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Nano Medicine Advancements in Addressing Rare Neurological Disorders: A Focus on Globoid Cell Leukodystrophy (Krabbe's Disease) Treatment

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ABSTRACT:

Because of the intricate architecture of brain tissue and the blood–brain barrier's selective permeability, the brain continues to be one of the most difficult therapeutic targets. Nanomedicines are being extensively researched as means of delivering therapeutics into the brain, despite their relatively large size when compared to small molecules and nucleic acids. Here, we expand on the potential applications of nanomedicines for treating uncommon neurodevelopmental disorders, utilizing the case of Globoid cell leukodystrophy (Krabbe disease) as a framework. The lessons gained from studying nanoparticle delivery to the brain in the context of Globoid cell leukodystrophy (GCL) can have a wider impact on the treatment of various other neurodevelopmental and neurodegenerative disorders because Globoid cell leukodystrophy is a monogenetic disorder and lysosomal storage disease affecting the nervous system.

In this review, we provide an overview of the genetic foundation and epidemiology of Globoid cell leukodystrophy,talk (GCL) about the disease's current in vivo and in vitro models, and address approved and clinically developed therapeutic approaches. Next, we go into more detail about the difficulties in getting particles to the brain, focusing on ways to get nanomedicines past the blood-brain barrier. We emphasize the use of nanoparticles in delivering.

Keywords: Globoid cell leukodystrophy,talk (GCL), BBB Blood–brain barrier, BPN Brain penetrating nanoparticle, CED Convection-enhanced delivery, CNS Central nervous system, dH Hydrodynamic diameter, DNA Deoxyribonucleic acid, DSPE 1,2-distearoyl-sn-glycero-3-phosphoethanolamine.

1. Introduction

Globoid Cell Leukodystrophy (Krabbe's Disease) is a rare monogenetic disease that is classified as a lysosomal storage disease. Early-onset GCL is currently incurable, and untreated infants rarely survive past the age of two. Scientists are looking for long-term, effective ways to combat the debilitating effects of GCL, an illness that primarily affects the nervous system.

Researchers are looking for ways to use nanomedicines against pathological targets other than SARS-Cov-2 after lipid nanoparticles demonstrated efficacy in protecting against [1,2]. Researchers are trying to figure out how to use nanomedicines against other disease targets. Globoid Cell Leukodystrophy is one potential target condition for nanoparticle-mediated therapy. Another approach is the engineering of nanoparticles to cross the blood–brain barrier (BBB) for the treatment of neurological diseases [3–9]. Here, we will discuss the potential of nanomedicines in the treatment of neurodevelopmental disorders, with a focus on lysosomal storage disease (KD) as a framework for investigation. Even though Globoid Cell Leukodystrophy is a unique monogenic disease, knowledge about how nanoparticles can be used to treat it can help with the treatment of other disorders that are specific to the brain.

The pathophysiology of Globoid Cell Leukodystrophy will be discussed in detail in this review, along with how nanomedicine is used to treat it. This will elaborate on clinical trials for treating Globoid Cell Leukodystrophy and examine in vitro and in vivo models for preclinical studies of Globoid Cell Leukodystrophy (such as the flicker mouse model). Additionally, in the context of Globoid Cell Leukodystrophy, targeting and delivery strategies for delivering nanomedicines across the blood–brain barrier will be covered. Lastly, a detailed discussion of the applications of nanoparticles in gene therapy, enzyme replacement therapy, and small molecule delivery will be provided, with particular emphasis on the management of lysosomal storage disorders and Globoid Cell Leukodystrophy.

Globoid Cell Leukodystrophy (Krabbe's Disease)

One of the well-known genetic leukodystrophies, globoid cell leukodystrophy, or Krabbe's disease, was initially reported by Krabbe in 1916.1. It is a recessive autosomal disorder that is inherited.

It is usually a disease that affects infants at a young age, progresses quickly, and always results in death. Almost all of the symptoms are related to white matter involvement. But more often than not, patients with the slower-moving juvenile and adult forms are being found. Pathologic hallmarks of the disease include infiltration of the distinct "globoid cells," reactive astrocytic gliosis, and rapid and total loss of myelin and oligodendrocytes2. In 1970, globoid cell leukodystrophy in humans and dogs was found to be caused by a genetic deficiency of galactosylceramidase.3.

Additionally, it was shown early on that patients with Krabbe's disease did not exhibit overt accumulation of galactosylceramide in their brains. This was in contrast to an equivalent disease called metachromatic leukodystrophy, where abnormal sulfatide accumulation had been well-established since Jatzkewitz's initial report.Six Even though the two disorders are metabolically related, there were additional observations in globoid cell leukodystrophy that offered a perplexing contrast with those in metachromatic leukodystrophy. Metachromatic leukodystrophy observations are more in line with the theory of Hers' Inborn Lysosomal Disorder,7 a storage disorder caused by a genetic lysosomal hydrolase deficiency. In order to explain the distinct features of Krabbe's disease, which are not shared by metachromatic leukodystrophy, a disease that is conceptually equivalent and closely related, the psychosine hypothesis was proposed.8. The hypothesis, which was first met with skepticism, has gained acceptance over the course of 30 years and is now widely acknowledged as a crucial pathogenetic mechanism underlying globoid cell leukodystrophy.Nine Several disease-causing mutations and functionally silent polymorphisms have been found since the human galactosylceramidase complementary DNA was cloned in 1993–1994,10,11. The standard comprehensive review, which was published in 2001, is intended to be updated by this presentation.twelve A recent review has also expressed some of the aspects that align with my current perspective on the pathogenetic mechanisms.13.

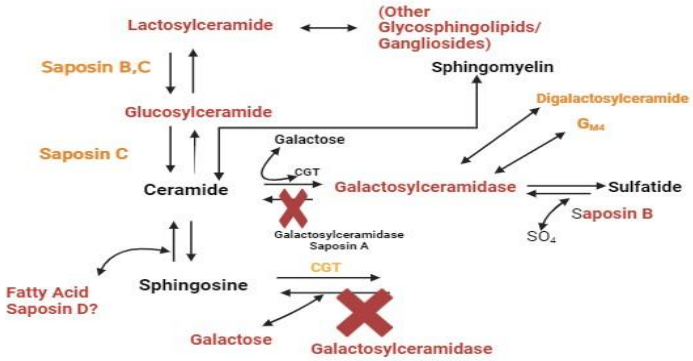
Classification

Originally, Krabbe disease was described as a pediatric disorder marked by spasticity and a rapidly progressing neurological degeneration that ultimately resulted in death [29]. The disease's infantile form affects over 85% of KD patients, with the remaining 10-15% having a later-onset form that can show symptoms in infancy or adulthood. While 3 to 10 years of age account for the majority of cases of late-onset KD, some patients have maintained good health well into their forties or even 60 years of age. Clinical manifestations of KD vary greatly in infantile, juvenile, and adult forms [31].

Epidemiology

Originally, it was estimated that the incidence of Globoid Cell Leukodystrophy was 1:100,000 [34]. A later study by Wasserstein et al. [35,36] using data from the New York State Newborn Screening Program revealed an actual incidence of 1:394,000. An Incidence in the US was estimated with greater accuracy at 1:250,000. as established through death certificate analysis [37]. Still, KD was found in a sizable Druze population with an extremely high incidence (6/1,000 live births). Israelite relatives [38]. The Globoid Cell Leukodystrophy incidence is most likely that of the inevitably deadly early infantile variation, most likely cited as a contributing factor of death, and the early Krabbe deletion is more prevalent in nations in Northern Europe [39].

The human galc gene contains 17 exons and is found on chromosome 14 (14q31.3) [40, 41]. Six putative N-glycosylation sites on the 669 amino acid GALC protein interact with the mannose-6-phosphate receptor to facilitate lysosomal trafficking [42]. Although notable interfamilial variability in clinical manifestations has been reported, the amount of residual galactocerebrosidase activity and clinical severity generally has an inverse relationship [43]. The condition is inherited as an autosomal recessive characteristic. The Human Gene Database contains reports of over 200 galc mutations, including numerous small deletions, insertions, and point mutations [31, 44–46]. There are very few genotype-phenotype correlations that have been found [47]. There is no information on whether the.86 infantile pathogenic variants that have been found specifically correlate with the early-infantile or late-infantile phenotype [48–50].



Important metabolic pathways for substances connected to galactosylceramide are shown in Figure 1. The synthetic pathway starts with sphingosine being acylated to ceramide, which is subsequently galactosylated by uridine diphosphate (UDP)-galactose:ceramide galactosyltransferase (CGT) to form galactosylceramide. Psychosine can be directly produced by the same enzyme by galactosylating sphingosine. Psychosine and galactosylceramide are broken down by the enzyme galactosylceramidase, which is genetically deficient in Krabbe's disease. The sphingolipid activator protein saposin A and the enzyme must both break down galactosylceramide in vivo. Galactosylceramide is further sulfated to produce sulfatide. Due to impaired degradation, the two classic genetic leukodystrophies, metachromatic leukodystrophy and globeid cell leukodystrophy, are caused by the characteristic myelin glycolipids galactosylceramide and sulfatide. Reproduced with permission from Suzuki.Thirteen

Biochemistry

Galactosylceramidase deficiency is the genetic cause of all known human cases of Krabbe's disease (Figure 1). The lysosome is home to the hydrolytic enzyme galactosylceramidase, which has an acid pH optimum. Accordingly, the illness falls within the original Hers

definition of an inborn lysosomal disorder.7. The enzyme is particular to some glycolipids that have an anomeric configuration of the terminal galactose moiety. Galactosylceramide is the main naturally occurring substrate and is almost exclusively found in the myelin sheath. Other recognized naturally occurring substrates include monoglyctosyldiglyceride, psychosine (galactosylsphingosine), and the precursor of seminolipid (1-alkyl, 2-acyl-, 3-galactosyl glycerol). Galactosylceramidase and an activator protein called saposin A are needed for the in vivo breakdown of these substrates. Another natural substrate of galactosylceramide is lactosylceramide.

GM1-ganglioside-galactosidase, another lysosomal \-galactosidase, can, nevertheless, also break it down.16–17 Contrary to expectations based on the enzymatic defect, the distinctive biochemical feature of Krabbe's disease is an absence of abnormal accumulation of galactosylceramide in the brain.18–19 The unique location of galactosylceramide in the myelin sheath and the sudden and early death of the myelinating cells during the course of the disease provide a phenomenological explanation for this paradoxical phenomenon. As the myelinating cells vanish, the source of galactosylceramide synthesis is eliminated, so the accumulation of this molecule is limited to the level reached during the early stages of myelination.

Genetics

The human galc gene contains 17 exons and is found on chromosome 14 (14q31.3) [40, 41]. Six putative N-glycosylation sites on the 669 amino acid GALC protein interact with the mannose-6-phosphate receptor to facilitate lysosomal trafficking [42]. The amount of residual galactocerebrosidase activity and clinical severity generally correlate inversely, however there is a notable interfamilial variability in clinical manifestations[43]. The condition is inherited as an autosomal recessive characteristic. The Human Gene Database contains reports of over 200 galc mutations, including numerous small deletions, insertions, and point mutations [31, 44–46]. There are very few genotype-phenotype correlations that have been found [47].

There is no information on whether the 86 infantile pathogenic variants that have been found specifically correspond with the early-infantile or late-infantile phenotype [48–50].

2. In vitro models of Globoid Cell Leukodystrophy(GCL)

Patient-specific fibroblasts [51, 52], hematopoietic cells [53], or epithelial cell lines with induced galc mutations [54, 55] are examples of GLD human cellular models. However, these models hardly replicate the metabolic and functional characteristics of neural cells. However, studies of various processes, including neurotoxicity, inflammation, and neuroprotection, as well as the selection of novel therapeutic approaches for the treatment of neurodegenerative disorders, including GCL, have been conducted using primary cultures and cell lines of neurons, microglia, astrocytes, and oligodendrocytes. More recently, the creation of novel GCLcell models has made it possible to identify pathogenic cascades that are neurologically significant, one of which is the major function of elevated psychosine levels.

In vitro research has been done on psychosine's function in the formation of globoid cells. Specifically, using primary glial cultures, psychosine activates and transforms microglia into globoid cells, but not macrophages [57]. Extracellular protease matrix metalloproteinase-3 was found to be the mediator of this transformation into globoid cells.

These results were expanded upon by Claycomb et al. [58], who found that oligodendrocytes and oligodendrocyte progenitor cells are toxic to psychosine-activated microglia and globoid cells developed in this in vitro model system.

Similarly, to further elucidate the role of inflammation in , psychosine has been added to neuronal, oligodendrocyte, Schwann, and/or fibroblast cell cultures. By obtaining brain samples from twitcher mice, the natural mouse model with GALC deficiency, and immortalizing the primary neuroglial cultured cells with SV40 large T antigen, Ribbens et al. [51] developed and characterized a new cell model for GCL, producing the 145 M-Twi and the 145C-Wt cell lines from twitcher and control mice, respectively. Neuroglial cells derived from twitchers exhibited higher levels of psychosine, and both control and twitcher-derived cells were positive for markers suggestive of oligodendrocytes. Further, the neuroglial cells derived from the ticker displayed reduced GALC activity and a relative increase in the lysosomal compartment.

In order to evaluate corrective measures and examine disease pathogenesis in a patient's unique genetic background, human induced pluripotent stem cells, or iPSCs, have been employed. Therapeutic screening and disease modeling of the central nervous system (CNS) have been enhanced by the differentiation ofiPSCs in neural cells [59]. GLD patient-specificiPSC lines were developed by Mangiameli et al. [60] as a trustworthy human model to clarify the pathophysiology of GLD and assess the effectiveness of gene therapy in pertinent neural cell types. In order to achieve this, they transformed GLDiPSCs into neural progenitor cells, produced progeny (oligodendrocytes, neurons, and astrocytes), and tracked the development of primary and secondary defects that were unique to each patient's cell type. They demonstrated a clear distinction in the lipid profiles of cells derived from GLD patients and those from healthy donors.

Thus, in vitro testing has been helpful in studying GCL pathogensis (i.e., the cellular pathogenesis of GCL and the formation of globoid cells from microglia) and clarifying the role of psychosine in GCL-related toxicity. These in vitro cell cultures can shed light on the pathogenic development of GCL, but they are hardly suitable for assessing the efficacy of potential treatment approaches. Even though preliminary in vitro screenings can determine whether a therapeutic agent can lower the concentration of the substrate, or galactosylceramidase, or reconstitute GALC activity, the majority of therapy research employs well-established in vivo models because these models are better able to replicate the complex interactions between biological systems and the progression of disease.

In vivo models of Globoid Cell Leukodystrophy

By 1990, five different mammalian species—mice, cats, dogs, sheep, and rhesus monkeys—were known to naturally harbor GCL. The disease-causing mutations were discovered in mice, dogs, and rhesus monkeys by 1997 [63, 64]. The first report of the spontaneously arising murine model of GCL (twitcher) dates back to 1980 [65]. When the active myelination period begins, affected mice exhibit clinical symptoms. If they are not treated, they will die in approximately 35 days. After 15–20 days, pathological differences between twitcher mice and wild-type mice become apparent. Twitcher mice show tremors, become less active, and fail to gain weight. In addition, terminal stage mice show a rapid loss of motor functions and paralysis, especially of the neck and hindlimb muscles [66]. The pathology is strikingly similar to what is seen in patients who are human.

Histological images of the brain matter of rhesus macaques affected by GCL in Figs. 1C-D [62] and electron microscopy images of the sciatic nerves of twitcher and wild-type mice in Figs. 1A-B [61] show characteristic large globoid cells, indicating phenotypic changes on a tissue-level. Similarly, Wilson et al. [67] investigated the peripheral nervous system, or sciatic nerve, using electron microscopy and histology. Thirty-five-day-old twitcher mice

displayed higher levels of endoneurial connective tissue and more dispersed (less organized) nerve fibers with a high concentration of mononuclear cells than wild-type mice.
Collagen was deposited between the extra Schwann cell processes that formed around axons during ultrastructural observations using electron microscopy. Macrophages were seen in the interstitial space and surrounding nerves at 25 days, and they played a role in the disintegration of the nerve architecture. Twitcher mice at the terminal stage (35 days old) had sciatic nerve cross-sectional areas that were, on average, two times larger than those of wild-type mice. Immunofluorescent staining revealed a gradual increase in CD68+ macrophages in sciatic nerve cross-sections from twitcher mice, along with noticeably slower and generally lower myelin development starting 21 days after birth when compared to wild-type mice. These findings demonstrate the striking neuroinflammation seen in twitcher mice, in addition to the notable degeneration and anatomical alterations to the nervous system that occur with the advancement of the illness.
Future research in these crucial areas will be crucial for the development of new therapies. These areas include understanding the cellular mechanisms that initiate inflammation, the primary cells that initiate and respond to inflammatory stimuli, and the identification of key immune signaling pathways involved in the progression of disease.

3. Therapy

The development of new treatments will depend heavily on future research in these important fields. These domains encompass comprehending the cellular processes that trigger inflammation, identifying the principal cells that initiate and react to inflammatory stimuli, and identifying crucial immune signaling pathways implicated in the advancement of illness.
The only proven treatment for GCL is hematopoietic cell transplantation (HCT), which produces cells that are believed to transfer GALC to myelinating cells. Variations in the genotype-phenotype relationships among these patients complicate the evaluation of the effectiveness of cord blood transplantation. Additionally, transplantation must be done before symptoms appear in children with the infantile-onset phenotype for treatment to be effective [93]. However, at this age, HCT is linked to a 20% mortality rate. For this reason, a precise diagnosis and prognosis are critical to the treatment of GCL patients.
Another strategy that has demonstrated promise in the treatment of lysosomal storage disorders (LSDs), including type I Gaucher's disease, is substrate reduction therapy (SRT) [94,95]. SRT aims to reduce lysosomal dysfunction brought on by the decreased pathogenic load and the accumulation of pathogenic substrates by reducing the synthesis of the primary enzyme substrate. In order to reduce the rate at which the accumulating glycolipids synthesize, SRT has been studied in twitcher mice.
Mice have been subjected to substrate reduction therapy with L-cycloserine, an inhibitor of 3-ketodihydrosphingosine synthase. Subcutaneous injections of 75 mg/kg L-cycloserine or phosphate-buffered saline (PBS) were administered to the litters. L-cycloserine treatment reduced pathological signs, postponed the onset of clinical symptoms, and increased longevity by roughly 31% in twitcher mice [74]. For nearly 20 years, stem cell transplantation and/or gene therapy have been developed in mouse models, with the increasing therapeutic benefit of gene therapy occurring in tandem with advancements in next-generation adeno-associated virus (AAV) vectors. Retroviral vectors containing the galc cDNA were used to in vitro correct the enzyme deficiency in fibroblasts, glial cells, astrocytes, and oligodendrocytes from twitcher mice [96–99]. These investigations showed that retrovirally mediated gene transfer and enzyme uptake can be used to biochemically and phenotypically correct twitcher mouse oligodendrocytes in vitro.

In particular, twitcher mice used in AAV experiments exhibited improvements like longer life spans, lower psychosine levels, increased body weight, and improved performance in behavioral tests [78,79,82]. However, these mice also died with symptoms resembling those of the untreated mice.

More recently, an AAV2 genome construct that expressed mouse GALC was packaged in an AAVrh10 capsid. Compared to untreated mice, treated twitcher mice showed slower symptom progression and remained active and symptom-free for up to eight months of age [100]. Notably, studies have also demonstrated that combining gene therapy with bone marrow transplantation extends life expectancy even more than either treatment does on its own, suggesting that immune modulation and GALC enzymatic activity replacement work best together [81].

Additionally, lentiviral vectors (LV) were used to effectively transduce proliferating and post-mitotic oligodendroglia in the brains of twitcher newborn mice, thereby transferring a functional copy of the galc gene [101]. Lately, this endeavor has demonstrated exceptional effectiveness in the canine rendition of theillness by one group that administered AAVrh10 or AAV9 via systemic or cerebrospinal fluid (CSF) [102]. According to these authors, a translationally feasible single dose of AAVhu68.

Table

Clinical trial interventions for Krabbe disease

Indication(s)	**Treatment**	**Status**	**Clinical Trial Identifier/Ref.**
Krabbe disease	FBX-101 single infusion (AAVrh10 carrying the GALC gene, following conventional hematopoietic stem cell transplantation	Phase I/II (recruiting	NCT04693598
Krabbe disease	PBKR03 injection (AAVhu68 carrying the GALC gene)	Phase I (recruiting)	NCT04771416
Inherited metabolic disorders	PBKR03 injection (AAVhu68 carrying the GALC gene)	Phase I (recruiting	NCT02254863
Inherited metabolic disorders	Busulfan and fludarabine conditioning prior to hematopoietic stem cell transplantation	Phase II (recruiting)	NCT02171104
Inherited metabolic disorders	Hydroxyurea, Campath-1H, Fludarabine, Melphalan, Thiotepa with umbilical cord blood, matched unrelated donor bone marrow transplant, or	Phase I/ II (active)	NCT01962415

	peripheral blood stem cell transplant		
Inherited metabolic disorders	Hydroxyurea, Campath-1H, Clofarabine, Cyclosporine A, Mycophenylate Mofetil, Melphalan, Antithymocyte Globulin and total body irradiation administered in days leading up to hematopoietic stem cell transplantation	Phase II/III (completed)	NCT01586455
Inherited metabolic disorders	Melphalan, Antithymocyte Globulin and total body irradiation administered in days leading up to hematopoietic stem cell transplantation	Phase II (completed)	NCT01372228
Inherited metabolic disorders	Campath-1H, Busulfan, Cyclophosphamide, Cyclosporine A and Mycophenolate Mofetil administered in days leading up to hematopoietic stem cell transplantation	Phase II (completed)	NCT00668564, NCT00383448, NCT00176904, [
Inherited metabolic disorders	MGTA-456 (CD34+ cell therapy) with hematopoietic stem cell transplantation	Phase II (completed)	NCT01043640

In the mouse and canine models, expressing GALC into the CSF could reduce the majority of Krabbe disease symptoms, and this cleared the way for the first-ever human trial of AAVhu68.hGALC-administered intra-cisterna magna to GCL patients in infancy [102]. Additionally, in vivo combination therapies based on the effects of bone marrow transplantation, AAV-mediated CNS-directed gene therapy, and SRT with L-cycloserine have been proposed [87]. This triple combination not only produced notable and long-lasting behavioral improvements in twitcher mice, but it also increased their median life span from about 35 days to about 300 days. Preclinical studies using a different methodology and a

greater number of animals were carried out in a canine model of GCL [103]. There were no obvious therapeutic benefits from the intravenous (given at 3 days of age) or intracerebroventricular (given at 6 weeks of age) injections of AAV of serotype rh10 (AAVrh10), which target the peripheral and central nervous systems, respectively.

In a canine GCL model, a more recent study using intrathecal delivery of AAV9 demonstrated a definite dose- and time-dependent effect [104]. Here, a single intrathecal injection of high- or low-dose AAVV9-encoding canine GALC was given in conjunction with an immunosuppressive dosage of prednisone at the presymptomatic (2 weeks) or symptomatic (6 weeks) stages.

Surprisingly, dogs given a single high dose during the presymptomatic phase demonstrated 100% survival for up to 16 weeks. Six of these dogs, whose neurological conditions remained normal for up to 1.5 years, were kept for extended periods of observation.

But there was very little increase in life expectancy or motor skills [101,105]. When the study was done on rhesus macaques, the same outcomes were seen, but within three months of therapy, there were notable increases in neuromuscular strength, and the animals' scores were on par with those of age-matched normal animals [106]. However, AAV (~20 nm) are preferred due to the large size of LV (~100 nm) and their decreased ability to diffuse after injection [107]. AAV have been specifically used as vectors to restore GALC activity on Twi-trs, twitcher mice, and a Globoid Cell Leukodystrophy model in dogs. Regardless, aside from AAV9, very few viral vectors are able to pass through the BBB [108].

After performing a quick search for clinical trials using AAV to treat lysosomal storage diseases (also known as "adeno-associated virus" and "lysosomal storage disease"), demonstrates that the majority of treatments are administered intravenously (44%), closely followed by direct intracranial/intracerebroventricular injection (40%), intramuscular injections (25%), and follow-up studies from prior treatments (2%). AAVs are therefore actively sought after as gene delivery vehicles; however, to increase brain-targeting specificity, there is a propensity to inject directly into the brain, which is a more invasive and technically challenging process [109,110].

Nanoparticles to target Globoid Cell Leukodystrophy

The blood-brain barrier (BBB) is a significant barrier to the entry of drugs, macromolecules, nanoparticles, and other substances into the brain when it comes to the delivery of nanomedicine. The basement membrane, specialized endothelial cells with tight junctions, and a variety of supporting cells, such as pericytes, astrocytes, neurons, and microglial cells, make up the neurovascular unit (Fig. 2), which functions in concert to control brain homeostasis, restrict the diffusion of small and large molecules, and mediate the inflammatory response [111–113]. Thus, when thinking about neurological disorders, the integrity of the BBB is especially important [114].

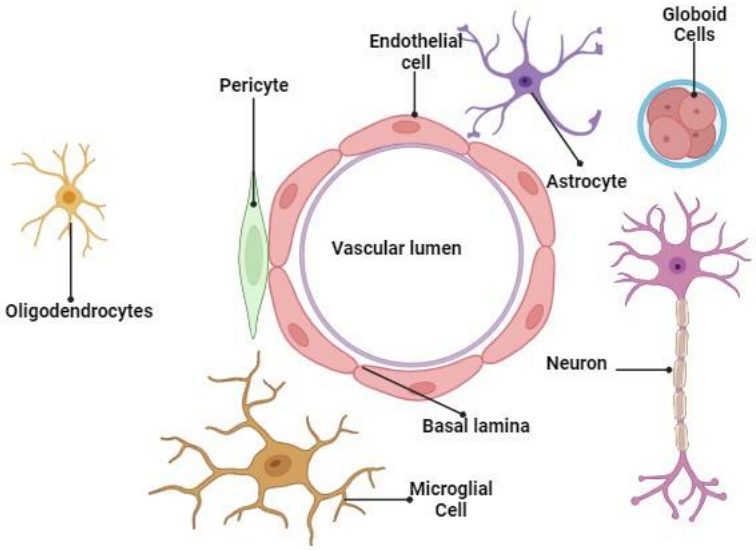


Fig. 2. Cross-sectional representation of the neurovascular unit (i.e. blood–brainbarrier) showing the effects of Krabbe disease – The build up of psychosineleads to toxicity in oligodendrocytes and Schwann cells, leading todemyelination and axonal dysfunction

To treat GCL, it is also crucial to determine the appropriate physiological and cellular therapeutic targets and goals, such as producing GALC in the central nervous system and peripheral nervous system and reducing the neurodegenerative and neurodevelopmental effects brought on by GALC deficiency. Freshly synthesized GALC is transported via the endoplasmic reticulum, the trans-Golgi network, early/late endosomes, and finally lysosomes [115]. Therefore, the cellular targets of GCL would be those (such as myelin-forming Schwann cells and oligodendrocytes) where a deficiency in GALC causes an accumulation of psychosine. As a result, getting therapeutic nanoparticles past the BBB continues to be a challenging task. Additionally, GCL-specific abnormalities in the brain's endothelium and microvascular architecture must be taken into account.

Giacomini et al. [116] used immunoreactivity, quantitative RT-PCR, microvascular corrosion casting followed by scanning electron microscopy, and other techniques to examine alterations in the frontal cortex angioarchitecture of twitcher mice relative to wild type mice. They discovered that the brain endothelium of twitcher mice expressed much less CD31+, or platelet endothelial cell adhesion molecule (PECAM-1). Twitcher mice's brain vasculature also displayed evidence of persistent neuroinflammation, and electron microscopy pictures of the brain vasculature revealed dilated vessels and frequent variations in vessel diameter. The results of quantitative RT-PCR indicated an upregulation of CD45, tnf-α, IL-1, cxcr4, fgf2, and cxcl-1 mRNA. In twitcher mice, immunofluorescent studies revealed perivascular pericyte disarray and a subsequent decrease in endothelial coverage, indicating alterations in the effectiveness of the blood-brain barrier. y. Li et al.'s histological analyses [117] revealed globoid cells and disordered myelin in the cerebellum of 36-day-old twitcher mice, along with a broad distribution of CD68+ (such as microglial) cells.

Li et al.'s histological analyses [117] revealed globoid cells and disordered myelin in the cerebellum of 36-day-old twitcher mice, along with a broad distribution of CD68+ (such as microglial) cells. The cerebral microvasculature displays swelling of astrocytic end-feet around vessels, enlarged perivascular space, macrophage infiltration, dilated vascular lumen, and irregularly shaped endothelium, according to a review of endothelial cell dysfunction in Krabbe disease [118].

Twitcher mice in a 1987 study by Kondo et al. [119] did not exhibit increased permeability of the blood-brain barrier to horseradish peroxidase. However, a histopathological study on samples from a patient with Krabbe disease who was 2.5 years old revealed significant abnormalities in cerebral vascularization, including irregular endothelium, decreased corical microvascularization, and decreased smooth muscle coverage [120]. .. Therefore, it is evident that neuroinflammation is a hallmark symptom of Krabbe disease, even though it is still unclear if this condition is linked to increased BBB permeability [121]. As with the pathology, the effects of neuroinflammation on BBB permeability vary according to the type, location, and cause of inflammation [122]. Although the precise cause of the neuroinflammation associated with Globoid Cell Leukodystrophy is unknown, it is most likely caused by the cytotoxic accumulation of lysosomal psychosine. More research is required to determine whether nanoparticles can exploit this neuroinflammation to enhance translocation across the blood-brain barrier.
When combined, nanoparticles may be able to take advantage of changes in BBB efficiency caused by GALCdeficiency and target receptors associated with neuroinflammation or those discovered to be elevated in the vasculature of GCL. The delivery of nanoparticles across the BBB can be facilitated by a variety of techniques, as several outstanding reviews have demonstrated [6,9,123,124].

3.1 Particle targeting approaches

Therapeutics are transported across the blood-brain barrier (BBB) by means of either active (transcytosis mediated by receptors or adsorptive mediated transcytosis) or passive (transcellular and paracellular diffusion) methods. Numerous potential targeting molecules for localizing particles at or across the BBB have been discovered by researchers [125–128]. Here, we go over a few of these tactics in relation to neurodevelopmental illnesses (more especially, lysosomal storage disorders like GCL), where there may be changes to the blood-brain barrier that could make it easier for nanoparticles to enter the brain. The cell surface glycoprotein known as intercellular adhesion molecule 1 is found on endothelial cells and controls leukocyte extravasation at inflammatory sites [129]. In healthy C57BL/6 or acid sphingomyelinase (ASM) knock-out mice, Solomon et al. [130] systematically examined the biodistribution after intravenous administration of nanoparticles targeted against various cell surface receptors or proteins, such as intercellular adhesion molecule 1 (ICAM-1), transferrin receptor (TfR), or monosialotetrahexosylganglioside. The ASM knock-out mouse model mimics Niemann Pick disease, a lysosomal storage disease characterized by an ASM deficiency.and the ASM knock-out mouse model thus reproduces this. After injecting radiolabeled and targeted polystyrene nanoparticles (~200 nm), the accumulation in the brain could be measured using the localization ratio (i.e., the ratio of the localization ratio for targeted versus non-targeted nanoparticles in the brain versus liver) and the specificity index (i.e., the percent of injected dose/g, or % ID/g, in the brain compared to the % ID/g in the blood). Anti-ICAM-1 targeted nanoparticles demonstrated a significantly higher specificity index (6.4 versus 2.4, respectively) and brain localization ratio in ASM knock-out mice (0.18) when compared to control mice (0.09).

Transferrin receptors are a class of transmembrane proteins expressed on BBB endothelial cells that are in charge of delivering transferrin into the brain parenchyma transcytotically [132–134]. Transferrin is an essential glycoprotein that is responsible for the cellular transport of iron [131]. It was demonstrated by Clark and Davis [135] that 80 nm gold nanoparticles labeled with transferrin via an acid-cleavable linkage could penetrate the brain tissue and exit the brain vasculature. The study's acid-cleavable linker was essential because

it allowed the gold nanoparticle core to be isolated from the targeting molecule during transcellular transport, preventing receptor protein recycling or destruction.

It was further demonstrated that ligand avidity targeting was crucial. Targeting ligand affinity, valency, and density are crucial elements for brain targeting, as confirmed by Johnsen et al. [136, 137]. It was demonstrated that in the brain parenchyma, brain capillaries, and whole brain homogenate, a higher accumulation of gold nanoparticles was correlated with a lower affinity [136].

Another endogenous transporter that helps target particles to the brain is the insulin receptor, which also controls the transport of glucose into cells. They found that at two hours after injection, about 5% of the ID had reached the brain using flame atomic absorption spectroscopy—a ten-fold increase over non-targeted PEGylated gold nanoparticles.

It is noteworthy, though, that when insulin-targeting nanoparticles were used, there was also a noticeably greater distribution of particles to the pancreas and liver. They found 0.6% ID of insulin-labeled gold nanoparticles in the brain even 48 hours after injection, but no PEGylated gold nanoparticles of the control were found. In a subsequent study, male BALB/c mice were intravenously injected with insulin receptor-targeted gold nanoparticles of different sizes (20, 50, and 70 nm) via the tail vein [141].

Two hours after injection, there was a roughly two-fold increase in 20 nm gold nanoparticles in the brain (per gram tissue) compared to 50 nm gold nanoparticles. It was observed that smaller insulin-targeted particles (20 nm) had the highest brain accumulation.

The rabies virus is known to be neuro-invasive because nicotinic acetylcholine receptors on brain microvascular endothelial cells and neurons specifically bind to the rabies virus glycoprotein (RVG) [142,143]. Researchers have tried to co-opt RVG's functionality in order to target nanoparticles across the BBB [143,145–147] because it has been demonstrated to enhance the retrograde axonal transport of the rabies virus once it has entered the CNS [144]. . To target thermosensitive Pluronic-based nanoparticles (~60 nm) to the brain, Kim et al. [148] used RVG29, a 29 amino acid peptide derived from RVG. After intravenous tail vein administration, the biodistribution of particles in C3H/HeN athymic nude mice was observed over a 48-hour period using a fluorescent in vivo imaging system. Ex vivo brain imaging revealed that although the combination of RVG and chitosan significantly increased the amount of particles that appeared to accumulate in the brain when compared to bare or chitosan-only functionalized nanoparticles, both types of particles appeared to accumulate more.

According to study You et al. [143], the surface decoration of poly(lactide-co-glycolide)-poly(ethlyene glycol) (PLGA-PEG) nanoparticles (~170 nm) with RVG29 markedly enhanced brain targeting in comparison to untargeted PLGA-PEG. Furthermore, in vivo experiments demonstrated that brain targeting for RVG29-labeled particles tripled in comparison to non-targeted particles six hours after tail vein injection into C57BL/6 mice.

Since brain endothelium has been shown to have elevated levels of low-density lipoprotein (LDL) receptors [149,150], it has been suggested that this large endocytic receptor could be a possible route for medication delivery to the brain. Together with tissue plasminogen activator, LDL receptors control BBB permeability and are expressed in neurovascular unit cells [151].

A group of studies exploring the possibility of using solid-lipid nanoparticles (SLNs) functionalized with apolipoprotein E (ApoE), a fat-binding protein involved in lipid metabolism, to target LDL receptors for delivery to the brain were reported by Neves et al. [152–154]. Using biotin-avidin conjugation, two distinct particle components (DSPE-avidin or palmitate-avidin) were used to conjugate ApoE to the SLN surfaces [152]. By using dynamic light scattering, the resulting lipid nanoparticles were found to be 150 nm (bare SLNs) to 190 nm (targeted SLNs). ApoElabeled stem cell lines (SLNs) demonstrated a

significantly higher apparent permeability (1.5-fold increase) in permeability studies using transwell cell culture devices and hCMEC/D3 human brain microvascular endothelial cells as compared to non-labeled SLNs. .. By using dynamic light scattering, the resulting lipid nanoparticles were found to be 150 nm (bare SLNs) to 190 nm (targeted SLNs). ApoElabeled stem cell lines (SLNs) demonstrated a significantly higher apparent permeability (1.5-fold increase) in permeability studies using transwell cell culture devices and hCMEC/D3 human brain microvascular endothelial cells as compared to non-labeled SLNs. Later research revealed that ApoE-labeled SLNs could improve the delivery of resveratrol across hCMEC/D3 monolayers, a naturally occurring polyphenol found in plants [153]. Lipid nanoparticles (LNPs) with artificial ApoE adsorbed to the particle surface were able to increase brain targeting after intravenous injection, as demonstrated by Dal Magro et al. [155].

In order to achieve brain targeting, cell membranes have also been used to coat or form nanoparticles [156]. The reasoning behind this is that certain peripheral cells in systemic circulation have the ability to respond to endothelium-based cell signals in order to exit circulation and move toward a target site. Monodisperse nanoparticles (190 nm) derived from "neutrophil" membranes (i.e., HL60 human promyelocytic leukemia cells differentiated into neutrophillike cells) were successfully formed by Dong et al. [157]. Next, Resolvin D2, a metabolite that can lessen leukocyte contact with endothelial cells and lower cytokine production, was added to these liposome-like nanovesicles. Ex vivo fluorescence imaging demonstrated that nanovesicles could effectively target the inflamed brain tissue in a stroke model in male C57 mice, Ex vivo fluorescence imaging demonstrated that nanovesicles could effectively target the inflamed brain tissue. When compared to free fluorophore and nanovesicles made from non-differentiated cells, this targeting effect was noticeably stronger. The neutrophil-derived nanovesicles located in the brain capillaries could also be captured by real-time fluorescence imaging of the mouse brain vasculature. In an alternative investigation, neural stem cell membranes that had been initially engineered to overexpress C-X-C chemokine receptor type 4 (CXCR4), a receptor for the lymphocytic chemotactic molecule stromal-derived-factor-1, were coated onto ~150 nm PLGA nanoparticles [158].

They demonstrated that coating PLGA nanoparticles in the neural stem cell membrane improved particle localization at the stroke site using a stroke model in male C57BL/6 mice. However, this effect was amplified twofold when the neural stem cells were initially engineered to overexpress CXCR4. These nanoparticles could also be loaded with glyburide, a diabetic drug that is being studied for treating stroke. When compared to both free glyburide and glyburide-loaded, membrane-coated nanoparticles (without CXCR4 overexpression), particles targeted with CXCR4 were demonstrated to significantly increase mouse survival as well as the measured infarct volume from the stroke model.

3.2. Non-receptor mediated approaches

There are other interesting approaches to transport nanoparticles into the brain besides receptor-mediated targeting. This can include specific engineering of nanoparticle physico-chemical properties [159,160], or physically and transiently disrupting the BBB to facilitate particle transport [161,162]. A classical approach in nanomedicine to alter particle biodistribution, pharmacokinetics, and general interactions with the biological environment is to specifically engineer particle physico-chemical properties. One such parameter is nanoparticle shape. Baghirov et al. [163] investigated the brain distribution of rod-shaped (300 nm long × 100 nm wide) mesoporous silica nanoparticles. It was shown that after intravenous tail vein injection in C57BL/6 mice, these particles were detected on the luminal side of the brain vasculature via two-photon microscopy. However, the rod-shaped nanoparticles were not found to cross into the brain parenchyma.

This finding supported earlier findings from the Mitragotri group [164], which demonstrated that brain accumulation was increased seven times by transferrin receptor-targeted, rod-shaped polystyrene nanoparticles (500 nm long × 120 nm wide) as opposed to similarly targeted spherical nanoparticles (200 nm diameter). It is crucial to remember that the brain vascular endothelium was the target of these studies because the rod-shaped particles' preferred localization in the brain vasculature over spherical particles was made possible by their shape and targeting molecule. The BBB in any case hindered the nanoparticles. Nonetheless, these findings highlight a crucial piece of the puzzle: particle shape can help with brain localization.

The size and surface of the particles are significant additional factors.

There are several methods to modify the surface of a nanoparticle: modifying the functional groups to change the zetapotential (surface charge of the particle), modifying the chemical groups of the particle surface to change the constituent proteins of the so-called protein corona, or modifying the hydrophobicity/hydrophilicity of the particle. Using multiple particle tracking in rodent and human brain tissue slices, Justin Hanes' group has methodically investigated the impact of particle size, particle surface charge, and PEGylation density on nanoparticle diffusivity and penetration in brain parenchyma [165], this would replicate the nanoparticles' mobility once within the brain.

The 40, 100, or 200 nm-diameter polystyrene particles were functionalized with a dense PEGylation or a negative (-COOH) surface. By measuring the mean square displacement of the PEGylated particles, they demonstrated in ex vivo human brain slices that the PEGylated particles, in all sizes, had much higher mobility than the bare, negatively charged particles. It was also demonstrated—rather logically, I suppose—that smaller particles were more mobile than larger ones. Subsequent research using ex vivo rat brain slices made it abundantly evident that size (i.e., <120 nm) and high density PEGylation were the most important factors in ex vivo brain diffusivity. These findings were validated through in vivo studies involving intracranial injections of nanoparticles and real-time fluorescence microscopy monitoring of particle diffusion.

Convection-enhanced delivery (CED) is a technique employed to enhance the targeted delivery of therapeutics within the brain parenchyma. This method involves applying a continuous pressure gradient directly at the injection site, allowing for localized administration in the brain. While this approach involves a direct injection into the brain, its advantage lies in the ability to effectively transport larger agents or hydrophobic compounds into the brain parenchyma, where their diffusion is naturally limited [175].

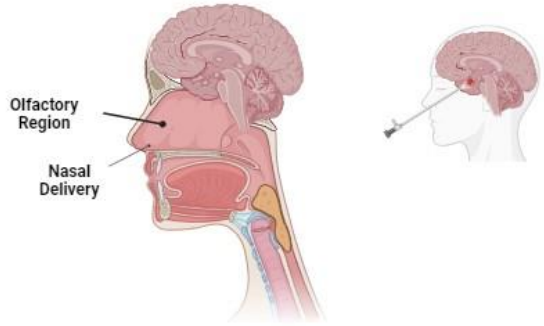


Figure 5: Diagram illustrating the direct pathway from the nose to the brain through the nasal cavity's olfactory region. Therapeutics can be transported via intracellular axonal pathways into the olfactory bulb or through the olfactory epithelium movement within the smell receptors

Primarily investigated for treating brain tumors due to their localized nature, CED has shown promise in increasing the retention of therapeutic agents in the brain. For instance, Xi et al. conducted a study using CED to deliver doxorubicin coupled with nanodiamonds in healthy Fisher 344 rats. This resulted in significantly improved retention of the drug at the injection site compared to free doxorubicin, lasting up to 72 hours post-injection [176]. Similarly, CED has been utilized for the delivery of PLGA nanoparticles in healthy Sprague Dawley rats and liposomes, such as those loaded with temozolomide or Gd-DTPA as an MRI contrast agent. In the case of Gd-DTPA-loaded liposomes, enhanced MRI contrast persisted for up to 14 days post-CED, highlighting the effectiveness of this approach [179].

PEGylation, a process involving the attachment of polyethylene glycol, played a crucial role in enhancing the retention of liposomes in the brain. In a search of clinical trials related to "convection-enhanced delivery," two studies were found employing CED with liposomal irinotecan for the treatment of brain cancers (NCT03086616, NCT02022644). Additionally, four studies investigated CED for Parkinson's treatment, and one focused on treating aromatic L-amino acid decarboxylase deficiency.

In summary, CED represents a well-established and translational approach to improve the delivery of therapeutic particles to the brain, particularly in the context of treating brain tumors. Clinical trials further underscore the potential of CED for various neurological conditions, showcasing its versatility in targeted drug delivery within the intricate environment of the brain.

It has been suggested that intranasal delivery, as an alternative to intravenous or localized injection, can decrease operational invasiveness and improve patient compliance for direct nose-to-brain delivery [181–185]. This method makes use of the direct connection that exists between the nervous system and the olfactory region of the nasal cavity (Fig. 5). But there are particular difficulties with this strategy. Mistry et al. [186] offer a thorough analysis of the specifics of nanoparticle delivery to the brain via the nasal route. In contrast to systemic administration through intravenous injections, nanoparticles administered via the nasal route must overcome mucus and the nasal epithelium to gain access to either systemic circulation or the nervous system.

Nonetheless, to maximize nasal medication delivery, it is important to take into account particular nanoparticle design attributes. For instance, research has demonstrated that shape is crucial for the passage of nanoparticles through mucus at the nanoscale. A comparison between two different sized silica nanospheres (80 nm or 140 nm core diameter, dH 100 nm or 200 nm, respectively) and silica nanorods (80 nm × 240 nm, dH 200 nm, aspect ratio = 3) revealed that in fresh mucus isolated from Sprague Dawley rat intestines, nanorods displayed significantly higher mobility compared to spherical particles [187]. In a similar vein, functionalization of the particle surface can significantly affect mucus penetration.
As a result, one must weigh the trade-off between mucoadhesion and nanoparticle mobility through the nasal mucosa, which may lengthen the time that particles interact with the olfactory region's mucosa (thereby raising the likelihood that they will be absorbed).

3.3. Summary of brain targeting approaches for Krabbe disease

Limited literature exists on the use of nanoparticles in the treatment of KD. Nonetheless, the aforementioned methods can offer valuable perspectives on potentially efficacious paths for administering therapeutic nanoparticles to the brain for gene therapy (such as DNA or RNA delivery), enzyme replacement therapy (like GALC delivery), or small molecule therapy to address symptoms associated with KD. Active targeting strategies can take advantage of established pathways (like ICAM-1) or receptors that have been demonstrated to be upregulated in KD (like CXCR-4). Targeting downregulated receptors (like PECAM-1) in KD endothelium would also be ineffective.
Del Grosso et al.'s study [192] examined the use of peptides targeting angiopep-2, glyco-heptapeptide g7, and TfR to deliver PLGA nanoparticles containing cross-linked enzyme aggregates of GALC to twitcher mice's brains. They discovered that targeted nanoparticles outperformed non-targeted nanoparticles in their ability to restore GALC activity in the brains of twitcher mice, regardless of the targeting molecule. This suggests that improving the therapeutic efficacy of particle-mediated treatment of KD requires a focus on particle targeting.
To clarify whether this is because of enhanced cell-specific delivery of GALC in the brain, whether targeting molecules enhance therapeutic particle translocation into the brain parenchyma, or whether targeting prolongs the time that particles stay localized in the brain, more research is needed. To find out if non-active targeting strategies can boost particle-mediated therapy's effectiveness in KD, more research is needed. Densely PEGylated particles, for instance, have been demonstrated to have a greater capacity to permeate the brain parenchyma (Nance et al., 165), and smaller particle sizes have also been demonstrated to have a significant impact on particle localization in brains that display neuroinflammation. Given the developmental alterations to the angioarchitecture and endothelium in KD, it would be prudent to investigate whether methods using, for example, CED or MRI-FUS, would better facilitate particle delivery in KD.

4.Nanoparticles and therapeutic payloads to treat Krabbe disease

Although there is a dearth of research on the use of nanomedicines in the treatment of monogenetic neurological disorders, it may be possible to understand how nanoparticles can be used to treat Krabbe disease by examining how nanomedicines are used to treat other neurological disorders. One can approach Krabbe disease from three different perspectives when it comes to nanoparticle therapy: i. gene therapy, which aims to treat the genetic mutation in the GALC gene; ii. enzyme therapy, which replaces the deficiency of GALC; and iii. small molecule therapy, which manages and treats the symptoms associated with this

lysosomal storage disease. Table research on using nanoparticles to deliver the three distinct payloads to the brain; Fig. 6 illustrates the various particle and payload types.

4.1. Nanoparticles for gene delivery

Since its first discovery, gene therapy holds great promise for the treatment of so-called undruggable diseases. In recent years, this promise has started to be delivered thanks to the approval of several new therapies, marking the start of a "Golden Age" for the field. The approved medicines treat a wide range of clinical indications and tissue targets, including the first oligonucleotide-based therapies (Spinraza, Exondys, Vyondys), three cell therapies (Kymriah, Yescarta, Tescartus), and two in vivo gene therapies (Luxturna and Zolgensma), as well as the first RNA-based drug (i.e. Onpattro and the SARS-CoV-2 vaccines). On one hand these are life-changing for the affected patients, and on the other demonstrate a more general way forward by laying the foundations upon which treatments for many other conditions can be developed.

This is the case of KD, which being a recessive monogenic disorder, is an obvious candidate for gene therapy. In fact, despite the significant challenges, gene replacement, silencing, or editing are perhaps the most functionally straightforward options for the treatment of diseases caused by a single gene defect.

In this instance, KD is a recessive monogenic disease. condition, is a clear choice for gene therapy. In actuality, despite the major obstacles, editing, silencing, or gene replacement are possibly the most practically simple choices for treating illnesses brought on by a single gene defect. Regardless of the specific differences in the chemical structure, target site, or mechanism of action of the nucleic acids used, crossing the cell membrane and localizing into the appropriate subcellular compartment are well-known barriers to the clinical translation of nucleic acid-based therapies. This, along with the very limited stability and need to limit side effects due to off-target action, makes the development of an appropriate carrier for the delivery of nucleic acid-based therapeutics pivotal. Consequently, the common bottleneck in the translation to the clinic is the need for a carrier that could protect the genetic payload and deliver the nucleic acid at the target site. The gene transfer agent, or nanoparticle, must be carefully selected based on the cell type to be targeted, the number of treatments needed (one dose versus repeated administration), and the size and type of nucleic acid to be delivered. It is well known that no single vector is suitable for all applications. Because of their natural adaptability and efficiency of delivery, viruses are more commonly considered for gene therapy even though non-viral vectors are more straightforward and do not carry some of the risks associated with viral systems. Viruses make effective gene therapy tools because they can "naturally" insert genetic material into host cells to replicate.

4.2. Nanoparticles for enzyme replacement therapy

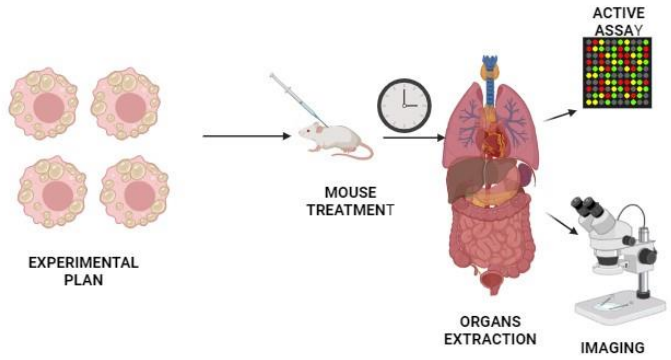
Galactocerebrosidase, a lysosomal enzyme, is deficient in Krabbe disease on a cellular and molecular level. Hemopoietic stem cell transplantation has been the traditional treatment for this, and most therapeutic clinical trials for Krabbe disease focus on pharmaceutic regimens to enhance donor engraftment, or the body's acceptance of the donor cells (Table 1). Therefore, one would anticipate that the effects of this genetic disorder would be lessened if a drug delivery system was able to regularly replenish this deficiency in some way. An outstanding review that was published recently addresses the use of nanomedicines to support enzyme replacement therapy (ERT) in the treatment of lysosomal storage diseases [201].

Generally speaking, one must take into account both the therapeutic payload and the target when logically designing nanoparticle drug delivery systems [202]. ERT aims to deliver enzymes specifically to the lysosomal compartment in the context of Krabbe disease and other lysosomal storage diseases. Due to the difficulty of accumulating more in the brains of

Idua knock-out mice than in those of wild type mice, conventional ERT can be difficult. In a similar vein, the brains of IdS knock-out mice had higher concentrations of targeted particles than those of wild type mice. The significance of targeting in the delivery of enzymes to the brain is highlighted by these two studies.

For instance, due to inadequate brain biodistribution of particles, PLGA nanoparticles with arylsulfatase B conjugated to the particle surface were unable to efficiently increase enzyme activity in the brain [204]. Furthermore, a 1985 study by Umezawa et al. [205] where β-galactosidase encapsulating liposomes were injected in twitcher mice for ERT is worth taking into consideration, even though it is not a direct comparison. They were unable to demonstrate that the exogenous enzyme had a discernible impact on the removal of lipid buildup in the brains of twitcher mice. The authors stress the difficulty and urgency of figuring out how to get their liposomes through the BBB even in 1985.

Even though there aren't many papers on using nanoparticles to treat Krabbe disease, some broad conclusions can be drawn by considering nanoparticles for enzyme replacement therapy. The nanoparticles must first be able to load enzymes, which have a relatively high MW payload. As was mentioned, cross-linked enzyme aggregates or recombinant human enzymes can be used for this. Enzyme delivery will therefore typically be facilitated by liposomes or polymeric nanoparticles. Second, to ensure particle localization in the brain, additional measures must be taken or nanoparticles must be modified with a targeting ligand in order to cross the blood-brain barrier. Lastly, since ERT is a temporary fix, strategies for optimizing particle dosage and delivery method must be thought of.



PLGA nanoparticles containing cross-linked GALC aggregates and labeled with a targeting molecule were given to Twitcher mice (Fig. 7). Four hours after injection, GALC activity was measured in several organs. It was found that targeted particles could restore up to 40% of the GALC activity in twitcher mice's brains when compared to wild type mice, which was comparable to heterozygous, non-pathological mice.

4.3. Nanoparticles for small molecule delivery

There are currently no approved small molecule interventions for treating Krabbe disease, and small molecules in clinical trials are mainly focused on improving stem cell transplantation outcomes. Consequently, the prospects for delivering a therapeutic small molecule to address Krabbe disease are suboptimal. The potential approach involves nanoparticle-mediated delivery of a drug to manage severe symptoms such as demyelination and chronic neuroinflammation associated with Krabbe disease.

Remyelination is a critical area of research for various neurodegenerative and neurodevelopmental diseases. Myelin, the protective sheath around axons, plays a crucial role

in supporting axonal metabolism and facilitating nerve signaling. Oligodendrocytes, responsible for forming myelin, are the cellular target for remyelination therapies. In vivo studies have demonstrated that certain small molecules, like fingolimod, can facilitate remyelination. Fingolimod, an active drug against the sphingosine 1-phosphate receptor, significantly increased myelin levels in twitcher mice, reduced immobility, twitching severity, and extended survival time.

Fingolimod has also been encapsulated in PLGA nanoparticles and, when delivered locally with neural stem/progenitor cells, promoted motor function recovery following spinal cord injury. Other studies have explored drugs like miconazole and clobetasol, identified for their ability to enhance the generation of mature oligodendrocytes, ultimately increasing myelination. Additionally, clemastine, an antihistamine, has shown potential for remyelination, with ongoing clinical trials investigating its effectiveness.

While clemastine is typically administered orally, limited literature exists on nanoparticle formulations. Nevertheless, supercritical antisolvent precipitation has been employed to formulate clemastine nanoparticle crystals. Nanoparticles have been extensively studied for managing neuroinflammation. One innovative approach involved nanoparticles acting as a sponge to absorb the neurotoxic sphingolipid psychosine, a characteristic feature of Krabbe disease. Lecithin/chitosan nanoparticles recovered key proteins in cerebellar organotypic cultures, providing a potential strategy for controlling neuroinflammation.

In contrast, conventional approaches have explored the use of small molecules to mitigate neuroinflammation. For instance, Luzi et al. investigated anti-inflammatory drugs such as ibuprofen, indomethacin, and minocycline in a mouse model of Krabbe disease, revealing improved survival times. Indomethacin, known to enhance remyelination, has also been formulated into nanoparticles in previous studies.

4.4. Challenges in nanoparticle therapy for Krabbe disease

The application of nanomedicine in treating neurodevelopmental and neurodegenerative disorders has garnered attention. Although there is limited research on Krabbe disease (KD) as a therapeutic target, nanomedicine holds potential to make a significant impact. As a recessive monogenic disorder, gene therapy stands out as the primary therapeutic approach. However, nanoparticle-mediated gene therapy faces challenges in manufacturing, scalability, and targeting. Additionally, non-viral gene therapy effects can be transient, necessitating repeated administrations, posing a challenge for KD treatment due to the need to overcome the blood-brain barrier (BBB) with each administration.

Enzyme-based therapies, such as enzyme replacement therapy (ERT) or substrate reduction therapy (SRT), would also require multiple administrations. While gene therapy could potentially address the underlying cause of KD—the recessive mutation on the galc gene—ERT or SRT aims to supplement GALC in deficient tissues or reduce psychosine precursors in the tissue. Thus, ERT relies on repeated administrations to maintain normal GALC levels in the central nervous system (CNS) and peripheral nervous system.

In contrast, small molecule therapy aims to alleviate the effects of GALC deficiency, such as prolonged neuroinflammation and demyelination. Although this approach does not resolve the fundamental cause of KD, using nanomedicines for delivering therapeutic small molecules could potentially treat neuroinflammation, reverse demyelination, and assist or halt the development of disorganized angioarchitecture in the brain. Clinical trials investigating therapeutics like clemastine for remyelination exist, but few have explored nanoparticles as a delivery vehicle. Other small molecules, like clobetasol or miconazole, have shown promise in increasing oligodendrocyte proliferation. Systematic studies are needed to determine if

packaging these therapeutics into a nanoparticle drug delivery system, possibly with a brain-specific targeting approach, could enhance their utility in treating KD-related symptoms.

It is evident that a multi-pronged approach is necessary to treat KD, and nanomedicine may play a role. While viral-based therapies show success in clinical trials, nanomedicine could assist either through ERT or by delivering drugs that repair some of the damage caused by KD-related GALC deficiency.

Summary and conclusion

Krabbe disease, as a lysosomal storage disease and rare orphan disorder, presents a unique opportunity for exploring the application of nanomedicines in its treatment. This neurodegenerative condition is characterized by chronic neuroinflammation, a common feature shared with many neurodevelopmental disorders, and is caused by numerous mutations on a single gene.

This review has outlined the causes and current clinical treatments of Krabbe disease, shedding light on existing in vitro and in vivo models for the disease. Additionally, it has provided an overview of various approaches, including gene therapy, enzyme replacement therapy, and small molecule delivery, in the context of treating Krabbe disease. Collectively, these studies offer valuable insights into the complex landscape of nanomedicines for neurological disorder treatment.

Key findings emphasize the importance of considering the physico-chemical properties of particles concerning their delivery routes. For intravenous delivery, higher aspect ratio particles, such as rods or discs, demonstrate better margination in cerebral vasculature but may require assistance in crossing the blood-brain barrier (BBB) due to their shape and size. Similarly, small, highly PEGylated rod-shaped particles may enhance penetration in nasal delivery by overcoming mucus barriers. High-density PEGylation is linked to improved particle penetration in the brain parenchyma.

Optimizing particle distribution to the brain, especially for neurodegenerative disorders, often requires auxiliary delivery methods such as MRI-FUS or CED. While these methods enhance particle distribution, their invasive nature, particularly with CED, necessitates careful consideration of potential complications. The efficacy of targeting ligands for facilitating nanoparticle transport across the BBB in treating Krabbe disease is an area that warrants further exploration, as clinical testing of such approaches remains limited.

Despite ongoing clinical trials testing viral vectors and small molecules for Krabbe disease treatment, the potential of nanomedicine in improving therapeutic outcomes is an avenue that requires exploration. Pre-clinical in vivo studies are essential to assess the ability of nanomedicines to cross the BBB and effectively deliver gene therapies, enzyme supplements, or small molecules. Successfully treating Krabbe disease involves achieving persistent and lasting results, as there is currently no cure for this devastating condition. These systematic studies evaluating nanomedicines have broader implications for understanding and potentially addressing a multitude of disorders affecting the brain and central nervous system.

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