories Inc., Plymouth Meeting, PA) that permitted 360° movement of the conscious rat during the infusion procedure. Swivels were connected with tubing to a Hamilton gastight syringe mounted on a Harvard Apparatus infusion pump (Harvard Apparatus, Holliston, MA) set to deliver enzyme at a rate of 20 μl/h for 4 h. Fifteen minutes after the completion of the infusion the rats were euthanized (as described above) and processed for tissue collection.

Non-human primate studies

Two male Rhesus macaques (NHP#1035: 15 kg, 8 years 5 months; NHP#683: 11.5 kg, 8 years 6 months) were obtained and housed at Valley Biosystems (Sacramento, CA). Studies were reviewed and approved by the institutional animal care and use committee. Test articles were administered by ICV infusion (see below). Body weights were measured pre-ICV cannulation and at the time of euthanasia. Vital signs (body temperature, heart rate, blood pressure, respiration rate, blood oxygen levels) were monitored during surgical procedures and ICV administration. Cage side observations were performed on a daily basis after ICV infusion of MRI contrast material. Immediately after ICV administration of rhASM, each animal was transferred from the surgery