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

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, siRNA 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 nanocarrier; nanoparticles; nanotechnology; nonviral vectors; peptide-mediated nanocarrier; polymer-based nanocarrier; 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 mortality 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
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). Argonaute 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.

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 nucleas 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).

Potential delivery systems for siRNA include viral and nonviral delivery systems. Viral vectors are preferred due to their ability to transfect 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.

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 siRNA expression with target-specific gene knockdown in vivo. Lentivirus (LV), adenovirus (AV), adenovirus-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 disease (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). LVs are well tolerated 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 discovered a significant reduction 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, AAV 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. AAV- induced Hpx overexpression in the brain decreased hematoma volume, lipid peroxidation, astrocyte activation, and improved neurological deficits suggesting a clinical-grade possibility of AAV-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 AAV 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 ampiclon vectors. Recombinant HSV vectors have been used in clinical trials to treat brain tumors, whereas ampiclon HSV vectors showed promising results only in animal studies (Manservig et al., 2010). HSV’s were studied and modified in various disease conditions targeting the potential molecules of interest. HSV-mediated overexpression of glucose transporter 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 limits their use as siRNA delivery systems. 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 is a significant challenge (Shao et al., 2010). Currently, AAV 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.

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### Table 1

| LV | AV | AAV |
|----|----|-----|
| virus | vector | vector |
| LV vectors use for siRNA delivery | | |
Table 1  |  Advantages and disadvantages of viral vectors

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

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

| Nonviral vectors | Advantages                                                                 | Disadvantages                                                                                     | References                           |
|-----------------|----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|--------------------------------------|
| Polymer-based-nanoparticles (Chitosan) | - Biodegradable                                                           | - High molecular weight limited their use in clinical trials                                   | Alameh et al., 2012; Lee et al., 2013 |
|                 | - Biocompatible                                                           | - Low solubility at physiological pH                                                              |                                      |
|                 | - Low toxicity                                                            |                                                                                                   |                                      |
|                 | - Have several free amine groups that are available for crosslinking      |                                                                                                   |                                      |
|                 | - Improve membrane absorption and cellular interaction                     |                                                                                                   |                                      |
|                 | - Low cost of production                                                  |                                                                                                   |                                      |
| Polymer-based-nanoparticles (PLGA) | - Biodegradable                                                           | - 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 |
|                 | - Biocompatible                                                           | - The high cost of production                                                                   |                                      |
|                 | - Nontoxic                                                                | - Low solubility                                                                                 |                                      |
|                 | - FDA approved                                                            |                                                                                                   |                                      |
|                 | - High solubility                                                          |                                                                                                   |                                      |
|                 | - Prolong controlled release                                               |                                                                                                   |                                      |
|                 | - Improve endosomal escape                                                 |                                                                                                   |                                      |
| Peptide-mediated nanoparticles | - Low toxicity                                                             | - Immunogenicity                                                                                 | Martinez-Veracochea and Frenkel, 2012; Marqus et al., 2017; Jeong et al., 2018 |
|                 | - High binding strength (high potency)                                     | - The high cost of production                                                                   |                                      |
|                 | - High target selectivity                                                  | - Low solubility                                                                                 |                                      |
|                 | - Increase circulation time                                                |                                                                                                   |                                      |
|                 | - Protect from RNase degradation                                           |                                                                                                   |                                      |
| Dendrimers | - Biodegradable                                                           | - Toxicity of cationic dendrimer with terminal poly(L-lysine) and poly(propylene imine) group     | Li et al., 2017; Zhu et al., 2019     |
|                 | - Monodispersity                                                          |                                                                                                   |                                      |
|                 | - Higher water solubility                                                  |                                                                                                   |                                      |
|                 | - Ability to link drugs                                                   |                                                                                                   |                                      |
|                 | - Encapsulation ability                                                    |                                                                                                   |                                      |
|                 | - Presence of surface functionalizable groups like –NH₂, –COOH, and –OH groups |                                                                                                   |                                      |
| Lipid-based nanoparticles (Solid lipid nanoparticles) | - Biodegradable                                                           | - The high cost of production                                                                   | Liu and Zhang, 2011b; Dizaj et al., 2014b |
|                 | - Biocompatible                                                           | - Toxicity at high dose                                                                          |                                      |
|                 | - Low toxicity                                                            |                                                                                                   |                                      |
|                 | - Ease of production without organic solvents                             |                                                                                                   |                                      |
|                 | - Ease of large-scale production                                           |                                                                                                   |                                      |
|                 | - Good storage stabilities                                                 |                                                                                                   |                                      |
| Lipid-based nanoparticles (Liposomes) | - Biodegradable                                                           | - The high cost of production                                                                   | Liu and Zhang, 2011a; Ramos-Cabrera and Campos, 2013; Ozpolar et al., 2014; Xia et al., 2016 |
|                 | - Biocompatible                                                           | - Toxicity at high dose                                                                          |                                      |
|                 | - Nonotoxic                                                               |                                                                                                   |                                      |
|                 | - Low immunogenicity                                                       |                                                                                                   |                                      |
|                 | - Ease of large-scale production                                           |                                                                                                   |                                      |
|                 | - FDA approved (Onpattro®)                                                 |                                                                                                   |                                      |
|                 | - Prolong controlled release                                               |                                                                                                   |                                      |
|                 | - Improve endosomal escape                                                 |                                                                                                   |                                      |
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., 2015). NPs 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 hold 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 surface modification, entrapment, conjugation to 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
Polymer NPs have shown to be promising carriers for siRNA brain delivery. Polymer 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 (Teleau et al., 2019). The only FDA-approved polymers employed to design NPs include natural polymers such as 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-ODER 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 and 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 mixed findings about 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 anti-atherosclerotic and neuroprotective effects in animal stroke models (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 complications, 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 another 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., 2020), and enhances cellular uptake. 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 (Teleau et al., 2019). The only FDA-approved polymers employed to design NPs include natural polymers such as 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-ODER 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.

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 (Oliver-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, endothose interruption, and targeted delivery, qualities that are critical for the targeted delivery of siRNA. Fiediba et al. (2010) used complexing fluorescent-labeled methylated polyarginine peptides to siRNA for entry into a liposomal factor complex that was complexed 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 (Iedriba 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 a polyethylene glycol (PEG)–PEI conjugate enhanced the antiviral activity of the drug.PEG–PEI conjugate enhanced the antiviral activity of the drug.

siRNA delivery to the brain and, in particular, to ICH. More work is needed to improve the potential of siRNA delivery systems for treating neurological diseases (Malhotra et al., 2013). Another delivery method involves intranasal administration. The results indicate efficient delivery of siRNA to the brain with no apparent systemic toxicity or immune response (Rungta et al., 2015). These studies highlight the potential of lipid-based NPs for siRNA delivery in CNS diseases. More studies are needed to prove clinically, the results from in vitro and in vivo studies extracellular space promise and provide hope for improved treatment in the future. Specifically, the intranasal 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.

NP systems for siRNA can help endosomal 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 transport (TP-RVG), and a targeting peptide, N-terminal targeting peptide (RVG) (Wong et al., 2016). This strategy has not been explored extensively in the brain after injection and remained intact after crossing the BBB. This dual functional ability was evaluated by QSH. This study concluded that CQ/siRNA crosses the BBB and remains intact after systemic administration. The results showed that the peptide treatment and drug delivery to the brain.

4.20% of target protein, p42-MAPK (Stenström et al., 2007). 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 +ve charged groups. Dendrimers can be delivered systemically or intranasally for the delivery of drugs to the brain.

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 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.

Dendrimers Dendrimers are star-shaped nanoconstructs 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 negative charges of these 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 (G0, G1, G2, G3, and G4 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 +ve 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, fluorescence-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 delivery (Kim et al., 2012). Similarly, CQ dendrimers were shown to increase their affinity towards transferrin receptor, highly expressed on both BBB and glioma, was a successful strategy for dual glioma targeting. G3 dendrimers (dendrified-grafted poly-l-lysine) surface modified with HAiYPHR (77 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 (G0–G4 generations) based on 2,2-bis(methyl)propionic acid (bis-MPA) for siRNA delivery in glioma (C6) and glioblastoma (U87) cell lines. No cytotoxicity was observed in astrocytes, C6, and U87. G1, G2, and G4 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 extracellular space promise and provide hope for improved treatment 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.

Cationic solid lipid nanoparticles Cationic solid lipid nanoparticles 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 (Dizaji 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. Because cationic NPs used in cationic solid lipid NPs are more hydrophobic and nontoxic. In recent studies, 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 examinations of the lungs, kidneys, 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 investigate the toxicity of cationic NPs 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, 2011). Liposomes have been widely studied 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-Cabrera 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 liposomes are typically stable, and the circulation time is longer than that of cationic 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 ephocnigen 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 (Akin 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. Szwarzugski et al. (2020) investigated whether MPI works in visualizing the hematoma expansion in ICH mice. After mice were subjected to intra-striatal collagease injection-induced ICH, an MRI-tailored tracitats 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 (Szwarzugski 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 had a beneficial effect on endogenous inflammatory mediators associated 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 intraperitoneal 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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