Small interfering RNAs based therapies for intracerebral hemorrhage: challenges and progress in drug delivery systems

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Daniyah A. Almarghalani[1, 2], Sai H.S. Boddu[3], Mohammad Ali[2], Akhila Kondaka[2], Devin Ta[2], Rayyan A. Shah[2], Zahoor A. Shah[2, *]

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Abstract

Intracerebral hemorrhage (ICH) is a subtype of stroke associated with higher rates of mortality. Currently, no effective drug treatment is available for ICH. The molecular pathways following ICH are complicated and diverse. Nucleic acid therapeutics such as gene knockdown by small interfering RNAs (siRNAs) have been developed in recent years to modulate ICH's destructive pathways and mitigate its outcomes. However, siRNAs delivery to the central nervous system is challenging and faces many roadblocks. Existing barriers to systemic delivery of siRNA limit the use of naked siRNA; therefore, siRNA-vectors developed to protect and deliver these therapies into the specific-target areas of the brain, or cell types seem quite promising. Efficient delivery of siRNA via nanoparticles emerged as a viable and effective alternative therapeutic tool for central nervous system-related diseases. This review discusses the obstacles to siRNA delivery, including the advantages and disadvantages of viral and nonviral vectors. Additionally, we provide a comprehensive overview of recent progress in nanotherapeutics areas, primarily focusing on the delivery system of siRNA for ICH treatment.

Key Words: intracerebral hemorrhage; lipid-based nanoparticle; nanoparticles; nanotechnology; nonviral vectors; peptide-mediated nanoparticle; polymer-based nanoparticle; siRNA therapeutics; siRNA-barriers; viral vectors

Introduction

Intracerebral hemorrhage (ICH) is a common subtype of hemorrhagic stroke and accounts for the incidence of 10–15% in Europe, 20–30% in the United States, and 20–30% in Asia (van Asch et al., 2010; Feigin et al., 2018). ICH is more common in; Asians, old age people, males, and low to middle-income countries. ICH fatality rate is high, 40% reported within a month and 54% within a year (An et al., 2017). ICH occurs by rupture of the arteries or arterioles, leading to bleeding in the brain parenchyma. ICH-led brain injury is characterized by "primary damage" arising from hemorrhage and hematoma and "secondary damage" caused by a pathophysiologic response to the hematoma and its expansion (Zhao et al., 2020). Although drug discovery and development advancements have touched new heights, ICH management is limited to supportive care and surgical removal/evacuate the blood (An et al., 2017; Zhao et al., 2020).

Since the discovery of RNA interference (RNAi) gene silencing by double-stranded RNA in nematode Caenorhabditis Elegans (C. Elegans) (Manzari et al., 2021), small interfering RNAs (siRNA) were investigated extensively in the laboratories for achieving therapeutic purposes. siRNA is a short double-stranded molecule produced by a specific ribonuclease enzyme called Dicer. It cleaves an endogenous long double-stranded RNA (dsRNA) to smaller fractions known as short dsRNA or siRNA (21 to 23 base pairs in length with low molecular weight ~14 kDa). siRNA consists of sense and antisense strands forming complexes by binding to RNA-induced silencing complex (RISC). The sense strand cleaves away from the antisense strand by Argonaut 2 (AGO-2). The antisense strand guides the RISC to target complementary mRNA for consequent cleavage by the AGO-2-RISC-siRNA complex, thereby knocking off the translation process (Rogers and Phillips, 2020) (**Figure 1**). siRNA's initiate mRNA degradation, gene silencing, and downregulation of the target-specific proteins (Song and Rossi, 2017). The success of the simple siRNA gene silencing technique encouraged basic scientists to translate it into clinical practice and use it as therapeutics for CNS diseases.

siRNA therapy faces various obstacles and problems, such as; stability/degradation in the circulation, rapid excretion by the kidneys, and possible induction of off-target effects (Gavrilov and Saltzman, 2012). Researchers have made considerable development in identifying and testing various delivery systems to overcome these challenges and maximize siRNA therapeutic potency. The success of siRNA therapeutics is exclusively dependent on the delivery systems, classified as viral and nonviral vectors (Marquez et al., 2018). Viral and nonviral vectors differ by their properties: viral vectors are modified viruses designed to carry the desired nucleic acids into their viral genome, such as; lentivirus (Conniot et al., 2021), adenovirus (Li and Wang, 2017), adeno-associated virus (Conniot et al., 2021), and herpes simplex virus (White et al., 2011). Viral vectors of short RNA (shRNA) have shown successful gene silencing properties for various

[1]Department of Pharmacology and Experimental Therapeutics, [2]Department of Medicinal and Biological Chemistry, College of Pharmacy and Pharmaceutical Sciences, University of Toledo, Toledo, OH, USA; [3]Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Ajman University, Ajman, United Arab Emirates

*Correspondence to: Zahoor A. Shah, PhD, zahoor.shah@utoledo.edu.
https://orcid.org/0000-0001-8977-1618 (Zahoor A. Shah)

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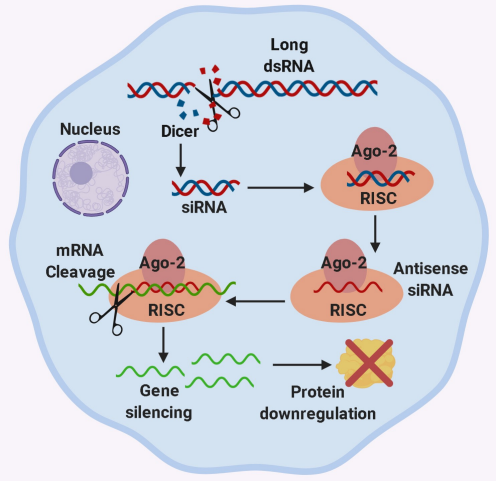

Figure 1 | Schematic representation of molecular mechanism of siRNA-mediated gene silencing.
Long double-stranded RNA (dsRNA) generates small-interfering RNA (siRNA) by the ribonuclease enzyme Dicer. siRNA enters a protein complex called the RNA-induced silencing complex (RISC). Argonaut 2 (AGO-2) cleaves the sense strand away from the antisense strand. The free antisense strand guides the RISC to target complementary mRNA and interfere with the translational process of a specific protein. Eventually, leads to gene silencing and down-regulation of a particular protein.

targets. However, viral vectors are attributed to immunogenicity, manufacturing, and safety concerns (Monty et al., 2021). In contrast, nonviral vectors are based on nanocarriers but use a similar mechanism as viruses to enter the target cell. Therefore, nanoparticle (NP)-based technology has been utilized and modified to overcome the disadvantages and limitations faced with viral vectors (Hardee et al., 2017; Xiang et al., 2017).

siRNA-based therapeutics is projected to treat various diseases, target cells, and/or genetic components (Hajj and Whitehead, 2017). The Food Drug Administration (FDA) and the European Medicines Agency (EMA) approved patisiran (Onpattro®) as a new class of siRNA drugs for the treatment of hereditary amyloidogenic transthyretin amyloidosis (Akinc et al., 2019; Huang, 2019). Recently, the FDA and EMA also approved givosiran (Givlaari®) as the second RNAi-based therapeutics to treat acute hepatic porphyria in adult patients (Agarwal et al., 2020; Scott, 2020). Therefore, in this review, we focus on the use and challenges of siRNA therapeutics and their delivery using viral and nonviral vectors. We also discussed the progress and prospect of NP-based siRNA therapeutics for ICH.

Search Strategy and Selection Criteria

We used the PubMed search from 2010 to the present and selected articles related to siRNA vectors, including viral and nonviral delivery systems.

Challenges and Barriers to siRNA Delivery

Several studies have shown promise in developing RNAi-based therapy for different diseases (Dong et al., 2019) and have emerged as a novel therapeutic approach by silencing the harmful genes using a complementary siRNA (Dua et al., 2019). Local delivery of siRNA has fewer obstacles compared to systemic delivery. Naked siRNA finds its use for local delivery, and its systemic delivery to target-specific genes is challenging (Takahashi et al., 2018). There are many limitations to using systemic delivery of siRNA (**Figure 2**). After intravenous (i.v.) injection, naked siRNA gets rapidly degraded by the nuclease enzyme (RNase) in the blood (Daka and Peer, 2012). Its degraded products stimulate innate immune responses by initiating the release of inflammatory cytokines in a sequence-specific manner. Immune stimulation by siRNA can be reduced by its chemical modifications, such as incorporating 2′-O-methyl or 2′-fluoro groups into ribose or phosphorothioate, which protects siRNA from RNase degradation (Ayadi et al., 2019). Additionally, naked siRNA is removed by glomerular filtration and excreted by the kidneys into the urine within 1 hour of administration (Gargano et al., 2021).

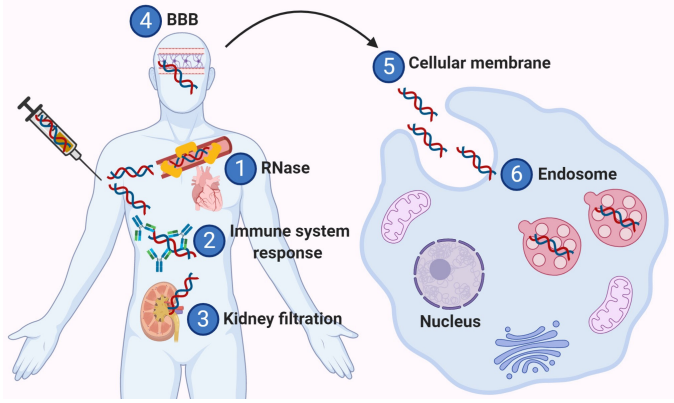

Figure 2 | Schematic illustration of the barriers to siRNA delivery to the brain after systemic administration.
① Degraded in the blood by nucleases (RNase); ② stimulated innate immune response; ③ excreted by the kidney after glomerular filtration; ④ inhibited the transporter to the brain by the blood brain barriers; ⑤ unable to cross the lipid bilayer cell membrane; ⑥ unable to escape the endosome. BBB: Blood-brain barrier; RNase: Ribonuclease; siRNA: small interfering RNAs.

The blood-brain barrier (BBB) is considered the most challenging obstacle for delivering siRNA into the brain. The BBB is a unique structure composed of brain capillary endothelial cells forming a physical barrier due to tight endothelial junctions, which control the import and export of substances in and out of the brain. Lipid soluble molecules with a molecular weight of < 400 Da crosses the BBB, whereas hydrophilic siRNAs with a molecular weight of ~14 kDa do not (Abbott et al., 2010; Banks, 2016). siRNA has to cross the cell membrane of target cells and undergo internalization by an endocytosis process, which encapsulates siRNA into endocytic vesicles to fuse with endosomes. siRNA is released from the endosome into the cytoplasm to bind with RISC and interact with the RNA interference machinery (Neuberg and Kichler, 2014). Cell membranes only allow small hydrophobic or neutral molecules to enter the cells. Therefore, naked siRNA cannot diffuse passively across cellular membranes due to its high molecular weight (14 kDa) and negatively charged phosphate backbone, which produces electrostatic repulsion from the anionic cell membrane surface (Villar-Alvarez et al., 2019). Releasing siRNA from the endosomes is considered a significant challenge to siRNA delivery into the target brain tissue. Thus, siRNA needs to escape from the endosome to avoid hydrolytic degradation. The off-target silencing effect of siRNA is another significant limitation for siRNA therapy. For these reasons, siRNA-based delivery strategies require overcoming the various biological barriers to reach the target cell/tissue and promote its therapeutic effect.

Viral and Nonviral Vector-Based siRNA Delivery for Intracerebral Hemorrhage Therapeutics

The current management of ICH includes supportive therapy and open surgery to evacuate the hematoma (Rajashekar and Liang, 2020). Therefore, there have been attempts to develop an alternative therapy approach as siRNA therapeutics to modulate pathological cascade after ICH. Various delivery approaches are utilized to avoid the difficulties/limitations of siRNA delivery (Schroeder et al., 2010). Viral and nonviral vectors developed over the years have shown differences in safety and efficacy profile. For an ideal siRNA delivery system into the brain, it should be nontoxic, low immunogenic, easy to manufacture, target specific tissue/cell, and efficiently knock down the target-specific gene (Ramachandran et al., 2013). In the next section, we summarize various viral and nonviral delivery systems, including different virus vectors and NP-based nanotechnology, commonly used as siRNA therapeutics for ICH.

Viral vectors

Several viral vectors are used for siRNA delivery into the brain, and multiple viral therapies have been utilized in cerebrovascular diseases, including ischemic stroke, ICH, and SAH. Viral vectors can achieve long and stable shRNA expression with target-specific gene knockdown *in vivo*. Lentivirus (LV), adenovirus (AV), adeno-associated virus (AAV), and herpes simplex virus (HSV) vectors are extensively researched and studied (Gan et al., 2013).

LV

LV is a subclass of the retrovirus family used as a vector for brain gene therapy by integrating into the host cell genome and maintaining long-term gene expression. LV vectors used for siRNA delivery have shown promising outcomes in the preclinical gene therapy in mouse models of Alzheimer's diseases (AD) and amyotrophic lateral sclerosis (Piguet et al., 2021). LV vectors also showed potential therapeutic usage for ICH (Mao et al., 2017; Zhang et al., 2017; Jin et al., 2020) and ischemic stroke (Hu et al., 2011; Mao et al., 2017).

LV-based overexpression to study the role of the different genes in disease conditions has gained prominence. To this end, microRNAs were reviewed and modulated, as they are highly conserved RNA molecules that regulate protein expression and play an essential role in ICH-induced inflammation and microglia activation. Overexpression of miR-132-encoded-lentivirus vectors attenuated the neurological deficit and reduced brain edema in a mouse model of ICH. miR-132 decreased microglia activation, inflammatory cytokines, and cell death and restored BBB integrity (Zhang et al., 2017). A recent study showed that LV-mediated overexpression of miR-26a in the ICH mouse model improved behavioral deficits and decreased proinflammatory cytokines, such as tumor necrosis factor-α, interleukin-1β and interleukin-6 (Jin et al., 2020).

Another molecule modulated by LVs is heat shock protein B8 (HSPB8), which is neuroprotective and maintains BBB stability after ischemic stroke (Li et al., 2019). LV-induced overexpression of HSPB8 significantly improved neurobehavioral outcomes and decreased brain edema after ICH. HSPB8 overexpression reserved BBB integrity by increasing p-AKT, pGSKβ, and β-catenin following ICH (Hou et al., 2020). Overall, LV vectors seem to provide stable and long-term gene overexpression with minimum toxicity and immunogenicity (**Table 1**), making them ideal for ICH therapeutic interventions.

AV

AV vectors are easy to prepare. They can be replicated up to 1013 viral units/ml, making them the most used viral vectors in the clinical trials for cancer gene therapy (Wang et al., 2019). Currently, no clinical trials of AV vectors are going on for ICH. AV vectors-mediated transient gene expression can integrate into the host genome (Nidetz et al., 2020).

The first study using AV vectors identified iron and its modulation. Iron, a hemoglobin degradation product of erythrocyte lysis, activates ICH-induced inflammation and secondary injury. Iron accumulates in the brain after ICH causing neurotoxicity by forming hydroxyl radicals and stimulating oxidative stress (Takahiko et al., 2019). Serum iron or ferritin is used to assess clinical outcomes in ICH patients (Yang et al., 2016). Hepcidin is responsible for maintaining the homeostasis of iron and plays a central role in host defense mechanisms (Prchal, 2017). Yang et al. (2021) tried AV-mediated overexpression of hepcidin and observed a significant decrease of iron in the brain after ICH by suppressing iron transport protein expression, which resulted in reduced brain edema, cell death, and ROS, and improved cognitive functions.

AVs were also tried to stop neuroinflammation, as inflammation is the main culprit in ICH-induced secondary injury (Tschoe et al., 2020). Another study demonstrated that AV vector-mediated overexpression of the interleukin-1 receptor antagonist attenuated brain edema and thrombin-induced neuroinflammation after ICH (Wilkinson et al., 2018).

AAV

AAV is used as a vector for brain gene therapy by providing transduction efficiency in ependymal cells (Hocquemiller et al., 2016), endothelial cell (Merkel et al., 2017), neurons (Naso et al., 2017), glia (Gray et al., 2011), and astrocytes (Zhang et al., 2011). AAV has emerged as a preferred viral vector for suppressing a single mutant gene in Huntington disease (Zhang and Friedlander, 2011) and showed a good safety profile in Parkinson's diseases (Eberling et al., 2008; LeWitt et al., 2011) and late infantile neuronal ceroid lipofuscinosis (Souweidane et al., 2010). Currently, AVV is the most commonly used viral vector for CNS clinical trials (Choudhury et al., 2017). However, no clinical trials are reported so far for ICH, and the studies are limited to the preclinical stage only.

In one of the studies, heme, a component of hemorrhage, was targeted using AAVs. Heme toxicity is part of secondary injury after ICH causing irreversible brain damage and neurological deficits (Leclerc et al., 2015). Hemopexin (Hpx), an endogenous glycoprotein that binds heme and neutralizes its proinflammatory properties, fails to reduce the vast heme overload after ICH (Smith and McCulloh, 2015). Leclerc et al. (2018) evaluated the neuroprotective effect of high Hpx levels after ICH using a recombinant AAV vector. rAAV-induced Hpx overexpression in the brain decreased hematoma volume, lipid peroxidation, astrocyte activation, and improved neurological deficits suggesting a clinical-grade possibility of rAAV-induced Hpx for ICH treatment.

AAV technique was also tried to modulate peroxiredoxin 1 (PRDX1), an RNA-binding protein vital for post-transcriptional regulation of inflammation (Liu et al., 2014) and apoptosis (Lu et al., 2019). Yang et al. (2020) investigated the role of AAV-mediated overexpression of PRDX1 in a rat model of ICH. Overexpression of PRDX1 in the striatum significantly decreased the inflammation and apoptosis after ICH. Altogether, these studies showed that the AVV vector delivery system is an efficacious method in preclinical ICH studies.

HSV

There are two types of herpes simplex virus (HSV) vectors: recombinant virus vectors and amplicon vectors. Recombinant HSV vectors have been used in clinical trials to treat brain tumors, whereas amplicon HSV vectors showed promising results only in animal studies (Manservigi et al., 2010). HSV's were studied and modified in various disease conditions targeting the potential molecules of interest. HSV-mediated overexpression of glucose transport improved striatal neuronal survival after transient focal cerebral ischemia in rats (Gan et al., 2013). A study showed that HSV vectors expressing 72-kD heat shock protein with lacZ as a reporter after cerebral ischemia significantly attenuated the ischemic injury and improved the survival of striatal neurons (Kim et al., 2018). Besides, HSV overexpression of Bcl-2 also protected neurons from focal cerebral ischemia (Rhim et al., 2013). However, HSV vector-mediated gene therapy in ICH is yet to be published.

Limitations of viral vectors

Although viral vectors provide excellent tissue-specific RNAi efficiency, difficulties in achieving high-scale manufacture and potential toxicity limit their use as siRNA vectors and jeopardize their safety profile (**Table 1**; summarizes the advantages and disadvantages of viral vectors). Therefore, nonviral vectors were utilized and became famous, and wifely used alternatives in no time due to their safety and ease of being prepared and delivered on a large scale (Patil et al., 2019).

Nonviral vectors

Most of the interventional studies used viral vectors for siRNA delivery. These vectors have several drawbacks; thus, nonviral vectors were tried and exploited to avoid these drawbacks (Chen et al., 2020b). Nonviral vectors have many advantages over viral ones, including low toxicity, no risk of infection, and low possibility of immunogenicity (Nayerossadat et al., 2012). Nonviral vectors are biocompatible, nontoxic, able to protect siRNA from RNase, provide immune response interaction, and avoid renal filtration. Furthermore, nonviral vectors can cross the cell membrane and escape the endosomal degradation to achieve the target-specific gene knockdown (Raftery et al., 2013). They have an excellent safety profile to achieve efficient-specific targeted delivery and have efficacy similar to viral vectors. A wide variety of nonviral delivery systems such as polymer-based NPs (chitosan, and poly-lactide-co-glycolide (PLGA), peptide-based NPs, dendrimers, and lipid-based NPs (cationic solid lipid NPs and liposomes) are in use for the delivery of siRNA into the brain. The studies related to nonviral vectors used as potential carriers for siRNA delivery, their advantages and disadvantages are given in **Table 2**.

The highly dynamic and complex ICH damage mechanisms require a better target and efficient treatment to ensure proper therapeutic utilization for ICH patients. Primary and secondary injury phases further complicate brain damage, and developing a therapy for ICH poses a significant challenge (Shao et al., 2019). Recently, gene therapy using naked siRNA emerged as a promising treatment option for targeting ICH in animal studies (Zeng et al., 2017; Zhu et al., 2018; Wu et al., 2019; Chen et al., 2020a; Fu et al., 2020; Wang et al., 2020). However, limitations of naked siRNA's stability and delivery have limited its clinical applicability. Therefore, developing sophisticated technology such as nonviral vectors to avoid siRNA barriers will improve the systemic siRNA delivery. In the next section, we review the progress of NP-based siRNA therapeutics for ICH treatment.

Table 1 | **Advantages and disadvantages of viral vectors**

Viral vectors	Advantages	Disadvantages	References
Lentivirus (LV) 	- Low toxicity - Low immunogenicity - Infect both mitotic and non-mitotic cells - Long term expression - Stable gene expression	- Potential insertional mutagenesis - Gene disruption risk due to large size and complexity - Medium genomic integration (7–8 kb of genetic capacity)	Hutson et al., 2014
Adenovirus (AV) 	- No insertional mutagenesis - Long term expression - Infect both dividing and non-dividing cells (efficient penetration into differentiated cells)	- High immunogenicity - Transient gene expression - Dose-dependent hepatotoxicity - Medium genomic integration (genetic capacity: 20 kb for recombinant AV and up to 36 kb for gutless AV)	Wold and Toth, 2013
Adeno-associated virus (AAV) 	- Low toxicity - Low immunogenicity - Stable gene expression - Infect both dividing and non-dividing cells - Long term expression - Non-pathogenic	- High immunogenicity - Slow onset of gene expression - Low genomic integration (4.7 kb of genetic capacity)	Borel et al., 2014; Zinn and Vandenberghe, 2014
Herpes simplex virus (HSV) 	- Low toxicity - Low immunogenicity - Moderately-prolonged transgene expression - Safe to use in immunocompromised patients	- Display cytopathic effects due to IE gene products - High genomic integration (genetic capacity: 40 kb for recombinant HSV and 150 kb for amplicon HSV)	Neve, 2012; Vannucci et al., 2013

Table 2 | **Advantages and disadvantages of nonviral vectors: nanoparticle-based nanotechnology**

Nonviral vectors	Advantages	Disadvantages	References
Polymer-based-nanoparticles (Chitosan) 	- Biodegradable - Biocompatible - Low toxicity - Have several free amine groups that are available for crosslinking - Improve membrane absorption and cellular interaction - Low cost of production	- High molecular weight limited their use in clinical trials - Low solubility at physiological pH	Alameh et al., 2012; Lee et al., 2013
Polymer-based-nanoparticles (PLGA) 	- Biodegradable - Biocompatible - Nontoxic - FDA approved - High solubility - Prolong controlled release - Improve endosomal escape	- Lower electrostatic interactions between poly(L-lactide-co-glycolide)-nanoparticles and siRNA (low efficacy)	Singha et al., 2011; Yuan et al., 2011; Anderson and Shive, 2012; Nitta and Numata, 2013
Peptide-mediated nanoparticles 	- Low toxicity - High binding strength (high potency) - High target selectivity - Increase circulation time - Protect from RNase degradation	- Immunogenicity - The high cost of production - Low solubility	Martinez-Veracoechea and Frenkel, 2012; Marqus et al., 2017; Jeong et al., 2018
Dendrimers 	- Biodegradable - Monodispersity - Higher water solubility - Ability to link drugs - Encapsulation ability - Presence of surface functionalizable groups like $-NH_2$, –COOH, and –OH groups	- Toxicity of cationic dendrimer with terminal poly(L-lysine) and poly(propylene imine) group	Li et al., 2017; Zhu et al., 2019
Lipid-based nanoparticles (Solid lipid nanoparticles) 	- Biodegradable - Biocompatible - Low toxicity - Ease of production without organic solvents - Ease of large-scale production - Good storage stabilities	- The high cost of production - Toxicity at high dose	Liu and Zhang, 2011b; Dizaj et al., 2014b
Lipid-based nanoparticles (Liposomes) 	- Biodegradable - Biocompatible - Nontoxic - Low immunogenicity - Ease of large-scale production - FDA approved (Onpattro®) - Prolong controlled release - Improve endosomal escape	- The high cost of production - Toxicity at high dose	Liu and Zhang, 2011a; Ramos-Cabrer and Campos, 2013; Ozpolat et al., 2014; Xia et al., 2016

Nanoparticle-based nanotechnology

Nanotechnology-based carrier systems such as NPs are considered efficient carriers for transporting drugs into the brain (Shakeri et al., 2020). NPs made of polymeric materials are solid particles ranging in size from 1 to 100 nm in at least one dimension (Bachu et al., 2018). NPs made of biodegradable and biocompatible polymers are nontoxic, nonimmunogenic, and remain in the blood for a longer time when surface-modified with proper ligands (Zhou et al., 2018). The size and surface charge of particles affect their cellular uptake and cytotoxicity. Particles of 200 nm length with a positive charge are more suitable for cell uptake. In contrast, particles smaller than 5 nm are not ideal as they are eliminated from the circulation by renal excretion. Recently, a breakthrough study reported siRNA brain delivery through nanotechnology in both animals and humans. Davis et al. conducted the first siRNA clinical trial in melanoma patients with systemic administration of siRNA loaded on a targeted nanoparticulate system. This study concluded that nano-delivery systems have the potential for gene therapy for various diseases (Davis et al., 2010).

Nanotechnology holds a grand promise for making siRNA a successful therapeutic modality for ICH. An advantage of nanostructured particles is their malleability for siRNA loading and protection, such as redesigning size, shape, and surface for a long half-life in the blood circulation. They can further be bio-functionalized with appropriate ligands for precise targeting. Nano-formulations of NPs are created by electrostatic interaction, entrapment, or conjugation. Modifying nano-formulation surfaces and adding delivery carriers assist naked siRNAs in crossing the BBB. Delivery carriers protect siRNA against nuclease break down by impeding the interaction between siRNA and RNase enzymes in the blood (Resnier et al., 2013; Zheng et al., 2018). Different types of NPs developed as carriers for siRNA delivery are; polymer-based NPs, peptide-based NPs, dendrimers, and lipid-based NPs. In the next section, we present several NP delivery systems used in treating CNS diseases, including ICH.

Polymer-based nanoparticles

Polymeric NPs have shown to be promising carriers for siRNA brain delivery. Polymeric NPs can potentially encapsulate siRNA, hence protecting it from RNase degradation and renal excretion and delivering them across the BBB to target desired cells by surface functionalization (Teleanu et al., 2019). The only FDA-approved polymers employed to design NPs include; natural polymers (e.g., chitosan) and synthetic polymers (e.g., poly (butyl cyanoacrylate), poly(lactic acid) (PLA), and PLGA. PLGA is a copolymer of PLA and polyglycolic acid. Polymer-based NPs carrying siRNA, siG12D-LODER combined with chemotherapy reached the clinical trials in patients with locally advanced pancreatic cancers (Varghese et al., 2020). However, polymer encapsulated siRNA in ICH has not moved beyond preclinical studies.

Chitosan

In recent years, chitosan's exciting properties have led to various siRNA-loaded chitosan NP delivery systems. Chitosan is a biocompatible, biodegradable cationic polymer with low immunogenicity. It has many functional groups for targeting ligand modification (Zhang et al., 2016). Chitosan uses in gene delivery are primarily due to its ability to form electrostatic interactions between positively charged amino groups of chitosan and negatively charged nucleic acid such as siRNA (Cao et al., 2019). Studies indicate a high transfection potential of chitosan NPs for siRNA delivery due to their ability to escape endosomes in the cytosol (Cao et al., 2019). The properties of chitosan NPs are further modified through graft copolymerization with polyethylene glycols (PEG). Chitosan NPs and PEGylated chitosan NPs also find their use in brain gliomas, AD, Parkinson's disease, and cerebral ischemia (Yu et al., 2019). Chitosan has mucoadhesive and permeation-enhancing properties, which makes it suitable for increasing the nose-to-brain transport drugs. Guo et al. (2019) reported that chitosan encapsulated nicardipine dispersed in blending hyaluronan and methylcellulose gel showed a neuroprotective effect in ICH mouse model. Nicardipine, a calcium channel inhibitor, has an anti-inflammatory effect on microglial activation and provides neuroprotection in mice challenged with lipopolysaccharide (Huang et al., 2014). Intranasal administration of chitosan-nicardipine gel reduced brain edema and neuronal apoptosis, suggesting a rapid drug transport from nose to brain and providing a new and efficient delivery route (Guo et al., 2019).

Similarly, intranasal administration of chitosan-based NPs loaded with anti-HTT siRNA was observed to be safe and effective in lowering HTT gene expression by at least 50% in a transgenic YAC128 mouse model of HD (Sava et al., 2020). Thus, intranasal administration of chitosan-based-NPs carrying siRNA can become a promising therapeutic strategy for CNS diseases and ICH. Nevertheless, over the last few years, limited progress has been made in treating neurologic conditions, including ICH with intranasal administration of chitosan NPs. Therefore, more preclinical and clinical studies are needed to determine the safety and efficacy of siRNA-loaded chitosan NPs.

PLGA

PLGA-NPs have found their use as nanocarriers for genetic material such as plasmid DNA and siRNA. PLGA-NPs can encapsulate both hydrophobic and hydrophilic drugs, depending on the NP preparation method (Sharma et al., 2016). The advantages of PLGA-NPs to deliver siRNA are; low toxicity, ability to undergo endocytosis in target tissues, and sustained release of drugs. In one study, solid lipid NPs and PLGA NPs were compared for their efficacy in delivering siRNA to the brain. PLGA NPs (size ~115 nm) showed 50% of siRNA association efficiency, whereas SLNs (size ~150 nm) exhibited 52% association efficiency. Functionalization of NP surface with a peptide-binding transferrin receptor resulted in better interaction of NPs with brain endothelial cells. The interaction of surface-modified NPs with brain endothelial cells increased 4-fold for PLGA NPs and 3-fold for SLNs. *In vitro* study demonstrated the safety and efficacy of NPs to deliver siRNA to human brain endothelial cells while protecting them from enzymatic degradation (Gomes et al., 2017). Two approaches are in wide use for uploading siRNA into PLGA NPs: encapsulation into the NP core and adsorption onto the NP surface via electrostatic interactions. The hydrophobic nature of PLGA reduces the entrapment of hydrophilic siRNA inside NP and inhibits the PLGA electrostatic interaction with siRNA. Coating PLGA-NPS with polymers such as chitosan and polyethyleneimine (Singha et al., 2011) can help to overcome the limitation. Polymer coating increases the electrostatic interactions, particle stability controls drug release (Wang et al., 2013), and enhances cellular uptake of the PLGA-NPs (Chronopoulou et al., 2013; Zhao et al., 2014; Pandit et al., 2017). For example, poly (ethylene glycol)-block-poly (D,L-lactide) (PEG_{5K}-PLA_{8K}) loaded with C3-siRNA effectively penetrated the BBB and reduced the expression of C3 in microglial cells, and effectively crossed the BBB. PEG_{5K}-PLA_{8K} loaded with C3-siRNA effectively decreased the inflammatory cells and pro-inflammatory markers in the penumbra, resulting in improved behavioral and pathological outcomes after brain ischemia/reperfusion injury (Wang et al., 2018). Surface modification of PLGA NPs with fusion proteins that target tissue factors, a plasma membrane glycoprotein, is another promising strategy for siRNA delivery. Chen et al. (2013) studied PLGA NPs surface modification with EGFP-EGF1 (ENPs) for targeted delivery of TF-siRNA to the injured brain microvascular endothelial cells. The study concluded that TF-siRNA-loaded ENPs had minimal toxicity (~96% of cells viable 24 hours after transfection), whereas only 75% of cells survived when transfected with lipofectamine (Chen et al., 2013). Another study demonstrated that encapsulated tissue plasminogen activator into Fe_3O_4-based PLGA-NPs coated with cRGD grafted chitosan showed improved thrombolytic activity (Hassanpour et al., 2020). The versatile nature of PLGA NPs makes them suitable for siRNA delivery in treating ICH and other CNS diseases. The area of ligand conjugation requires further progress. Ligands such as low-density lipoprotein, cholesterol, insulin, iron-transferrin, and metabolic nutrient transporters, conjugated onto the NP surface to achieve the unlimited potential of PLGA NPs for delivering siRNA across the BBB is needed (Shakeri et al., 2020). To date, no studies investigated the efficacy and safety of PLGA NPs for the treatment of ICH in either *in vitro* and *in vivo* models.

Peptide-mediated nanoparticles

Peptides have gained increasing popularity as a siRNA delivery system in CNS diseases. They are less toxic, avoid degradation and provide targeted delivery of drugs by circumventing rapid efflux mechanisms in the brain to cross the BBB (Oller-Salvia et al., 2013). Brain drug delivery can be enhanced by combining nanocarriers with peptide shuttles. Peptides are small molecules that display various functions such as siRNA binding, membrane infiltration, endosome interruption, and targeted delivery, qualities that are critical for the targeted delivery of siRNA. Ifediba et al. (2010) used complexing fluorescent-labeled myristoylated polyarginine peptides to siRNA for entering the primary cells. This complex facilitated probe penetration into the cells while avoiding degradation from serum RNase. Using this complex directly against c-Src, a protein associated with stroke pathology, reduced endogenous mRNA of c-Src in primary cortical

neurons, brain endothelial cells, and astrocytes. These results suggest that siRNA-peptide probes can be a beneficial tool for imaging and therapeutic applications (Ifediba et al., 2010). A study showed for the first time that a self-assembling peptide nanofiber dramatically achieves homeostasis when injected/applied directly into the brain, spinal cord, liver, or skin (Zhang et al., 2021). Moreover, Alvarez-Erviti et al. (2011) showed that siRNA encapsulation within an exosome fused to rabies virus glycoprotein (RVG) peptide showed a significant increase siRNA uptake and gene knockdown within the targeted neuronal cells without facing host rejection. The exosomes were collected from mouse dendritic cells and engineered to express an exosomal membrane protein (Lamp2b). The exosome fuses with the neuron-specific RVG peptide for targetability.

NPs have displayed their potential ability to conjugate with peptides to improve their functions and provide synergistic effects. As a result, peptide-NP conjugates have emerged as a vital tool for various therapeutic applications (Tai and Gao, 2017; Jeong et al., 2018). Peptide-chitosan systems increase siRNA accumulation in the brain. For example, a peptide derived from RVG and linked to the siRNA/trimethylated chitosan (TMC) complex through a PEG linker to siRNA was tested for targeted delivery to the brain. The RVG-siRNA-TMC-PEG complex showed efficient binding to acetylcholine receptors of neuron cells and was associated with high cellular uptakes, high serum stability, and no toxicity. *In vivo* imaging showed accumulation of Cy5-siRNA in the mouse brain after administration of RVG-siRNA-TMC-PEG complexes (Gao et al., 2014). A different study confirmed the successful delivery of siRNA-peptide tagged PEG-chitosan NPs to the thalamus, hypothalamus, hippocampus, and Purkinje cells after intranasal administration. The results indicate efficient delivery of siRNA to the brain with no cellular toxicity, suggesting the potential of siRNA-peptide tagged PEG-chitosan NPs to treat neurological diseases (Malhotra et al., 2013). Another delivery method involves encapsulating the siRNA within an NP that expresses specific targeting peptides. For example, a previous study has shown that transferrin receptor monoclonal antibody conjugated chitosan nanosphere was used as an efficient delivery system of the peptide into the brain. The results showed that the peptide treatment decreased the infarct volume, caspase-3, and neurological deficits after ischemic stroke (Gosselet et al., 2021).

Peptide-based NP systems for siRNA can help endosomals escape after cellular internalization (Ren et al., 2012). A NP system is constructed by complexing siRNA with a peptide with two components [a membrane-interactive peptide, N-terminal transportation (TP-RVG), and a targeting peptide, N-terminal targeting peptide (RVG-TP)]. Kwon et al. (2016) demonstrated the effectiveness of peptide-based NPs to deliver siRNA in traumatic brain injury. By engineering NPs to contain neuron targeting peptides derived from RVG, Kwon et al. (2016) showed that i.v. administration of NPs crosses the already damaged BBB due to brain injury and precisely targets the neurons.

Mann et al. (2016) found similar results by using NPs coated in the tetra-peptide, CAQK, to deliver siRNA to the injured brain using two animal models; penetrating brain injury and traumatic brain injury. After systemic administration, the CAQK peptide recognized the injury areas in both models, suggesting its high efficiency and targeting across brain injury tissues (Mann et al., 2016). Zheng et al. (2017) formed a hybrid system for amyloid plaques targeted siRNA to help treat AD. The introduction of hybrid of PEGylated poly (2-N, N-(dimethylamino) ethyl methacrylate) (PEG-PDMAEMA) was conjugated with two d-peptides; a QSH-peptide for β-amyloid binding and a CGN-peptide for BBB penetration. The amount of CGN-PEG-PDMAEMA and QSH-PEG-PDMAEMA used in making the hybrid was optimized to achieve the highest BBB penetration. It showed low cytotoxicity, high transfection efficiency, and protected siRNA from enzyme break down. This study concluded that CQ/siRNA crosses the BBB with assistance from CGN and binds to plaques with the help of QSH. This dual functional ability was evaluated *in vivo*, and as expected, it complexes with CGN and accumulates at higher levels in the brain after injection and remained intact after crossing the BBB. Thus, the dual-functional complex has the potential to deliver siRNA into the lesions associated with AD accurately (Zheng et al., 2017). Re-engineering of NPs using a peptide ligand appears to be a promising approach. This strategy has not been explored extensively for siRNA delivery to the brain and, in particular, to ICH. More work is needed to identify newer BBB shuttle peptides that can efficiently cross BBB along with siRNAs and provide treatment options for ICH.

Dendrimers

Dendrimers are star-shaped nano-constructs made up of poly (amidoamine) (PAMAM) polymers. They have unique physical and chemical properties such as monodispersity, higher water solubility, ability to conjugate drugs, encapsulation ability, and the presence of surface functionalize groups like –NH2, –COOH, and –OH groups. The surface groups can be functionalized for the sustained delivery of both lipophilic and hydrophilic molecules (Natesan et al., 2020). These properties make dendrimers a perfect platform for the delivery of drugs to the brain. PAMAM polymers allow the formation of various generations of "G" dendrimers (G_0, G_1, G_2, G_3, G_4, G_5, and so on). The molecular weight, the number of atoms, terminal primary amine groups of PAMAM dendrimers increase exponentially for each generation, while the increase in radius is linear (~10 Å/generation) (Araújo et al., 2018). One limitation related to PAMAM dendrimers is their cytotoxicity, which can easily be terminated with +vely charged groups. Dendrimers can be delivered systemically or intranasally for the delivery of drugs to the brain.

Kim et al. (2012) studied the therapeutic effect of siRNA delivery via intranasal administration in the postischemic rat brain using high mobility group box 1 (HMGB1) as a target gene. PAMAM dendrimer was used as a gene vector through complexation with siRNA. After 1 hour following intranasal administration, fluorescent-labeled siRNA was found in the cytoplasm of glial cells of amygdala, cerebral cortex, and striatum. Moreover, HMGB1 siRNA decreased the cerebral infarct volume in rats by ~43%, showing dendrimers' potential in nasal siRNA drug delivery to the brain (Kim et al., 2012). Surface modification of dendrimers to increase their affinity towards transferrin receptor, highly expressed on both BBB and glioma, was a successful strategy for dual glioma targeting. G3 dendrimers (dendri-grafted poly-l-lysine) surface modified with HAIYPRH (T7) peptide as a ligand showed high transfection efficiency and low toxicity and stability in glioblastoma (U87) cells (Kuang et al., 2013). In a more recent study, Stenström et al. (2018) explored amino-functional polyester dendrimers (G_1–G_4 generations) based on 2,2-bis(methylol)propionic acid (bis-MPA) for siRNA delivery in glioma (C6) and glioblastoma (U87) cell lines. No cytotoxicity was observed in astrocytes, C6, and U87 cells. G_3 and G_4 exhibited concentration-dependent toxicity in primary neurons, whereas G_2 showed no toxicity in primary neurons with any of the tested concentrations. G_2–G_4 proved to be capable of siRNA complexation and protection against RNase-mediated degradation. Gene transfection proved successful in both rat C6 cells and human U87 with G_3 and G_4 dendrimers as indicated by the reduction in ~20% of target protein, p42-MAPK (Stenström et al., 2018). Dendrimer technology has paved a new path for delivering small molecules, macromolecules, genes, and oligonucleotides to the brain in a controlled fashion. Although dendrimer-based systems are yet to be proven clinically, the results from *in vitro* and *in vivo* studies exhibit great promise and provide hope for a new delivery system in the future. Specifically, the intranasal dendrimer delivery system holds promise as the best delivery system to treat CNS diseases and ICH and circumvents the degradation of the cargo when given by systemic administration.

Lipid-based nanoparticles

Lipid-based NPs such as cationic solid lipid NPs and liposomes are the most widely studied gene vectors due to their phenomenal biodegradability and biocompatibility (Torrecilla et al., 2014). These lipid-based NPs were initially designed to overcome conventional drug delivery system challenges, mainly poor solubility. Lipid-based NPs can protect siRNA from RNase and increase their circulation time, resulting in higher efficiency for target-specific gene knockdown (Gomes-da-Silva et al., 2012). The first validation of lipid-based NPs for delivering siRNA to neurons was demonstrated in cell cultures and animal brains following intracranial injection. The *in-vivo* results indicated that siRNA-lipid-based NPs downregulated the target genes either within the injection site or in widespread regions with no apparent systemic toxicity or immune response (Rungta et al., 2013). Another study also showed that knockdown of SLC26A1 (a voltage-gated channel activated upon neuronal depolarization, cause neuronal swelling and cytotoxic brain edema) by siRNA-lipid-based NPs significantly attenuated the neuronal swelling and cell death (Rungta et al., 2015). These studies highlight the potential of lipid-based NPs for siRNA delivery in CNS diseases. More studies are warranted to identify the potential of lipid-based NPs in the treatment of ICH.

Cationic solid lipid nanoparticles

Cationic solid lipid NPs are considered the most effective lipid-based

NP vectors for systemic administration of siRNA-based therapy. They are composed of highly compatible lipids as surfactants surround the core as covered shells, which condense DNA into colloidal NPs (Dizaj et al., 2014). Cationic solid lipid NPs used for DNA delivery are now used for siRNA delivery (Yonezawa et al., 2020). The positive charge of cationic lipids interacts electrostatically with the siRNAs negative charge (Schroeder et al., 2010). As a carrier for siRNA delivery, cationic solid lipid NPs offer several advantages: ease of manufacturing process and large scale production, low toxicity, biocompatibility, biodegradability, and chemically modified PEG prolonged circulation and increased target-specific cellular uptakes (Schroeder et al., 2010). For example, Jin et al. (2011) demonstrated the utility of cationic solid lipid NPs, produced by the natural components of low-density lipoprotein conjugated with PEG, as c-Met siRNA carrier. The PEG-c-MET-siRNA solid lipid complex significantly down-regulated the expression of c-Met (an oncogene overexpressed in glioblastoma patients) in both *in vitro* and *in vivo* models. After i.v. administration in a mouse model of glioblastoma (ortho-topic U-87MG xenograft tumor model), the PEG-c-MET-siRNA solid lipid complex crossed the BBB, reached the tumor tissue, and inhibited tumor growth with no apparent systemic toxicity (Jin et al., 2011). Cationic solid lipid NPs are considered effective nanocarriers for the delivery of genetic material and low water-soluble compounds. Besides, excipients used in cationic solid lipid NPs are considered safe and nontoxic. However, in a recent study, the toxicological profile of cationic solid lipid NPs was assessed in male Wistar rats after 24 and 72 hours of a single i.v. injection. Solid lipid NPs were made of stearic acid, DOTAP, and pluronic. Hematological, biochemical, and histopathological evaluations of the spleen, lungs, and liver indicated short-lived alterations in neutrophilia, increased macrophages, and migration of circulating neutrophils into the inflamed tissue, and a decrease in blood urea nitrogen. The presence of cationic solid lipid NPs was found in the brain parenchyma without any sign of damage to the BBB (Mendonça et al., 2020). These findings suggest the need to reinvestigate the toxicity of excipients used in cationic solid lipid NPs before these are tested for CNS diseases and ICH.

Liposomes

Bangham discovered liposomes in 1965, which were considered the first generation of nano-drug carriers in 2001 (Bozzuto and Molinari, 2015). Nowadays, liposomes are the most widely studied vectors for siRNA delivery for systemic administration due to their efficient biodegradability, biocompatibility, low toxicity, and BBB permeability (Ozpolat et al., 2014). Liposomes can encapsulate both lipophilic and hydrophilic drugs and increase their circulation life-time (Ramos-Cabrer and Campos, 2013). The factors that play a role in the liposomal siRNA delivery include; the liposomal size and surface charge, the degree of PEGylation, the structure of targeting ligands, and the fusogenicity of liposomes. The surface charge of liposomes determines their circulation time in the blood. Neutral liposome carriers are typically stable, and the circulation time is longer than that of charged liposomes. However, during the cellular uptake and endosomal escape stage of cancer cells, cationic liposomes show better results than neutral and anionic liposomes, which show the least efficiency. PEGylation of liposomes is achieved either by incorporating PEG-conjugated lipids while preparing liposomes or mixing PEG-lipid aqueous dispersions with preformed liposomes. PEGylation of liposomal carriers increases stability and circulation time to meet siRNA delivery requirements (Verma and Stellacci, 2010; Xia et al., 2016).

PEGylated liposomes have shown significant improvement in the neuroprotective activity of various drugs in animals following ischemic stroke. Peng et al. (2013) developed Xenon (Xe) liposomes containing echogenic liposomes. The Xe-liposomes provided neuroprotection after middle cerebral artery occlusion in rats. Xe-liposomes reduced infarct size and neuronal cell death and increased BDNF, assisting in the survival of existing neurons (Peng et al., 2013). Another study demonstrated the efficacy of PEGylated liposomes encapsulating FK506 after i.v. administration in the rat middle cerebral artery occlusion model. PEGylated-FK506-liposomes significantly decreased oxidative stress, cell death, and motor deficits, suggesting a neuroprotective effect in ischemia (Fukuta et al., 2015). The FDA and the EMA approved Onpattro® as the first chemically modified TTR-siRNA formulated in liposomes in August 2018 to treat hereditary amyloidogenic transthyretin amyloidosis in adult patients (Akinc et al., 2019; Huang, 2019). Despite unlimited advantages with nanotechnology to enhance neuroprotection of siRNA based drugs, particularly with liposomes, no nanomedicine is currently available in the clinic to manage CNS diseases, including ICH. Nevertheless, these studies have shown the effectiveness of functionalized liposomes in siRNA delivery to the brain. The unique characteristic of liposomes, such as the BBB's high permeability with low immunogenicity and toxicity, makes them an effective nonviral vector for siRNA delivery for ICH treatment.

Current Status and Future Perspectives of Nanoparticle-Based Therapeutics in Intracerebral Hemorrhage

In the perspective of ICH therapy, notable studies have been highlighted showing the worth of NP-based nanotechnology in diagnosis and therapeutic applications for ICH. Thus, the researchers have employed NP-based nanotechnology at the preclinical level to improve diagnostic and therapeutic outcomes in ICH (Siaw-Debrah et al., 2017). For example, magnetic particle imaging (MPI), a novel imaging technique that uses free-radiation tomographic imaging, provides an excellent resolution for monitoring whole-brain perfusion in humans. Szwargulski et al. (2020) investigated whether MPI works in visualizing the hematoma expansion in ICH mice. After mice were subjected to intra-striatal collagenase injection-induced ICH, an MPI-tailored tracer was administered i.v. to detect the ICH. ICH location was found in less than 3 min, and hematoma expansion monitoring was performed in real-time. Multi-contrast MPI was applied to differentiate the area of blood coagulation within the hematoma, providing critical information in making the surgical decision. The study provided a new diagnostic method for monitoring ICH expansion using MPI nanotechnology (Szwargulski et al., 2020).

Additionally, a recent clinical trial investigated the effect of NP-encapsulated edaravone in patients with ICH who underwent minimally surgical removal of hematoma. The results showed that the NP-encapsulated edaravone group had significantly enhanced neurological benefits and decreased peripheral inflammatory cytokines after 15 days of treatment compared with the edaravone alone treatment group. It was concluded that NP-encapsulated edaravone has a beneficial effect on the neurological functions of patients with ICH (Dang et al., 2021). Furthermore, Marques et al. investigated the neuroprotective effect of free curcumin and nanoemulsion-loaded curcumin in rats with ICH. The results show that two intraperitoneally injections of nano-emulsified curcumin at 24 and 48 hours after ICH improved the behavioral functions, reduced hematoma size, and modulated the antioxidant effect of ICH. The nanoemulsion-loaded curcumin also decreased the weight loss caused by ICH and showed no toxicity to the kidney and liver. The study concluded that nanoemulsion-loaded curcumin could be a promising formulation strategy for ICH treatment (Marques et al., 2020).

Until now, no studies are reported using siRNA-NP in the treatment of ICH. Therefore, more studies employing the use of siRNA-based NP are required. NP-based nanotechnology can help in progressing siRNA systemic delivery to the brain after ICH without any complications.

Conclusions

Gene silencing with siRNA represents a promising new therapeutic approach for ICH. The complex pathological cascade of primary and secondary injury phases after ICH poses roadblocks for finding effective therapy. siRNA has the potential to target any ICH-related genes, which represent a new therapeutic class for the treatment of ICH. However, several challenges are associated with siRNA delivery into the brain. Thus, in this review, we discussed the molecular mechanisms of siRNA and the barriers to siRNA delivery to the brain. We focused on the different strategies, including viral and nonviral vectors, to overcome the challenges and improve siRNA delivery following systemic or spot administration.

Moreover, we highlighted the most recent research related to NP-based nanotechnology delivery system of siRNA in various CNS diseases and ICH. We also discussed the current research status of NP-based nanotechnology for ICH treatment. Further studies on both viral and nonviral vector safety and toxicity to deliver siRNA into the brain are required to develop new methods to target specific proteins in the brain. Therefore, we projected siRNA therapeutics as a viable strategy for providing beneficial effects by decreasing neurologic dysfunctions associated with primary and secondary injury following ICH.

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designed figures, performed data search and collection. SHSB wrote the nanoparticle-based technology part. MA, AK, DT, and RAS contributed to different portions of the manuscript. ZAS performed critical review and corrections, and proposed changes. All authors approved the final version of the manuscript.

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References

Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ (2010) Structure and function of the blood–brain barrier. Neurobiol Dis 37:13-25.

Agarwal S, Simon AR, Goel V, Habtemariam BA, Clausen VA, Kim JB, Robbie GJ (2020) Pharmacokinetics and pharmacodynamics of the small interfering ribonucleic acid, givosiran, in patients with acute hepatic porphyria. Clin Pharmacol Ther 108:63-72.

Akinc A, Maier MA, Manoharan M, Fitzgerald K, Jayaraman M, Barros S, Ansell S, Du X, Hope MJ, Madden TD (2019) The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. Nat Nanotechnol 14:1084-1087.

Alameh M, DeJesus D, Jean M, Darras V, Thibault M, Lavertu M, Buschmann MD, Merzouki A (2012) Low molecular weight chitosan nanoparticulate system at low N: P ratio for nontoxic polynucleotide delivery. Int J Nanomedicine 7:1399-1414.

Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ (2011) Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol 29:341-345.

An SJ, Kim TJ, Yoon BW (2017) Epidemiology, risk factors, and clinical features of intracerebral hemorrhage: an update. J Stroke 19:3-10.

Anderson JM, Shive MS (2012) Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Deliv Rev 64:72-82.

Araújo RVd, Santos SdS, Igne Ferreira E, Giarolla J (2018) New advances in general biomedical applications of PAMAM dendrimers. Molecules 23:2849.

Ayadi L, Galvanin A, Pichot F, Marchand V, Motorin Y (2019) RNA ribose methylation (2′-O-methylation): Occurrence, biosynthesis and biological functions. Biochim Biophys Acta Gene Regul Mech 1862:253-269.

Bachu RD, Chowdhury P, Al-Saedi ZH, Karla PK, Boddu SH (2018) Ocular drug delivery barriers—role of nanocarriers in the treatment of anterior segment ocular diseases. Pharmaceutics 10:28.

Banks WA (2016) From blood-brain barrier to blood-brain interface: new opportunities for CNS drug delivery. Nat Rev Drug Discov 15:275-292.

Borel F, Kay MA, Mueller C (2014) Recombinant AAV as a platform for translating the therapeutic potential of RNA interference. Mol Ther 22:692-701.

Bozzuto G, Molinari A (2015) Liposomes as nanomedical devices. Int J Nanomedicine 10:975-999.

Cao Y, Tan YF, Wong YS, Liew MWJ, Venkatraman S (2019) Recent advances in chitosan-based carriers for gene delivery. Mar Drugs 17:381.

Chen C, Mei H, Shi W, Deng J, Zhang B, Guo T, Wang H, Hu Y (2013) EGFP-EGF1-conjugated PLGA nanoparticles for targeted delivery of siRNA into injured brain microvascular endothelial cells for efficient RNA interference. PLoS One 8:e60860.

Chen S, Peng J, Sherchan P, Ma Y, Xiang S, Yan F, Zhao H, Jiang Y, Wang N, Zhang JH, Zhang H (2020a) TREM2 activation attenuates neuroinflammation and neuronal apoptosis via PI3K/Akt pathway after intracerebral hemorrhage in mice. J Neuroinflammation 17:168.

Chen W, Hu Y, Ju D (2020b) Gene therapy for neurodegenerative disorders: advances, insights and prospects. Acta Pharm Sin B 10:1347-1359.

Choudhury SR, Hudry E, Maguire CA, Sena-Esteves M, Breakefield XO, Grandi P (2017) Viral vectors for therapy of neurologic diseases. Neuropharmacology 120:63-80.

Chronopoulou L, Massimi M, Giardi MF, Cametti C, Devirgiliis LC, Dentini M, Palocci C (2013) Chitosan-coated PLGA nanoparticles: a sustained drug release strategy for cell cultures. Colloids Surf B Biointerfaces 103:310-317.

Conniot J, Talebian S, Simões S, Ferreira L, Conde J (2021) Revisiting gene delivery to the brain: silencing and editing. Biomater Sci 9:1065-1087.

Daka A, Peer D (2012) RNAi-based nanomedicines for targeted personalized therapy. Adv Drug Deliv Rev 64:1508-1521.

Dang L, Dong X, Yang J (2021) Influence of nanoparticle-loaded edaravone on postoperative effects in patients with cerebral hemorrhage. J Nanosci Nanotechnol 21:1202-1211.

Davis ME, Zuckerman JE, Choi CHJ, Seligson D, Tolcher A, Alabi CA, Yen Y, Heidel JD, Ribas A (2010) Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature 464:1067-1070.

Dizaj SM, Jafari S, Khosroushahi AY (2014) A sight on the current nanoparticle-based gene delivery vectors. Nanoscale Res Lett 9:252.

Dong Y, Siegwart DJ, Anderson DG (2019) Strategies, design, and chemistry in siRNA delivery systems. Adv Drug Deliv Rev 144:133-147.

Dua K, Wadhwa R, Singhvi G, Rapalli V, Shukla SD, Shastri MD, Gupta G, Satija S, Mehta M, Khurana N (2019) The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress. Drug Dev Res 80:714-730.

Eberling JL, Jagust WJ, Christine CW, Starr P, Larson P, Bankiewicz KS, Aminoff MJ (2008) Results from a phase I safety trial of hAADC gene therapy for Parkinson disease. Neurology 70:1980-1983.

Fu S, Luo X, Wu X, Zhang T, Gu L, Wang Y, Gao M, Cheng Y, Xie Z (2020) Activation of the melanocortin-1 receptor by NDP-MSH attenuates oxidative stress and neuronal apoptosis through PI3K/Akt/Nrf2 pathway after intracerebral hemorrhage in mice. Oxid Med Cell Longev doi:10.1155/2020/8864100.

Fukuta T, Ishii T, Asai T, Sato A, Kikuchi T, Shimizu K, Minamino T, Oku N (2015) Treatment of stroke with liposomal neuroprotective agents under cerebral ischemia conditions. Eur J Pharm Biopharm 97:1-7.

Gan Y, Jing Z, Stetler RA, Cao G (2013) Gene delivery with viral vectors for cerebrovascular diseases. Front Biosci (Elite Ed) 5:188-203.

Gao Y, Wang Z-Y, Zhang J, Zhang Y, Huo H, Wang T, Jiang T, Wang S (2014) RVG-peptide-linked trimethylated chitosan for delivery of siRNA to the brain. Biomacromolecules 15:1010-1018.

Gargano G, Oliviero A, Oliva F, Maffulli N (2021) Small interfering RNAs in tendon homeostasis. Br Med Bull doi: 10.1093/bmb/ldaa040 .

Gavrilov K, Saltzman WM (2012) Therapeutic siRNA: principles, challenges, and strategies. Yale J Biol Med 85:187-200.

GBD 2016 Lifetime Risk of Stroke Collaborators, Feigin VL, Nguyen G, Cercy K, Johnson CO, Alam T, Parmar PG, Abajobir AA, Abate KH, Abd-Allah F, Abejie AN, Abyu GY, Ademi Z, Agarwal G, Ahmed MB, Akinyemi RO, Al-Raddadi R, Aminde LN, Amlie-Lefond C, Ansari H, et al. (2018) Global, regional, and country-specific lifetime risks of stroke, 1990 and 2016. N Engl J Med 379:2429-2437.

Gomes-da-Silva LgC, Fonseca NA, Moura V, Pedroso de Lima MC, Simões Sr, Moreira JoN (2012) Lipid-based nanoparticles for siRNA delivery in cancer therapy: paradigms and challenges. Acc Chem Res 45:1163-1171.

Gomes MJ, Fernandes C, Martins S, Borges F, Sarmento B (2017) Tailoring lipid and polymeric nanoparticles as siRNA carriers towards the blood-brain barrier – from targeting to safe administration. J Neuroimmune Pharmacol 12:107-119.

Gosselet F, Loiola RA, Roig A, Rosell A, Culot M (2021) Central nervous system delivery of molecules across the blood-brain barrier. Neurochem Int 144:104952.

Gray SJ, Matagne V, Bachaboina L, Yadav S, Ojeda SR, Samulski RJ (2011) Preclinical differences of intravascular AAV9 delivery to neurons and glia: a comparative study of adult mice and nonhuman primates. Mol Ther 19:1058-1069.

Guo T, Guo Y, Gong Y, Ji J, Hao S, Deng J, Wang B (2019) An enhanced charge-driven intranasal delivery of nicardipine attenuates brain injury after intracerebral hemorrhage. Int J Pharm 566:46-56.

Hajj KA, Whitehead KA (2017) Tools for translation: non-viral materials for therapeutic mRNA delivery. Nat Rev Mater 2:1-17.

Hardee CL, Arévalo-Soliz LM, Hornstein BD, Zechiedrich L (2017) Advances in non-viral DNA vectors for gene therapy. Genes 8:65.

Hassanpour S, Kim HJ, Saadati A, Tebon P, Xue C, van den Dolder FW, Thakor J, Baradaran B, Mosafer J, Baghbanzadeh A, de Barros NR, Hashemzaei M, Lee KJ, Lee J, Zhang S, Sun W, Cho HJ, Ahadian S, Ashammakhi N, Dokmeci MR, et al. (2020) Thrombolytic agents: nanocarriers in controlled release. Small 16:e2001647.

Hocquemiller M, Giersch L, Audrain M, Parker S, Cartier N (2016) Adeno-associated virus-based gene therapy for CNS diseases. Hum Gene Ther 27:478-496.

Hou Y, Hu Z, Gong X, Yang B (2020) HSPB8 overexpression prevents disruption of blood-brain barrier after intracerebral hemorrhage in rats through Akt/GSK3β/β-catenin signaling pathway. Aging 12:17568-17581.

Hu Q, Chen C, Khatibi NH, Li L, Yang L, Wang K, Han J, Duan W, Zhang JH, Zhou C (2011) Lentivirus-mediated transfer of MMP-9 shRNA provides neuroprotection following focal ischemic brain injury in rats. Brain Res 1367:347-359.

Huang BR, Chang PC, Yeh WL, Lee CH, Tsai CF, Lin C, Lin HY, Liu YS, Wu CYJ, Ko PY (2014) Anti-neuroinflammatory effects of the calcium channel blocker nicardipine on microglial cells: implications for neuroprotection. PLoS One 9:e91167.

Huang YY (2019) Approval of the first-ever RNAi therapeutics and its technological development history. Prog Biochem Biophys 46:313-322.

Hutson TH, Foster E, Moon LD, Yánez-Munoz RJ (2014) Lentiviral vector-mediated RNA silencing in the central nervous system. Hum Gene Ther Methods 25:14-32.

Ifediba MA, Medarova Z, Ng SW, Yang J, Moore A (2010) siRNA delivery to CNS cells using a membrane translocation peptide. Bioconjug Chem 21:803-806.

Jeong WJ, Bu J, Kubiatowicz LJ, Chen SS, Kim Y, Hong S (2018) Peptide–nanoparticle conjugates: a next generation of diagnostic and therapeutic platforms? Nano Converg 5:38.

Jin J, Zhou F, Zhu J, Zeng W, Liu Y (2020) MiR-26a inhibits the inflammatory response of microglia by targeting HMGA2 in intracerebral hemorrhage. J Int Med Res doi:10.1177/0300060520929615.

Jin J, Bae KH, Yang H, Lee SJ, Kim H, Kim Y, Joo KM, Seo SW, Park TG, Nam D-H (2011) In vivo specific delivery of c-Met siRNA to glioblastoma using cationic solid lipid nanoparticles. Bioconjug Chem 22:2568-2572.

Kim ID, Shin JH, Kim SW, Choi S, Ahn J, Han PL, Park JS, Lee JK (2012) Intranasal delivery of HMGB1 siRNA confers target gene knockdown and robust neuroprotection in the postischemic brain. Mol Ther 20:829-839.

Kim JY, Han Y, Lee JE, Yenari MA (2018) The 70-kDa heat shock protein (Hsp70) as a therapeutic target for stroke. Expert Opin Ther Targets 22:191-199.

Kuang Y, An S, Guo Y, Huang S, Shao K, Liu Y, Li J, Ma H, Jiang C (2013) T7 peptide-functionalized nanoparticles utilizing RNA interference for glioma dual targeting. Int J Pharm 454:11-20.

Kwon EJ, Skalak M, Lo Bu R, Bhatia SN (2016) Neuron-targeted nanoparticle for siRNA delivery to traumatic brain injuries. ACS Nano 10:7926-7933.

Leclerc JL, Blackburn S, Neal D, Mendez NV, Wharton JA, Waters MF, Doré S (2015) Haptoglobin phenotype predicts the development of focal and global cerebral vasospasm and may influence outcomes after aneurysmal subarachnoid hemorrhage. Proc Natl Acad Sci U S A 112:1155-1160.

Leclerc JL, Santiago-Moreno J, Dang A, Lampert AS, Cruz PE, Rosario AM, Golde TE, Doré S (2018) Increased brain hemopexin levels improve outcomes after intracerebral hemorrhage. J Cereb Blood Flow Metab 38:1032-1046.

Lee JM, Yoon TJ, Cho YS (2013) Recent developments in nanoparticle-based siRNA delivery for cancer therapy. Biomed Res Int doi:10.1155/2013/782041.

LeWitt PA, Rezai AR, Leehey MA, Ojemann SG, Flaherty AW, Eskandar EN, Kostyk SK, Thomas K, Sarkar A, Siddiqui MS (2011) AAV2-GAD gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial. Lancet Neurol 10:309-319.

Li F, Yang B, Li T, Gong X, Zhou F, Hu Z (2019) HSPB8 over-expression prevents disruption of blood-brain barrier by promoting autophagic flux after cerebral ischemia/reperfusion injury. J Neurochem 148:97-113.

Li W, Wang Y (2017) Angiogenesis and Neurogenesis after ischemic stroke. In: Translational research in stroke (, ed), pp297-317. Berlin:Springer.

Li Z, Tan S, Li S, Shen Q, Wang K (2017) Cancer drug delivery in the nano era: an overview and perspectives (Review). Oncol Rep 38:611-624.

Liu C, Zhang N (2011) Nanoparticles in gene therapy: principles, prospects, and challenges. In: Progress in molecular biology and translational science (Lapchak PA, Yang GY, eds), pp509-562. Amsterdam:Elsevier.

Liu D, Mao P, Huang Y, Liu Y, Liu X, Pang X, Li Y (2014) Proteomic analysis of lung tissue in a rat acute lung injury model: identification of PRDX1 as a promoter of inflammation. Mediators Inflamm doi:10.1155/2014/469358.

Lu Y, Zhang XS, Zhou XM, Gao YY, Chen CL, Liu JP, Ye ZN, Zhang ZH, Wu LY, Li W (2019) Peroxiredoxin 1/2 protects brain against H2O2-induced apoptosis after subarachnoid hemorrhage. FASEB J 33:3051-3062.

Malhotra M, Tomaro-Duchesneau C, Saha S, Prakash S (2013) Intranasal, siRNA delivery to the brain by TAT/MGF tagged PEGylated chitosan nanoparticles. J Pharm (Cairo) doi:10.1155/2013/812387.

Mann AP, Scodeller P, Hussain S, Joo J, Kwon E, Braun GB, Molder T, She ZG, Kotamraju VR, Ranscht B, Krajewski S, Teesalu T, Bhatia S, Sailor MJ, Ruoslahti E (2016) A peptide for targeted, systemic delivery of imaging and therapeutic compounds into acute brain injuries. Nat Commun 7:11980.

Manservigi R, Argnani R, Marconi P (2010) HSV recombinant vectors for gene therapy. Open Virol J 4:123-156.

Manzari MT, Shamay Y, Kiguchi H, Rosen N, Scaltriti M, Heller DA (2021) Targeted drug delivery strategies for precision medicines. Nat Rev Mater 6:351-370.

Mao L, Li P, Zhu W, Cai W, Liu Z, Wang Y, Luo W, Stetler RA, Leak RK, Yu W, Gao Y, Chen J, Chen G, Hu X (2017) Regulatory T cells ameliorate tissue plasminogen activator-induced brain haemorrhage after stroke. Brain 140:1914-1931.

Marques M, Cordeiro M, Marinho M, Vian C, Vaz G, Alves B, Jardim R, Hort M, Dora C, Horn A (2020) Curcumin-loaded nanoemulsion improves haemorrhagic stroke recovery in wistar rats. Brain Res 1746:147007.
Marquez AR, Madu CO, Lu Y (2018) An overview of various carriers for siRNA delivery. Oncomedicine 3:48-58 .
Marqus S, Pirogova E, Piva TJ (2017) Evaluation of the use of therapeutic peptides for cancer treatment. J Biomed Sci 24:21.
Martinez-Veracoechea F, Frenkel D (2012) Designing super-selectivity in multivalent nano-particle binding. APS 2012:X39.002.
Mendonça MCP, Radaic A, Garcia-Fossa F, da Cruz-Höfling MA, Vinolo MAR, de Jesus MB (2020) The in vivo toxicological profile of cationic solid lipid nanoparticles. Drug Deliv Transl Res 10:34-42.
Merkel SF, Andrews AM, Lutton EM, Mu D, Hudry E, Hyman BT, Maguire CA, Ramirez SH (2017) Trafficking of adeno-associated virus vectors across a model of the blood–brain barrier; a comparative study of transcytosis and transduction using primary human brain endothelial cells. J Neurochem 140:216-230.
Monty MA, Islam MA, Nan X, Tan J, Tuhin IJ, Tang X, Miao M, Wu D, Yu L (2021) Emerging role of RNA interference in immune cells engineering and its therapeutic synergism in immunotherapy. Br J Pharmacol 178:1741-1755.
Naso MF, Tomkowicz B, Perry WL, 3rd, Strohl WR (2017) Adeno-Associated Virus (AAV) as a vector for gene therapy. BioDrugs 31:317-334.
Natesan S, Boddu SH, Krishnaswami V, Shahwan M (2020) The role of nano-ophthalmology in treating dry eye disease. Pharm Nanotechnol 8:258-289 .
Nayerossadat N, Maedeh T, Ali PA (2012) Viral and nonviral delivery systems for gene delivery. Adv Biomed Res 1:27.
Neuberg P, Kichler A (2014) Recent developments in nucleic acid delivery with polyethylenimines. In: Advances in genetics (Kumar D, ed), pp263-288. Amsterdam:Elsevier.
Neve RL (2012) Overview of gene delivery into cells using HSV-1-based vectors. Curr Protoc Neurosci doi: 10.1002/0471142301.ns0412s61..
Nidetz NF, McGee MC, Tse LV, Li C, Cong L, Li Y, Huang W (2020) Adeno-associated viral vector-mediated immune responses: Understanding barriers to gene delivery. Pharmacol Ther 207:107453.
Nitta SK, Numata K (2013) Biopolymer-based nanoparticles for drug/gene delivery and tissue engineering. Int J Mol Sci 14:1629-1654.
Oller-Salvia B, Teixidó M, Giralt E (2013) From venoms to BBB shuttles: synthesis and blood–brain barrier transport assessment of apamin and a nontoxic analog. Biopolymers 100:675-686.
Ozpolat B, Sood AK, Lopez-Berestein G (2014) Liposomal siRNA nanocarriers for cancer therapy. Adv Drug Deliv Rev 66:110-116.
Pandit J, Sultana Y, Aqil M (2017) Chitosan-coated PLGA nanoparticles of bevacizumab as novel drug delivery to target retina: optimization, characterization, and in vitro toxicity evaluation. Artif Cells Nanomed Biotechnol 45:1397-1407.
Patil S, Gao YG, Lin X, Li Y, Dang K, Tian Y, Zhang WJ, Jiang SF, Qadir A, Qian AR (2019) The development of functional non-viral vectors for gene delivery. Int J Mol Sci 20:5491.
Peng T, Britton GL, Kim H, Cattano D, Aronowski J, Grotta J, McPherson DD, Huang SL (2013) Therapeutic time window and dose dependence of xenon delivered via echogenic liposomes for neuroprotection in stroke. CNS Neurosci Ther 19:773-784.
Piguet F, de Saint Denis T, Audouard E, Beccaria K, André A, Wurtz G, Schatz R, Alves S, Sevin C, Zerah M (2021) The challenge of gene therapy for neurological diseases: strategies and tools to achieve efficient delivery to the central nervous system. Hum Gene Ther 32:349-374.
Prchal JT (2017) Ironing out the role of hepcidin in infection. Blood 130:233-234.
Raftery R, O'brien FJ, Cryan SA (2013) Chitosan for gene delivery and orthopedic tissue engineering applications. Molecules 18:5611-5647.
Rajashekar D, Liang JW (2021) Intracerebral Hemorrhage. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK553103/.
Ramachandran PS, Keiser MS, Davidson BL (2013) Recent advances in RNA interference therapeutics for CNS diseases. Neurotherapeutics 10:473-485.
Ramos-Cabrer P, Campos F (2013) Liposomes and nanotechnology in drug development: focus on neurological targets. Int J Nanomedicine 8:951-960.
Ren Y, Hauert S, Lo JH, Bhatia SN (2012) Identification and characterization of receptor-specific peptides for siRNA delivery. ACS Nano 6:8620-8631.
Resnier P, Montier T, Mathieu V, Benoit J-P, Passirani C (2013) A review of the current status of siRNA nanomedicines in the treatment of cancer. Biomaterials 34:6429-6443.
Rhim T, Lee DY, Lee M (2013) Drug delivery systems for the treatment of ischemic stroke. Pharm Res 30:2429-2444.
Rogers AK, Phillips CM (2020) RNAi pathways repress reprogramming of C. elegans germ cells during heat stress. Nucleic Acids Res 48:4256-4273.
Rungta RL, Choi HB, Lin PJ, Ko RW, Ashby D, Nair J, Manoharan M, Cullis PR, MacVicar BA (2013) Lipid nanoparticle delivery of siRNA to silence neuronal gene expression in the brain. Mol Ther Nucleic Acids 2:e136.
Rungta RL, Choi HB, Tyson JR, Malik A, Dissing-Olesen L, Lin PJ, Cain SM, Cullis PR, Snutch TP, MacVicar BA (2015) The cellular mechanisms of neuronal swelling underlying cytotoxic edema. Cell 161:610-621.
Sava V, Fihurka O, Khvorova A, Sanchez-Ramos J (2020) Enriched chitosan nanoparticles loaded with siRNA are effective in lowering Huntington's disease gene expression following intranasal administration. Nanomedicine 24:102119.
Schroeder A, Levins CG, Cortez C, Langer R, Anderson DG (2010) Lipid-based nanotherapeutics for siRNA delivery. J Intern Med 267:9-21.
Scott LJ (2020) Givosiran: first approval. Drugs 80:335-339.
Shakeri S, Ashrafizadeh M, Zarrabi A, Roghanian R, Afshar EG, Pardakhty A, Mohammadinejad R, Kumar A, Thakur VK (2020) Multifunctional polymeric nanoplatforms for brain diseases diagnosis, therapy and theranostics. Biomedicines 8:13.
Shao Z, Tu S, Shao A (2019) Pathophysiological mechanisms and potential therapeutic targets in intracerebral hemorrhage. Front Pharmacol 10:1079.
Sharma N, Madan P, Lin S (2016) Effect of process and formulation variables on the preparation of parenteral paclitaxel-loaded biodegradable polymeric nanoparticles: a co-surfactant study. Asian J Pharm Sci 11:404-416.
Siaw-Debrah F, Nyanzu M, Ni H, Lin X, Xu Z, Ruan L, Zhuge Q, Huang L (2017) Preclinical studies and translational applications of intracerebral hemorrhage. Biomed Res Int doi:10.1155/2017/5135429.
Singha K, Namgung R, Kim WJ (2011) Polymers in small-interfering RNA delivery. Nucleic Acid Ther 21:133-147.
Smith A, McCulloh RJ (2015) Hemopexin and haptoglobin: allies against heme toxicity from hemoglobin not contenders. Front Physiol 6:187.
Song M-S, Rossi JJ (2017) Molecular mechanisms of Dicer: endonuclease and enzymatic activity. Biochem J 474:1603-1618.
Souweidane MM, Fraser JF, Arkin LM, Sondhi D, Hackett NR, Kaminsky SM, Heier L, Kosofsky BE, Worgall S, Crystal RG (2010) Gene therapy for late infantile neuronal ceroid lipofuscinosis: neurosurgical considerations. J Neurosurg Pediatr 6:115-122.
Stenström P, Manzanares D, Zhang Y, Ceña V, Malkoch M (2018) Evaluation of amino-functional polyester dendrimers based on Bis-MPA as nonviral vectors for siRNA delivery. Molecules 23:2028.
Szwargulski P, Wilmes M, Javidi E, Thieben F, Graeser M, Koch M, Gruettner C, Adam G, Gerloff C, Magnus T (2020) Monitoring intracranial cerebral hemorrhage using multicontrast real-time magnetic particle imaging. ACS Nano 14:13913-13923.
Tai W, Gao X (2017) Functional peptides for siRNA delivery. Adv Drug Deliv Rev 110:157-168.
Takahashi T, Nakano Y, Ui-Tei K (2018) Current status for application of RNA interference technology as nucleic acid drug. In: Gene Expression and Regulation in Mammalian Cells: Transcription From General Aspects (Uchiumi F, ed), pp149. London: IntechOpen Limited.
Takahiko I, Sena I, Tasuku H, Hideko N, Nakamura S, Masamitsu S, Hideaki H (2019) Intracellular Fe 2+ accumulation in endothelial cells and pericytes induces blood-brain barrier dysfunction in secondary brain injury after brain hemorrhage. Sci Rep 9:6228.
Teleanu DM, Negut I, Grumezescu V, Grumezescu AM, Teleanu RI (2019) Nanomaterials for drug delivery to the central nervous system. Nanomaterials 9:371.
Torrecilla J, Rodríguez-Gascón A, Solinís MÁ, del Pozo-Rodríguez A (2014) Lipid nanoparticles as carriers for RNAi against viral infections: current status and future perspectives. Biomed Res Int 2014:161794.
Tschoe C, Bushnell CD, Duncan PW, Alexander-Miller MA, Wolfe SQ (2020) Neuroinflammation after intracerebral hemorrhage and potential therapeutic targets. J Stroke 22:29-46.
van Asch CJ, Luitse MJ, Rinkel GJ, van der Tweel I, Algra A, Klijn CJ (2010) Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis. Lancet Neurol 9:167-176.
Vannucci L, Lai M, Chiuppesi F, Ceccherini-Nelli L, Pistello M (2013) Viral vectors: a look back and ahead on gene transfer technology. New Microbiol 36:1-22.
Varghese AM, Ang C, Dimaio CJ, Javle MM, Gutierrez M, Yarom N, Stemmer SM, Golan T, Geva R, Semenisty V (2020) A phase II study of siG12D-LODER in combination with chemotherapy in patients with locally advanced pancreatic cancer (PROTACT). J Clin Oncol 38:15_suppl TPS4672.
Verma A, Stellacci F (2010) Effect of surface properties on nanoparticle–cell interactions. Small 6:12-21.
Villar-Alvarez E, Leal BH, Martinez-Gonzalez R, Pardo A, Al-Qadi S, Juárez J, Váldez MA, Cambón A, Barbosa S, Taboada P (2019) SiRNA silencing by chemically modified biopolymeric nanovectors. ACS Omega 4:3904-3921.
Wang D, Tai PWL, Gao G (2019) Adeno-associated virus vector as a platform for gene therapy delivery. Nat Rev Drug Discov 18:358-378.
Wang L, Zheng S, Zhang L, Xiao H, Gan H, Chen H, Zhai X, Liang P, Zhao J, Li Y (2020) Histone deacetylation 10 alleviates inflammation after intracerebral hemorrhage via the PTPN22/NLRP3 pathway in rats. Neuroscience 432:247-259.
Wang Y, Li P, Kong L (2013) Chitosan-modified PLGA nanoparticles with versatile surface for improved drug delivery. Aaps Pharmscitech 14:585-592.
Wang Y, Li SY, Shen S, Wang J (2018) Protecting neurons from cerebral ischemia/reperfusion injury via nanoparticle-mediated delivery of an siRNA to inhibit microglial neurotoxicity. Biomaterials 161:95-105.
White E, Bienemann A, Megraw L, Bunnun C, Gill S (2011) Evaluation and optimization of the administration of a selectively replicating herpes simplex viral vector to the brain by convection-enhanced delivery. Cancer Gene Ther 18:358-369.
Wilkinson DA, Pandey AS, Thompson BG, Keep RF, Hua Y, Xi G (2018) Injury mechanisms in acute intracerebral hemorrhage. Neuropharmacology 134:240-248.
Wold WS, Toth K (2013) Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. Curr Gene Ther 13:421-433.
Wu X, Fu S, Liu Y, Luo H, Li F, Wang Y, Gao M, Cheng Y, Xie Z (2019) NDP-MSH binding melanocortin-1 receptor ameliorates neuroinflammation and BBB disruption through CREB/Nr4a1/NF-κB pathway after intracerebral hemorrhage in mice. J Neuroinflammation 16:192.
Xia Y, Tian J, Chen X (2016) Effect of surface properties on liposomal siRNA delivery. Biomaterials 79:56-68.
Xiang Y, Oo NNL, Lee JP, Li Z, Loh XJ (2017) Recent development of synthetic nonviral systems for sustained gene delivery. Drug Discov Today 22:1318-1335.
Yang G, Hu R, Zhang C, Qian C, Luo QQ, Yung WH, Ke Y, Feng H, Qian ZM (2016) A combination of serum iron, ferritin and transferrin predicts outcome in patients with intracerebral hemorrhage. Sci Rep 6:1-10.
Yang G, Qian C, Zhang C, Bao Y, Liu MY, Jiang F, Li W, Liu Y, Ke Y, Qian ZM (2021) Hepcidin attenuates the iron-mediated secondary neuronal injury after intracerebral hemorrhage in rats. Transl Res 229:53-68.
Yang GQ, Huang JC, Yuan JJ, Zhang Q, Gong CX, Chen Q, Xie Q, Xie LX, Chen R, Qiu ZM, Zhou K, Xu R, Jiang GH, Xiong XY, Yang QW (2020) Prdx1 reduces intracerebral hemorrhage-induced brain injury via targeting inflammation- and apoptosis-related mRNA stability. Front Neurosci 14:181.
Yonezawa S, Koide H, Asai T (2020) Recent advances in siRNA delivery mediated by lipid-based nanoparticles. Adv Drug Deliv Rev 154-155:64-78.
Yu S, Xu X, Feng J, Liu M, Hu K (2019) Chitosan and chitosan coating nanoparticles for the treatment of brain disease. Int J Pharm 560:282-293.
Yuan X, Naguib S, Wu Z (2011) Recent advances of siRNA delivery by nanoparticles. Expert Opin Drug Deliv 8:521-536.
Zeng J, Chen Y, Ding R, Feng L, Fu Z, Yang S, Deng X, Xie Z, Zheng S (2017) Isoliquiritigenin alleviates early brain injury after experimental intracerebral hemorrhage via suppressing ROS- and/or NF-κB-mediated NLRP3 inflammasome activation by promoting Nrf2 antioxidant pathway. J Neuroinflammation 14:119.
Zhang H, Yang B, Mu X, Ahmed SS, Su Q, He R, Wang H, Mueller C, Sena-Esteves M, Brown R (2011) Several rAAV vectors efficiently cross the blood–brain barrier and transduce neurons and astrocytes in the neonatal mouse central nervous system. Mol Ther 19:1440-1448.
Zhang N, He L, Wu W (2021) Self-assembling peptide nanofibrous scaffolds in central nervous system lesions. In: Nanomedicines for brain drug delivery (Morales J, Gaillard P, ed), pp103-117. Berlin: Springer.
Zhang X, Yang X, Ji J, Liu A, Zhai G (2016) Tumor targeting strategies for chitosan-based nanoparticles. Colloids Surf B Biointerfaces 148:460-473.
Zhang Y, Friedlander R (2011) Using non-coding small RNAs to develop therapies for Huntington's disease. Gene Ther 18:1139-1149.
Zhang Y, Han B, He Y, Li D, Ma X, Liu Q, Hao J (2017) MicroRNA-132 attenuates neurobehavioral and neuropathological changes associated with intracerebral hemorrhage in mice. Neurochem Int 107:182-190.
Zhao K, Zhang Y, Zhang X, Shi C, Wang X, Wang X, Jin Z, Cui S (2014) Chitosan-coated poly (lactic-co-glycolic) acid nanoparticles as an efficient delivery system for Newcastle disease virus DNA vaccine. Int J Nanomedicine 9:4609-4619.
Zhao W, Wu C, Stone C, Ding Y, Ji X (2020) Treatment of intracerebral hemorrhage: Current approaches and future directions. J Neurol Sci 416:117020.
Zheng M, Tao W, Zou Y, Farokhzad OC, Shi B (2018) Nanotechnology-based strategies for siRNA brain delivery for disease therapy. Trends Biotechnol 36:562-575.
Zhou Y, Peng Z, Seven ES, Leblanc RM (2018) Crossing the blood-brain barrier with nanoparticles. J Control Release 270:290-303.
Zhu Q, Enkhjargal B, Huang L, Zhang T, Sun C, Xie Z, Wu P, Mo J, Tang J, Xie Z, Zhang JH (2018) Aggf1 attenuates neuroinflammation and BBB disruption via PI3K/Akt/NF-κB pathway after subarachnoid hemorrhage in rats. J Neuroinflammation 15:178.
Zhu Y, Liu C, Pang Z (2019) Dendrimer-based drug delivery systems for brain targeting. Biomolecules 9:790.
Zinn E, Vandenberghe LH (2014) Adeno-associated virus: fit to serve. Curr Opin Virol 8:90-97.

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