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Intrathecal delivery of protein therapeutics to the brain: A critical reassessment

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ABSTRACT

Disorders of the central nervous system (CNS), including stroke, neurodegenerative diseases, and brain tumors, are the world's leading causes of disability. Delivery of drugs to the CNS is complicated by the blood–brain barriers that protect the brain from the unregulated leakage and entry of substances, including proteins, from the blood. Yet proteins represent one of the most promising classes of therapeutics for the treatment of CNS diseases. Many strategies for overcoming these obstacles are in development, but the relatively straightforward approach of bypassing these barriers through direct intrathecal administration has been largely overlooked. Originally discounted because of its lack of usefulness for delivering small, lipid-soluble drugs to the brain, the intrathecal route has emerged as a useful, in some cases perhaps the ideal, route of administration for certain therapeutic protein and targeted disease combinations. Here, we review blood–brain barrier functions and cerebrospinal fluid dynamics and their relevance to drug delivery via the intrathecal route, discuss animal and human studies that have investigated intrathecal delivery of protein therapeutics, and outline several characteristics of protein therapeutics that can allow them to be successfully delivered intrathecally.

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1. Introduction

The World Health Organization has called neurological disorders one of the greatest threats to public health, making their treatment a critical unmet need in the current healthcare environment (World Health Organization, 2006). It is estimated that over 1 billion people worldwide suffer from a neurological disorder, including brain tumors, epilepsy, cerebrovascular diseases, neurodegenerative disorders, depression, multiple sclerosis, autoimmune encephalopathy, and chronic

Abbreviations: BBB, blood–brain barrier; CSF, cerebrospinal fluid; CNS, central nervous system; GAG, glycosaminoglycan; HNS, heparan *N*-sulfatase; I2S, iduronate-2-sulfatase; ICV, intracerebroventricular; IDU, α-L-iduronidase; ICV, intracerebroventricular; ISF, interstitial fluid; IT, intrathecal; IV, intravenous; MC, meningeal carcinomatosis; MRI, magnetic resonance imaging.

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neuropathic pain (World Health Organization, 2006; Bray et al., 2012). Effective treatment of most of these and other neurological conditions may require the use of drugs with sites of action within the central nervous system (CNS). However, the blood–brain barrier (BBB), a protector of the CNS and a major regulator of its environment, impedes the blood-to-brain entry of most potential therapeutics.

Strategies under investigation to overcome the problem of the BBB can be roughly divided into two broad categories. The first category comprises techniques that allow or facilitate the crossing of drugs through the BBB (e.g. molecular Trojan horses, proton-coupled oligopeptide transporters, exosomes, liposomes, nanoparticles, chimeric peptides, prodrugs), while the second category consists of techniques that bypass the BBB altogether via direct delivery to the CNS (Smith et al., 2004; Alam et al., 2010; Tan et al., 2010; Guo et al., 2012; Vlieghe & Khrestchatisky, 2013). In the second category, several techniques have been investigated, including BBB disruption, and intrathecal (IT), intracerebroventricular (ICV), and intranasal delivery. This review focuses specifically on IT delivery. Because promising reports for ICV and intranasal delivery and BBB disruption have been extensively described (Alam et al., 2010; Rajadhyaksha et al., 2011; Tayebati et al., 2013; Zhao et al., 2013), we will not recapitulate them here. Instead, this review is intended to re-examine the potential of IT drug delivery to allow penetration of protein therapeutics to the brain parenchyma, a role that has been largely discounted in the past. We begin with a discussion of endogenous BBB mechanisms and CNS fluid flow dynamics, then examine current data demonstrating effective IT delivery of particular classes of therapeutic proteins to the brain. We conclude with a consideration of ideal molecules for IT delivery and promising future applications of this technology.

2. Barriers to the delivery of drugs to the brain and cerebrospinal fluid

Drug delivery to the CNS is complicated by complex biological barriers generally termed the blood–brain barriers, including the vascular blood–brain barrier (BBB), blood–cerebrospinal fluid barrier, and specialty barriers such as the blood–retinal barrier (Neuwelt et al., 2008). These barriers serve many functions for their dependent tissue beds. The most widely known function, especially for the vascular BBB, is the prevention of the unregulated movement of substances from the blood into the CNS. Generating and maintaining stable resting potentials, action potentials, and synaptic transmission, together with the massive spatial and temporal summation of nerve impulses necessary for CNS function, requires an extreme degree of control over ionic, protein, and neurotransmitter concentrations in CNS fluids. The BBB thus affects and even regulates many of the complex interactions between the peripheral tissues and the CNS that are mediated through the blood stream, including neuroimmune interactions (Quan & Banks, 2007), feeding and energy balance (Banks, 2008), and even those affecting cognition (Banks, 2012).

The simplest mechanism by which molecules can cross the BBB is passive transmembrane diffusion. The degree to which a substance can enter by this mechanism is dictated by its lipid solubility and molecular weight, with small, lipid-soluble substances crossing more efficiently than large, hydrophilic substances. Steroid hormones are good examples of endogenous substances that can cross the BBB in this manner (Banks, 2012). Most small molecule recreational drugs also cross the BBB by this mechanism, including morphine, heroine, and ethanol (Becker & Greig, 2010). Exploitation of passive transmembrane diffusion in drug delivery has been hampered, however, by the presence of CNS-to-blood (efflux) saturable transport systems (Begley, 2004). Efflux transporters at the BBB serve critical functions, controlling electrolyte levels and limiting CNS exposure to endogenous and exogenous neurotoxins and to other endogenous biologics, including enkephalins and immunoglobulin G molecules, but they complicate the delivery of potential protein therapeutics to the CNS (Begley, 2004; Banks, 2005). We have shown, for example, that efflux of the neurotrophic peptide, pituitary adenylate cyclase-activating polypeptide 27, limits it accumulation in the brain and that inhibition of the efflux transporter allows intravenously administered peptide to accumulate in the brain to therapeutic levels (Dogrukol-Ak et al., 2009).

Influx transporters are also located at the BBB in large numbers, including those for glucose, amino acids, organic acids, vitamins, minerals, electrolytes, nucleic acids, peptides, feeding hormones, immune cells, and cytokines (Oldendorf, 1971; Davson & Segal, 1996; Engelhardt, 2008). Use of an influx transporter by a substance can increase its brain uptake to 4- to 30-fold over what would be predicted from entry via a passive transmembrane route (Oldendorf, 1971). A few drugs are known to use endogenous, saturable influx transport systems to enter the CNS, including L-dopa, donepezil, valproic acid, and gabapentin (Pardridge, 2007). Utilizing influx transporters for drug delivery of proteins is fraught with its own special difficulties, however. For example, the proteins which transport certain ligands across the BBB are not always the same proteins which act as receptors within the CNS (Pan & Kastin, 1999). As such, a modification that enhances transport of an endogenous ligand across the BBB can also have the unwanted effect of reducing its binding affinity to its CNS receptor. Further, recombinant proteins are unpredictably sensitive to being modified. Encapsulation, alteration to the base sequence (fusion proteins), and slight changes to glycosylation patterns can cause recombinant proteins to fold improperly, lose stability, lose enzymatic activity, or become more immunogenic (Jorgensen et al., 2006; Jorgensen & Nielson, 2009; Tan et al., 2010).

Many drug delivery techniques are in development with the aim of facilitating BBB crossing, as mentioned above, although a full discussion of these is outside the scope of this review. A different strategy is direct injection of protein therapeutics into the cerebrospinal fluid (CSF) in order to bypass the BBB altogether (Patel et al., 2009; Alam et al., 2010; Rajadhyaksha et al., 2011). Administration into CSF is accomplished by injection into the lateral ventricles of the brain (ICV administration), the subarachnoid space at the level of the cisterna magna, or the lumbar spine (IT administration). Direct CNS administrations have been successfully employed in instances where a local effect of the delivered therapeutic is desired, such as in pain management, treatment of spasticity, and cancer chemotherapy. A larger extent of penetration beyond the site of injection, which is needed to treat neurodegenerative disease, is influenced primarily by the flow dynamics of the CSF.

3. Cerebrospinal and interstitial fluid flow and dynamics: Implications for drug delivery

A thorough understanding of the fluid flow dynamics in the brain is critical when considering the distribution patterns for protein drugs administered directly into the CNS. In the body, interstitial fluid (ISF), containing sugars, salts, lipids, amino acids, coenzymes, hormones, and cellular waste products, bathes nearly every cell, including those of the brain. ISF flow plays a key role in nutrient and waste transportation, intercellular signaling, immune regulation, and the maintenance of cellular homeostasis throughout the body. The regulation of both colloidal osmotic pressure and fluid volume is dependent upon this efficient removal of soluble proteins and waste products from the ISF (Scallan et al., 2010; Wiig & Swartz, 2012).

Within the brain ventricles, a second CNS fluid, the CSF, is continuously produced. The major direction of CSF flow is between the sites of production in the choroid plexuses (Johanson, 1988) and the major sites of reabsorption in the arachnoid villi and in the primitive lymphatic system located at the cribriform plate (Knopf et al., 1995; Boulton et al., 1999). The total volume of human CSF is about 150 mL, and the human brain produces approximately 500 mL of CSF per day (Johanson et al., 2008). It has been calculated that while the ISF is replaced relatively slowly (every 20 hours), the rate of turnover of CSF is faster and dependent on the size of the brain, with the mouse turning over its CSF

compartment every 1.87 hours and the human every 4.39 hours (Cserr & Patlak, 1992; Davson & Segal, 1996; Begley et al., 2000; Johanson et al., 2008; Begley, 2012).

Although somatic ISF drains into lymphatic vessels, such vessels are notably lacking in the CNS, despite the fact that the intense metabolic activity of neural tissue and its exquisite sensitivity to changes in the extracellular environment would seem to demand an efficient lymphatic vasculature. The absence of such vasculature led to early work whereby the role of CSF, which flows throughout the ventricular system in the CNS, was clarified as critical to ISF solute clearance from the brain via paravascular pathways (Rennels et al., 1990, 1985). Recent elegant experiments in mice have expanded our understanding of CNS fluid flow, with resulting implications for drug delivery (Begley, 2012; Iliff et al., 2012). Iliff and colleagues investigated the flow of CSF into and through the brain interstitium through a series of ex vivo and in vivo experiments in mice using fluorescent tracers of different molecular weights. In the first set of experiments, tracers of three different molecular weights (759 Da, 3 kDa, and 2000 kDa) were infused into subarachnoid space of anesthetized mice, from which point they entered the brain along paravascular spaces (Iliff et al., 2012). The large 2000 kDa tracer was confined to the paravascular spaces and did not enter the interstitial space, but the 3 kDa tracer entered the interstitium from the paravascular space and from the pial surface. The smallest tracer of 759 Da rapidly disseminated throughout the interstitial spaces of the brain, leaving only a small amount in the paravascular spaces through which it had entered. Building upon these findings, the investigators used two-photon laser scanning microscopy through a closed cranial window in anesthetized mice to conduct real-time imaging of the movement of the same 3 kDa and 2000 kDa tracers after intracisternal injection (Iliff et al., 2012). After injection, both of the tracers rapidly entered the brain along the outside of cortical surface arteries and penetrating arterioles. Movement from the paravascular spaces into the interstitium occurred readily for the 3 kDa tracer, but the 2000 kDa tracer remained confined to the paravascular space. The movement of the 3 kDa tracer into the interstitium was dependent on the aquaporin-4 water channel localized in perivascular astrocytic endfeet, and its movement was abolished in Aqp4-null mice.

Ex vivo techniques were then used to map the exit pathway from the brain of a 45 kDa tracer after intracisternal injection. After injection, the 45 kDa tracer moved rapidly inward along penetrating arteries and arterioles to reach the terminal capillary beds throughout the brain. The largest influxes were seen along large ventral perforating arteries of the basal ganglia and thalamus. Exit from the brain appeared to occur along both the medial internal cerebral veins and the lateral-ventral caudal rhinal veins. Interestingly, tracer injected intraparenchymally into the cortex, striatum, or thalamus was cleared along the same pathways. The authors concluded that the ISF and CSF moving through the brain parenchyma share the same paravenous drainage pathways. Because the brain-wide fluid transport that the authors observed in their series of experiments is dependent upon astroglial water flux, and because it serves a lymphatic-like function in clearing solutes from the interstitial space, the authors coined the term “glymphatic pathway” to describe their findings (Iliff et al., 2012). This glymphatic pathway may help IT-administered substances that reach the subarachnoid CSF to distribute more deeply into the brain than would be predicted were penetration to depend on diffusion only.

4. Direct delivery of protein therapeutics to the CNS

Early human studies of proteins administered by the ICV route found that they were rapidly cleared from the CSF and brain into the blood, presumably failing to penetrate deeply into the brain parenchyma (Greene et al., 1969; Ghersi-Egea et al., 1996). Some later studies were similarly disappointing. For example, glial cell line-derived neurotrophic factor, a peptide that promotes survival of dopamine neurons, failed to produce any improvements in Unified Parkinson’s Disease Rating Scale scores when delivered into the ventricles of patients with advanced Parkinson’s disease by an implanted catheter and access port (Nutt et al., 2003). A later animal study investigating insulin-like growth factor-1 for its potential neuroprotective effects in an Alzheimer’s disease model was no more encouraging. After unilateral ICV injection in the rat model, nearly all of the insulin-like growth factor-1 was cleared from the brain by 1 hour after injection, and very little penetrated into the parenchyma (Nagaraja et al., 2005).

Despite these well-known disappointing results, studies have documented successful ICV or IT delivery of large protein therapeutics to the brain parenchyma. One of the first examples of successful delivery in an animal model was with leptin, which after lumbar IT administration into the CSF of baboons and dogs was shown to reach the hypothalamus and so influence feeding (LeBel et al., 1999; McCarthy et al., 2002). This early work rekindled the interest in direct CNS administration of biologics, which has since been shown in animal studies to be a potentially viable therapeutic approach in several diseases, as discussed below.

4.1. IT Nogo-A Antibodies for Stroke

One factor affecting functional recovery after stroke is the neurite-outgrowth inhibitory environment present in the adult CNS. Proteins such as Nogo-A appear to prevent fiber regeneration, sprouting, and new network formation, contributing to the failure of CNS axons to regrow and reconnect after injury (Pernet & Schwab, 2012). IT-administered anti–Nogo-A antibody has been shown to penetrate into the corpus callosum and the striatal white matter in rat models and has resulted in improvements in measures of functional recovery (Tsai et al., 2007). In a stroke model, macaque monkeys implanted with osmotic pumps delivering anti–Nogo-A antibody to the IT and subdural spaces showed recovery on the Brinkman box task, which tests reaching and grasping. Recovery ranged from 73% to 89.6% of pre-lesion levels in treated animals, compared with below 50% recovery in the control animals (Hamadjida et al., 2012). The treated animals, but not controls, also exhibited an enhanced callosal connectivity with the ipsilesional premotor cortex, based on normalized numbers of retrogradely labeled neurons in the intact hemisphere.

4.2. IT enzyme replacement for neuropathic lysosomal storage diseases

The lysosomal storage diseases are inherited disorders characterized by a deficiency in one or more specific lysosomal enzymes, resulting in the accumulation of undigested or partially digested macromolecules within cells and tissues (Winchester et al., 2000). Of the approximately 50 lysosomal storage diseases, two-thirds manifest neurological and cognitive declines (Hemsley & Hopwood, 2009). Peripheral administration of the missing enzyme can result in recovery of the peripheral tissues, but it does little to reverse CNS manifestations of the disease. Studies in animal models of several neuropathic lysosomal storage disorders have demonstrated that IT or ICV administration of the missing enzyme is feasible and potentially effective. Rodent studies are summarized in Table 1. In general, these studies have found penetration of recombinant enzyme into the brain parenchyma; in some cases localization to the lysosomes of neuronal cells has been verified via co-immunostaining with lysosomal-associated membrane protein 2 and neuronal nuclear antigen. Significant reduction or normalization of storage material levels in the brain as well as improvements in lifespan or cognitive performance testing have also been seen. Probably of great significance for the IT administration of lysosomal enzymes is the phenomenon of cell uptake of the recombinant proteins by neurons and glia, mediated by mannose-6-phosphate receptors or other uptake mechanisms (Calias et al., 2012), thus effectively rescuing them from the extracellular fluid and CSF and reducing the extent of clearance by bulk flow.

Large animal studies of brain delivery of recombinant enzymes have been conducted for several lysosomal diseases, including

Table 1
Studies of intracerebroventricular (ICV) and intrathecal (IT) administration of recombinant enzymes in rodent models of lysosomal storage diseases.

Study	Disease	Organism	Protein	Delivery	Findings in Treated Animals
Cabrera-Salazar et al. 2010	Gaucher disease	Mouse	Glucocerebrosidase	Bilateral ICV injection	Enzyme detected in parenchyma. Significant reduction in storage material. Lifespan increased by about 50%.
Lee et al., 2007	Globoid cell leukodystrophy (Krabbe disease)	Mouse	Galactocerebrosidase	Unilateral ICV injection	Enzyme detected in parenchyma. Subcellular staining pattern consistent with a lysosomal/endosomal distribution. Lifespan increased by about 20%.
Stroobants et al., 2011	Metachromatic leukodystrophy	Mouse	Arylsulfatase A	Unilateral ICV continuous infusion	Enzyme detected in parenchyma co-localized with neuronal cell markers and lysosomal markers. Significant reduction in storage material. Normalization of motor incoordination and reduced gait homogeny in gait analysis.
Tsuji et al., 2011	Tay-Sachs disease and Sandhoff disease	Mouse	β-hexosaminidase with a high mannose 6-phosphate-type-*N*-glycan content	Bilateral ICV injection	Enzyme detected in parenchyma, co-localized with neuronal cell markers. Significant reductions in storage materials in neural cells. Delay in onset of motor dysfunction as well as a 12% increase in lifespan.
Chang et al., 2008	Late infantile neuronal ceroid lipofuscinosis	Mouse	Tripeptidyl peptidase 1 proenzyme	Unilateral ICV continuous infusion	Enzyme detected in parenchyma. Improvements in mean tremor amplitude.
Xu et al., 2011	Late infantile neuronal ceroid lipofuscinosis	Mouse	Recombinant tripeptidyl peptidase 1	IT via lumbar puncture	Decline in gait analysis significantly delayed. Lifespan increased by about 30% to 40%.
Belichenko et al., 2005	MPS I	Rat	α-L-iduronidase	Unilateral ICV injection	Enzyme detected in parenchyma.
Calias et al., 2012	MPS II	Mouse	Iduronate-2-sulfatase	IT via lumbar puncture	Enzyme detected in parenchyma. Reduction in storage inclusions in neurons in gray matter and vacuolation in oligodendrocytes in white matter.
Higuchi et al., 2012	MPS II	Mouse	Iduronate-2-sulfatase	Unilateral ICV injection	Normalization of alternation behavior and arm entry in Y-maze test.
Hemsley, 2007	MPS IIIA	Mouse	Heparan *N*-sulfatase	Cerebellomedullary cistern injection	Reduction in storage material level throughout brain. Reduction in storage vesicles in neuronal and glial cells in cerebellum, cerebral cortex, and spinal cord. Slower decline on open-field testing over time. Normalization of gait.
Hemsley et al.,, 2008	MSP IIIA	Mouse	Heparan *N*-sulfatase	Cerebellomedullary cistern injection	Reduction in storage material level throughout brain. Reduction in markers of brain inflammation.
Dodge et al., 2009	Niemann–Pick A	Mouse	Acid sphingomyelinase	Unilateral ICV infusion	Enzyme detected in parenchyma. Reductions in storage material throughout brain. Improved performance on foot fault test. Improved gait analysis parameters.
Ziegler et al., 2011	Niemann–Pick A	Rat	Acid sphingomyelinase	Unilateral ICV infusion	Enzyme detected in parenchyma.

Abbreviations: GAG, glycosaminoglycan; HNS, heparan *N*-sulfatase; I2S, iduronate-2-sulfatase; IDU, α-L-iduronidase; MPS, mucopolysaccharidosis.

mucopolysaccharidosis I, II, and III, late infantile neuronal ceroid lipofuscinosis, and Niemann-Pick A disease (Table 2). After IT or cisterna magna administration, recombinant enzymes reached the hippocampus, basal ganglia, thalamus, caudate, and periventricular white matter of these large animals (Kakkis et al., 2004; Dickson et al., 2007; Hemsley et al., 2009; Dierenfeld et al., 2010; Chen et al., 2011; Crawley et al., 2011; Vuillemenot et al., 2011; Calias et al., 2012; Dickson et al., 2012). Penetration was dose dependent, and regions of the brain that are closest to the CSF surface accumulated the highest enzyme levels. Detectable enzymatic activity in various CNS cell types and improvements in neuropathology have been reported in some of the large animal studies (Kakkis et al., 2004; Dickson et al., 2007; Hemsley et al., 2009; Chen et al., 2011; Crawley et al., 2011; Vuillemenot et al., 2011; Calias et al., 2012; Dickson et al., 2012; Pfeifer et al., 2012). For example, enzymatic activity was detected in neurons and glia in all six neuronal layers of the cerebral cortex and in white matter oligodendrocytes in non-human primates and beagles that received lumbar IT or ICV doses of iduronate-2-sulfatase (the enzyme that is deficient in mucopolysaccharidosis II) (Calias et al., 2012). Similarly, weekly intrathecal administration of recombinant human α-L-iduronidase to mucopolysaccharidosis I model dogs revealed 2- to 5-fold elevated levels of enzyme in brain specimens (Kakkis et al., 2004). Levels of pathologic glycosaminoglycan storage material were normalized in the brains of these treated dogs, and neuronal glycosaminoglycan storage was significantly reduced as seen on electron microscopy. Although improvement in cognitive function and memory has been suggested by murine studies (Table 1), only one study included testing for cognitive improvement in a large animal model of lysosomal storage disease; that study had a sample size of one treated animal which developed an immune response to the administered protein, and no improvement or stabilization of cognitive function was seen (Vuillemenot et al., 2011).

Investigational use of IT treatment in a human patient has been reported in a case study of a male patient with mucopolysaccharidosis I-Scheie (Munoz-Rojas et al., 2008). At the age of 38 years, the patient, while presenting with near-normal intellectual function, developed unstable and progressive spinal cord compression with resulting gait ataxia and lower extremity numbness, tingling, and pain. The patient refused standard surgical treatment. He was treated with IT injections of laronidase (Aldurazyme®, BioMarin Pharmaceutical/Genzyme Corporation, Cambridge, Massachusetts) via lumbar puncture at 1-month intervals for a total of 4 months. After 6 months, his 12-minute walk test distance increased by 14%, pulmonary function parameters improved between 18% and 56%, a consistent right ankle clonus disappeared, and temperature sensation in the feet showed improvement. Spinal canal stenosis remained stable on MRI. The patient reported decreased numbness and tingling, increased stability when rising from a chair and when walking, and decreased need for pain medication. There was a reduction of 22.5% in CSF levels of glycosaminoglycan substrate from baseline such that by month 4, the levels were normal.

Lysosomal enzymes may be particularly well suited for IT or ICV delivery, as several viral vector studies have supported the idea that lysosomal enzymes may undergo axonal transport, being transported to and from a neuron cell body along microtubules through the cytoplasm of axons. A similar transport throughout the brain may occur

Table 2
Studies of IT administration of recombinant enzymes in large animals.

Study	Disease	Organism	Protein	Delivery	Findings in Treated Animals	Tolerability in Treated Animals
Kakkis et al., 2004	MPS I	Dog	IDU	IT injection weekly for 4 weeks	IDU levels increased 2.7 to 5.9-fold normal levels depending on dose. Brain GAG levels normalized. Neuronal GAG storage significantly reduced.	Well tolerated. All treated dogs developed variable accumulations of immune cells in meninges of spinal cord, areas of spinal dura, and around brain. No clinical effects observed.
Dickson et al., 2007	MPS I	Dog	IDU	IT injection either once monthly or once every 3 months	IDU widely distributed throughout brain tissue. Brain GAG levels normalized. Neuronal GAG storage moderately reduced. One dog in monthly group with baseline gait disturbances improved on therapy.	Well tolerated. One dog in monthly group developed a moderate neutrophilic meningitis. One dog died from a large brain stem hematoma caused by a traumatic injection.
Felice et al., 2011	MPS II	Cynomolgus monkey	I2S	IT injection monthly for 6 months	I2S staining was observed both in surface neurons next to meninges and in neurons adjacent to white matter in a dose-dependent manner.	Well tolerated. Leukocyte infiltrates observed in meninges of brain in treated and control groups without notable tissue damage. Anti-idursulfase antibodies detected in two-thirds of treated animals.
Calias et al., 2012	MPS II	Cynomolgus monkey	I2S	IT injection monthly for 6 months	I2S detected in neurons of cerebrum, cerebellum, brainstem, and spinal cord of all groups in a dose-dependent manner. Enzyme was specifically detected within lysosomes of oligodendrocytes and neurons.	Not reported.
Calias et al., 2012	MPS II	Dog	I2S	Single IT injection	I2S detected in all 6 neuronal layers of cerebral cortex, as well as the cerebellar cortex, hippocampus, thalamus, and caudate nucleus.	Not reported.
Hemsley et al., 2009	MPS III	Dog	HNS	IT injection weekly for up to 4 weeks	Increased HNS activity detected in the dorsal, lateral and ventral cortical regions of cerebral cortex. Reductions in GAG-derived oligosaccharides were found in cerebellum and in surface samples from brainstem, spinal cord, and dura.	Well tolerated. One treated dog euthanized because of development of a non-infectious meningitis. All treated dogs developed antibodies against HNS, and levels increased over time.
Pfeifer et al., 2012	MPS III	Juvenile cynomolgus monkeys	HNS	IT injection every other week for 6 months	Elevated HNS activity seen in surface as well as periventricular areas of brain in treated animals. HNS immunostaining seen in meningeal and perivascular macrophages of brain and spinal cord, in adjacent glial and neuronal cell populations, and in cerebellum.	Well tolerated. One female had to be sacrificed early due to leaking device. All monkeys tested positive for anti-HNS antibodies in serum and CSF at one or more time points. Eosinophilic infiltrates were present in brain and spinal cord of some animals but did not cause morphological changes.

Abbreviations: CSF, cerebrospinal fluid; GAG, glycosaminoglycan; HNS, heparan *N*-sulfatase; I2S, iduronate-2-sulfatase; IDU, α-L-iduronidase; MPS, mucopolysaccharidosis.

via tanycytes and astrocytic processes (Begley, 2012). Such studies used viral vectors that transduce cells only at the site of injection, but showed detectable levels of enzyme activity and some correction of storage in distal sites, including the contralateral hemisphere (Skorupa et al., 1999; Bosch et al., 2000; Consiglio et al., 2001; Passini et al., 2002). Passini et al. directly tested the axonal transport hypothesis by injection of a viral vector expressing beta-glucuronidase, a lysosomal enzyme that can be visualized in tissue sections, into mouse brain structures that have defined axonal connections to other structures (Passini et al., 2002). The authors found that after injection of the vector unilaterally into the hippocampus of beta-glucuronidase–deficient mice, gene expression was detected only at the site of injection, but cells were strongly positive for beta-glucuronidase activity in both hemispheres of the hippocampus and in the septum. Only regions with axonal connections to the site of transduction were enzyme positive. Storage lesions in the distal structures were also reversed, suggesting that the enzyme was correctly targeted to the lysosomal compartment and remained enzymatically active after transport.

IT and ICV treatment with recombinant human enzymes not surprisingly can be associated with an inflammatory response. As noted above, this may be related to the reabsorption of CSF by way of routes previously discussed (Cserr & Knopf, 1992; Knopf et al., 1995). Homozygous null animals, such as the mucopolysaccharidosis I canine model, show variable accumulations of B lymphocytes, plasma cells, and other lymphocytes in the meninges of the spinal cord, areas of the spinal dura, and around the brain (Kakkis et al., 2004). The inflammatory cells in the meninges appeared more prominent in animals that were treated with frequent doses or that developed immunoglobulin G antibodies against the human protein (Dickson et al., 2007). Specific antibodies were found in serum and CSF in these animals, and in dogs, cats and monkeys receiving intra-CSF doses of other recombinant human enzymes (Kakkis et al., 2004; Dickson et al., 2007; Hemsley et al., 2009; Crawley et al., 2011; Felice et al., 2011; Vuillemenot et al., 2011; Dickson et al., 2012; Pfeifer et al., 2012). Antibody formation may be partly due to cross-species reactivity; however, titers in wild-type animals were lower than those seen in null animal models (Vuillemenot et al., 2011). While in some cases the appearance of antibodies in the serum and CSF coincided with the development of infusion-related reactions (Hemsley et al., 2009; Vuillemenot et al., 2011), the majority of studies reported that no adverse effects were observed (Kakkis et al., 2004; Dickson et al., 2007; Crawley et al., 2011; Felice et al., 2011; Ziegler et al., 2011; Dickson et al., 2012; Pfeifer et al., 2012). In the future, blocking antibody production by inducing immune tolerance (Dickson et al., 2012) or by neonatal exposure to the enzymes (Vogler et al., 1999; Dierenfeld et al., 2010; Crawley et al., 2011) might enhance the effectiveness of IT delivery of protein therapeutics.

4.3. IT Trastuzumab for breast cancer brain metastases

About 5% to 6% of patients with breast cancer develop brain metastases (Schouten et al., 2002; Schouten et al., 2002; Barnholtz-Sloan et al., 2004) at which point survival is about 7 months (Altundag et al., 2007). In about 40% of patients with CNS metastases, the tumors are positive for human epidermal growth factor receptor 2 (HER-2) (Altundag et al., 2007). Trastuzumab, a humanized anti–HER-2 monoclonal antibody, has been shown in several large, prospective, controlled

Table 3
Case reports of IT delivery of trastuzumab in patients with MC from breast cancer.

Report	Patient	Treatment	Outcome
Laufman and Forsthoefel, 2001	48-year-old female with mid-back pain and sudden paralysis	3 IT doses of trastuzumab escalating from 5 to 20 mg over 2 weeks. Concomitant treatment with IT thiotepa and/or methotrexate.	Treatment well tolerated. No benefits seen after first 2 doses. After third dose, patient remained neurologically stable for 30 days, then declined and died of respiratory arrest.
Stemmler et al., 2006	39-year-old female with dizziness, hearing loss, and headache.	4 IT doses of trastuzumab escalating from 5 to 20 mg over 2 weeks followed by another 20 mg dose 3 weeks later. Concomitant treatment with IV trastuzumab and chemotherapy every 3 weeks continued.	Treatment well tolerated. Neurological symptoms improved within 2 weeks from last dose. Tumor cell count in CSF remained low for 11 months from first diagnosis of MC. Progression of MC occurred after 11 months.
Platini et al., 2006	41-year-old female with vertigo, memory and sleep problems, and difficulty concentrating.	IT trastuzumab escalating from 20 mg to 25 mg given weekly for about 1 year. IT 25 mg prednisone and 10 mg thiotepa added after 1 year. Total of 46 IT injections over 17 months. Concomitant treatment with IV trastuzumab and chemotherapy continued.	Treatment well tolerated. Patient developed vision disturbances after 1 year of IT trastuzumab. These stabilized after addition of prednisone and thiotepa. Progression was again seen 6 months later with nausea, vomiting, and exacerbation of vision problems.
Stemmler et al., 2008	48-year-old female with overflow incontinence and paraparesis of the legs.	4 IT doses 12 mg methotrexate and 20 mg trastuzumab.	Treatment well tolerated. Tumor cell count in CSF decreased within 2 weeks. Patient's general condition improved significantly (Eastern Cooperative Oncology Group 3 to 1) and she was able to walk again without assistance. Tumor cell count was negative by the end of treatment. After 1 month, patient died due to rapid progression of lung and liver metastases, with no signs of MC.
Mir et al., 2008	59-year-old female with mid-back pain, cerebellar ataxia and headaches.	6 IT doses of trastuzumab escalating from 20 to 100 mg given weekly.	Treatment well tolerated. MRI at 6 weeks showed stable MC. 2 months later patient died of rapidly progressing brain metastasis to parenchyma.
Ferrario et al, 2009	38-year-old female with visual impairment, right ptosis, right hemi-facial hypoesthesia, left foot drop, and paralysis of the left facial nerve.	6 IT doses of trastuzumab given weekly, escalating from 20 to 30 mg, followed by 2 IT doses of 30 mg trastuzumab plus 10 mg methotrexate given weekly. IT doses of 40 mg trastuzumab given every 3 weeks for 8 months. IT doses of 40 mg trastuzumab plus 10 mg thiotepa given every 3 weeks for 6 months. IT doses of 50 mg trastuzumab plus 12 mg thiotepa given every 3 weeks for 7 months. Concomitant treatment with IV chemotherapy every 3 weeks continued.	Treatment well tolerated. MRI revealed that all the lesions were stable or slightly decreased after 40 mg trastuzumab plus 10 mg thiotepa. Significant improvement, with some lesions significantly decreasing in size, and other lesions no longer visualized, seen after 50 mg trastuzumab plus 12 mg thiotepa. Patient was fully functional at end of treatment.
Mego et al., 2011	43-year-old female with dizziness and cranial nerve palsies.	6 IT doses of 15 mg methotrexate, 24 mg cytarabine, and 24 mg hydrocortisone plus escalating dose of trastuzumab up to 40 mg given weekly. Systemic trastuzumab continued.	Treatment well tolerated. CSF protein level remained elevated but CSF tumor cell counts were negative. Symptoms improved significantly. 2 months later presented with impaired level of consciousness but refused further treatment. Overall survival was 13.5 months from diagnosis of MC.
Mego et al., 2011	39-year-old female with vision disorders, headaches, dizziness, cranial nerve palsies, and sleepiness.	6 IT doses of 15 mg methotrexate, 24 mg cytarabine, and 24 mg hydrocortisone plus escalating dose of trastuzumab up to 100 mg given weekly.	Treatment well tolerated Tumor cell count in CSF decreased, then was negative by last infusion. Symptoms improved significantly. Lesions were reduced on MRI. 6 months after last IT infusion, died due to progression of liver metastases, free of symptoms of MC.
Oliveira et al., 2011	44-year-old female with headache, gait disturbance, neck stiffness, and reduced flexion of lower limbs.	67 weekly IT doses of 25 mg trastuzumab plus 25 mg prednisolone. Systemic trastuzumab plus chemotherapy continued.	Treatment well tolerated. After 3 doses, recovered lower limb motion and resumed daily physical activities. Tumor cell count in CSF was negative after third dose and thereafter. The patient died from *Listeria* meningitis 27 months after diagnosis of MC. No evidence of MC was seen on autopsy.
Brandt, 2012	49-year-old female with headache, mental status changes, seizures, and lower limb weakness.	IT doses of 25 mg trastuzumab given every other day for 3 weeks, followed by doses given weekly for 43 months, followed by doses given biweekly to present time. Systemic trastuzumab plus capecitabine and lapatinib continued.	Treatment well tolerated. Improvement in mental status after 2 doses. After 3 months, all neurological compromises abated and have remained so to date.

Abbreviations: CSF, cerebrospinal fluid; IT, intrathecal; IV, intravenous; MC, meningeal carcinomatosis; MRI, magnetic resonance imaging.

studies to improve survival for patients with primary and metastatic HER-2–positive breast cancer (Slamon et al., 2001; Smith et al., 2007) and is used as part of the first-line treatment regimens in these populations. Unfortunately, peripherally administered trastuzumab does not reach therapeutic levels in the brain because of poor penetration of the BBB (Stemmler et al., 2007). A number of case reports documenting the use of IT delivery of trastuzumab in patients with leptomeningeal carcinomatosis have suggested that this route of administration is well tolerated (Table 3). IT trastuzumab improved clinical status and neurological complaints, and the overall survival appeared to be greater than what would be expected in this patient population. The safety of IT trastuzumab was evaluated in a formal toxicology study performed in cynomolgus monkeys. Animals received weekly IT trastuzumab at doses of 0, 3, or 15 mg for four weeks, and no drug-related effects on body weight, clinical signs, neurological function, clinical pathology, or anatomic pathology were seen, even at CSF concentrations that exceeded those reported in patients in the IT case studies (Braen et al., 2010). Phase I/II trials in human patients with leptomeningeal carcinomatosis are in progress (NCT01373710, NCT01281696, NCT01645839, NCT01325207).

5. Drugs delivered by the intrathecal route: Characteristics of the ideal molecule

The studies reviewed here illustrate that, contrary to traditional dogma, some molecules administered by the IT route can exert therapeutic effects on the CNS. The traditional view was based on and is largely true for small, lipid-soluble molecules. The molecules that are able to reach the CNS in therapeutic amounts after IT delivery tend to share a number of characteristics. Ideal molecules are usually proteins with little or no brain-to-blood passage at the level of the blood–brain barriers and are relatively resistant to degradation by the enzymes found within the CSF. Although the CSF has been shown to contain hundreds of proteins (Bora et al., 2012; Holtta et al., 2012) and may have a lower enzymatic activity than blood, enzymatic susceptibility should be evaluated for each drug candidate individually during the development process. Distribution to deep brain tissues after IT delivery can also be aided by receptor mediated cellular uptake and intercellular transfer. The lysosomal enzymes, for which some success in IT delivery has been reported, are examples of protein drugs with these characteristics. Without such intracellular and intercellular distributions, another characteristic of the ideal molecule would be a site of action within diffusible distance of a CSF-contacting area or accessed by the deep subarachnoid circulation recently described by Iliff and colleagues (Iliff et al., 2012). Another advantage of IT delivery of a protein therapeutic with a high therapeutic index is that the drug may be able to treat not only the neurological but also the peripheral symptoms of the disease. In most cases, CNS delivery results in the drug entering the blood stream as the CSF is reabsorbed.

There are also several disadvantages of IT delivery of therapeutic proteins. One is the development of antibodies that tend to reduce the effectiveness of the drugs. Another is that it may be difficult to predict the percent of an IT dose of a drug that will reach the brain and cranial CSF space; this may be caused by the variations in rates of CSF and metabolic free water production and reabsorption. Chronic IT delivery would be easier to achieve with the placement of indwelling catheters rather than repeated lumbar punctures; however, treatment would then be dependent on reliable catheter placement and stability. Some disease conditions may complicate catheter placement or stability, such as obesity or rapid growth rates in children (Follett et al., 2003). Infection is a risk that must be managed as well, with rates of infection with implanted IT catheters reported to be between 4% and 9% (Du Pen, 2005; Holmfred et al., 2006; Aprili et al., 2009; Taira et al., 2013; Motta & Antonello, 2014). Thus, even in the best of circumstances, catheters require periodic replacement.

6. Conclusions

Whereas early experiences with small, lipid-soluble molecules resulted in the IT route for drug administration being discounted, new evidence demonstrates that IT delivery may be an ideal route of administration for large biomolecules. Numerous studies in small and large animal models of stroke and the neurodegenerative lysosomal storage diseases show that IT delivery of protein therapeutics can result in widespread penetration into the brain parenchyma, improved signs of neuropathology, and improved clinical and behavioral outcomes. Tolerability to treatment was generally good in these studies. Similarly, case reports and studies in human patients with a neurodegenerative lysosomal storage disease or breast cancer metastases to the CNS have suggested generally acceptable tolerability and therapeutic effectiveness with few adverse events after the IT delivery of enzymes or antibodies. A number of clinical trials of IT-administered protein therapeutics are underway for patients with mucopolysaccharidosis I (NCT00638547, NCT00852358), mucopolysaccharidosis II (NCT00920647, NCT01506141) and mucopolysaccharidosis III (NCT01155778, NCT01299727); leptomeningeal carcinomatosis (NCT01373710, NCT01281696, NCT01645839, NCT01325207) and intraventricular hemorrhage (NCT01810302). It is anticipated that results from these trials will help guide clinical implementation of IT drug delivery on a larger scale and will inform therapeutic efforts for a variety of neurological disorders.

Conflict of interest

P. Calias is an employee of Shire. W. A. Banks has received research grants and honoraria from Shire. D. Begley has received research grants, travel grants, and honoraria from Shire and is a consultant for ArmaGen Technologies, Inc., and Synageva BioPharma. M. Scarpa has received research grants, travel grants, and honoraria from Shire. P. Dickson receives research and educational support from BioMarin Pharmaceutical and Genzyme Corporation, and is a consultant for ArmaGen Technologies, Inc., Isis Pharmaceuticals, Inc., and Shire.

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