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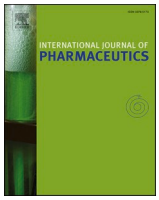

Review

The use of nanocarriers in treating Batten disease: A systematic review

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ABSTRACT

The neuronal ceroid lipofuscinoses, commonly known as Batten disease, are a group of lysosomal storage disorders affecting children. There is extensive central nervous system and retinal degeneration, resulting in seizures, vision loss and a progressive cognitive and motor decline. Enzyme replacement and gene therapies are being developed, and mRNA and oligonucleotide therapies are more recently being considered. Overcoming the challenges of the blood–brain barrier and blood-ocular barrier is crucial for effectively targeting the brain and eye, whatever the therapeutic approach. Nanoparticles and extracellular vesicles are small carriers that can encapsulate a cargo and pass through these cell barriers. They have been investigated as drug carriers for other pathologies and could be a promising treatment strategy for Batten disease. Their use in gene, enzyme, or mRNA replacement therapy of all lysosomal storage disorders, including Mucopolysaccharidoses, Niemann-Pick diseases, and Fabry disease, is investigated in this systematic review. Different nanocarriers can efficiently target the lysosome and cross the barriers into the brain and eyes. This supports continued exploration of nanocarriers as potential future treatment options for Batten disease.

1. Introduction

Batten disease (BD), also known as the neuronal ceroid lipofuscinoses (NCL), is a group of devastating inherited neurological diseases primarily affecting children and part of a larger group of lysosomal storage disorders (LSDs). These paediatric monogenic biallelic disorders lead to dysfunction in a variety of lysosomal and extralysosomal proteins (Butz et al., 2020; Cooper et al., 2022; Gardner and Mole, 2021). BD is characterised by the accumulation of autofluorescent storage material within cells throughout the body, and progressive neuronal cell death. Clinical manifestations include seizures, vision loss, and progressive cognitive and motor decline. Each genetic type has a characteristic age of onset and disease progression, and there is further variation according to the impact of the underlying genetic defects on gene function (Gardner and Mole, 2021). While various palliative therapeutic approaches, including anticonvulsants, help to manage symptoms, definitive cures for the different genetic types of BD remain elusive (Mole et al., 2019). Enzyme replacement therapy (ERT) is a promising strategy

Abbreviations: **ASA**, Arylsulfatase A; **ASM**, Acid sphingomyelinase; **ATP**, Adenosine triphosphate; **BBB**, Blood-brain barrier; **BD**, Batten disease; **CAM**, Cell adhesion molecule; **CNS**, Central nervous system; **CRISPR**, Clustered regularly interspaced short palindromic repeats; **EC**, Endothelial cell; **ERT**, Enzyme replacement therapy; **EV**, Extracellular vesicle; **FDA**, U.S Food and Drug Administration; **g7 peptide**, H2N-Gly-L-Phe-D-Thr-Gly-L-Phe-LLeu-L-Ser(O—D-Glucose)–CONH2; **GAG**, Glycosaminoglycans; **GALC**, Galactosylceramidase; **GALNS**, Galactosamine (N-Acetyl)-6-Sulfatase; **GLA**, Alpha-galactosidase A; **GM1**, monosialotetrahexosylganglioside; **ICAM-1**, Intracellular adhesion molecule 1 receptor; **Ids**, iduronate-2-sulfatase; **IDIA**, Alpha-L-iduronidase; **IVT-mRNA**, In vitro transcribed m-RNA; **JAMs**, Junctional adhesion molecule; **JNCL**, Juvenile neuronal ceroid lipofuscinosis; **LDL**, Low-density lipoprotein; **LINCL**, Late infantile neuronal ceroid lipofuscinosis; **LNP**, Lipid nanoparticle; **LSD**, Lysosomal storage disorder; **M6PR**, Mannose-6 phosphate receptor; **MLD**, Metachromatic leukodystrophy; **MPS**, Mucopolysaccharidosis; **MPS-IH**, Mucopolysaccharidosis type I-Hurler syndrome; **MRT**, mRNA replacement therapy; **NC**, Nanocarrier; **NCL**, Neuronal ceroid lipofuscinosis; **NP**, Nanoparticle; **NPD-B**, Niemann-Pick Disease Type B; **NT-lipidoids**, neurotransmitter lipidoids; **PEGPLA**, Polyethylene glycol-b-polylactic acid; **PICO**, Population, Intervention, Comparison, Outcome; **PLGA**, Polylactic-co-glycolic acid; **PLVAP**, Plasmalemma vesicle-associated protein; **PRISMA**, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; **PS-BMP**, Phosphatidylserine (PS)- Bis(monoacylglycero)phosphate; **rh-ASM**, recombinant human acid sphingomyelinase; **SGSH**, N-sulphoglucosamine sulphohydrolase; **SLN**, solid lipid-based nanoparticles; **TJ**, Tight Junction; **TPP1**, Tripeptidyl peptidase 1; **VLPs**, Virus like particles; **α-Gal**, α-galactosidase; **β-CD**, β-cyclodextrin.

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for those types of BD where the defective gene encodes a lysosomal enzyme (Johnson et al., 2019). Notably, Brineura, which treats neuronal ceroid lipofuscinosis type 2 (CLN2) disease by delivering the recombinant TPP1 enzyme directly into the brain is now in the clinic (Schulz et al., 2024; Schulz et al., 2018). However, its invasive and regular intraventricular administration and high costs (£703 K per year in the UK) support continued exploration of alternative treatment avenues (Cost Comparison, 2019). Further, delivery into the brain does not prevent the loss of vision.

Effective drug delivery must reach the affected tissues and cells, which, for BD, is primarily the brain and eye. The major challenge for BD is overcoming the blood–brain barrier (BBB) (Kadry et al., 2020; Patel and Patel, 2017). The BBB is a critical physiological barrier that regulates the exchange of substances between the central nervous system (CNS) and the bloodstream (Daneman and Prat, 2015). This barrier, primarily composed of endothelial cells (ECs) with tight junctions, maintains CNS homeostasis by tightly controlling the passage of molecules, ions, and cells. Various mechanisms enable substances to traverse this barrier; these include transmembrane diffusion, endocytosis by ECs, saturable transporters, and extracellular routes. Efflux transporters and lipid solubility significantly influence the permeability of substances across the BBB. Larger molecules are more impeded in their ability to cross the BBB (Banks, 2009). A second challenge in treating BD is ensuring that drugs pass the blood ocular barrier (BOB) and reach the retina when administered systemically or topically. Current experimental approaches by direct injection into the eye – vitreous or subretinal – pose high risk of ocular complications when done in the clinic. Understanding the complex structure and transport systems of the BBB and BOB is fundamental for the development of effective alternative drug delivery strategies.

One such treatment strategy involves nanocarriers, which possess the capacity to encapsulate therapeutic agents and navigate the BBB for targeted drug delivery (Saraiva et al., 2016). Nanoparticles (NPs), ranging from 1 to 1000 nm in size, offer promising opportunities due to their small size and ability to act as carriers. They can enhance drug delivery by exploiting various transport mechanisms, including transcytosis, tight junction modulation, and endocytosis. NPs enable targeted drug delivery to the brain, potentially reducing dosages and side effects while improving patient comfort (Saraiva et al., 2016). Extracellular vesicles (EVs), natural cell-secreted NPs, are emerging as versatile carriers (Witwer and Wolfram, 2021). With complex lipid bilayer structures and diverse cargo, EVs offer a multifaceted platform for intercellular communication and therapy. Their ability to traverse biological barriers, including the BBB, holds promise for delivering therapeutics to the CNS for LSDs.

The aim of this review is to examine the current state of development and application of engineered NPs and EVs in enzyme replacement therapy, gene and mRNA therapy for Batten disease. We assess their potential to target the lysosome for enzyme replacement therapy and bypass the lysosome for gene and mRNA therapy and explore how these carriers interact with the BBB and BOB to facilitate CNS access. Through a comprehensive and systematic analysis of existing literature for their utilisation in LSDs, we aim to shed light on the applicability and challenges of nanocarriers in Batten disease treatment.

2. Methodology

A systematic review of the use of NPs and EVs in all LSDs was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidance (Page et al., 2021).

2.1. Sources of information and research strategy

For the systematic review, comprehensive searches of the PubMed and Scopus databases were conducted periodically, beginning in December 2022 and concluding in February 2024. Our search strategy in PubMed used the prompt "(lysosomal storage diseases) AND ((nanoparticle) OR (nanocarrier) OR (nanotechnology) OR (nanoparticles drug delivery) OR (microcarrier) OR (extracellular vesicle))". In Scopus, we employed the search prompt "(ALL ("lysosomal storage diseases")) AND (ALL ("nanoparticle") OR ALL ("nanocarrier") OR ALL ("nanotechnology") OR ALL ("nanoparticles drug delivery") OR ALL ("microcarrier") OR ALL ("extracellular vesicle"))" to identify relevant papers pertaining to nanoparticle utilisation in LSDs. To refine our search for mRNA-related literature in Scopus, we appended the prompt with "AND (ALL ("RNA therapy") OR ALL ("mRNA Therapy") OR ALL ("messenger RNA therapy"))". This search methodology ensured the thorough identification of relevant studies. Further, relevant clinical trials were identified from clinicaltrials.gov.uk and summarised in the **Supplementary Material 2**. We noted that none were assessing efficacy of therapy using nanocarriers.

2.2. Inclusion criteria

The systematic review had specific inclusion criteria according to the PICO (Population, Intervention, Comparison, Outcome) system. These included studies focused on the use of rodents and *in vitro* cell models for LSDs, therapeutic compounds/drugs, and gene/mRNA therapy in combination with nanocarriers or different NPs for transport-related outcomes, and experimental studies that mentioned NPs/EVs used for drug/gene delivery purposes in the therapy of LSDs. These criteria applied to prospective primary research articles published over a period of 18 years, between 2006 and February 2024. Only records in English were considered, owing to the language ability of the authors.

Research papers that did not investigate nanotechnology for drug or gene delivery for the treatment of LSDs, as well as book chapters and review articles, were excluded.

2.3. Data Extraction

The articles were extracted and stored in the Zotero Referencing Manager, for subsequent analysis using Microsoft Excel; a summary of articles reviewed is available in **Supplementary Material 1.**

2.4. Outcomes of the studies

Searching PubMed identified 176 articles. 46 articles were eliminated as they were review articles. Due to the inability to access three articles in full text, they were also excluded from the review. Exclusion and inclusion criteria were applied, and 87 articles that did not satisfy the research aims were removed. 40 research papers were included in the review. Searching Scopus, 665 primary research papers were identified related to nanoparticle studies and mRNA research. 29 were duplicates with the PubMed search and removed. Of the remaining 763 papers, 697 (87 from PubMed + 610 from Scopus) were deemed ineligible based on our criteria and excluded, and the 4 remaining reviews were eliminated. A total of 62 papers (40 from PubMed + 22 from Scopus) were reviewed, of which 45 (25 PubMed and 20 Scopus) papers are cited in this paper.

The information flow is summarised in Fig. 1.

3. Results and discussion

NPs and EVs hold promise as nanocarrier treatment options for Batten disease if they fulfil four crucial requirements: either targeting the lysosome for enzyme replacement therapy or delivering a functional gene or gene editing tool or mRNA to a functional location in the cell, and efficiently crossing the BBB or BOB to access the CNS or eye (Fig. 2). The current ability of nanocarriers to satisfy these requirements is discussed in the following sections. The relevance to LSDs and specifically to BD is included as appropriate.

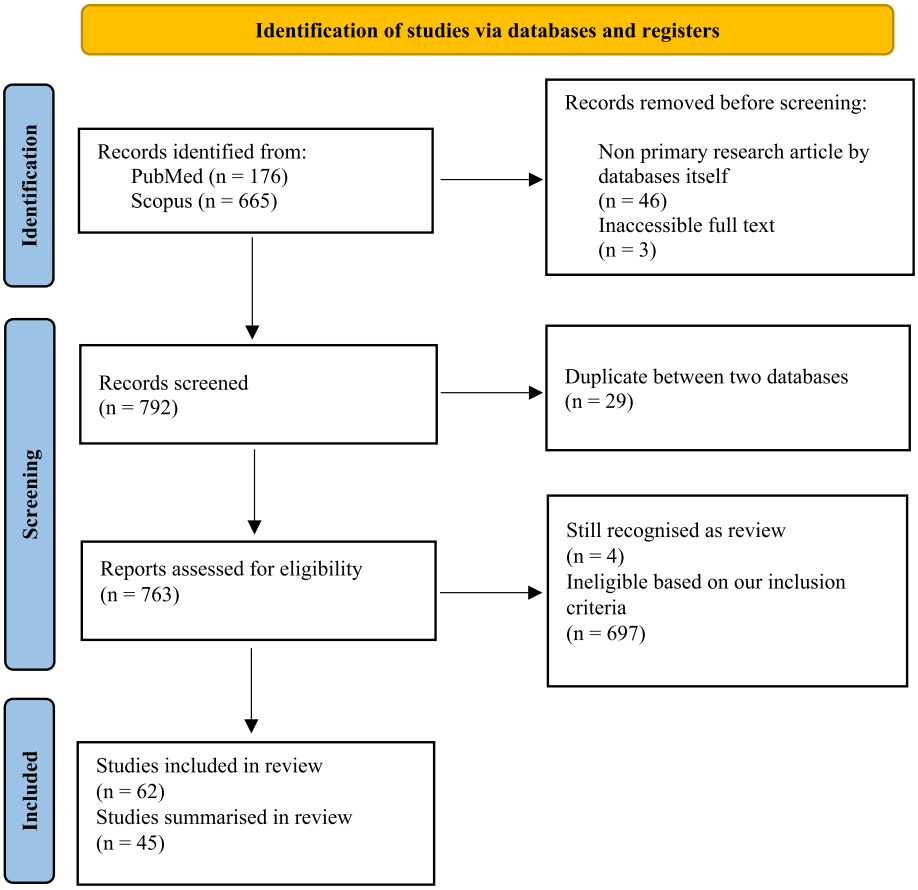

Fig. 1. The PRISMA 2020 flow diagram. The diagram follows the guidelines for reporting systematic reviews (Page et al., 2021).

3.1. *Types of nanocarriers*

In drug delivery systems, carriers play pivotal roles in ensuring efficient and targeted delivery. The following includes brief descriptions of two prominent carriers that are most suitable for reaching the CNS and eye; these are nanoparticles and extracellular vesicles (Fig. 3**)**.

3.1.1. *Nanoparticles*

NPs represent a diverse class of drug carriers characterised by their small size, typically ranging from 1 to 1000 nm. These carriers can be fabricated from various materials including polymers, lipids, metals, and ceramics, each offering unique advantages in terms of biocompatibility, stability, and drug loading capacity (Yih and Al-Fandi, 2006). Traditional NPs, such as polymeric NPs and liposomes, have been extensively studied and utilised for drug delivery applications. Polymeric NPs, composed of biodegradable polymers like poly(lactic-co-glycolic acid) (PLGA) or polyethylene glycol (PEG), offer controlled release kinetics and tuneable surface properties, making them suitable for targeted drug delivery (de Castro et al., 2022. Several studies have reported that PLGA-based NPs can cause instability and denaturation of encapsulated proteins due to the formation of acidic by-products (Fu et al., 2000; van de Weert et al., 2000). In contrast, liposomes provide enhanced protection against protein denaturation and destabilisation. Liposomes are lipid-based vesicles consisting of phospholipid bilayers that can encapsulate hydrophilic and hydrophobic drugs within their aqueous core and lipid membrane, respectively. These carriers exhibit excellent biocompatibility and versatility, with the ability to modify their surface properties for enhanced targeting and prolonged circulation (Al-Jamal and Kostarelos, 2007). Additionally, liposomes improve targeted delivery and minimise off-target effects of protein cargos. For instance, liposomes have been used to prevent the off-target degradation of sphingomyelin by free rh-ASM during ERT for Niemann-Pick disease B (NPD-B). This approach demonstrated a 71 % reduction in sphingomyelin levels in NPD-B fibroblasts after treatment with rhASM-loaded PS-BMP liposomes, compared to a 55 % reduction with free enzyme treatment. Furthermore, the degradation of sphingomyelin caused by free enzyme treatment decreased by 61 % when using rhASM-loaded PS-BMP liposomes (Aldosari et al., 2019). Unmodified liposomes tend to have relatively low lysosomal delivery efficiency. Modifications, such as GNeosomes, which are stearyl-Gneo decorated liposomes, have shown promise in delivery of alpha-L-iduronidase (IDUA) to lysosomes of MPS I human fibroblasts (Hamill et al., 2016).

Hybrid nanoparticles, formed by combining two or more distinct NPs or nanoparticle-liposome blends, offer a strategy to amplify advantageous traits and potentially address limitations of single-material NPs (Ma, 2019). The potential of hybrid nanoparticles is exemplified by phospholipid-polysaccharide compositions (lecithin/chitosan) as effective CNS drug delivery platforms. These hybrids lower psychosine accumulation in Krabbe's disease *ex vivo* cell models (Clementino et al., 2021). Hybrid liposomes functionalised with cationic miristalkonium chloride surfactant and loaded with GLA exhibit promising safety profiles and enhanced efficacy in *in vitro* and *in vivo* contexts for Fabry disease treatment, warranting further exploration in preclinical and clinical trials (Tomsen-Melero et al., 2022).

Lipid nanoparticles (LNPs) are lipid-based spherical vesicles, and while similar to liposomes, they differ in function, composition and structure (Joun et al., 2022). Their unique characteristics derive from organic, water-insoluble compounds, enabling them to self-assemble into well-defined structures similar to cell membranes (van Meer et al., 2008). They can be used as carriers for RNA. The formation of LNP-RNA systems involves hydrophobic and electrostatic interactions. The initial use of fixed cationic lipids has now transitioned to ionisable

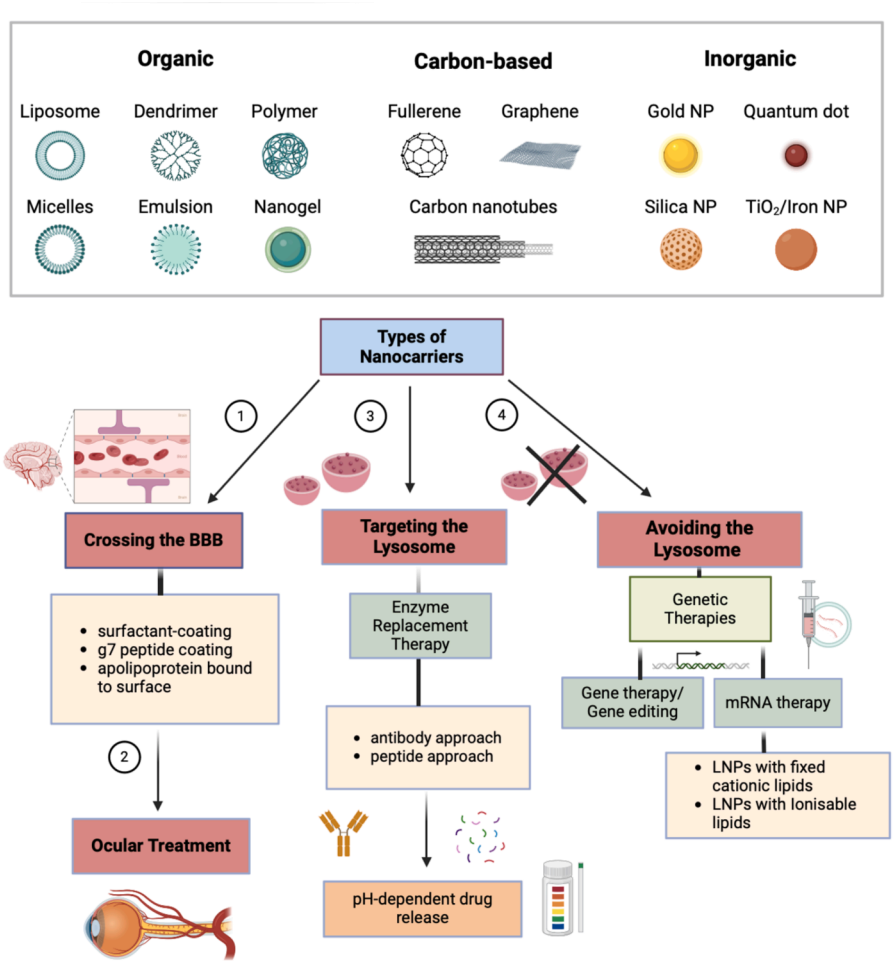

Fig. 2. Schematic summary of the potential of nanocarriers to treat Batten disease. Diverse types of nanocarriers and extracellular vesicles (or a hybrid form) are able to (1) cross the BBB, (2) facilitate ocular treatment, (3) efficiently target the lysosome or (4) avoid the lysosome to enable gene therapy, gene editing, and mRNA therapy. Created in BioRender. Mole, S. (2024) https://BioRender.com/s36c651.

cationic lipids due to reduced toxicity (Yi Xue et al., 2015). Ionisable cationic lipids exhibit a positive charge at low pH for RNA binding then turning neutral at physiological pH, contributing to decreased toxicity of LNP-RNA complexes *in vivo* (Bessodes et al., 2019; Cullis and Hope, 2017). Polyethylene glycol is added to improve stability and extend circulation time by preventing serum protein binding (Suk et al., 2016).

3.1.2. *Extracellular Vesicles*

EVs are small membranous structures naturally released by cells into the extracellular environment, playing crucial roles in intercellular communication by transferring biomolecules, including proteins, nucleic acids and lipids. As important signal carriers they are delivered to target cells where they contribute to maintaining homeostasis across various cellular processes, including differentiation during cell development (Herrmann et al., 2021).

EVs are classified into different subtypes based on their size and biogenesis, with the most well-known subtypes being exosomes and microvesicles. Exosomes, ranging from 30 to 150 nm in diameter, originate from the endosomal pathway and are released from cells following the fusion of multivesicular bodies with the plasma membrane. Microvesicles are larger, at 100 to 1000 nm in diameter, and shed directly from the plasma membrane of cells (Kosanović et al., 2021).

The growing interest in EVs is attributed to their selective uptake by recipient cells and their ability to induce phenotypic changes through the transfer of their molecular cargo (Ginini et al., 2022). EVs from various sources have been reported to exhibit preferential interactions with specific cell types, a process influenced by the lipid, glycan, and protein composition of their membranes (Ginini et al., 2022). For instance, the presence of distinct integrin complexes on EVs enables targeted delivery to tissues such as the liver, brain, or lungs. Several mechanisms have been identified for EV uptake by target cells, including clathrin-dependent and clathrin-independent endocytosis (Tian et al., 2014), macropinocytosis (Fitzner et al., 2011; Nakase et al., 2015), phagocytosis (Feng et al., 2010), and direct fusion with the plasma membrane (Montecalvo et al., 2012). Once within a cell, EVs tend to localize within lysosomes. This, combined with their natural ability to cross the BBB, underscores their potential application in the treatment of LSDs and neurological diseases (Do et al., 2019; Flanagan et al., 2021; Seras-Franzoso et al., 2021).

EVs offer several advantages over synthetic liposomes, such as better biocompatibility, lower immune clearance, the ability to target specific tissues and their natural ability to traverse biological barriers, including the BBB. Moreover, EVs can be engineered to display targeting ligands on their surface and to be loaded with therapeutic cargo, making them promising candidates for targeted drug delivery and regenerative medicine applications (Rufino-Ramos et al., 2017). Using EVs for drug delivery offers further advantages by providing stability to encapsulated enzymes, protecting them from protease degradation (Haney et al., 2019). However, they also present challenges, including size heterogeneity, batch-to-batch variations, and difficulties in large-scale production. The process of re-engineering EVs for drug delivery involves removing their native contents and loading them with drugs using

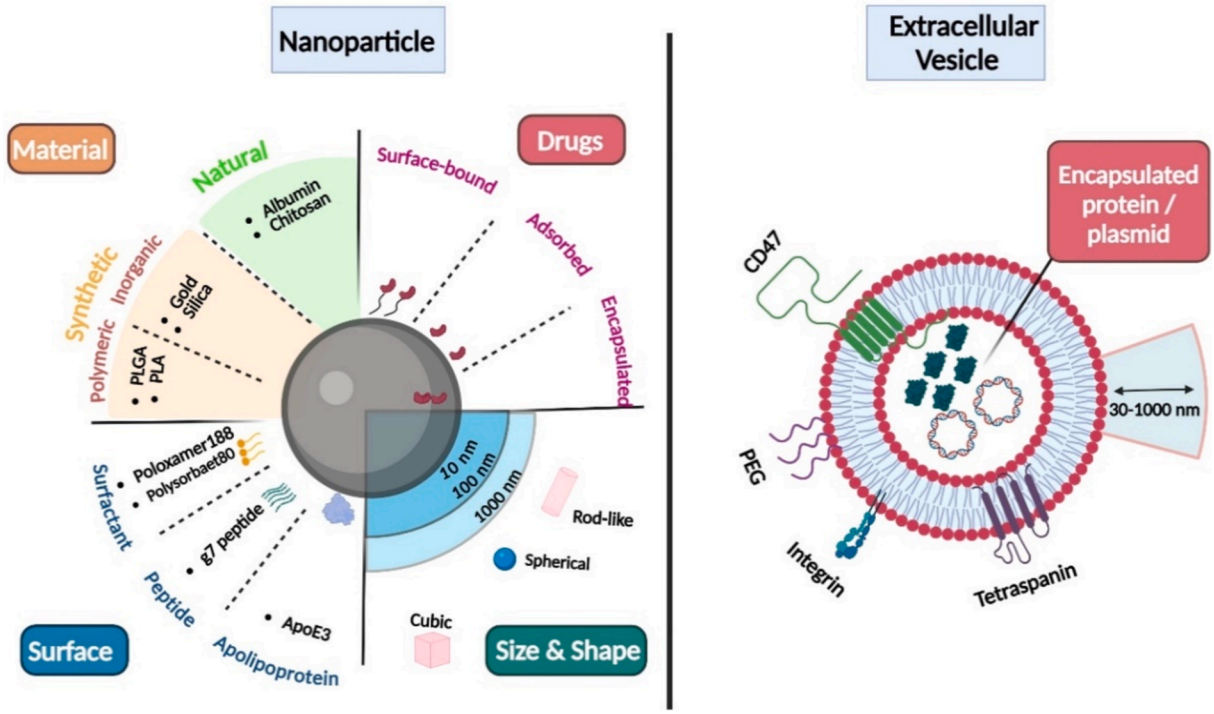

Fig. 3. Comparative structure and properties of nanoparticles (left) and extracellular vesicles (right). Nanoparticles exhibit diverse materials, sizes (10–1000 nm), and geometries (e.g., cubic, spherical, rod-like), while accommodating drugs via surface-binding, adsorption, or encapsulation, with potential surface modifications for lysosomal targeting or enhanced BBB penetration. In contrast, drug or plasmid encapsulation within extracellular vesicles involves distinct surface molecules such as CD47, integrin, tetraspanins, or PEG. Figure inspired by (Murphy et al., 2019; Saraiva et al., 2016). Created in BioRender. Mole, S. (2024) https://BioRender.com/s35f579.

techniques like sonication, electroporation, saponin treatment, or passive incubation. Alternatively, drugs can be incorporated endogenously into EVs through genetic modification of their parent cells. More detailed discussions of these manufacturing methods are available (Herrmann et al., 2021; Kumar et al., 2024; Murphy et al., 2019).

3.2. Strategies to cross the BBB

There are various administration routes into the brain for nanocarriers, including systemic, intranasal, intrathecal and intracranial injections. To treat BD, NPs and EVs need to be able to cross the BBB. The effect of BD on BBB composition has not been extensively studied in children, BD patients or animal disease models, and this may differ between NCL subtypes. The CLN1 (Ppt1) disease mouse model showed increased permeability and inflammation in the brain. $CD4^+$ T-helper 17 lymphocytes (T_H17) produce IL-17A which promotes production of matrix metalloproteinase and breakdown of the TJ proteins that maintain the integrity of the BBB. Treatment with resveratrol reduced the number of IL-17A positive T_H17 cells and increased TJ proteins which ameliorated the BBB disruption and neuroinflammation (Saha et al., 2012). The severely affected CLN10 (Ctsd) mouse model had oxidative damage to pericytes located in the brain capillaries, dilated blood vessels, and impaired BBB function with increased permeability of the BBB to small molecules and the infiltration of peripheral blood mononuclear cells into the brain parenchyma (Okada et al., 2015). Increased BBB permeability is a hallmark of various neurological disorders and is generally harmful, however, early BBB leakage could be exploited for the administration of drugs to the CNS to treat the disease.

3.2.1. NP and BBB

Three important approaches to facilitate the penetration and crossing of the BBB by NPs include their coating with surfactants, peptides, or apolipoproteins (see Fig. 3).

3.2.1.1. Surfactant coating approach.

Polymeric NPs have been utilised with great success to transport a wide range of small compounds and proteins through the BBB. The surfactant coating of nanocarriers with poloxamer 188 or polysorbate 80 is believed to be the key to their ability to penetrate the brain (Joseph et al., 2021) (Fig. 4). Although the mechanism of *trans*-endothelial transport is still being studied, surfactant coating appears to facilitate the binding of endogenous apolipoproteins from the bloodstream to the surface of the nanoparticle. These apolipoproteins may then attach to the low-density lipoprotein (LDL) receptor family proteins that are present on the apical surface of capillary ECs in the brain, leading to complete nanoparticle transcytosis (Kreuter et al., 2002).

Polysorbate 80-coated monoolein NPs can traverse the BBB and concentrate in lysosomes in mice. NPs loaded with β-cyclodextrin (β-CD), with an average size of 120 nm, were taken up by lysosomes, reducing cholesterol accumulation in Niemann-Pick type C 1 (NPC1) fibroblasts (Donida et al., 2020). In an NPC mouse model, they exhibited deeper penetration into the mouse brain than other organs and showed potential to decrease brain damage of the disease (Donida et al., 2020; Donida et al., 2018). A more recent study explored the synergistic effects of combining β-CD in nanoparticulate form with antioxidants, namely, N-Acetylcysteine (NAC) and Coenzyme Q10 (CoQ10). The β-CD NPs effectively reduced cholesterol accumulation and mitochondrial oxidative stress in NPC1 fibroblasts, and this effect was significantly enhanced when the NPs were used in combination with the antioxidants NAC and CoQ10 (Hammerschmidt et al., 2023). These promising findings support the potential of a combined approach using β-CD-loaded NPs and antioxidants for treating NPC1 disease.

Another study investigated the capacity of NPs coated with a surfactant (Fig. 4) to improve brain delivery of arylsulfatase A (ASA) for the treatment of metachromatic leukodystrophy (MLD). Although the biodistribution of nanoparticle-bound ASA in peripheral organs was changed, there was no increase observed in the brain. The ASA

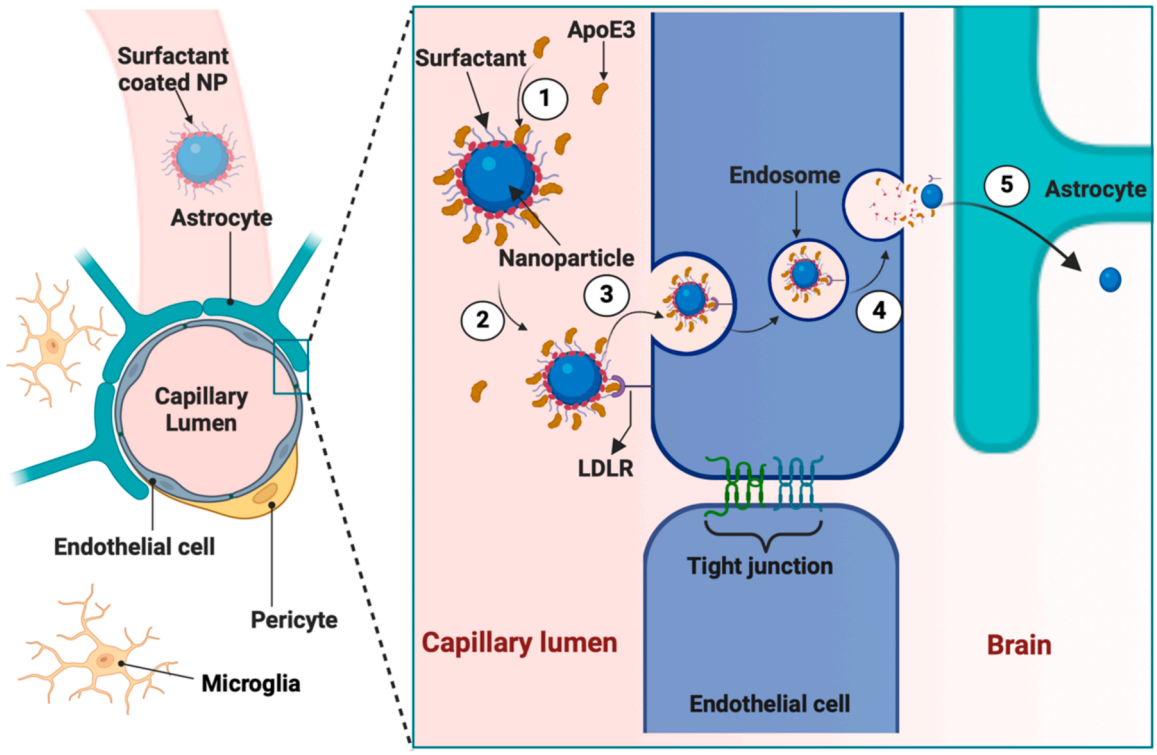

Fig. 4. Mechanism of BBB crossing by NPs coated with surfactant. Injected NPs in the bloodstream interact with ApoE3 (step 1). This interaction directs the NP-surfactant complex to LDL receptors (LDLR) on the luminal membrane of ECs (step 2), initiating transcytosis (step 3). Subsequently, the NPs reach the abluminal membrane of ECs in the neurovascular unit, where they are released to reach astrocytes (shown), microglia, neurons and other inner parts of the brain (steps 4 and 5). Created in BioRender. Mole, S. (2024) https://BioRender.com/e44n788.

glycoprotein may disrupt the mechanisms responsible for delivering NPs coated with surfactant to endothelial cells in brain capillaries (Schuster et al., 2017). The potential of poly(butyl cyanoacrylate) NPs (PBCA NP) coated with polysorbate 80 to deliver ASB and ASA across BBB for treatment of MPS VI and MLD, respectively, has been examined. These *in vitro* studies demonstrated high release efficiency of the freeze-dried enzyme and nearly 100 % enzyme activity recovery (Mühlstein et al., 2014; Mühlstein et al., 2013). Previous studies have proved the potential of PBCA NPs for delivering large proteins across BBB *in vivo* (Kurakhmaeva et al., 2009).

For BD, the primary therapeutic goal is to effectively target neurons. However, the fate of NPs after crossing the BBB and being taken up by neuronal cells remains a crucial but largely underexplored area. Recent findings suggest that NPs may be transported between different brain cells, independent of surface properties, with significant implications for their distribution, toxicity, and therapeutic efficacy (Guo and Yi, 2023; Tosi et al., 2014). Other cell-specific targeting mechanisms have been described, with strategies tailored to microglia, astrocytes, oligodendrocytes, and other CNS cells and we refer the reader to this review (Guo and Yi, 2023). Despite these advances, mechanisms to avoid clearance by microglia, the brain's primary immune cells, remain unclear and represent a critical challenge in NP-based therapies. Further research is essential to optimise targeting strategies and enhance the precision of treatments for neurodegenerative conditions like BD.

3.2.1.2. G7 peptide coating. PLGA NPs, which showed promise in delivering deficient lysosomal enzymes like rhGGA for Pompe disease (Brunella et al., 2015), can be chemically modified to cross the BBB and access the CNS. PLGA-NPs chemically altered with the g7 peptide (H_2N-Gly-L-Phe-D-Thr-Gly-L-Phe-Lleu-L-Ser(O—D-Glucose)–$CONH_2$ may penetrate the BBB effectively without causing damage. The localisation of g7-NPs has been studied in cultured neural cells and animals. The clathrin and Rab-5 pathways have been implicated in NP absorption and trafficking into neural cells (Salvalaio et al., 2016). Further, g7-NPs accumulate in the lysosome, which makes them a promising treatment option for LSDs.

When introduced intravenously, PLGA NPs coated with g7 and loaded with the high molecular weight compound FITC albumin can cross the BBB in mouse models of mucopolysaccharidosis (MPS) type I and II (Salvalaio et al., 2016). However, the amount of g7-NPs/Albumin reaching the brain is lower in the mouse model of MPS II compared to MPS I. This could be due to differences in BBB permeability or altered mechanisms in BBB passage routes, highlighting the importance of studying BBB permeability in different pathologies. Iduronate-2-sulfatase-loaded PLGA NPs can reduce GAG levels in a mouse model of MPS II, but not to healthy levels. To be clinically relevant, the NP formulation needs to be optimised for higher efficacy (Rigon et al., 2019).

One study investigated the ability of different peptide-coated PLGA NPs encapsulating GALC enzyme to cross the BBB to treat Krabbe disease in a murine model. G7 peptide-coated NPs had a higher loading efficiency (74 %) than Angiopep-2 and transferrin binding 2 peptides, and GALC enzyme reached the brain and was released at acidic pH in the lysosome (Del Grosso et al., 2019).

3.2.1.3. Apolipoprotein E3 binding to the surface. PEGylation of NPs increases their half-lives by reducing the binding of undesirable proteins, preventing non-specific cellular uptake and phagocytosis (Kelly et al., 2017). Polyethylene glycol-b-polylactic acid (PEGPLA)-coated NPs also bound to apolipoprotein E3, a BBB penetrating protein, aided the delivery and pH-responsive release of active galactosidase in an *in vitro* GM1 gangliosidosis model (Kelly et al., 2017).

3.2.1.4. Magnetoliposome. Magnetoliposomes, combining magnetic NPs with lecithin-based liposomes, showed promise *in vitro* in the CRISPR/nCas9 delivery system for MPS IVA treatment, as this

nanocarrier significantly elevated GALNS enzyme levels (5–88 %) in MPS IVA fibroblasts (Leal et al., 2022).

3.2.2. EV and BBB

Approaches for EVs to cross the BBB are different to those of NPs. EVs have minimal immunogenicity as they contain the CD47 receptor, which interacts with SIRP to create a "don't eat me" signal in phagocytes (de Jong et al., 2020). The use of macrophage-derived EVs is beneficial as they are inflammatory response cells that offer the opportunity for site-specific delivery of cargo, and most patients with LSD show signs of neuroinflammation. The exact mechanism of lysosome localisation is unclear, but it is thought that EVs cross the BBB through the interactions of LFA-1 protein on the macrophage membrane with ICAM-1 receptors overexpressed in inflamed endothelial cells (Yuan et al., 2017).

Chitosan polyelectrolytes (Giannotti et al., 2011), liposomes (Cabrera et al., 2016), and polystyrene NPs have all been explored as ERT carriers in Fabry disease (Hsu et al., 2014). Other biological nanocarriers, such as NPs composed of 30Kc19 protein and albumin (Lee et al., 2016), have been investigated. None of these synthesised NPs have yet reached clinical testing in LSDs. EVs isolated from human embryonic kidney (HEK) cells and Chinese hamster ovary (CHO) cells have been investigated for their ability to deliver GLA and SGSH enzymes to treat murine models of Fabry and Sanfilippo A diseases. The EV formulations did reach the brain in Fabry mice when introduced via intravenous administration, although intra-arterial administration is proposed to be more efficient (Seras-Franzoso et al., 2021).

Macrophage-derived EVs containing tripeptidyl peptidase 1 (TPP1) have been tested for their ability to deliver this enzyme to lysosomes in a CLN2 (Tpp1) mouse model. TPP1 was incorporated into EVs through two methods: transfecting parent cells with plasmid DNA encoding TPP1 or loading empty EVs with TPP1 protein using either sonication or saponin permeabilisation of the EVs membrane. Sonication proved more effective than saponin permeabilisation (Haney et al., 2019). Intraperitoneal injection of TPP1-EVs resulted in EV carriers accumulating in the brain, extending the lifespan of the mouse model. The treatment efficacy was more pronounced in younger mice (Haney et al., 2019). Additional benefits included reduced inflammation, enhanced neuronal survival, and effective elimination of lipofuscin aggregates in lysosomes through the activation of autophagy (El-Hage et al., 2023).

3.3. Strategies to cross the BOB

The eye is one of the most accessible organs in the human body, however, delivery to ocular tissues poses significant challenges. Similar to the brain, the eyes are considered immune-privileged as part of the CNS, due to complex ocular barriers, including the tear film, cornea, Sclera and Bruch's-choroid complex, vitreous, blood-aqueous barrier (BAB), and blood-retinal barrier (BRB); these are reviewed elsewhere (Han et al., 2023). Particularly relevant for BD treatment are the BRB and vitreal barrier, which hinder drug permeation to the retina and the posterior segment of the eye.

There is retinal degeneration in BD, and there may also be changes in the optic nerve and visual projection regions of the brain (Ouseph et al., 2016). It will be important to understand all underlying pathways to design treatment strategies that fully prevent vision loss for each type of BD.

Overcoming ocular barriers for drug delivery in BD represents a critical avenue for improving treatment outcomes and preserving vision in affected individuals, including those receiving brain-directed therapy (Fig. 5). Alternative delivery strategies, particularly nanoparticle-based approaches, offer promising solutions to address the challenges associated with traditional methods. These require further research and clinical exploration.

Intravitreal injection is utilised to achieve therapeutic

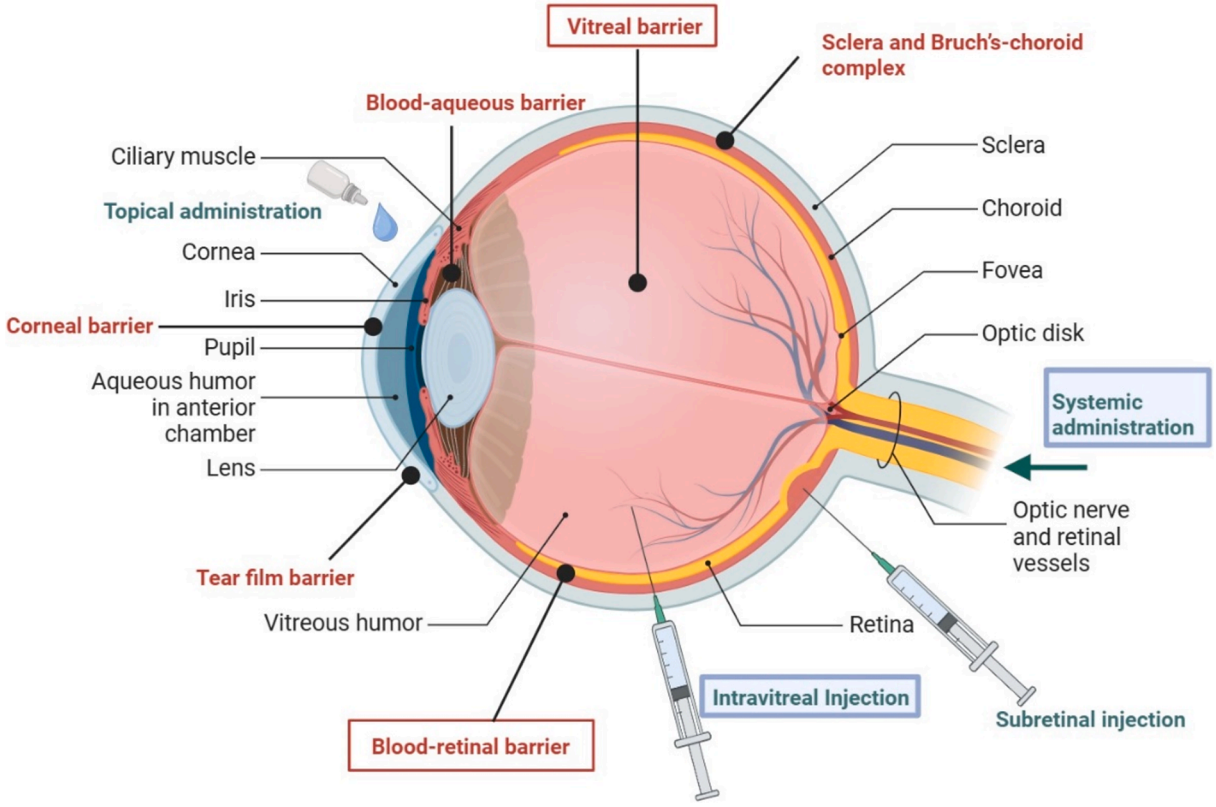

Fig. 5. Ocular barriers and drug delivery routes. The barriers most relevant to BD are marked in red boxes, including the blood-retinal and vitreal barrier, and administration routes in blue. Systemic administration and intravitreal injection (boxed) both hold potential for the use of nanoparticles in safer and directed drug delivery. Created in BioRender. Mole, S. (2024) https://BioRender.com/a97a583.

concentrations in the posterior segment of the eye. This method, including both direct intravitreal injection and implantable devices, has emerged as the primary approach for treating vitreoretinal diseases in recent years (Choonara et al., 2010). It enables the administration of various therapeutics, including anti-vascular endothelial growth factor (VEGF), steroids, genes, and stem cells, thereby enhancing drug concentrations in the vitreous and retina. However, it poses a significant risk of ocular complications, including bleeding, vitreous haemorrhage, retinal holes, elevated intraocular pressure, cataracts, secondary glaucoma, optic nerve damage, endophthalmitis, and retinal toxicity (Han et al., 2023).

Intravitreal enzyme replacement therapy has shown high efficacy in canine models for BD (Kick et al., 2023). However, for BD, including CLN2 disease, outer retinal degeneration occurs (Orlin et al., 2013; Wawrzynski et al., 2024), so an intravitreal route may not be suitable. Nevertheless, in BD and some LSDs, direct injection of naked genes or enzymes into ocular tissues is currently under investigation. The safety and efficacy of delivering recombinant human tripeptidyl peptidase-1 (rhTPP1) was investigated intravitreally for CLN2 disease. Eight children receiving intracerebroventricular ERT (Brineura) also underwent intravitreal injection of rhTPP1 into the right eye every 8 weeks over a period of 12–18 months, with the left eye serving as a control. The study found no severe adverse reactions. Efficacy was assessed using paracentral macular volume (PMV) measured by spectral domain OCT. In the three children who were still actively losing vision, the rate of PMV loss was slower in the treated eye compared to the untreated eye, suggesting the potential efficacy of intravitreal rhTPP1 in slowing retinal degeneration (Wawrzynski et al., 2024). The study underscores the importance of early intervention for optimal treatment outcomes.

Although changing the route to subretinal administration is challenging due to the need for repeated pars plana vitrectomy, it may be possible to increase TPP1 concentration in photoreceptors by administering a higher intravitreal dose. The inner retinal layers may not act as an absolute barrier to TPP1, as transport through the retinal layers is known to occur via mannose 6-phosphate dependent uptake and transcytosis (Wawrzynski et al., 2024).

Alternative administration strategies aided by NPs, such as systemic treatment through intravenous, oral or periocular routes, offer safer approaches to reach the retina and overcome ocular barriers. NPs exhibit properties conducive to retinal delivery, including charge effects influencing vitreal dispersion and retinal bioavailability, particle size affecting penetration through ocular barriers, and surface modifications enhancing mucoadhesion and cellular uptake. Positively charged NPs have shown improved retention in the vitreous, while smaller particles can diffuse more readily through the vitreous humour (Xu et al., 2013). Within the blood-retinal barrier (BRB), small lipophilic molecules can penetrate the retinal pigment epithelium (RPE) more effectively compared to hydrophilic ones. This is because of their ability to diffuse through the intracellular pathway. Additionally, NPs can bypass the blood-ocular barrier (BOB) by utilising transporters or exploiting receptor-mediated endocytosis mechanisms, allowing for targeted drug delivery to the retina. NP formulations for the targeting of the posterior segment of the eye are reviewed in more detail elsewhere (Alshaikh et al., 2022; Kompella et al., 2013).

Overcoming ocular barriers for drug delivery in BD represents a critical avenue for improving treatment outcomes and preserving vision in affected individuals. Alternative delivery strategies, particularly NP-based approaches, offer promising solutions to address the challenges associated with traditional methods, emphasising the need for further research and clinical exploration in this field.

3.4. Strategies to target the lysosome

3.4.1. Conventional targeting of receptors and pathways

Some types of BD are caused by defects in genes encoding lysosomal enzymes (e.g. PPT1/CLN1, TPP1/CLN2, CLN5, CTSD/CLN10, CTSF/CLN13) (Cooper et al., 2022; Gardner and Mole, 2021). For these, therefore, delivering recombinant enzyme directly to the lysosome is a valid treatment aim. The mannose-6-phosphate (M6P) receptor is important for clathrin-dependent endocytosis and improved lysosomal enzyme delivery, as its expression on the cell surface allows 'cross-correction' whereby enzymes in the extracellular milieu can be picked up and internalised. These enzymes may be secreted by cells treated through gene therapy or delivered as a recombinant enzyme replacement therapy, as for Brineura treatment for CLN2 disease. However, the clathrin-mediated pathways are impaired in numerous LSDs including Niemann-Pick diseases (Willenborg et al., 2005), and Pompe disease which have lower expression levels of the M6P receptor (Bonam et al., 2019; Spada et al., 2018). For these particular LSDs the clathrin-dependent endocytosis routes cannot be exploited for ERT as the uptake of cargo via M6P is inefficient.

There is limited data on M6PR-related transport in NCLs, however reduced M6PR expression is likely for at least some types of BD. Loss of CLN3 leads to the mis-trafficking of the cation independent M6PR (CI-M6PR), followed by its degradation. CLN3 has been identified as a pivotal factor in the proper sorting of CI-M6PR, establishing a connection between this process and autophagic-lysosomal reformation. This may explain the widespread lysosomal dysfunction observed in CLN3 disease (Calcagni' et al., 2023). Further, inflammation in NCL brain tissues may contribute to disease progression. Intercellular cell adhesion molecule 1 (ICAM-1) is a protein that coregulates the immune response and is upregulated in response to inflammatory signals (Bui et al., 2020). There is some evidence that ICAM-1 expression is increased in the brains of juvenile CLN3 disease patients due to disease-associated inflammation (Hersrud et al., 2016). This elevation in the ICAM-1 receptor could be exploited for nanoparticle delivery as it may render targeting of the lysosome more efficient.

New strategies to bypass endogenous pathways for lysosomal enzyme delivery include targeting of ICAM-1 and CD44, the main receptor for hyaluronic acid (HA), by NPs to enable internalisation of cargo by endocytosis (Muro et al., 2006). ICAM-1 transports cargo/ligands to the lysosome in a manner independent of glycosylation and clathrin. Both ICAM-1 and CD44 are overexpressed in oxidative stress (Książek et al., 2010; Whelan et al., 2017). Two main approaches are being followed that involve targeting ICAM-1 or CD44 either via antibodies or peptides for intracellular transport of NPs to the lysosome.

Towards treatment for Niemann-Pick disease types A and B, nanocarriers coated in ICAM-1 antibody localise in the lysosome and deliver rh-ASM, providing consistent activity of rh-ASM and reducing lipid build-up in lysosomes (Muro et al., 2006). Anti-ICAM-1 coated polystyrene NPs penetrate the blood–brain barrier in studies conducted in Fabry disease, Pompe disease and Niemann Pick disease mouse models (Hsu et al., 2012; Hsu et al., 2011). However, polystyrene NPs are non-degradable, so they are not clinically relevant. Hence, more suitable biocompatible NPs, which are biodegradable and non-toxic, such as polylactic-co-glycolic acid (PLGA), need to be developed (Garnacho and Muro, 2017).

Uptake of HA-grafted NPs involves lipid raft-mediated endocytosis, resulting in NP localisation within lysosomes (Qhattal and Liu, 2011). While HA-coated NPs have primarily been utilised for drug delivery in cancer studies, a study reported that polymersomes coated with HA can efficiently deliver β-galactosidase (βgal) in a cellular model of GM1 Gangliosidosis. This resulted in the fusion of lysosomes and autophagosomes returning to normal levels following 24 h of treatment (Paruchuri et al., 2022).

The use of antibodies for lysosomal delivery can be associated with an elevated immune response. Therefore, recent studies explored the use of peptides to target the ICAM pathway as peptides are invisible to the immune system (Mitchell et al., 2021). ICAM-1 targeting, functional activity and intracellular trafficking of PLGA NPs coated with a fibrinogen-derived peptide have also been investigated for the treatment of Niemann-Pick B disease. In cell studies, these nanocarriers are

effectively internalised, transported to lysosomes and able to restore cholesterol and sphingomyelin levels in lysosomes, reducing them to around 95 % of the elevated disease levels. Further, there is selective binding of fibrinogen to ICAM-1 under inflammatory conditions. This fibrinogen-derived ICAM-1-targeting peptide has significant potential for lysosomal ERT in clinical applications (Garnacho and Muro, 2017).

Two-dimensional graphene-based materials offer a range of advantages, including their large surface area, exceptional biocompatibility, and the ability to target lysosomes through multiple endocytic pathways. A recent investigation conducted on fibroblasts derived from patients with LSD highlights the enhanced enzyme delivery capabilities of defect-free graphene nanomaterials (Chen et al., 2023). Remarkably, all graphene-based materials remained biocompatible even at concentrations of up to 100 μg/mL in the tested cell lines. Moreover, the study has unveiled a promising approach involving positively charged graphene flakes as carriers for transporting the arylsulfatase B enzyme to the lysosomes of individuals with Mucopolysaccharidosis VI (MPS IV). When arylsulfatase B formed complexes with these cationic graphene flakes, its enzymatic activity was preserved, exhibiting nearly twice the biological efficacy compared to when arylsulfatase B was administered in isolation, thereby facilitating the removal of the substrate within MPS VI fibroblasts. This research serves as a foundation for further exploration of the potential application of graphene-based materials as vehicles for enzyme replacement therapy in LSDs (Chen et al., 2023).

Virus-like particles (VLPs), NPs made from self-assembling viral capsid proteins but lacking viral genetic material, can encapsulate cargo protein inside the hollow nanostructure for drug delivery, or alternatively be coated with enzymes to enhance their stability and functionality (González-Davis et al., 2023). Specifically, decoration of *in vitro* assembled parvovirus B19-derived VLPs with α-glucosidase Ima1p resulted in a three-fold and a 10 °C increase in the catalytic rate and optimum temperature, respectively (Cayetano-Cruz et al., 2018). VLPs from the brome mosaic virus were used to encapsulate the enzyme glucocerebrosidase for therapy of Gaucher disease. Their surface was functionalised with mannose groups to target macrophages, and significant GCase catalytic activity was seen in the VLP nanoreactors, which were also efficiently internalised by macrophage cells. It is significant that these VLP-based targeted nanoreactors improve GCase stability, which is essential for an extended blood circulation half-life and lowers the frequency of injections and overall treatment costs (Chauhan et al., 2022).

3.4.2. pH-dependent release mechanisms

Cargo needs to be released at the right place and time in the cell. As lysosomes have a lower pH than the rest of the cell, this can be exploited in the mechanism for release of cargo from the NP.

The lysosomal pH of fibroblasts from patients with some types of BD, such as juvenile CLN3 disease, may be less acidic than normal, while in other types, like late infantile CLN2 disease and CLN8 disease, lysosomal pH remains unaffected (Holopainen et al., 2001; Vidal-Donet et al., 2013). A change of lysosomal pH in disease conditions could interfere with the potential use of pH-sensitive nanogels (see below). Further, PLGA acidic NPs, that can translocate to the lysosome, have been shown to restore defects in lysosomal acidification (Bourdenx et al., 2016), an approach that could be used to restore defective lysosomal pH in disease.

In promising *in vitro* studies, a polymeric nanogel consisting of tetraethylene glycol has been developed for the delivery of acid-α-glucosidase in Pompe disease treatment. The nanogel incorporates a thiopropionate cross-linker, which provides pH sensitivity as the cross-linker is degraded at low pH causing swelling of the nanoparticle followed by the release of the encapsulated drug. While the enzyme is fully inactivated when encapsulated, approximately 75 % of its activity is recovered upon reducing the pH to 5.0 (Molla et al., 2014). Such polymeric nanogels have the benefit of being concentration independent since they are stable at large dilutions and do not have the need for the critical aggregation concentration required by amphiphilic assemblies such as vesicles and micelles. The β-thioester crosslinker is stable at neutral pH and hydrolyses slowly at lower pH (~5.3) which ensures a long-term release of the cargo (Molla et al., 2014).

Another promising investigation towards treatment of MPSIVA exploited injectable and biodegradable PEG-based hydrogels made of 4-arm PEGAc (polyethylene glycol tetra-acrylate) macromer and PEG-diSH (poly (ethylene glycol) dithiol) crosslinker loaded with rhGALNS. The hydrogel degraded fully in ~ 28 days when incubated with a release buffer at 37°C. Furthermore, their release study demonstrated continuous release of rhGALNS for at least 7 days (>30 % released by day 7). This efficient sustained release did not impact the enzyme activity which remained high (75 %) by day 7 (Jain et al., 2020).

3.5. Strategies that avoid the lysosome

3.5.1. Genetic therapy

Gene therapy (GT) and gene editing (GE) are promising approaches in the management of multiple LSDs as alternatives to enzyme replacement therapy which requires repeated applications. Using nanocarriers to deliver a functioning copy of the disease gene as part of a plasmid vector or tools for editing of the endogenous gene, leading to expression of a functioning gene product in cells could reduce lysosome effects and alleviating the symptoms in the patient. In this approach, NPs are targeted to the nucleus as a non-viral gene therapy vector. Non-viral vectors are being suggested as a safer, less restrictive, and more cost-effective alternative since they have no limitations in terms of the size of DNA they can transport. Their use has been investigated in LSDs, including Fabry disease and MPS I and IVA.

Protamine solid lipid-based nanoparticles (SLNs) loaded with pR-M10-aGal A plasmid that encodes a-Gal enzyme were able to correct α-Gal levels in human hepatocellular carcinoma cells (Ruiz de Garibay et al., 2012). SLNs have been used for treatment of LSDs including Fabry disease and MPS VIA (Álvarez et al., 2019; de Garibay et al., 2015). Other studies demonstrated that cationic nanoemulsions containing the plasmid encoding the IDUA protein were able to reach the brain, lung and liver tissues in Idua-knockout mice via nasal, intravenous or intra-articular administration, therefore establishing their potential use as a gene therapy vector to treat the neurological impairment of MPS I (Bidone et al., 2018; Fraga et al., 2015). Nanoemulsions, combined with the CRISPR/Cas9 system and a donor oligonucleotide, could transfect cells from MPS patients and promote IDUA synthesis (Schuh et al., 2018). Positively charged liposome NPs incorporating the CRISPR/Cas9 system and a IDUA donor plasmid were investigated as an efficient approach to treat neurological and somatic features through nasal route in a murine model of MPS I. This led to increased IDUA activity in the serum, the main organs and especially in brain areas (mostly the olfactory bulb) with the reduction of GAG levels in urine, tissues, serum and brain cortex (Vera et al., 2022).

Nanocarriers can reach the brain and are potential non-viral gene therapy vectors for a variety of LSDs including NCLs. Various viral-based gene therapies are under development for the treatment of NCLs, with clinical trials for CLN2, CLN3, CLN5, CLN6 and CLN7 diseases listed on clinicaltrials.gov (**Supplementary Material 2**), though none using nanocarriers. Gene therapy approaches may be suitable for more types of NCL than enzyme replacement therapies, which are only suitable for NCLs caused by defects in enzymes, and currently only approved for CLN2 disease.

Two primary challenges hinder the clinical application of mRNA replacement therapy (MRT). The first barrier is elicitation of the innate immune response due to the presence of uridine in the RNA structure (Heil et al., 2004). Substituting uridine (U) with pseudouridine (Ψ) to make a modified mRNA (modRNA) diminishes this innate immune reaction (Karikó et al., 2008). Other modifications on *in vitro* transcribed messenger RNA (IVT-mRNA) including modifications to the 5′ cap, 5′ and 3′ untranslated regions (UTRs), poly(A) tail (Strenkowska et al., 2016) and codon optimisation (Zuber et al., 2018) enhances stability

and translatability, as well as reducing immunogenicity. The second challenge is the rapid degradation of IVT-mRNA by extracellular ribonucleases in the blood, extracellular matrix (ECM), and cerebrospinal fluid (CSF) (Probst et al., 2006). Therefore, the efficacy of mRNA therapy requires a secure and effective delivery mechanism. Lipid-based NPs are among the most efficient and promising (Okay et al., 2020) and have been approved for clinical use as carriers for RNA therapeutics of rare genetic disorders (Belliveau et al., 2012; Semple et al., 2010). Other types of NPs incorporating polyethyleneimine (PEI), polyesters and chitosan, exhibit high nucleic acid affinity; however, they are hindered by cytotoxicity, limited target capability, and poor water solubility, respectively (Žak and Zangi, 2021), or have only been utilised for assessing MRT efficacy in *in vitro* studies, as for lipofectamine (Furtado et al., 2022).

The administration of IVT-mRNA is gaining prominence as an innovative form of medical treatment with applicability across a diverse range of conditions, including rare monogenic disorders. Recent advancements in mRNA technology and LNP-based delivery methods are expanding the therapeutic potential of mRNA, with clinical trials for several cancer types (Phase I/II), genetic disorders (Phase I/II), including Methylmalonic acidaemia, Propionic acidaemia, Ornithine transcarbamylase, Cystic fibrosis, and Transthyretin amyloidosis, and clinical treatment for viral infections, including SAR-CoV-2 (Koeberl et al., 2024). The efficacy of the MRT-LNP approach for LSDs is being tested *in vivo* for Fabry disease and MPS-IH and *in vitro* using Lipofectamine for Niemann-Pick disease (Furtado et al., 2022; Martini and Guey, 2019; Palanki et al., 2023).

Work is ongoing to develop suitable nanocarriers for DNA and RNA delivery.

3.5.2. Lipid nanoparticles with fixed cationic lipids

Cationic lipids are amphiphilic small molecules that can be categorised into three main components: a positively charged polar head, a linking bond, and a hydrophobic tail. The positively charged head group possesses one or more positive charges, allowing it to efficiently interact with the negatively charged phosphate group in nucleic acids. This interaction is driven by electrostatic attractions, leading to the formation of complexes that consist of condensed nucleic acids (Niculescu-Duvaz et al., 2003).

Despite their high potential for entrapping nucleic acids due to their positive charge, cationic lipids have several drawbacks in drug delivery. Systemic delivery of LNPs with a permanent positive surface charge leads to interaction with negatively charged serum proteins, causing rapid clearance from circulation. Cationic LNPs have demonstrated toxicity towards phagocytic cells *in vitro*, and their systemic delivery triggers a robust immune response, activating interferon type I response and inducing the expression of inflammatory cytokines (Filion and Phillips, 1998; Lonez et al., 2012). Although excessive immune reactions to LNPs can lead to life-threatening conditions, carefully designed immune response activation can be utilised as an adjuvant in RNA-LNP-based vaccines (Pizzuto et al., 2018). To date, no studies have reported the utilisation of cationic lipids to construct LNPs for mRNA or gene delivery for LSDs due to these drawbacks.

To address some of the disadvantages of fixed cationic LNPs, pH-sensitive ionisable cationic LNPs have been developed for more effective RNA delivery.

3.5.3. LNP with ionisable lipids

At present, the primary LNPs employed for systemic nucleic acid delivery consist of ionisable cationic lipids, helper phospholipids, cholesterol, and PEG. The development of ionisable LNPs aimed to address the toxicity associated with permanently cationic lipids used in earlier LNP-RNA systems, facilitating their therapeutic use (Wang et al., 2021).

The design of ionisable cationic lipids involves a delicate balance based on their pKa value. The pKa value needs to be high enough so that at low pH levels the lipids become positively charged, facilitating the binding with negatively charged RNA molecules and the formation of LNPs. This positive charge at low endosomal pH enables interactions with endogenous anionic lipids, leading to the disruption of endosomal structure and the release of LNP cargo into the cytoplasm. Additionally, the pKa value of ionisable lipids should be low enough so that at physiological pH, the surface charge of the LNPs remains relatively neutral. This dual characteristic allows for the modulation of toxicity and immunogenicity of the resulting LNP, as well as an increase in their circulation time (Cullis and Hope, 2017; Patel et al., 2021).

At the cellular level, the efficient translation of LNP-RNA is hindered by the release of RNA cargo into the cytoplasm of target cells. One proposed mechanism involves the molecular structure hypothesis, wherein cationic ionisable lipids undergo protonation in acidic endosomal environments, leading to interactions with anionic lipids and the formation of non-bilayer hexagonal structures. These structures disrupt the endosomal bilayer, releasing LNP cargo into the cytoplasm. Different cell types exhibit varying endosomal escape mechanisms when transfected with LNP-mRNA, influencing transfection efficiency (Zheng et al., 2023).

3.5.4. Specific organ targeting

Whilst treatment for BD needs to target the brain and eye, future therapies may have to take into account deterioration in other organs, such as the heart (Østergaard et al., 2011). Except for antiviral vaccines, to date, nearly all developed LNPs for mRNA delivery specifically target the liver intravenously because of the natural tendency of LNPs to bind to LDL receptors on hepatocytes. Existing research indicates that Apolipoprotein E (ApoE) present in blood serum exhibits binding capabilities with intravenously injected LNPs. ApoE, essential for lipid transport and metabolism, plays a pivotal role in regulating lipoprotein and cholesterol levels in the plasma through high-affinity interactions with LDL receptors. Given that the liver is the primary organ responsible for clearing ApoE-binding lipoproteins, the systemic administration of LNPs is anticipated to facilitate binding with ApoE, thereby preferentially directing the LNPs to the liver (Fig. 6) (Wang et al., 2023).

While LNP-mRNA therapy is dependent on seroma protein ApoE for liver targeting, studies used different molecules to specifically target other organs, along with standard LNP components to deliver LNP-mRNA to other organs i.e. spleen and lung intravenously. Some studies indicated that incorporation of permanently positively charged 1,2-dioleoyl-3-trimethylammonium-propane lipid (DOTAP) in standard LNP formulation led to a shift in tissue tropism from the liver to the lungs (Lokugamage et al., 2021). Based on these outcomes, studies investigated the incorporation of other lipids with different charge. One such molecule tested was a negatively charged 1,2-dioleoyl-*sn*-glycero-3-phosphate (18PA). When incorporated at levels ranging from 10 % to 40 % in a LNP formulation, this molecule demonstrated specificity for the spleen (Pan et al., 2023). Although the LNP used in these studies have not demonstrated the ability to cross the BBB, recent research developed an LNP-based platform designed to deliver various types of cargo that naturally cannot permeate the BBB. By incorporating neurotransmitter-derived lipidoids (NT-lipidoids) into BBB-impermeable LNPs, several cargos were successfully transported into the mouse brain through systemic intravenous administration. These cargos included amphotericin B (AmB), antisense oligonucleotides (ASO) targeting tau, and the genome-editing fusion protein (−27) GFP-Cre recombinase. While the exact mechanism by which NT-lipidoids enable LNPs to cross the BBB and reach neuronal cells in the brain remains unclear, it is hypothesised that receptor-mediated transcytosis may play a role (Ma et al., 2020).

Numerous studies have demonstrated LNPs as promising carriers for delivering mRNA to treat various rare genetic disease models, such as acute intermittent porphyria (AIP), Methylmalonic Acidemia (MMA), Haemophilia B, cystinosis, and recently for Fabry disease (DeRosa et al., 2019; Martini and Guey, 2019) and MPS-IH (Palanki et al., 2023). Recent studies evaluated the efficiency of MRT using LNPs for Fabry

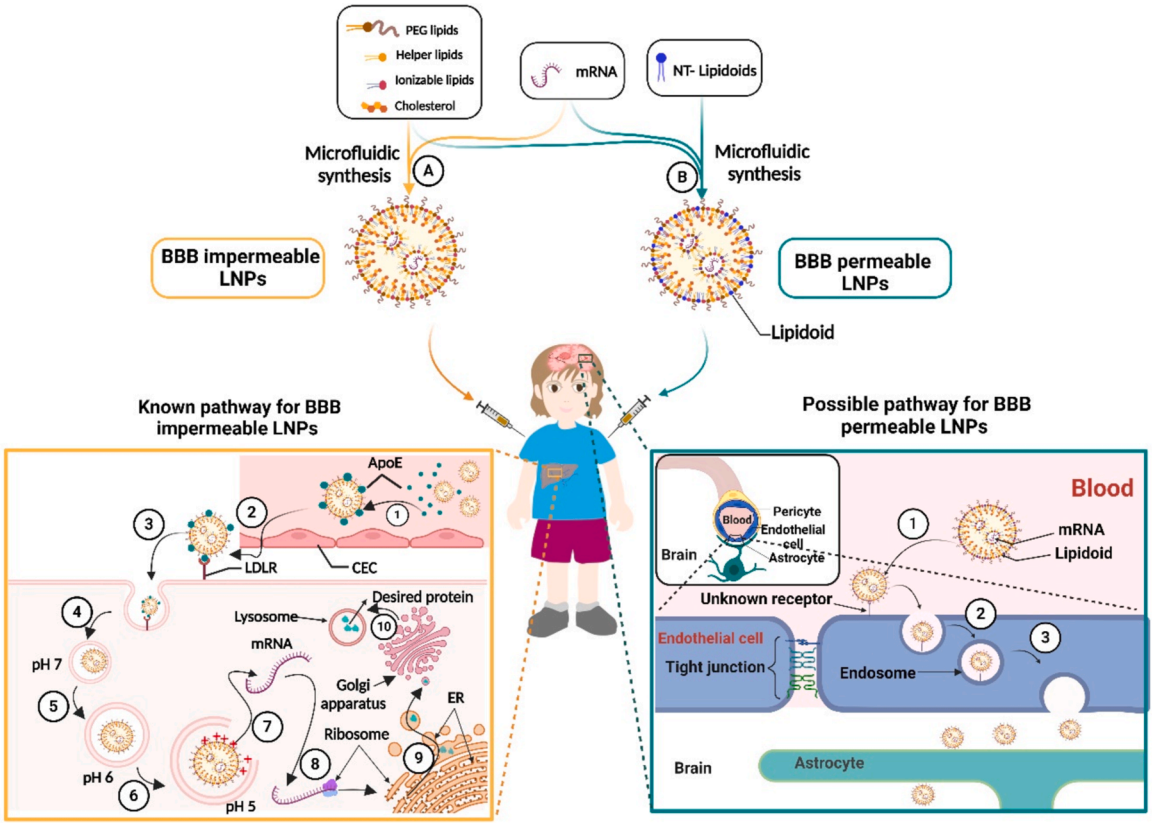

Fig. 6. Two types of LNP structures and their targeting mechanisms in Fabry and Batten diseases. BBB-impermeable LNPs (A) contain pegylated lipids, helper lipids, ionisable lipids, and cholesterol, while BBB-permeable LNPs (B) include additional lipidoids. LNP types can be assembled using microfluidic synthesis. The left orange box shows BBB-impermeable LNPs targeting hepatocytes via ApoE and LDL receptors (LDLR): ApoE directs LNPs to LDLR (steps 1 and 2), initiating endocytosis. Protonation of ionisable lipids by decreasing pH within endosomes disrupts the endosomal bilayer, releasing mRNA (steps 4 to 7), which restores enzyme activity in lysosomes after its translation within endoplasmic reticulum (ER) and reaching Golgi apparatus (steps 8 to 10). The right green box illustrates how lipidoids in LNPs enable BBB crossing: LNPs bind to an unknown neurotransmitter receptor (step 1), undergo transcytosis (step 2), and are released from the abluminal membrane of endothelial cells into the brain (step 3). Created in BioRender. Mole, S. (2024) https://BioRender.com/s08r157.

disease. They have confirmed the efficiency of MRT-LNP therapy for Fabry disease in mouse and non-human primate models as well as in iPSC-derived cardiomyocytes from Fabry-affected individuals (ter Huurne et al., 2023; Zhu et al., 2019).

An additional study investigated the efficiency of a library of LNPs in delivering a luciferase mRNA to the brain of a foetal cynomolgus macaque and neonatal BALB/c mice affected by Mucopolysaccharidosis type I-Hurler syndrome (MPS-IH). Despite the invasive nature of direct injection into the CNS, the study revealed promising results, particularly with the C3 LNP, leading to a 17-fold increase in mRNA expression in the foetal brain compared to the industry standard. The effectiveness of this approach for delivering α-L-iduronidase (IDUA) mRNA was further validated in human patient-derived brain cells affected by MPS-IH in the same study (Palanki et al., 2023).

However, direct CNS injection faces limitations, such as potential infection, tissue damage, diffusion distance, and rapid drug efflux. Although the formulation of LNPs to cross the BBB has not been investigated for LSDs including Batten disease, a recent study demonstrated that a combination of synthetic NT-lipidoids as helper lipids with LNPs presents a promising modality for crossing the BBB and delivering cargo, such as nucleic acids, to the CNS. Twelve NT-lipdoids were synthesised using combinations of three types of neurotransmitters including tryptamine (NT1), phenethylamine (NT2), and phenylethanolamine (NT3) and four types of bioreducible hydrophobic tails with 12 (O12B), 14 (O14B), 16(O16B), 18 (O18B) carbon atoms. It was concluded that the combination NT1-O12B resulted in efficient delivery of Dir fluorescent dye to the brain and bypassing BBB (Ma et al., 2020). Thus, this approach may be applicable to Batten disease.

4. Conclusion and future perspectives

In summary, the utilisation of nanoparticles, extracellular vesicles, and hybrid nanocarriers has demonstrated substantial promise across various lysosomal storage disorders. These nanocarriers have not only facilitated targeted drug delivery but have also extended the shelf life of therapeutic compounds. By employing diverse nanocarrier formulations in clinical practice, there is potential to enhance the quality of life of patients through more convenient administration routes, reduced drug dosages, and mitigated therapy-related side effects. This, in turn, could alleviate the substantial costs associated with other types of therapy, such as the direct enzyme replacement therapy for CLN2 disease which is the only treatment currently in the clinic for Batten disease. Furthermore, the ongoing development of numerous gene therapies for NCLs may benefit from the application of nanocarriers as non-viral vectors for gene delivery.

Despite these advances, several challenges must be addressed to translate nanocarriers into clinical solutions. Manufacturing hurdles such as batch-to-batch variability, low loading efficiency, and difficulties in achieving controlled drug release and stability require innovative solutions. The scalability of production and reproducibility of nanocarriers and extracellular vesicles remain critical bottlenecks. Moreover, logistical concerns related to storage and transport, such as the reliance on ultra-low temperatures (e.g., −20 to –80 °C) for lipid-based nanoparticles, pose additional challenges, increasing costs and complicating distribution (Herrmann et al., 2021; Mehta et al., 2023).

Moreover, nanocarriers have not yet gained approval as a clinical treatment option, primarily due to the need for more comprehensive

mechanistic studies that elucidate the precise mechanisms governing nanocarrier transport to and within cells. Additionally, potential long-term toxicity concerns remain unexplored, necessitating studies in larger animal models for Batten disease, such as sheep or dogs, before considering their application in human subjects.

While nanocarriers have exhibited success in delivering active compounds in multiple cell and murine models across various LSDs, variations in NP/EV transport and passage through the blood and brain or ocular barriers have been observed in different pathologies. Consequently, it is imperative to conduct preliminary investigations into the application of NPs or EVs in cell or murine models specific to Batten disease before drawing definitive conclusions regarding their clinical potential. The composition of the BBB or BOB in Batten disease patients has received limited scrutiny, and it is plausible that disease-related changes in their structure and function are linked to observed neuroinflammation. Moreover, pH alterations have been noted in certain NCLs, underscoring the importance of studying NP drug release mechanisms under these modified disease conditions. It is also crucial to recognise that the encapsulation and delivery success of NPs are contingent on specific drug characteristics, necessitating further research to explore the incorporation and delivery of drugs, perhaps beginning with cerliponase alfa (Brineura) for CLN2 disease.

Lastly, more sophisticated *in vitro* cell models hold promise for assessing the ability of nanocarriers to traverse the BBB or BOB. The application of co-culture cell models comprising endothelial cells, pericytes, and astrocytes may represent a crucial intermediary step before advancing to *in vivo* studies in mammalian disease models. These advancements collectively underline the potential of nanocarriers in revolutionising the treatment landscape of Batten disease and other LSDs, albeit with the recognition of the complexities and nuances inherent to their application.

CRediT authorship contribution statement

Larissa Henke: Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Ali Ghorbani:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation. **Sara E. Mole:** Writing – review & editing, Visualization, Supervision, Funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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We thank Shunping Han for helpful discussion. All figures are original. Figs. 2-6 created in https://BioRender.com have individual publication licenses, as indicated in the figure legends.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2024.125094.

Data availability

No data was used for the research described in the article.

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